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Pooled clustering of high-grade serous ovarian cancer gene expression leads to novel consensus subtypes associated with survival and surgical outcomes

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Abstract

Purpose—Here we assess whether molecular subtyping identifies biological features of tumors that correlate with survival and surgical outcomes of high-grade serous ovarian cancer (HGSOC).

Experimental Design—Consensus clustering of pooled mRNA expression data from over 2,000 HGSOC cases was used to define molecular subtypes of HGSOCs. This *de novo* classification scheme was then applied to 381 Mayo Clinic HGSOC patients with detailed survival and surgical outcome information.

Results—Five molecular subtypes of HGSOC were identified. In the pooled dataset, three subtypes were largely concordant with prior studies describing proliferative, mesenchymal, and immunoreactive tumors (concordance > 70%), and the group of tumors previously described as differentiated type was segregated into two new types, one of which (anti-mesenchymal) had down-regulation of genes that were typically upregulated in the mesenchymal subtype. Molecular subtypes were significantly associated with overall survival ($p < 0.001$) and with rate of optimal surgical debulking (< 1 cm, $p = 1.9 \times 10^{-4}$) in the pooled dataset. Among stage III-C or IV Mayo Clinic patients, molecular subtypes were also significantly associated with overall survival ($p = 0.001$), as well as rate of complete surgical debulking (no residual disease; 16% in mesenchymal tumors comparing to >28% in other subtypes; $p = 0.02$).

Conclusions—HGSOC tumors may be categorized into five molecular subtypes that associate with overall survival and the extent of residual disease following debulking surgery. Because mesenchymal tumors may have features that were associated with less favorable surgical outcome, molecular subtyping may have future utility in guiding neoadjuvant treatment decisions for women with HGSOC.

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Keywords

ovarian cancer; molecular subtypes; surgical outcome; prognostic; consensus clustering

Introduction

Ovarian cancer is the second most common and the most lethal gynecologic malignancy in the United States (1). Despite radical surgery and initial high response rates to platinum- and taxane-based chemotherapy (2), most patients experience a relapse, with an estimated 14,240 deaths in 2016 in the United States alone (3). Further understanding of the biology of ovarian cancer is required to improve clinical outcome. Microarray-based gene expression profiling has been used to classify high-grade serous ovarian carcinomas (HGSOC) into molecular subtypes but this has had limited clinical utility, in part due to lack of modifiable treatment options. Additionally, there have been multiple related but different subtype definitions proposed, each derived from individual studies with potential platform-specific biases (4–6), lack of detailed clinical data, and wide variation in surgical resection rates confounding survival outcomes. We sought to investigate whether HGSOC molecular subtypes associated with surgical outcomes as well as with survival. To improve power to discern molecular subtypes, we first pooled expression analysis of common genes across 14 expression studies with adjustment for batch-effects; we then established *de novo* molecular subtypes and examined associations with survival and surgical outcomes. In a smaller collection of Mayo Clinic patients with more detailed baseline surgical and updated clinical follow-up data, we further assessed association with molecular subtype and clinical outcomes.

Materials and Methods

Study Cohorts

Public cohort—Fourteen public expression datasets were retrieved from curatedOvarianData (7), an ovarian cancer transcriptome database; we excluded studies with less than 50 tumors due to minimum sample size for batch correction. We further restricted to HGSOC cases (grade>1, serous) (8, 9) and excluded chemo-treated tumors and duplicates with the Mayo Clinic collection, resulting in 2,103 cases (Supplementary Table 1). 1,017 deaths occurred, and among living cases mean follow-up was 42 months (range, 0–243). The extent of post-surgery residual disease was available as optimal debulking (<1cm, analogous to Mayo's RD0 and RD1 combined) and sub-optimal debulking (>1cm, analogous to Mayo's RD2).

Mayo Clinic cohort—Eligible cases (n=381) were ascertained at the Mayo Clinic between 1992 and 2009. A gynecologic pathologist (GLK) confirmed initial diagnosis of HGSOC and reviewed tissues to ensure >70% tumor content. Survival data were obtained from the Mayo Clinic Tumor Registry, electronic medical records, and active patient contact; 317 deaths occurred as of November 3, 2015. Among living cases mean follow-up was 101 months (range 30–202). The extent of post-surgery residual disease was available as RD0 (no macroscopic disease), RD1 (measurable but <1cm), and RD2 (>1cm). Clinical

characteristics along with associations with overall survival (hazard ratios, HRs; 95% confidence intervals, CIs), are shown as Supplementary Table 2. All cases provided informed consent for use of their tissues and data in research; all protocols were approved by the Mayo Clinic Institutional Review Board.

Gene Expression Analysis

The overall analytical flow is shown as Supplementary Figure 1.

Public cohort—The probe-set with the highest mean across all datasets of a platform was utilized when multiple probe-sets mapped a gene (10), resulting in 7,361 common genes across studies. Initial study-batch effects were corrected by ComBat (11). After adjustments, the residual batch differences by study and microarray platform were assessed by association analysis of the first 10 principal component (PC) projections, revealing the third and tenth PCs still significantly associated with studies and platform. The residuals from a linear model of the expression value for each gene and both PC3 and PC10 were computed. The expression mean value for each gene was added back to the residuals to obtain an adjusted expression value for each gene. After applying the linear regression-based method to eliminate per-study and/or per-platform batch effects across 14 datasets, we checked batch-effects according to PC analysis projections versus studies, found no residual batch differences associated with studies, shown as Supplementary Figure 2.

Mayo Clinic cohort—RNA extracted from fresh frozen tumors was assessed using Agilent Whole Human Genome 4x44K Expression Arrays as previously described (4, 12). Batch-effects among two different RNA preparations and three microarray profiling dates were corrected to adjust Cy5, Cy3 labeling differences observed among experimental batches (4, 12).

Genomic data of ovarian TCGA study

BRCAness data—BRCA1/2 germline and somatic mutations, and BRCA1 promoter methylation, collectively called as “BRCAness”, were retrieved from supplementary tables of a previous study summarizing whole-exome sequencing results from TCGA ovarian cohort (13).

Genomic scores—four genomic scores for ovarian TCGA cases were retrieved from a previous pan-Cancer study (14): (a) loss-of-heterozygosity (LOH): a genomic scarring score previously studied in HGSOC and defined by Abkevich et al. (15); (b) large scale transition (LST): a type of genomic scar associated with BRCA1 or BRCA2 status in triple negative breast cancer (16); (c) telomeric allelic Imbalance (TAI): another type of genomic scarring score which was found predictive of platinum-based chemotherapy response in both breast and ovarian cancers (17); (d) tumor mutation count: the total number of somatic mutations per tumor sample.

HGSOC molecular subtype assignments

De novo clustering and subtype assignment—To define molecular subtypes using genes with substantial expression variability, we chose 872 genes that expressed at least 1.5

log-scale fold-change differences across at least 200 samples, compared to its own median expression of 2,103 HGOSC cases in the public cohort. *De novo* clustering was evaluated using a consensus non-negative matrix factorization (NMF) approach (18). From cluster numbers $k=2$ to $k=9$, 50 independent NMF runs were performed to evaluate cluster stability. We found that $k=2$ to $k=5$ were comparably stable (Supplementary Figure 3) and selected $k=5$ as the stability of clustering solutions began to drop significantly starting from $k=6$. To select genes most predictive of subtypes, multinomial LASSO regression was adopted (19), and an optimal parameter was selected according to the so-called “one standard error” rule after 10-fold cross-validation (20). To achieve a parsimonious set of genes for future studies, the top 100 genes with largest average absolute LASSO coefficients were chosen as the molecular subtype signature. A *de novo* expression centroid for each molecular subtype was computed according to average expression of samples in each molecular subtype (Supplementary Table 3). For subtype assignment in public and Mayo Clinic data, Pearson correlation coefficients were computed for each subtype centroid and given sample, and the most highly correlated molecular subtype was assigned. No assignment was made if the highest coefficient was below 0.1.

Prior subtype assignments—For comparison, we also assigned molecular subtypes according to the TCGA (21) and Tothill et al (6). TCGA subtype assignment in public and Mayo data was done according to gene signatures and similar procedures as defined previously (21). Briefly, gene set activation scores for each subtype signature were computed using single-sample gene set enrichment analysis (ssGSEA) (22), and raw enrichment scores were expressed as relative z-scores to account for various sizes of signatures. Given one sample, ssGSEA assigned it to one of the four TCGA molecular subtypes with the highest enrichment z-scores. For the Tothill approach, data from the samples used for subtype assignments were retrieved from Tothill et al(6), and genes predictive of Tothill’s molecular subtypes were extracted from a related publication (23). Expression centroid per molecular subtype (C1/C2/C4/C5) was computed according to average expression of samples in a same subtype, with detailed expression centroids comprising of 841 genes shown as Supplementary Table 4. For a given sample, Pearson correlation coefficients were computed against subtype centroids, and the most highly correlated subtype in public and Mayo Clinic data was assigned. No assignment was made if the highest coefficient was below 0.1.

Differential Expression and Pathway Enrichment Analysis

For each gene, differential expression for each *de novo* molecular subtype compared to other molecular subtypes combined was evaluated using t-tests. Pathway enrichment analysis was done using Bioconductor package “GAGE” (24). Multiple hypothesis-testing corrected q-values were computed for both analyses (25).

Analysis of Clinical Features

Cox regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs), including multivariate step-wise variable selection. Univariate analysis of clinical features revealed that age, grade, stage and surgical debulking status significantly associated with survival with p-value <0.05. Following step-wise variable selection for the multivariate analysis, only stage and surgical debulking status remained significantly associated with

overall survival and therefore these two factors were adjusted for multivariate associations with overall survival.

Differential analysis of genomic scores

For each type of genomic scores (LOH, LST, TAI and tumor mutation count), the two-sided Wilcoxon rank sum tests were performed to test the score differences comparing s5.ANM subtype versus s4.DIF and all the other subtypes, respectively.

Results

Discovery of Five *de novo* Subtypes

Five stable *de novo* molecular subtypes (s1, s2, s3, s4, s5) of HGSOc were defined (Supplementary Table 3 and Supplementary Figures 1–3), using NMF-based consensus clustering of pooled data from 2,103 publicly available HGSOc expression profiles corrected for batch-effects. We developed a parsimonious 100-gene signature using LASSO-based analyses that discriminated these molecular subtypes, and assigned Mayo Clinic HGSOc tumors to the most likely subtype according to expression similarities as described in Methods. Independently in the public and Mayo Clinic datasets, we compared the assignments of *de novo* molecular subtypes with those of previous molecular subtyping systems defined by TCGA and Tothill studies (5, 6) (Figure 1). Overall, the *de novo* subtyping system was significantly associated with prior subtyping systems (chi-squared test $p < 2.2 \times 10^{-16}$, Supplementary Table 5). Three *de novo* molecular subtypes were largely consistent with previous molecular subtype definitions (s1.MES: “mesenchymal”; s2.IMM: “immunoreactive”; s3.PRO: “proliferative”). However, we report two *de novo* molecular subtypes emerging from “TCGA-differentiated/Tothill-C4” subtype. We examined differential expression patterns between each subtype vs. others, and found that the top differentially expressed genes of the novel s4 subtype was more consistent with the previously defined signatures of Tothill’s C3/TCGA-differentiated subtype (Supplementary Tables 6–10). Therefore, we refer to this as the s4.DIF (differentiated) subtype. The fifth novel subtype was named the “anti-mesenchymal” (s5.ANM) subtype because of its down-regulation of genes that were typically up-regulated in the mesenchymal subtype.

Subtypes’ Associations With Overall Survival

Classifications based on this five-level molecular subtyping system was significantly associated with overall survival of advanced stage patients in both public cohort (stage-III or IV; $p < 0.001$, Supplementary Figure 4), and Mayo Clinic cohort (stage-IIIC or IV; $p = 0.001$, Figure 2A). In univariate analysis of Mayo Clinic data, all three subtyping systems (five-level, Tothill, TCGA) showed significant associations with survival. For five-level subtyping system, the new s5.ANM subtype was observed with noticeably improved survival compared to s1.MES (Figure 2A). In multivariate analysis adjusting for age, stage and surgical outcome, the five-level molecular subtypes had the most significant associations with overall survival ($p = 0.0004$, Table 1). s2.IMM and s5.ANM tumors were significantly associated with longer survival time and lower death-risk compared to s1.MES (s2.IMM: HR=0.69, 95% CI=(0.49, 0.97), $p = 0.03$; s5.ANM: HR=0.43, 95% CI=(0.28, 0.64), $p = 4.6 \times 10^{-5}$). In contrast, the Tothill subtyping system did not have statistical significance in

multivariate analysis after adjusting for other clinical factors; for TCGA subtyping system, only the immunoreactive subtype was significantly associated with improved outcome compared to the mesenchymal subtype.

Among late-stage HGSOc patients with an optimal surgical debulking outcome (residual disease ≤ 1 cm), *de novo* molecular subtypes were significantly associated with overall survival in both the public (n=936, $p < 0.001$) and Mayo Clinic cohorts (n=250, $p = 0.014$) (Supplementary Figure 5). Among sub-optimally debulked late-stage patients, these molecular subtypes were still significantly associated with survival in the public cohort ($p = 0.005$) but not in the Mayo Clinic cohort ($p = 0.167$); however, the *de novo* s5.ANM subtype showed a non-significant trend toward increased survival compared to other molecular subtypes especially in Mayo Clinic cohort (median survival 25.8 months versus 19.6 months; Supplementary Figure 6). A potential advantage of Mayo Clinic cohort is the availability of complete cytoreduction data to investigate the relationship between molecular subtypes and residual disease on survival. Noticeably, molecular subtype was not associated with overall survival for patients who had no residual macroscopic disease (RD0) ($p = 0.23$, Figure 2B) or high volume (RD2) surgical outcome ($p = 0.167$, Figure 2D). We also evaluated debulking-survival associations within each molecular subtype, to determine if specific subtypes benefit more or less from surgical resection. We found unequivocal survival benefits of having RD0 surgical outcome regardless of *de novo* subtype membership (Supplementary Figure 7). Interestingly, s2.IMM tumors with RD1 and RD2 surgical outcomes resulted in similar survival (Supplementary Figure 7C), as did s4.DIF and s5.ANM tumors with RD0 and RD1 surgical outcomes (Supplementary Figure 7E and 7F). This implies surgical goals may not be the same for all subtypes and that molecular subtype may be tested in combination with neoadjuvant treatment.

Subtypes' Associations With Surgical Outcomes

Further analysis examined whether HGSOc molecular subtypes correlated with surgical debulking outcomes scored on a trinary scale in the Mayo Clinic cohort. We found that s1.MES subtype was associated with the lowest RD0 rates across different subtyping systems, ranging from 12%–16%, while the immunoreactive subtype consistently had the highest RD0 debulking rate, ranging from 40%–46% (Figure 3A and Supplementary Table 11). For 339 stage III-C or IV HGSOc patients in TCGA cohort with trinary scale of residual disease data, we again confirmed lowest RD0 rate in s1.MES subtype (6%) versus other subtypes ranging from 19% to 25% (Figure 3B). In the public cohort using binary surgical outcomes (optimal vs. sub-optimal debulking), surgical outcomes were also found to be significantly associated with HGSOc molecular subtypes ($p < 2.2 \times 10^{-16}$): the s1.MES/C1/mesenchymal subtype was again associated with lowest optimal-debulking rates ranging from 49.1%–51.6% across different subtyping systems.

Given our observation that tumors of the s1.MES molecular subtype showed less favorable surgical outcome, we hypothesized that the s1.MES subtype would also be associated with more complex surgical procedures than other molecular subtypes. To test this hypothesis, we enumerated surgical procedures in the Mayo Clinic cohort and computed surgical complexity scores (SCS) (26) for advanced-staged HGSOc patients with optimal debulking

outcomes (i.e. RD0 or RD1). We excluded sub-optimal/RD2 cases, which often involve unresectable cases and aborted surgical attempts, resulting in paradoxical lower SCSs despite more advanced disease. We found that the s1.MES subtype tended to have higher complexity scores than other molecular subtypes in RD0 and RD1 cases in the Mayo cohort, supporting the hypothesis (Figure 3B). Several recent reports have suggested specific gene signatures may be predictive of the ability to surgically resect HGSOC, potentially serving as biomarkers for surgical decisions (27–29). When expression levels of these genes were compared between the s1.MES subtype and others, nine out of ten genes in these previously defined surgical resection signatures were significantly up-regulated in the s1.MES subtype (Supplementary Table 12). This observation further supports the relationship between the s1.MES subtype and surgical debulking outcome.

Key Subtype-specific Genes and Pathways

In order to shed light on potential biological mechanisms underlying the relationship between molecular subtypes and outcomes, we performed differential expression analysis and pathway enrichment (Supplementary Figures 8–17, Supplementary Tables 7–11, Supplementary Tables 13–17). In these analyses, the s1.MES subtype showed strong up-regulation of cancer associated fibroblast signatures (e.g., *FAP*) and EMT/stem-cell drivers (e.g., *ZEB1* and *SNAIL2*), as well as significantly increased expression levels of fibronectin (*FN1*). The s2.IMM subtype showed significant up-regulation of antigen presentation machinery (e.g. *TAP1*, *PSMB8* and *PSMB9*); and its top subtype marker *TAP1* was highly correlated with expression of *PD-L1* ($R_{\text{Spearman}} = 0.72$; $p < 2.2\text{E-}16$, Supplementary Figure 18), a marker has recently been associated with increasing tumor infiltrating T-cells and favorable prognosis in HGSOC (30). Finally, our analysis involving unsupervised clustering showed that the TGF-beta pathway is up-regulated in the mesenchymal subtype ($q\text{-value} = 1.5 \times 10^{-4}$, Supplementary Table 13), which carries a worse prognosis (Figure 2A).

In order to shed some lights on novel s5.ANM subtype, we performed additional analysis to compare this novel subtype versus other subtypes in TCGA cases for which genomic data are available. We firstly examined distribution of BRCAness, including BRCA1/2 mutation and BRCA1 promoter methylation status, among these five *de novo* subtypes, shown as Supplementary Table 18. We found a trend that s3.PRO and s5.ANM subtypes had lower mutation rate in BRCA1 and BRCA2 (15.3% and 16.7%) comparing to all the other three subtypes (25%–29.6%). After accounting for BRCA1 methylation status, s3.PRO and s5.ANM had higher wild-type BRCA/2 rates (76.3% and 75% respectively), comparing to s1.MES (62.9%), s4.DIF (68.2%), and s2.IMM (57.4%). In TCGA cohort, we further evaluated associations between *de novo* subtypes and four tumor genomic scores summarized by a previous study (14). Among four genomic scores considered, s5.ANM subtype was associated with statistically higher telomeric allelic imbalance scores comparing to s4.DIF subtype ($p = 2.83\text{e-}2$). In addition, s5.ANM subtype was found associated with statistically lower tumor mutation count comparing to s4.DIF subtype ($p = 2.97\text{e-}3$), and comparing to all the other subtypes ($p = 4.89\text{e-}5$), shown as Supplementary Figure 19.

Discussion

The present study utilized transcriptional profiling from over 2,100 cases and unsupervised clustering to derive molecular subtypes of HGSOC, then applied this revised classification scheme to the original cases as well as a carefully annotated Mayo Clinic cohort to assess the relationship between subtype, surgical resectability and survival. To our knowledge the current study is the most comprehensive HGSOC molecular subtype analysis using a study- and platform-independent approach. This large-scale analysis confirmed the previously described mesenchymal (s1.MES), immunoreactive (s2.IMM), and proliferative (s3.PRO) subtypes as distinct entities. While our work was in progress, a cross-population analysis has also confirmed at least mesenchymal and proliferative subtypes were stable through clustering comparisons across individual expression studies (31). In the present study, a new subtype “anti-mesenchymal” (s5.ANM) was derived from TCGA-differentiated/Tothill-C4 subtype, with noticeable down-regulation of mesenchymal signature genes and better survival outcomes in both the public and Mayo Clinic HGSOC cohorts. To summarize our present analysis and linkage of the different subtyping systems, we made a schematic summary plot shown as Figure 4.

In view of the prognostic importance of surgical debulking for HGSOC and the modifiable use of neoadjuvant treatment, we examined the relationship between subtype and extent of residual disease. In the Mayo Clinic cohort, we found that the mesenchymal subtype had the lowest RD0 rates across different HGSOC subtyping systems. Among optimally debulked cases, we also observed that the mesenchymal subtype tumors had higher surgical complexity scores than other molecular subtypes. It does appear that the mesenchymal subtype is associated with higher rates of unsuccessful and/or more complex surgical resections. This is also supported by the fact that nine out of ten genes previously reported to be correlated with debulking outcome are significantly up-regulated in mesenchymal subtype (27–29). Collectively these observations suggest a potential role for subtyping in surgical planning or referral to expert centers. Not surprisingly, the *de novo* molecular subtypes were significantly associated with survival in both the public and Mayo Clinic cohorts when surgical debulking status was classified in a binary manner (≤ 1 cm versus >1 cm remaining). Theoretically, if tumors were completely removed, molecular subtypes should have no impact on survival; in fact, we observed that molecular subtypes were not associated with survival ($p=0.23$) in Mayo Clinic cases with no macroscopic diseases (RD0 using the trinary system, $n=97$). Because this observation was made in a small single-site population, further validation in additional cohorts is required to understand possible interactions between surgical efforts and tumor molecular subtypes in a clinical trial setting.

Although the molecular subtyping of HGSOC by transcriptional profile is appealing, how this subtyping can facilitate basic and clinical research into etiology, prognosis or therapeutic potential is still unclear (32–34). For example, existing prognostic signatures or molecular subtypes have not been able to identify platinum refractory HGSOC with sufficient accuracy to allow patients with a pertinent profile to be triaged to a clinical trial in place of standard chemotherapy (29). Nevertheless, HGSOC molecular subtyping could provide important biological insight and facilitate therapeutic studies. For example, the present study identified several subtype-specific pathways that were potentially targetable,

and therefore worthy of future study. Specifically, mesenchymal subtype cases in our analysis which carries a worse prognosis show that the TGF-beta pathway is up-regulated. Several other supervised expression studies have also reported that TGF-beta pathway activities are associated with worse clinical outcomes and ovarian cancer metastasis (29, 35–37). Therefore, tumors with the mesenchymal gene expression pattern might be considered for future trials containing TGF-beta inhibitors. With other developing therapeutic options such as PARP inhibitors and immune therapies for ovarian cancer (38, 39), it might also be important to consider treatment responses in the context of HGSOC molecular subtypes. Noticeably, laparoscopy assessment has been used in MD Anderson study to subjectively evaluate disease burden and resectability before cytoreduction surgery (40), making pre-surgery biopsy and molecular subtyping possible. In the future, knowledge of HGSOC molecular subtypes might help to identify patients for the most appropriate treatment options.

In order to utilize molecular subtyping clinically, a robust assay with reproducible subtype assignment is needed. There are examples of clinically implementing reliable and reproducible subtyping assays in other cancers, including breast cancer and lymphoma (41, 42). Noticeably, research evaluation of clinically feasible panels of ovarian subtypes is underway, pinpointing a 48-gene based NanoString panel as a promising platform also working for formalin-fixed paraffin-embedded samples (43). Once successfully implemented, molecular subtypes could be captured in prospective clinical trials to evaluate subtype-specific treatment benefit and serve as biomarkers for facilitating patient stratification. For example, we recently found PRO and MES molecular subtypes may derive a comparably greater benefit than DIF and IMM subtypes, in a retrospective study in a randomized controlled phase III trial of primary ovarian cancer with bevacizumab treatment (44).

In summary, unsupervised clustering of gene expression profiles from a large public HGSOC cohort (n=2,103) identified five molecular subtypes of HGSOC: three of these molecular subtypes, proliferative, mesenchymal and immunoreactive, were largely consistent across different subtyping systems (Tothill and TCGA), but two additional molecular subtypes were split from the previously described Tothill- C4/TCGA-differentiated subtype. Importantly, the novel anti-mesenchymal subtype, which was previously included in the differentiated subtype, is associated with improved survival in both the public and Mayo Clinic HGSOC cohort (n=381). Further analysis demonstrated the potential prognostic significance of this subtyping analysis. In particular, the mesenchymal subtype was associated with a much lower RD0 rate and increased surgical complexity in Mayo Clinic cohort and a lower optimal debulking rate in the public cohort. Future work in other clinically annotated HGSOC cases is needed to evaluate whether molecular subtyping can be used to guide treatment decisions in HGSOC (e.g., neo-adjuvant vs. adjuvant options).

Supplementary Legends

Refer to Web version on PubMed Central for supplementary material.

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Translational relevance

Despite growing knowledge about transcriptional profile-based molecular subtyping of high-grade serous ovarian cancer (HGSOC), molecular subtypes are not in current clinical use. Here we assess whether molecular subtyping identifies biological features of tumors that correlate with survival and surgical outcomes.

In this study, five molecular subtypes were identified in a large public cohort of and validated in Mayo Clinic patients. Molecular subtypes were significantly associated with surgical outcome and overall survival in both patient collections. The mesenchymal subtype of ovarian tumors generally showed the least favorable surgical outcome, with the lowest rate of no macroscopic disease (RD0) after primary surgeries. This result might suggest molecular subtyping should be clinically evaluated for future utility in guiding neoadjuvant treatment decisions for women with ovarian cancer.

Figure 1. Five *de novo* molecular subtypes and signatures comprised of 100 genes in the public HGSOC expression set (n=2,103) and Mayo Clinic HGSOC expression set (n = 381).

Figure 2.

Overall survival plots of five *de novo* molecular subtypes in the Mayo clinic cohort, for (A) all late-stage HGSOC patients, (B) late-stage patients with no macroscopic residual diseases (RD0), (C) late-stage patients with optimal-debulking but visible residual disease (RD1: 1cm), and (D) late stage patients with suboptimal diseases (RD2: >1cm).

Figure 3.

Surgical outcomes and complexity with versus tumor molecular subtypes in Mayo Clinic cohort. (A) and (B) are barplots of number and proportion of cases with trinary scale of surgical outcomes in Mayo Clinic cohort and TCGA cohort, respectively. (C–E) Boxplots of surgical complexity scores in optimally debulked cases of the mesenchymal subtype vs. other molecular subtypes according to the *de novo* molecular subtypes defined in this work (C), TCGA molecular subtypes (D) and Tothill molecular subtypes (E), respectively.

Figure 4. Schematic summaries of *de novo* molecular subtypes with respect to previous subtype systems and associated changes.

Table 1

Mayo Clinic HGSOc survival associated with three molecular subtype schemes (n=381).

		Univariate		Adjusted for age, stage, debulking*	
	N (%)	HR (95% CI)	p-value	HR (95% CI)	p-value
Five-level			2.74 x 10⁻⁵		0.0004
S1.MES	106 (28%)	REF		REF	
S2.IMM	82 (21.6%)	0.56 (0.41, 0.77)		0.69 (0.49, 0.97)	
S3.PRO	78 (20.6%)	0.74 (0.54, 1.00)		0.90 (0.66, 1.25)	
S4.DIF	66 (17.4%)	0.66 (0.47, 0.92)		0.77 (0.54, 1.08)	
S5.ANM	47 (12.4%)	0.40 (0.27, 0.59)		0.43 (0.28, 0.64)	
TCGA			4.58 x 10⁻³		0.0398
MES	101 (26.5%)	REF		REF	
IMM	86 (22.6%)	0.59 (0.43, 0.81)		0.70 (0.50, 0.98)	
PRO	106 (27.8%)	0.82 (0.61, 1.10)		1.01 (0.74, 1.37)	
DIF	88 (23.1%)	0.64 (0.47, 0.88)		0.73 (0.53, 1.01)	
Tothill			3.12 x 10⁻³		0.2006
C1/MES	119 (31.2%)	REF		REF	
C2/IMM	82 (21.5%)	0.58(0.43, 0.80)		0.77 (0.56, 1.08)	
C5/PRO	97 (25.5%)	0.76(0.56, 1.01)		0.97 (0.72, 1.31)	
C4/DIF	83 (21.8%)	0.64(0.47, 0.87)		0.76 (0.55, 1.04)	

* Covariates as age (continuous), stage (I, II, III, IV), and debulking (RD0, RD1, RD2).