

Extracellular Vesicles as biomarkers of the regenerative mechanisms induced by rehabilitation after heart transplantation

Aurora Mangolini¹, Silvia Picciolini¹, Paolo Pedersini¹, Alice Gualerzi¹, Francesca Di Salvo¹, Anastasia Toccafondi¹, Valentina Mangolini^{1,2}, Francesca Rodà^{1,3}, Luana Forleo¹, Rita Martino¹, Martina Gerli¹, Stefano Faggiano¹, Nuccia Morici¹, Marzia Bedoni¹.

¹ IRCCS Fondazione Don Carlo Gnocchi ONLUS, Milano, Italy

² Dept. Molecular and Translational Medicine (DMMT), University of Brescia, Brescia, Italy

³ Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy

Extracellular Vesicles (EVs) are particles naturally released by different kind of cells. Evidences suggest their involvement in cell interactions both in physiological and pathological conditions, including the regenerative processes.

Surface Plasmon Resonance imaging (SPRi) is a sensitive analytical technique that has been successfully applied for the simultaneous detection of multiple subfamilies of EVs in real time, providing remarkable advantages thanks to multiplexing and high throughput features. Here we propose the study of extracellular vesicles by SPRi to identify specific biomarkers useful to characterize patient after heart transplantation (HT), in order to choose the proper and personalized cardiac rehabilitation strategies to promote their recovery.

26 patients after HT were recruited at the Cardiac Rehabilitation Unit of IRCCS Fondazione Don Gnocchi of Milan (Italy). Serum was collected at admission (T0) and after two-four weeks of rehabilitative treatment (T1). EVs were isolated from serum by size exclusion chromatography and their concentration was measured by Nanoparticle Tracking Analysis (NTA).

Preliminary NTA data showed no difference in the concentration and size of EVs before and after rehabilitation. Besides, the SPRi biosensor was optimized to detect EVs populations coming from endothelium, platelets, cardiomyocytes and macrophages. The aim of SPR experiments is the verification of possible changes occurring in their relative amount in the blood stream before and after cardiac rehabilitation. Additionally we plan to verify the expression of HSP60 on membrane of VCAM1+ EVs, as its localization to the cell surface, correlated with increased cardiac myocyte apoptosis, occurring in heart failure.

Experiments are still ongoing, but our preliminary data confirm that this method offers multiple advantages: EV phenotyping needs limited amount of sample and reagents thanks to the intrinsic SPR enhancing property of their membrane, moreover the potential presence of contaminants in EV samples doesn't represent a limitation, because during the SPRi experiment sample undergoes an additional purification step.

Taking advantage by all these features, we expect to exploit the potential prognostic application of EVs as biomarker useful in cardiac rehabilitation.

References:

1. "The Exercise and Heart Transplant (ENEAT) trial - a registry-based randomized controlled trial evaluating the safety and efficacy of cardiac telerehabilitation after heart transplant" Pedersini P, Picciolini S, Di Salvo F, Toccafondi A, Novembre G, Gualerzi A, Cusmano I, Garascia A, Tavanelli M, Verde A, Masciocco G, Ricci C, Mannini A, Bedoni M, Morici N. *Contemp Clin Trials*. 136,107415 (2024).