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Proteomic Characterization of Exosomes as a Source of Biomarkers in Clinical Management of Patients with Prostate Cancer (PREsEnCE)*

María García[1] , Esther Masiá[2] , Manuel M. Sánchez[3] , José Rubio[4] , Juan Casanova[4] , Miguel Ramírez[4] , David Charbonnier[2] , María Jesús Vicent[2] , Antonio Pineda[5] , José Antonio López[1]

1) Laboratory of Molecular Biology, Instituto Valenciano de Oncología (IVO) 2) Polymer Therapeutics Laboratory of Centro de Investigación Príncipe Felipe (CIPF) 3) Department of Biochemistry, University of Valencia 4) Service of Urology, Instituto Valenciano de Oncología (IVO) 5) Instituto de Investigación Sanitaria La Fe (IIS La Fe)

Prostate cancer (PCa) is the first most common cancer in men and sixth cause of cancer death. Around twenty-five thousand cases are diagnosed every year in Spain. PCa is diagnosed by prostate biopsy which is oriented by serum levels of PSA and DRE (Digital Rectal Examination). The PSA has improved detection and management of this disease. However, and even though the PSA is a specific biomarker of prostate it is not cancer, leading to overtreatment, overdiagnosis and increased unnecessary biopsies that cause a problem of Public Health. For these reasons, there is an urgent need of new biomarkers in PCa that improves the performance of PCa diagnosis identifying specifically not only cancer cases but also those cases with the worst prognosis. Exosomes are small vesicles (30-100nm) containing DNA, RNA, miRNA, lipids and proteins. That are secreted by cells into the circulation in the different biological fluids. They play an important role in cell communication, progression and metastatic ability of some tumors. For all these, extracellular vesicles EVs are an ideal source of cancer biomarkers.

With this project (BIOChiP) we aim to contribute to the optimization of the clinical management of PCa patient on the basis of the proteomic characterization of exosomes derived from tumor cells, with the propose of developing new non-invasive diagnostic procedures (liquid biopsy). This study is designed in three phases: a) Phase 1 (discovery) in which we will identify those cancer-specific biomarkers and associated with tumor progression. The groups of patients that we have used include patients with PCa with localized disease, advanced disease and controls. b) Phase 2 (validation); in this phase we will evaluate the diagnostic performance of the identified biomarkers and we will undertake a functional and biological characterization of the most relevant biomarkers: c) Phase 3 (prospective validation), the performance of all these biomarkers will be evaluated in specific clinical scenarios.

Sample collection and pre-processing is being carried out at IVO-Biobank following normalized working protocol and the patients have signed their informed consent for the donation of samples. Matched plasma and urine was obtained from 152 men distributed in the following groups: 1) localized disease (Gleason <7) (n=69) ; 2) localized disease (Gleason >= 7)(n=36); 3) advanced disease without treatment (n=18); 4) advanced disease with treatment and hormonosensitive (n=8); and 5) healthy controls (n=21). The exosome isolation was performed at CIPF using a working protocol for serial ultracentrifugation specifically focused for exosome isolation. The vesicles were characterized by Nanoparticle Tracking Analysis (NTA) using NanoSight NS300 for quantification of particle number. Western Blotting was used to test exosome markers such as CD9, CD81, CD63 and Alix. Finally, electron microscopy was used to measure and characterize extracellular vesicles (EVs) in some of the cases. Once EVs were purified, protein content was measured using Bradford procedure. A fraction containing 20 mg of proteins was submitted to the Proteomics facility of SCSIE University of Valencia for proteomic characterization. A total of 40 samples were analyzed (4 x each patient group in plasma and urine). The protocol used was SWATCH-MS. Analysis of urine samples identified a total 1251 proteins. In parallel, 300 proteins were defined in exosomes extracted from plasma. A series of 186 proteins, involved in biological pathways such as protein activation cascade, blood coagulation, response to stress, migration and secretion were identified in EVs obtained from both plasma and urine. Thirty-five proteins in urine and fifty-seven from plasma were differentially expressed between cancer groups and healthy controls; indicating a signature that

might distinguish between cancer and no cancer with an AUC=0.888. Similar sets of proteins were also identified between groups 1 and 2 (localized disease) versus healthy controls, suggesting that these set of proteins might constitute a panel of diagnostic biomarkers in PCa.

Despite these preliminary results, herein we are presenting very promising data on candidate biomarkers that might be used in the diagnostic setting of PCa. Currently we are validating these data on an independent series of patients.

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