Phospho-Proteomics driven biomarker discovery for high-grade serous ovarian carcinoma from archived tissue specimens

Anjana Shenoy, Gali Arad, Dimitri Kovalerchik, Amit Manor, Nitzan Simchi, Dina Daitchman, Kirill Pevzner and Eran Seger
Protai Bio, Tel Aviv, Israel

American Association for Cancer Research
Abstract number: 1855

Introduction

High-Grade Serous Ovarian Carcinoma (HGSOC) is a highly aggressive and heterogeneous cancer with variable treatment response.

Current treatment strategies, including surgical resection and chemotherapy, are often ineffective due to a lack of molecular markers to predict drug sensitivity and patient prognosis.

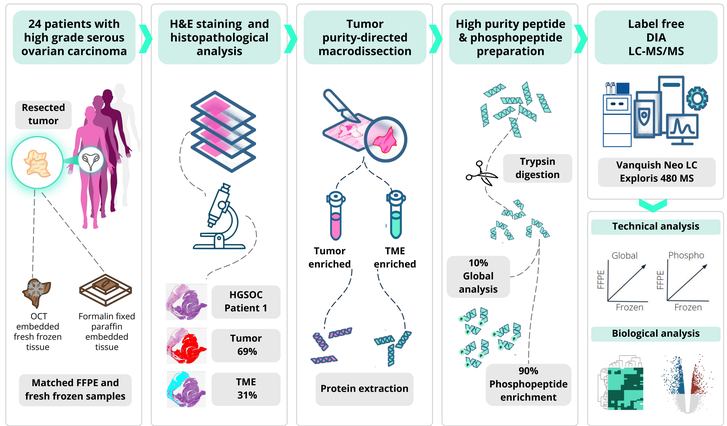
In this work, we conducted a comprehensive and deep global proteomics analysis of archived tissue specimens from 24 HGSOC patients (both fresh-frozen [FF] and formalin-fixed-paraffin-embedded [FFPE]) to associate proteomic profiles with patient outcomes.

- PreOp = pre operative
- PFS = progression free survival

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

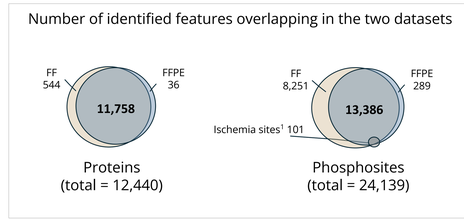
Clinical sample preparation workflow

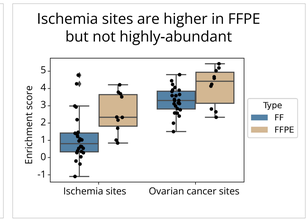
Single-shot data independent acquisition (DIA) analysis of tumor enriched and tumor microenvironment (TME) enriched tissue areas from archived samples result in quantification of more than 9,000 proteins in each clinical specimen.

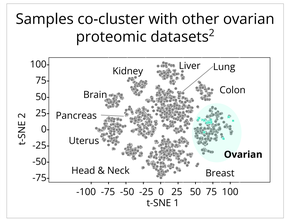


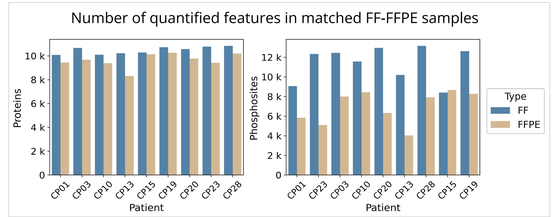
QC analysis shows comparable metrics in FF and FFPE samples

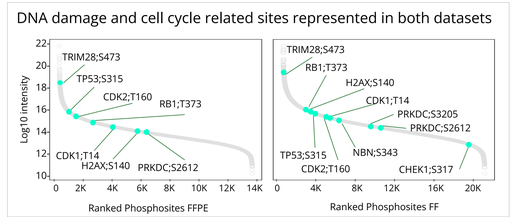
Comparison of matched FF and FFPE samples coming from the same patients show a large overlap on both proteome and phosphoproteome levels. Integration with Protai's clinical atlas[2] and comparison to pan cancer proteomic datasets shows enrichment of core ovarian cancer proteome.





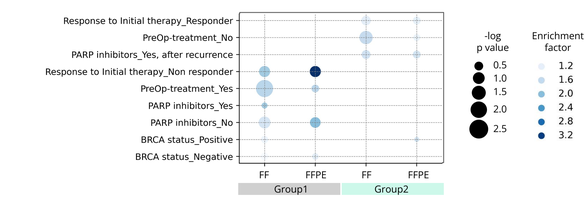




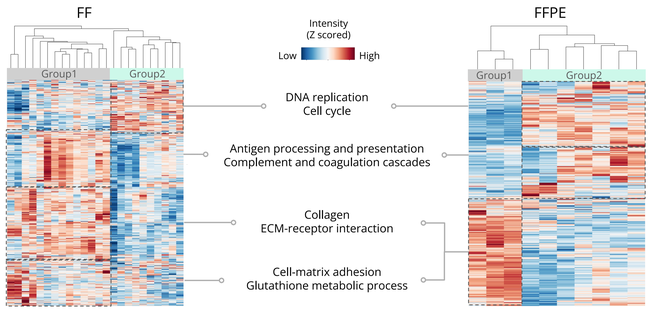


Global analysis highlights distinct proteomic subgroups

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

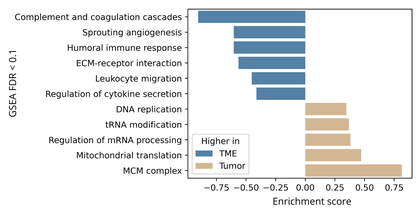


Untreated patients (Group2) are characterized by higher proliferation while pre-treated patients (Group1) are characterized by immune response, TME interactions and drug metabolism. A similar separation was also observed in the FFPE cohort (right).

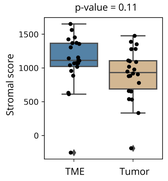


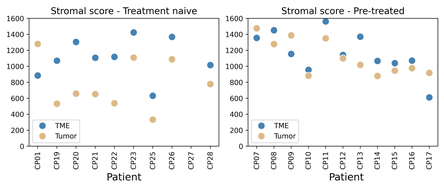
Macrodissection-based proteomics captures tumor & tumor microenvironment diversity

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

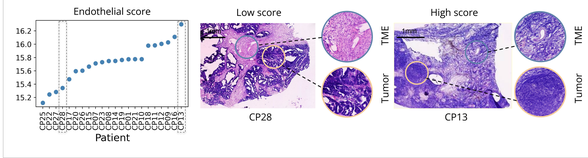


Tumor and TME samples differentiate according to their stromal signature score[3] (right). However, difference between tumor and TME changes as a function of treatment (bottom), showing higher similarity of tumor and TME in samples from treated patients.

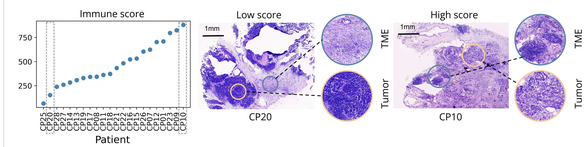




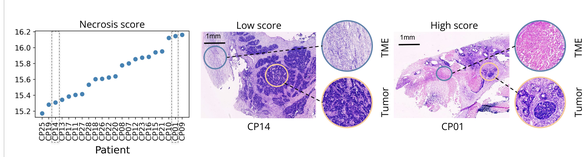
TME with higher vascularization show higher endothelial development pathway score (average expression of endothelial development-related gene ontology pathways)



TME with higher immune infiltration show higher immune score (calculated using ESTIMATE tool)[3]

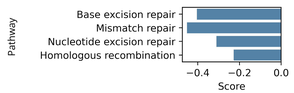


TME with higher necrosis show higher expression of necrosis signature (average expression of necrosis-related gene ontology pathways)

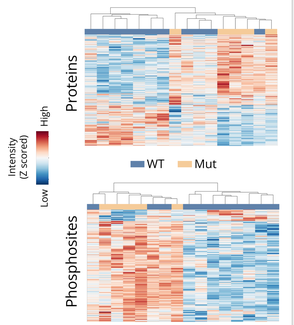


Proteomic markers associated with BRCA status

Proteomic differences between BRCA WT and mut include DNA repair processes such as BER and NER significantly higher in BRCA WT.



However, clustering of samples based on differentially expressed proteins (top) or phosphosites (bottom) there is a group of WT patients that are more similar to mutant than other WTs, suggesting indication expansion potential beyond BRCA mutation[4].



Summary

- We present an analysis of tumor and TME profiles using proteomics and phosphoproteomics from little starting materials of both FF and FFPE archived tissue samples.
- Our **AIMS™** platform enables robust protein identification and quantification with comparable results between FF and FFPE methods.
- We reveal high proteomic differences between treated and untreated tissues and show potential for proteomic biomarkers associated with DNA damage-related mutations.
- All data feeds into our clinical proteogenomic atlas for additional target/biomarker discovery.

References

1. Mertins et al. Mol. Cell. Proteomics (2014)
2. Simchi et al. A large scale proteogenomic atlas for precision oncology [abstract]. AACR; 2024. Abstract # 6241
3. Yoshihara et al. Nat. Commun. (2013)
4. Arad et al. Phospho-proteomic dynamics reveal DNA damage and repair signatures of drug sensitivity [abstract]. AACR; 2024. Abstract # 5130