UNIVERSITÀ
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Al collegio docenti del Dottorato in Medicina Molecolare

Candidato: Dr.ssa Marta Venturella

Tutor: Prof.ssa Antonella Naldini/ Dr. Davide Zocco

Ciclo XXXV

Attività scientifica svolta nel 1° anno di Dottorato, AA 2019/2020

INTRODUCTION

Extracellular vesicles (EVs) play a key role in intra- and intercellular communication, in health and disease. EVs contain biomolecules, such as proteins, DNA, mRNA, miRNA, ncRNA, protected by the double lipid membrane, that may be exploited for diagnostic or therapeutic purposes.
Hypoxia stress, the decreased oxygen availability, is a hallmark of many solid tumors and of the tumor microenvironment. My project's aim is to elucidate how hypoxia affects the production of EVs and their molecular cargo, especially hypoxia-induced proteins, in solid tumors. During the first year I was focused on analyzing the effects of hypoxia on three cancer cell lines, one human prostate cancer cell line, 22Rv1, and two human melanoma cell lines, A375 and SK-MEL-28.

MATERIALS AND METHODS

Within this year I performed Size-Exclusion Chromatography (SEC) to purify EVs from cell culture supernatants, obtained in normoxic and hypoxic conditions. I quantified EVs samples by Flow NanoAnalyzer instrument (NANOFCM), which allows the detection at a single-vesicle level. The EV's characterization was performed using different techniques: real time PCR and droplet digital PCR on the extracted RNA; ELISA assay and Western Blot assay for protein detection.

RESULTS

The particle size distribution analysis confirmed average nanoparticle size consistent with exosomes' reported size (70-120 nm). Exosome release from 22Rv1 cells increased under hypoxic condition. Instead, in melanoma cell lines the levels of exosomes released were similar under both the conditions. Interestingly, for A375 cell line I observed an increased expression of one target of interest under hypoxia. I demonstrated that this protein is associated to the membrane of exosomes, through a specific ELISA assay (this data will be confirmed by orthogonal methods). Further studies will investigate if A375-exosomes may have a functional effect on other cell lines, that have less invasive phenotype.

SEMINARS ATTENDED DURING THE FIRST YEAR:

- *Prolyl Hydroxylases: from oxygen sensors to therapeutic targets.* 7/10/2019. Prof. Cormac T. Taylor. University of Siena;
- *Impact of liquid biopsy in advanced HER2+ breast cancer tumors: a follow-up study.* 31/03/2020. Dr. Matteo Allegretti, PhD;
- *Accelerated Vaccine Research - Viral measurement by NanoFlowCytometry.* 15/04/2020. Dr. Ben Peacock, PhD, Senior Application Scientist at NanoFCM, Dr. Alice Law, PhD Application Scientist at NanoFCM;

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- *Droplet Digital™ PCR: Improved detection of EGFR mutations in cell-free plasma DNA from patients with non-small-cell lung cancer*. 21/04/2020. Dr. Maximilian Hochmair, Head of the Respiratory Oncology Unit in the Department of Respiratory and Critical Care Medicine at the Otto Wagner Hospital in Vienna;
- *La percezione pubblica della scienza: i giovani ricercatori di fronte a temi scientifici di forte interesse pubblico, politico e mediatico*. 27/04/2020-28/04/2020. Promoted by Prof. Elena Cattaneo, Department of Biosciences, University of Milan;
- *Modelli 3D: tecniche per coltura ed analisi di sferoidi e organoidi*. 05/05/2020. Dr. Alessandra Romano, Technical Sales Specialist CELL BIOLOGY ThermoFisher, Dr. Fabrizio Cozzani, Technical Sales Specialist IMAGING ThermoFisher;
- *Nano-Flow Cytometry: A Platform for Comprehensive EV Analysis*. 07/05/2020. Dr. Ben Peacock, PhD, Senior Application Scientist at NanoFCM, Dr. Alice Law, PhD Application Scientist at NanoFCM;
- *Covid-19: come si insegue e sconfigge un virus*. 28/05/2020-29/05/2020. Promoted by Prof. Elena Cattaneo, Department of Biosciences, University of Milan.

In June, July and September I attended the "Soft Skills for PhD" lessons, as provided by the Doctoral School program.

SCIENTIFIC PUBLICATIONS:

- Chiara Foroni, Natasa Zarovni, Laura Bianciardi, Simona Bernardi, Luca Triggiani, Davide Zocco, Marta Venturella, Antonio Chiesi, Francesca Valcamonico and Alfredo Berruti. *When less is more: specific capture and analysis of tumor exosomes in plasma increases the sensitivity of liquid biopsy for comprehensive detection of multiple androgen receptor phenotypes in advanced prostate cancer patients*. Published 22/05/2020. Biomedicines. 2020 May; 8(5): 13.

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