Integrative Proteomic Profiling in High-Grade Serous Ovarian Carcinoma: Unraveling Biomarkers and Therapeutic Targets

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Background

High-Grade Serous Ovarian Carcinoma (HGSOC) is a significant clinical challenge with a 30% five-year survival rate and limited targeted therapy options. PARP inhibitors exploit deficiency in DNA repair pathways which limit the benefits of this treatment to ~10% of HGSOC patients harboring BRCA mutations. Consequently, there is a pressing need for novel biomarkers and therapeutic targets to enhance patient care via precision medicine.

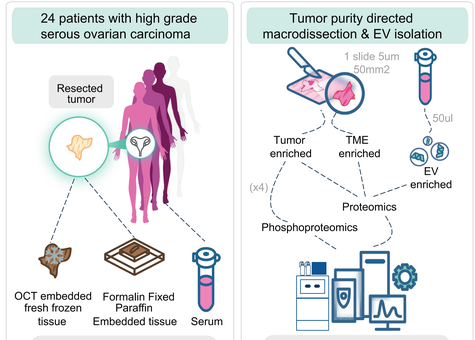
In this work, we conducted comprehensive and deep mass spectrometry based proteomic and phosphoproteomic analyses of archived matched fresh frozen (FF), formalin-fixed paraffin-embedded (FFPE) tissues from 24 HGSOC patients. Additionally, we analysed matched serum samples from the same patients and compared them to serum from healthy individuals to associate proteomic profiles with disease outcomes.

		FF n = 24 (%)	FFPE n = 9 (%)	Serum n = 24 (%)
Age	N	24 (100)	9 (100)	24 (100)
	Median	65	67	65
	Range	42-84	52-75	42-84
Smoking	N	23 (96)	8 (89)	23 (96)
	Yes	4	1	4
	No	19	7	19
BRCA status	N	16 (67)	8 (89)	16 (67)
	Mut	5	1	5
	WT	11	7	11
Neoadjuvant treatment	N	22 (92)	8 (89)	22 (92)
	Yes	11	3	11
	No	11	5	11
Response to Initial therapy	N	18 (75)	7 (78)	18 (75)
	Responder	14	6	14
	Non-responder	4	1	4
PFS (months)	N	16 (67)	6 (67)	16 (67)
	Median	17	17	17
	Range	1-96	7-96	1-96

PFS = progression free survival

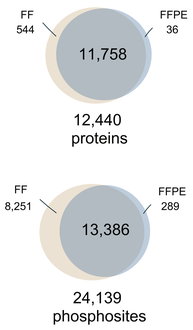
Clinical trial compatible proteomics workflow

- Tissue **macrodissection** from a **single slide** into **tumor-rich** and tumor **micro environment** (TME) regions for proteomic analysis.
- **Phosphoproteomics FFPE**- pooled macrodissected FFPE tissue samples (x4 slides).
- **Extracellular vesicle** (EV) enrichment from 50ul serum based on Mag-Net protocol[1].
- Data analysis using the **AIMS™ platform**[2].



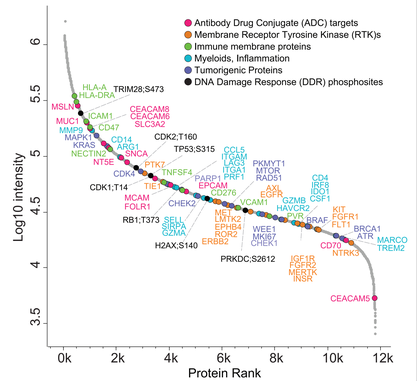
QC analysis shows comparable metrics in FF & FFPE

There is a high overlap between proteins and phosphosites when comparing fresh frozen and FFPE datasets.

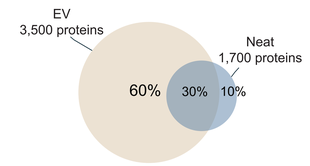


Known immune and tumor associated biomarkers and drug targets are well quantified in FFPE tissue samples. Important DDR phosphosites quantified in the phosphoproteomics dataset are highlighted.

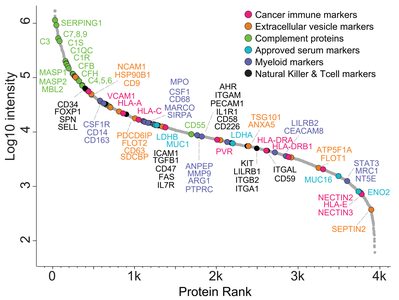
This enables the examination of multiple biological processes in a single study.

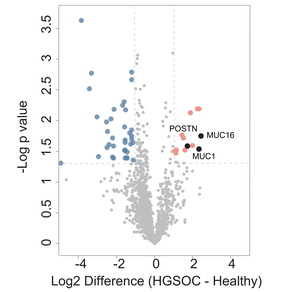


Enhanced serum protein detection with Extracellular Vesicle enrichment



EV enrichment allows 60% more protein identification compared to neat serum.



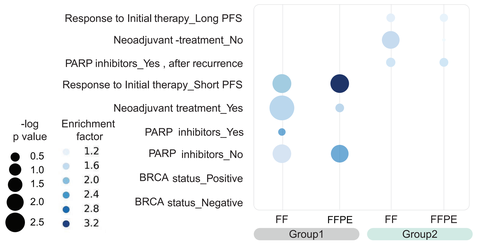


Different classes of serum, immune and tissue leakage proteins are well quantified in the Serum EV dataset making this a valuable approach for identifying novel biomarkers.

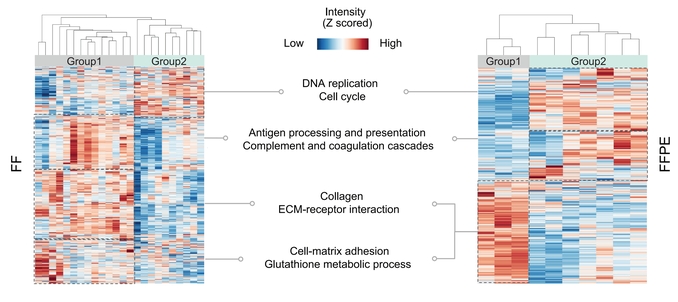
Well known diagnostic serum biomarker MUC16 (CA125) is significantly upregulated in tumor samples compared to healthy controls.

Proteomics highlights treatment associated changes in FF and FFPE

Global analysis of proteomics from FF samples identifies two patient subgroups, separated according to neoadjuvant treatment and poor response to initial therapy (defined by recurrence before 6 months from last treatment).

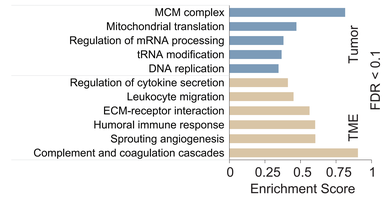


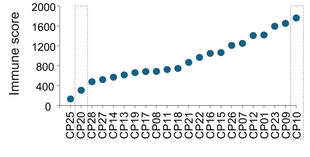
Untreated patients (Group2) are characterized by higher proliferation while pre-treated patients (Group1) are characterized by immune response, TME interactions and drug metabolism. A similar separation was also observed in the FFPE cohort (right).



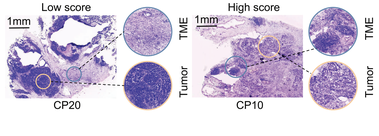
Macrodissection captures TME diversity

Comparing matched tumor and TME samples show enrichment of cellular and extracellular processes in tumors and TME samples respectively.



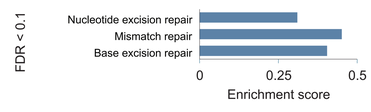


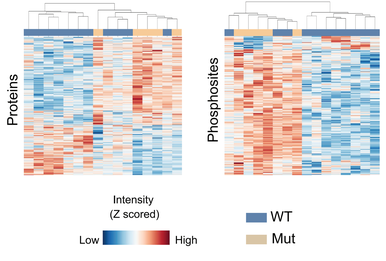
TME with higher immune infiltration show higher proteomic immune score (calculated using ESTIMATE tool[3]), thus validating the macrodissection procedure.



Clustering reveals proteomic BRCAness

Proteomic differences between BRCA WT and mut include DNA repair processes such as Base Excision Repair (BER) and Nucleotide Excision Repair (NER) significantly higher in BRCA WT.



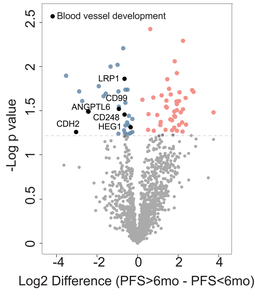


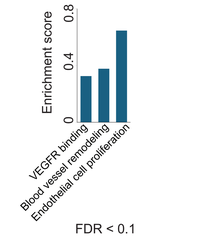
However, clustering of samples based on differentially expressed protein or phosphosites highlights a group of WT patients that are more similar to mutant than other WTs suggesting indication expansion for PARP inhibitors beyond BRCA mutation.

Angiogenesis reflected in both TME and serum proteomics

Analysis of progression free survival (PFS) status shows enrichment of ECM and angiogenesis related proteins in patients with short PFS (defined by recurrence before 6 months from last treatment).

Interestingly, this systemic difference is also reflected in the tissue TME proteomic profiles of patients with PFS<6mo compared to PFS>6mo.





Summary

- We present proteomic and phosphoproteomic analysis of macrodissected tumor and TME profiles from little starting materials of both FF and FFPE archived tissue samples compatible with standard clinical trial sample collection procedures.
- We reveal high proteomic and phosphoproteomic similarities between FF and FFPE tissues and show potential for proteomic biomarkers associated with DNA damage related mutations and PARP indication expansion.
- We show systemic changes using proteomic profiling of matched serum samples and its correlation to TME alterations in the tissue.
- We demonstrate the potential usefulness of clinical proteomics studies for system level precision medicine applications.

References
1. Wu et al. bioRxiv (2023)
2. Simchi et al. A large scale proteogenomic atlas for precision oncology ESMO; 2024. FPN # 190P
3. Yoshihara et al. Nat. Commun. (2013)

Disclosures I.W. has no COI to declare. A.S., G.A., D.K., A.M., S.H., N.S., K.P., E.S. are employees of Protai Bio and own stock in Protai Bio.

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