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Al collegio docenti del Dottorato in Medicina Molecolare

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Attività scientifica svolta nel 2° anno di Dottorato, Anno Accademico 2021/2022

INTRODUCTION:

Extracellular Vesicles (EVs) may be exploited for diagnostic or therapeutic purposes. In the first part of my PhD project I investigated the exosomal expression of a potential cancer biomarker, a carbonic anhydrase, under normoxic and hypoxic conditions.

In the second year of the PhD course, I focused on the characterization of Mesenchymal Stem Cells (MSC)-derived exosomes, which have been reported in literature to exert anti-inflammatory and anti-hypoxic effects in various disease models. Purified EVs will be tested to evaluate biological properties with 2/3 cell-based functional assays, related to regeneration, anti-inflammatory and/or tumor suppression.

MATERIALS AND METHODS:

EV isolation technique has an impact on the grade of purity of EVs and consequently on their biological effects. I compared the purification method established at Lonza, Size-Exclusion (SEC) chromatography, with density gradient ultracentrifugation (DG-UC), which is widely considered to be the gold-standard method for EV purification. EVs were characterized in terms of size, concentration, identity and integrity with single particle analysis instrument (NanoAnalyzer, NanoFCM), EV morphology and integrity by Transmission Electron Microscope (TEM), protein expression by Western Blotting, transcriptomic analysis of EV's cargo using HTG Edge Seq NGS platform.

RESULTS:

The particle size distribution analysis confirmed average size consistent with exosomes' reported size (50-100 nm). Integrity of EVs evaluated with TEM and with CFSE staining is consistent between the two purification methods (around 70%). Exosomal identity proteins evaluated by antibody staining and by Western Blot showed similar results for the two methods of purification, which confirmed the exosomal identity. RNA cargo from MSC-exosomes was analyzed for miRNA Whole Transcriptome Assay using HTG Edge Seq NGS. We found 187 miRNAs down-regulated and 1 miRNA up-regulated in exosomes purified with SEC, compared to exosomes from DG-UC.

SEMINARS ATTENDED DURING THE SECOND YEAR:

- *Webinar: GMP manufacturing for exosome-based therapies - the critical role of analytics and process design for clinical production and scale-up.* 28/09/2021. Dr. Marcos Langtry, Head of Allogeneic Cell Therapy Commercial Development-Lonza Pharma&Biotech; Dr. Davide Zocco, Research & Development Senior Manager-Lonza;
- *Development and optimization of 3D models: from culture to analysis.* 12/10/2021. Dr. Isha Dey, R&D Scientist, Biosciences; Dr. Bhaskar S. Mandavilli, PhD, Senior R&D Manager, Biosciences.
- *Minisimposi su Sperimentazione Animale in Biomedicina. Un percorso di scienza, storia, diritto, etica e medicina. "Dal topo all'uomo: aspetti evolutivi, fisiopatologici e traslazionali".* 29/10/2021. Università La Sapienza di Roma e Federico II di Napoli;

storia, diritto, etica e medicina. "Dal topo all'uomo: aspetti evolutivi, fisiopatologici e traslazionali". 29/10/2021. Università La Sapienza di Roma e Federico II di Napoli;

- *HypoxEU II*. 09/12/2021.
- *Avantor Webinar: Cell culture processes and cell types: from benchtop R&D to upscaled bioproduction*. Pär Larsson. 16/02/2022.
- *Vescicole extracellulari: dalla ricerca di base alle possibili applicazioni terapeutiche*. 23/03/2022. Prof.ssa Chiara Gentili, DIMES, University of Genova;
- *Webinar: Process development excellence to de-risk and accelerate commercialization of cell and gene therapies*. 05/05/2022. Dr. Behnam Ahmadian Baghbaderani, Global Head of Process Development Cell & Gene Technologies, Lonza.
- *Soft skill per i dottorandi: Ricerche bibliografiche e open access & science*. 19/05/2022. Silvia Aurigi- Cristina Mencarelli, UNISI.
- *Webinar: Stem Cell Culture - Make or Break / Find the Optimum for your MSCs and PSCs.* 09/06/2022. Dr. Jessica Wagener, Application Specialist Cell Handling, Eppendorf; Dr. Philipp Nold, Infield Application Specialist for Stem Cell, Bioprocess Center, Eppendorf.

SCIENTIFIC PUBLICATIONS:

- Marta Venturella, Mattia Criscuoli, Fabio Carraro, Antonella Naldini, Davide Zocco. *Interplay between Hypoxia and Extracellular Vesicles in Cancer and Inflammation.* Published 30/06/2021. Biology. 2021 Jul; 10(7): 606