QBIO490: Literature Presentation on Ovarian Cancer and Multi-Omics

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RESEARCH PAPER: Integrated Multi-Omic Analysis Reveals Immunosuppressive Phenotype Associated with Poor Outcomes in High-Grade Serous Ovarian Cancer

- Transcriptomics and Methylomics (RNA and methylation)
- Paper structure: Hypothesis, methodology, main findings, figures

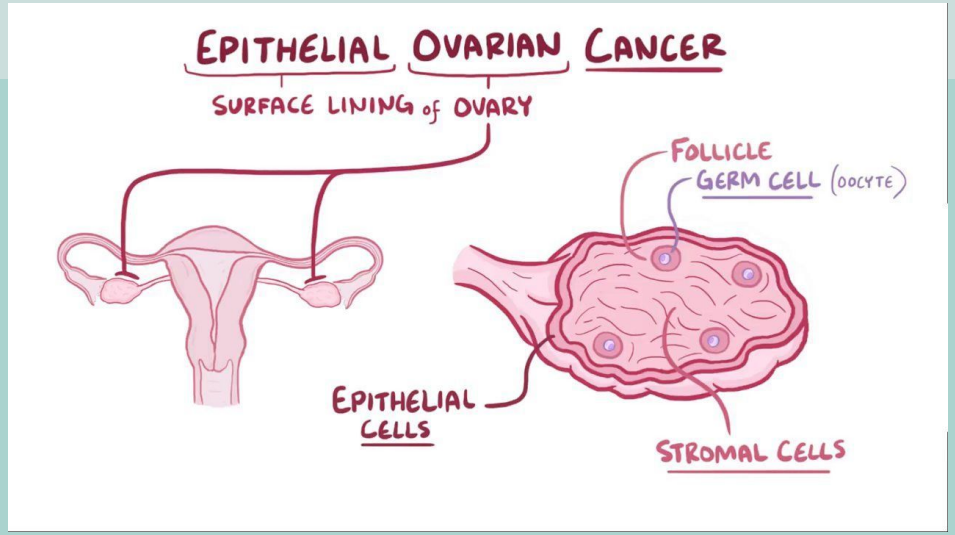
REVIEW: Recent Advances in Integrative Multi-Omics Research in Breast and Ovarian Cancer

- Genomics and Proteomics
- Paper structure: goals, methodology, conclusions of review paper

Our Questions

CONTEXT: Intro to High Grade Serous Ovarian Cancer

Epithelial ovarian cancer is the most common type of ovarian cancer. Other types include germ cell tumors and stromal cell tumors. About 90 out of 100 tumours of the ovary **(90%)** are epithelial (Cancer Research UK)



Epithelial ovarian cancer is divided into different subtypes, based on histology, which is the appearance of the tumor cells. The most common type is serous cancer, which may be high or low grade (Ovarian Cancer Research Alliance). High-grade serous ovarian carcinoma is the most common type of ovarian cancer — comprising approximately **75%** of epithelial ovarian cancers.

Integrated Multi-Omic Analysis Reveals Immunosuppressive Phenotype Associated with Poor Outcomes in High-Grade Serous Ovarian Cancer

Hypothesis:

Using multi-omic data and semi-biased clustering of HGSOC specimens will help to better understand the association between genomic features and response to treatment among 370 patients with newly diagnosed dHGSOC

- Specifically, to use transcriptomic and methylomic features from HGSOC tumors to find prognostic subtypes
    - Identify unique contributors to disease recurrence for HGSOC
    - Associate with different clinical outcomes

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Methodology:

Workflow consisted of 10 steps, we separated into prep (1-5) and analyses (6-10):

1. Data Collection: Obtained RNA-seq and methylation data for **n=374 HGSOC specimen**s from TCGA

2. RNA-sequencing data processing (count matrix):

3. Methylation data processing (beta values matrix)

4. Data Integration and Feature Selection: **1467 features** (methylation = 650 CpG probes, expression = 817 genes)

5. Cluster Analysis: Combined similarity network fusion (SNF) and consensus clustering (CC), **4 clusters**

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Methodology:

6. **Survival and recurrence analysis:** Used KM to estimate survival and time to recurrence

7. **Differential expression** with DESeq2 for cluster-wide gene expression and Methylation expression used with limma to compare responsive and nonresponsive clusters.

8. Multilayer Perceptron Neural Network: used *relu* and *softmax* functions for model accuracy

9. Gene set Enrichment Analysis: Genes of interest were isolated from model based on highest weights and aggregate bias

10. Correlational analysis: further differences between clusters, gene-level methylation and immune cell-type evaluations ran per cluster

11.Workflow validation and analysis: Repeated for 61 tumor samples from publicly available data, but made p-value cutoff more stringent (0.01 vs 0.05) to confirm methodology

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Main Findings:

- **Cluster 1: Mesenchymal n=101**
  - "Responsive"
  - Overall poor survival
- Cluster 2: Proliferative
  - Increased proliferation markers and WNT/β-catenin signaling
- **Cluster 3: Immunoreactive n=98**
  - "Nonresponsive"
  - Improved survival
  - Inflammatory and immune signaling pathways
- **Cluster 4: Differentiated n=111**
  - Nonresponsive/Hypomethylated
  - Dysregulated hormone signaling pathways
  - Increased expression of epithelial differentiation markers

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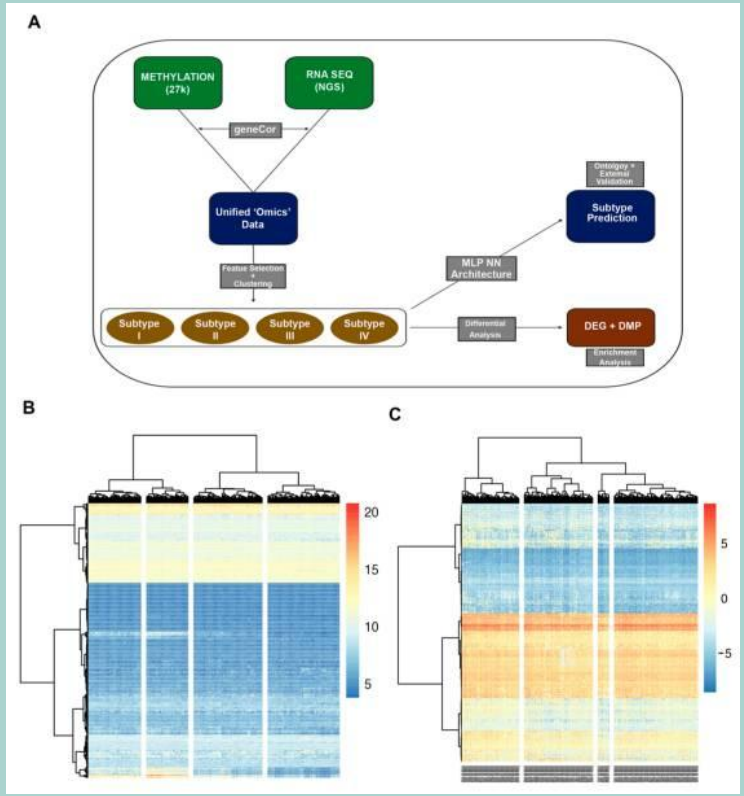
Figure 1: Analysis Workflow and Clustering analyses of methylomics and transcriptomics



A) Methylation array (27k) and RNA sequencing (-omics) data datasets were unified to n=370 patients
B) Ward clustering of unified RNA-seq
C) Ward clustering of methylation data

Result:
Cox regression resulted in **1467 features** selected for clustering and **four clusters** were set for analysis, however no major distinctive clusters were observed using this method

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Top 20 features out of the 1467 features
Highlighted: three smallest p-values (most significant) were chosen: **NFRKB** and **DPT** genes and the **TREML1** gene

Supplemental Table S1.

Feature ID	Gene	p-value
cg21022435	NFRKB	8.88E-06
cg10835876	DPT	3.33E-05
ENSG00000161911	TREML1	1.31E-04
cg13406768	C2orf33	2.37E-04
ENSG00000228058	LINC01736	2.40E-04
cg08278554	C15orf48	2.44E-04
ENSG00000238110		2.72E-04
cg06351503	RDBP	2.79E-04
cg25274750	HIPK2	3.63E-04
ENSG00000287012		3.76E-04
ENSG00000235437	LINC01278	3.87E-04
ENSG00000115592	PRKAG3	4.15E-04
ENSG00000256849	TCP1P3	4.16E-04
ENSG00000288302		4.39E-04
cg14802310	TUBA3	4.48E-04
cg08377000	TIGD2	4.82E-04
ENSG00000287680		4.86E-04
ENSG00000105131	EPHX3	6.16E-04
ENSG00000287255		6.42E-04
cg20655558	DNAJB7	6.99E-04

Supplemental Table S1. *Top 20 features selected by Cox regression model ($p < 0.05$) of integrated transcriptomic and methylomic TCGA data.*

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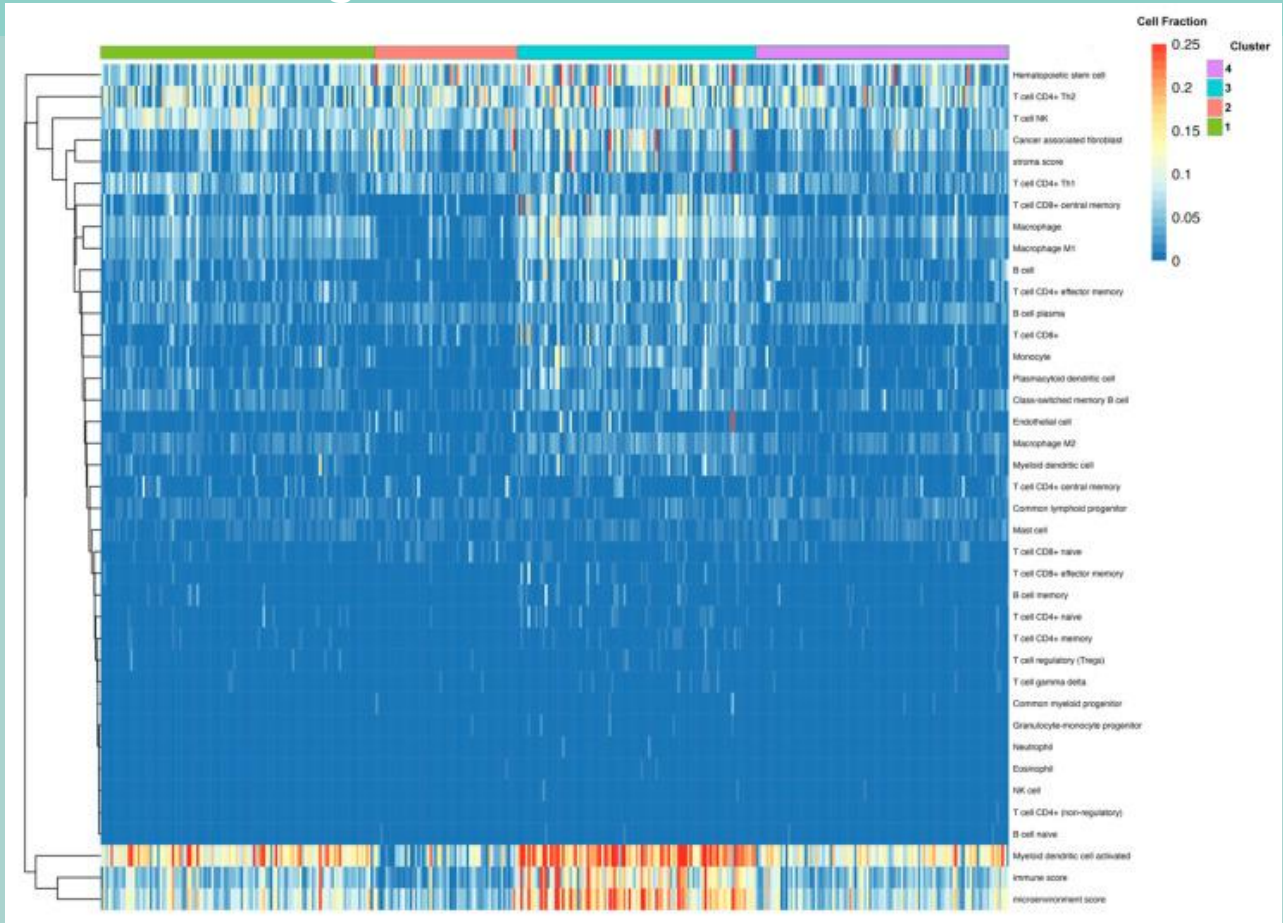
Figure 5: Characteristics of Identified Clusters

Fractional estimates of cell-type composition within each of the four identified clusters; a large, diverse set of immune cell types was found within Cluster 3



Recent Advances in Integrative Multi-Omics Research in Breast and Ovarian Cancer

Goals:

- This paper seeks to discuss:
  - how multi-omic analysis has classified different molecular types of both cancers
  - recent and historical advances in research of BC and OC
  - promote the use of these similarities and research advancements in generating new treatments

Recent Advances in Integrative Multi-Omics Research in Breast and Ovarian Cancer

Methodology:

- Different molecular subtypes of ovarian cancers listed in existing literature
  - 5 types vs 4 types?
- Advances in research that have allowed for better understanding of ovarian cancer
  - Single -cell omics
  - Integrative analyses (ex: MRKCA gene)
  - Single-cell RNA-sequencing for immunotherapeutic implications

➔ Aggregates relevant information on what has been learned about OC to point out inconsistencies in knowledge or new possible therapeutic approaches.

Recent Advances in Integrative Multi-Omics Research in Breast and Ovarian Cancer

Conclusions:

- Proteomics and genomics have allowed for novel advances in:
  - Molecular subtyping
  - Identifying oncogenes, tumor suppressor genes, and signaling pathways

- Disparities in data (ex: molecular typing) demonstrate the need for more clear, in-depth research on ovarian cancer

- The next step: use all known data to develop better diagnostic/treatment tools that will improve patient outcomes.

Connection to Review Paper and Multi-Omic Research

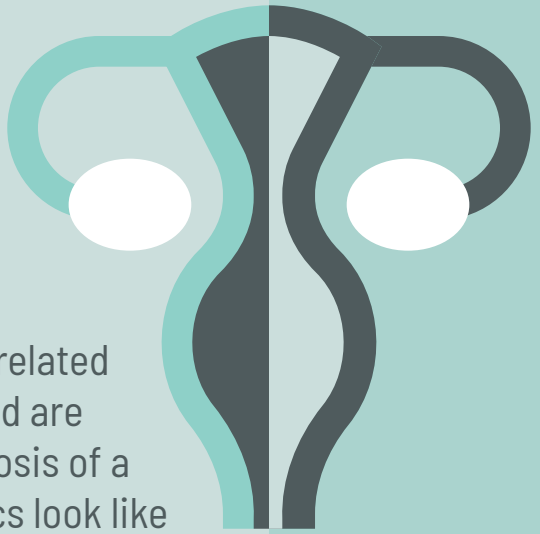
- In both review and research paper, the following focus on -omic analysis to further genetic transparency to conceptualize cancer cells
  - Ovarian cancer needs to be researched more in-depth in regards to treatment and alternative methods
- Genetic development is still being made focusing on genomic and transcription factors in regard to cancers that linked to women.
  - Cell population and clusters analysis is still in the works

Final Questions from both Papers

Research Paper

✦ In 2.5 - the methods section, 650 CpG probes were chosen for the methylation data and 817 genes for the expression data - why is there a discrepancy in data collection?

✦ Now that these methylation-related biomarkers have been found, and are insightful to give a better prognosis of a HGSOC, what would the ageomics look like when it comes to those biomarkers being detected after an age-related methylation event occurs?



Review

✦ If ovarian cancer and breast cancer are linked to similar genes, are methods being developed to predict which one a patient might develop?