Comparison between data- dependent analysis and data- indipendent analysis in EPS- urinary proteomics

Licia E. Prestagiacomo[a], Caterina Gabriele[a], Maria Antonietta Rota[b], Stefano Alba[b], Giovanni Cuda[a], Rocco Damiano[a], Marco Gaspari[a]

a) Department of Experimental and Clinical Medicine, Università degli studi Magna Graecia di Catanzaro, Italy;

b) Romolo Hospital, Rocca di Neto (KR), Italy

Abstract

Prostate cancer (PCa) is the second lethal cancer in men with 606.520 cancer deaths recorded in the United States in 2020 [1]. This pathology is silent and non-aggressive in some patients while in others shows a fast evolution and metastasis onset. Prostate cancer diagnosis, to date, is based on serum dosage of prostate specific antigen (PSA) and on digital rectal exam (DRE). Nevertheless, PSA has low specificity and does not help in discriminating between nonaggressive and aggressive disease.
In the effort of analysing urinary expressed prostatic secretion (EPS-urine) [2] to identify prostate cancer associated proteins, we tested two different label-free, MS-based approaches: data-dependent analysis (DDA) and data independent analysis (DIA).

Workflow

EPS-urine from PCa and benign prostatic hyperplasia (BPH) patients were digested using on filter digestion (FASP) and peptide mixtures were purified by sequential strong cation exchange (SCX) and C18 StageTips before LC-MS/MS analysis (Fig.1).

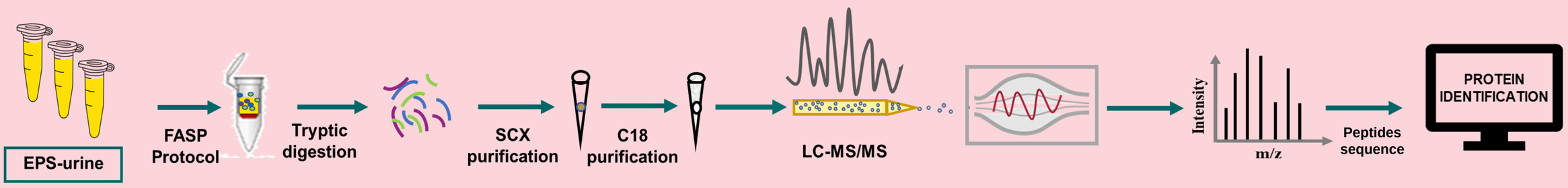

Fig.1: Workflow adopted for the analysis of EPS-urine samples.

DDA analysis

In the first phase, we have analysed 42 samples (20 PCa and 22 BPH) using DDA approach.
Raw files for label free quantification were processed by MaxQuant while statistical analysis was performed in Perseus.

VS

DIA analysis

To minimize the problem of missing values typical of DDA, we have analysed 80 (48 PCa and 32 BPH) samples using DIA approach. For spectral library generation, a pool of 22 EPS-urine samples was fractionated in 10 fractions by high pH reversed phase and analysed in DDA mode. DIA analysis was performed in Spectronaut 13,0.

Results

DDA analysis has cumulatively quantified 954 proteins in 42 samples. The list of identified proteins was compared to a panel of 135 prostate cancer-specific and tissue enriched proteins obtained from BioGPS (www.biogps.org) and Protein Atlas (www.proteinatlas.it) showing 36 common proteins (fig.3).

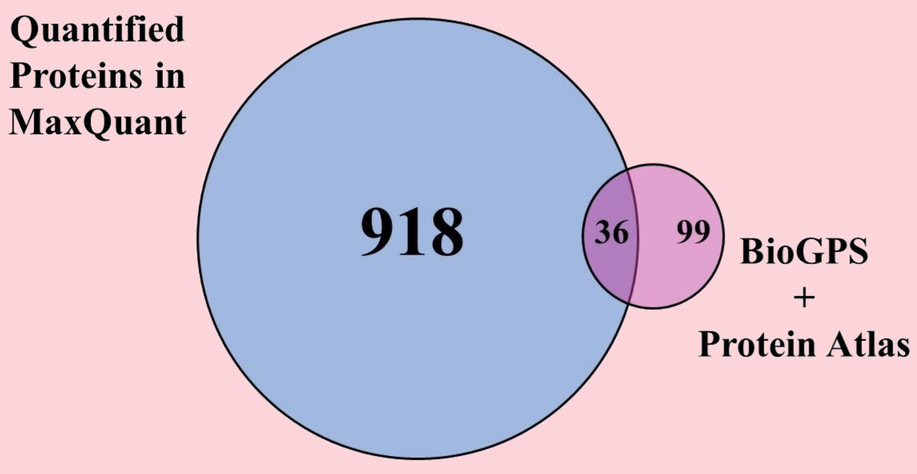

Fig.3: The comparison between the quantified proteins in MaxQuant and the panel of 135 proteins
.

DIA analysis led to the quantification of 1961 proteins in 80 samples showing a greater overlap to the list of 135 proteins respect to DDA. More than 50 % (72 proteins) of these prostate cancer-specific proteins were quantified in DIA analysis (Fig. 4).

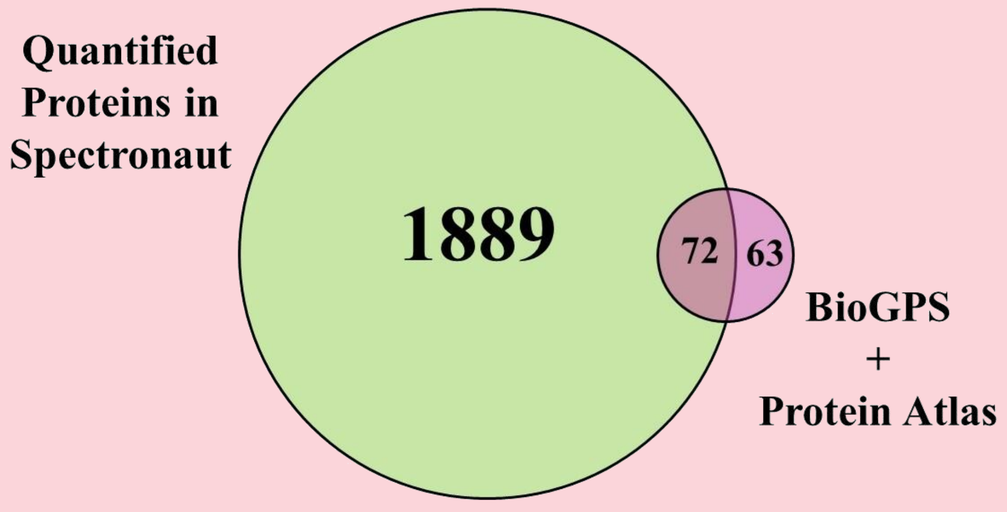

Fig.4: The comparison between the quantified proteins in Spectronaut and the panel of 135 proteins
.

Conclusions

The comparison between DDA and DIA has shown that DIA method is a promising approach to obtain a rich proteomic map of EPS-urine. Moreover, DIA analysis has identified a major number of prostate cancer- specific and tissue proteins respect to DDA analysis.
The increase in the number of protein identifications could raise the probability to detect cancer-associated proteins of potential interest.

References:

[1] . R. Siegel, K. Miller, A. Jemal; Cancer Journal for Clinicians, 70 (2020), pp 7-30

[2] Y. Kim, J. Jeon, S. Mejia, C.Q. Yao, V. Ignatchenko, J.O. Nyalwidhe, et al; Nature Communication, 7 (2016), pp 1-10.