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5-Hydroxymethylcytosine signals in serum are a predictor of chemoresistance in high-grade serous ovarian cancer

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Abstract

Objective.—The genome-wide profiling of 5-hydroxymethylcytosines (5hmC) on circulating cell-free DNA (cfDNA) has revealed promising biomarkers for various diseases. The purpose of this study was to investigate 5hmC signals in serum cfDNA and identify novel predictive biomarkers for the development of chemoresistance in high-grade serous ovarian cancer (HGSOC). We hypothesized that 5hmC profiles in cfDNA reflect the development of chemoresistance and elucidate pathways that may drive chemoresistance in HGSOC. Moreover, we sought to identify predictors that would better stratify outcomes for women with intermediate-sensitive HGSOC.

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Code availability

All software and packages used in this manuscript have been published previously. Code can be shared upon request.

CRediT authorship contribution statement

Melanie Weigert: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing - original draft, Writing - review& editing. **Xiao-Long Cui**: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing - review & editing. **Diana West-Szymanski**: Formal analysis, Methodology, Project administration, Writing - review & editing. **Xianbin Yu**: Data curation, Formal analysis, Methodology, Writing - review & editing. **Agnes Julia Bilecz**: Formal analysis, Methodology, Writing - review & editing. **Zhou Zhang**: Formal analysis, Methodology, Writing - review & editing. **Rohin Dhir**: Data curation, Resources, Writing - review & editing. **Mia Kehoe**: Data curation, Writing - review & editing. **Wei Zhang**: Conceptualization, Methodology, Software, Supervision, Writing - review & editing. **Chuan He**: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - review & editing. **Ernst Lengyel**: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing.

Methods.—Women diagnosed with HGSOC and known platinum sensitivity status were selected for this study. Nano-hmC-Seal was performed on cfDNA isolated from archived serum samples, and differential 5hmC features were identified using DESeq2 to establish a model predictive of chemoresistance.

Results.—A multivariate model consisting of three features (preoperative CA-125, largest residual implant after surgery, 5hmC level of *OSGEPL*), stratified samples from intermediate sensitive, chemo-naive women diagnosed with HGSOC into chemotherapy-resistant- and sensitive-like strata with a significant difference in overall survival (OS). Independent analysis of The Cancer Genome Atlas data further confirmed that high *OSGEPL1* expression is a favorable prognostic factor for HGSOC.

Conclusions.—We have developed a novel multivariate model based on clinico-pathologic data and a cfDNA-derived 5hmC modified gene, *OSGEPL1*, that predicted response to platinum-based chemotherapy in intermediate-sensitive HGSOC. Our multivariate model applies to chemo-naïve samples regardless if the patint was treated with adjuvant or neoadjuvant chemotherapy. These results merit further investigation of the predictive capability of our model in larger cohorts.

Keywords

High-grade serous ovarian cancer; Chemotherapy resistance; Biomarkers; Cell-free DNA, liquid biopsies

1. Introduction

High-grade serous ovarian cancer (HGSOC) is the most common subtype of ovarian cancer (OvCa), and, in its advanced stages, accounts for most OvCa-related mortality. Current standard treatment strategies for advanced-stage HGSOC are primary debulking surgery paired with taxane- and platinum-based chemotherapy or neoadjuvant chemotherapy (NACT) followed by interval debulking surgery (IDS). The initial response rate to platinum-based chemotherapy is high, with only 20–30% of women exhibiting primary resistance. However, most of the remaining 70–80% of women will acquire therapeutic resistance over time, relapse, and succumb to their disease [1].

Response to platinum-based adjuvant chemotherapy (AC) is clinically classified into four categories: (1) platinum-refractory (progression during treatment), (2) platinum-resistant (recurrence within less than six months), (3) intermediate-sensitive (recurrence between 6 and 12 months), and (4) platinum-sensitive (recurrence after 12 months) [2,3]. Multiple mechanisms involved in developing platinum resistance have been elucidated [4], but no biomarkers have yet been identified that can reliably predict which women will develop therapy resistance. Therefore, we continue to classify patient response as we did 30 years ago; by the timing of the first recurrence. A reliable biomarker, predictive of platinum response, should prospectively identify women who will respond or continue to respond to platinum-based chemotherapy after retreatment and allow clinicians to provide alternative options sooner to those who lack a durable response.

Malignant transformation is driven by genetic aberrations and wide-spread epigenetic changes such as DNA methylation [5,6]. Modifications of the DNA base cytosine in the

form of 5-methylcytosine (5mC) can create a repressed chromatin environment, leading to gene suppression. In contrast, the oxidation product of 5mC, 5-hydroxymethylcytosine (5hmC), likely creates an open chromatin environment that enables active gene transcription [7,8] (Fig. S1A). In 2011, the 5hmC-Seal method was developed to allow genome-wide profiling of 5hmC modifications on genomic DNA [9]. Later, the method was adapted for circulating cell-free DNA (cfDNA) and termed nano-hmC-Seal [10–12]. Since then, 5hmC modifications on genomic DNA have been studied using nano-hmC-Seal in a variety of solid and hematological cancers as diagnostic or prognostic biomarkers [13–15].

We report the genome-wide profiling of 5hmC on cfDNA isolated from serum samples from women with HGSOC at the time of surgery to determine the molecular features of HGSOC and to discover biomarkers predictive of carboplatin response. We found that 5hmC-modified genes and genomic regions can be used to predict chemoresistance in chemo-naïve samples from women with HGSOC.

2. Materials and methods

2.1. Inclusion criteria

Women diagnosed with HGSOC, undergoing surgical treatment by a gynecologic oncologist at the University of Chicago Medical Center and receiving carboplatin and paclitaxel-based first line adjuvant or neoadjuvant chemotherapy were included in this study. Please refer to Table S1 for patient meta data.

Study design, patient cohort, and definitions.

Women treated with adjuvant chemotherapty (AC) underwent upfront surgery followed by 6 cycles of carboplatin/paclitaxel-based chemotherapy. Women treated with NACT received between one to seven cycles of chemotherapy with carboplatin and paclitaxel, followed by interval debulking surgery (IDS) and postoperative chemotherapy. Clinico-pathological data were prospectively collected in a MS ACCESS database [16].

Platinum sensitivity was determined based on the number of days after the last cycle of chemotherapy until disease recurrence or progression. Women who recurred within 182 days were considered resistant, women who recurred within 182–365 days were considered intermediate-sensitive, and women who recurred after 365 days were considered sensitive [2,3]. Platinum refractory women were not included in this study. Platinum resistant days were calculated based on the date of the last chemotherapy treatment of the first line chemotherapy and the date the first recurrence/progression was diagnosed. Disease-free intervals (DFI) were calculated as days from the treatment start date (either surgery or the first cycle of chemotherapy) to the first recurrence or last follow-up date. Overall survival (OS) days were determined based on days between the treatment start date and the date of death. Recurrence was defined as either a) tripling in CA-125 (>100 U/mL), b) new disease detected on CT, MRI or sonographic imaging, or c) disease detected by clinical exam (preferentially with biopsy or presence of ascites). Disease-free intervals and OS, platinum resistance, disease recurrence, and subsequent chemotherapies were updated every three months by experienced clinicians (M. K.). Since most women in the NACT cohort had

no chemotherapy response score (CRS) associated with pathology, hematoxylin and eosin-stained omental tumor slides were retrospectively pulled and given a CRS score by a trained gynecologic pathologist (A. J. B.) using the International Collaboration on Cancer Reporting recommendations for CRS [17,18]. CA-125 ratio was determined for NACT women using the following formula: CA-125 ratio = pre-NACT CA-125/(pre-IDS CA-125+ post-IDS CA-125). CA-125 ratio was only determined for women who had all three CA-125 values recorded. The University of Chicago Institutional Review Board approved all protocols.

2.2. Blood samples and processing

Blood samples were prospectively collected from women with HGSOC before their surgery. For serum collection, blood was collected in red top tubes (without anti-coagulant) and allowed to clot for 30 min at room temperature. For plasma collection, blood samples were collected in purple top tubes (containing EDTA) and processed immediately. The blood was centrifuged at 3000 rpm at 4 °C for 11 min to separate serum from the clot or to separate the plasma respectively. Genomic DNA (gDNA) was isolated from whole human blood cells (WBC) using the Wizard Genomic DNA purification kit (Promega) according to the manufacturer's instructions. The serum, plasma and gDNA (WBC) were transferred to cryotubes and stored at −80 °C until further use. cfDNA was extracted using the QIAamp Circulating Nucleic Acid kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

2.3. Nano-hmC-Seal library preparation and sequencing

All samples were divided into equal assay batches so that each batch contained samples from all demographics and chemo-response categories. Technical and biological replicates were included in each batch for inter and intra-assay controls. Nano-hmC-Seal libraries were constructed from 6 to 10 ng of cfDNA or from 100 ng of gDNA as previously described [10–12,19]. Briefly, 1) 5hmC modifications on cfDNA were enzymatically labeled, 2) biotinylation was performed, 3) 5hmC-specific pull-down was accomplished using streptavidin Dynabeads, and 4) libraries were amplified by PCR. Libraries were sequenced using fifty base-pair, paired-end sequencing on an Illumina NovaSeq 6000. FASTQC [20], version 0.11.9 was used to assess sequence quality. Raw reads were trimmed using Trim Galore (RRID:SCR_011847) and mapped to the human genome (hg19) using Bowtie2 (RRID:SCR_016368), version 2.2.5 [21]. PCR duplications were removed by Samtools (RRID:SCR_002105), version 1.14[22]. Only uniquely mapped reads were retained for further analysis.

2.4. 5hmC distribution by genomic feature

Reads on genome-wide 5-kb sliding windows were counted by FeatureCounts, version 2.0.1. All sliding windows were annotated to different genomic features by bedtools (RRID:SCR_006646), version 2.30.0 DESeq2 (RRID:SCR_000154) -normalized read counts on different genomic features related to sliding windows were compared. The Kruskal-Wallis test was used to assess differences between normalized levels of 5hmC between groups according to a particular genomic feature type (e.g., gene body). CpG islands were downloaded from the, UCSC Genome Browser (University of California, Santa

Cruz) and CpG shores were defined as 2-kb regions adjacent to each CpG island. Enhancer regions were annotated according to the GeneHancer database (RRID:SCR_023953) [23].

2.5. Identification of genes with differential 5hmC and functional enrichment analysis

Aligned reads on gene bodies with a mapping quality score of 10 or higher were calculated using FeatureCounts [24]. Read counts were then normalized with corrected batch effects and compared by DESeq2 version 1.34.0 [25]. Pheatmap package, version 1.0.12 (R Project), was used to determine the distance matrix between samples for hierarchical clustering. Functional enrichment analysis was performed using a Metascape tool [26]. Differentially highly- and lowly hydroxymethylated genes with a p-value <0.05 were used to find relative enrichment of functional items according to the default settings of Metascape. Genes that had increased and decreased 5hmC within each treatment/ analysis group were used for functional enrichment analysis.

2.6. Generation and validation of the chemo-response prediction model

Normalized read counts for all chemo-naïve- or chemo-treated and chemo-sensitive or chemo-resistant samples were used to calculate the coefficient of variation. The top 500 age- and batch-corrected genes with the largest coefficients of variation were kept for further feature selection, together with additional clinical factors. Elastic net with 5-fold cross-validation, optimized for the area under the curve (AUC), was repeated 100 times, and the frequencies of each feature selected were calculated. The top three biomarkers were selected for the final model building. The final models were used on intermediate samples for classification. Kaplan-Meier survival analysis with log-rank test was performed using the Survminer package in R project to examine the OS difference across different groups. The model for chemo-naïve samples was built using the following three features: “Largest residual implant,” “Pre-operative CA-125 (PreOPCA125),” and “Top one gene,” while the model for chemotherapy-treated samples was built using the following three features: “Largest residual implant,” “CRS” and “Top one gene.”

2.7. Data analysis using cBioportal.org and GEPIA

The OncoPrint plot was generated using cBioPortal. The Ovarian Serous Cystadenocarcinoma (TCGA, PanCancer Atlas) cohort was selected for this analysis. Samples that were profiled for mutations, structural variants, putative copy-number alterations, and mRNA expression z-scores relative to diploid samples with a z-score threshold of ±1.5 were included. Only case sets with mRNA data were selected, resulting in a total cohort of 300 samples. Box plots, stage plots, and survival data were generated with GEPIA using a $|Log_2FC|$ cutoff of 1.5. Matching of TCGA normal and GTEx data was used. OSGEPL1 survival plots were created using the median gene expression cutoff.

2.8. Statistical analysis

All statistical analyses were performed in GraphPad Prism (version 10.0.0). Significance between cohorts and sensitivities for OS (Fig. S1B) was determined using the Kruskal-Wallis test, taking multiple comparisons into consideration. Wilcoxon signed-rank test was used to determine if CA-125 was significantly reduced before IDS (Fig. S1B).

Spearman correlation was used to determine positive and negative correlations between clinico-pathologic factors (Fig. S1D and Fig. 2A). For Spearman correlation, approximate *p*-values were reported. Only correlation coefficients with significance $p < 0.05$ were reported in the heatmap. Kaplan-Meier survival analysis with log-rank test was performed to examine the OS difference across different groups (Fig. 5C, F). All tests for clinical analysis were two-tailed, and $p < 0.05$ and $q < 0.05$ were considered statistically significant.

3. Results

3.1. Validation of nano-hmC-Seal using serum samples

Nano-hmC-Seal has been extensively studied using gDNA and plasma-derived cfDNA as an analyte, but its applicability to serum-derived cfDNA remains unexplored. To validate nano-hmC-Seal on serum samples, patient-matched serum, plasma and gDNA (WBC) samples, were analyzed. Cell-free DNA was isolated from plasma and serum samples showing no significant difference in cfDNA yield (Fig. 1A). Following nano-hmC-Seal and sequencing, the mapping ratio, non-redundant fraction, ratio of assigned reads, principal component analysis (PCA), saturation analysis and density plot analysis were performed to asses performance differences between analytes (Fig. S1). Analysis revealed no significant differences between 5hmC annotations from serum- or plasma-derived cfDNA and that cfDNA is different from gDNA. Spearman correlation coefficient analysis further confirmed that serum and plasma were highly correlated (Fig. 1B). This data demonstrates that serum-derived cfDNA may be used for nano-hmC-Seal.

3.2. Clinico-pathologic characteristics and genome-wide 5hmC profiling in high-grade serous ovarian cancer

To study the role of 5hmC in chemotherapy resistance, archived serum samples from women with HGSOC were selected for the study based on treatment approach (AC and NACT), chemotherapy treatment (chemo-naïve and -treated samples) and platinum sensitivity (Fig. 2A). The median follow-up for living women was 58 months. The final cohort included 58 chemo-naïve samples and 53 chemo-treated samples (Table S1–2). As expected, OS was significantly different between platinum-sensitive, –intermediate, and -resistant women, while there was no difference in treatment approach (AC or NACT) (Fig. S2B) [27,28]. NACT led to a significant reduction in CA-125 before IDS in all three platinum response subgroups (Fig. S2C), but did not correlate with chemotherapy response score (CRS) (Fig. S2D–E) [18]. Spearman correlation coefficient analysis of clinico-pathologic factors was performed to identify clinical factors that significantly correlate with survival and platinum sensitivity. Disease-free days, platinum-resistant days, platinum sensitivity, and the largest residual implant showed a significant positive correlation with OS, while pre-operative CA-125 showed a significant negative correlation with the factors mentioned above in the chemo-naïve and -treaded cohorts. Additionally, a significant positive correlation was observed for CRS and OS, while post-operative CA-125 and post-operative chemotherapy response showed a significant negative correlation in chemo-treated/ NACT (Fig. S2E).

Nano-hmC-Seal and next-generation sequencing (NGS) were performed to profile genome-wide 5hmC distributions [10–12]. The sample volumes, —age, and degree of hemolysis was

not correlated with clinico-pathologic data (Fig. 2B). Principle component analysis (PCA) confirmed that no known factors (i.e., disease characteristics, treatment variability, or batch effects) introduced significant bias to the dataset (Fig. 2C). Nano-hmC-Seal [11,12] was performed on all samples and 5hmC distribution (Fig. S2F) and distribution enrichment by genomic features (Fig. 2D) were assessed. Exons, promoters, transcription termination sites (TTS), 3′- and 5′-UTRs made up 13.51% of annotated 5hmC regions (Fig. S2F). Distribution enrichment by genomic features showed that 5hmC was reduced in non-coding regions (satellite, LINE, and simple repeats) (Fig. 2D). As expected, platinum sensitivity and treatment type did not affect global 5hmC distribution in genomic features (Fig. S2G). Taken together, this demonstrates that our nano-hmC-Seal data is suitable for further downstream discovery.

3.3. 5hmC patterns differ across women and undergo changes during treatment

Neoadjuvant chemotherapy treatment is used for women with advanced FIGO stage IIIC-IV HGSOC presenting with high tumor burden and multiple comorbidities [29]. If the patient is referred for IDS from a different provider or hospital, the acquisition of chemo-naïve samples may prove difficult. We attempted to assess if a signature can be identified and applied to samples from women that already received chemotherapy. To determine if differentially hydroxymethylated genes (DhMGs) reflect changes induced by chemotherapy treatment, we compared matched samples from women before NACT (i.e. chemo-naïve) and after 1–3 cycles of carboplatin and paclitaxel (but before IDS) (Table S3). We also compared the chemo-naïve and -treated cohorts (Table S1–2).

As expected, chemotherapy did not alter global 5hmC deposition patterns on genomic features (Fig. S3A and S3D). A differential comparison was performed to identify DhMGs that could separate chemo-naïve from chemotherapy-treated samples (Table S45). Using PCA and hierarchical clustering analyses, DhMGs of patient-matched serum separated samples by treatment (Fig. S3B), while unmatched chemo-naïve and -treated samples resulted only in partial separation (Fig. 3A and S3E). Next, functional pathway enrichment analysis was performed. Before chemotherapy, serum samples were enriched in pathways associated with neuron development, differentiation, and synapse organization (Table S4–5, Fig. 3B and D). After chemotherapy, serum samples were enriched in inflammation and immune cell regulation pathways (Fig. 3B and S3C).

3.4. Pathways driving chemoresistance in HGSOC

To assess if serum-derived DhMGs can separate chemotherapy-sensitive from -resistant women, PCA and hierarchical clustering were performed for the combined cohort (Fig. S4A, S4C and Table S6), the chemo-naïve cohort (Fig. 4A, Fig. S4A and Table S7), and for the chemo-treated cohort (Fig. 4C, Fig. S4A and Table S10). Hierarchical clustering identified DhMGs in chemo-naïve and -treated samples (Fig. 3A and C). Comparison of DhMGs in sensitive (>12 months DFI) and resistant women (<6 months DFI) revealed no overlap in identified DhMGs by chemotherapy status (Fig. S4B). Functional pathway enrichment analysis for the combined cohorts revealed that platinum-resistant disease was enriched in cellular response to stress (Fig. S4D and Table S6). chemo-naïve samples from-therapy resistant women were enriched in several metabolic pathways (e.g., monocarboxylic acid

metabolic process, pyruvate metabolism, etc.) and drug metabolism (e.g., drug ADME) (Fig. 4B). Chemo-treated samples from -resistant women were enriched in cellular stress response and canonical Wnt signaling pathways known to facilitate cancer stemness and chemo-resistance [30] (Fig. 4D and Table S8).

3.5. 5hmC is a predictive biomarker of chemo-response and overall survival in HGSOC

Given that intermediate-sensitive women develop recurrence within 6 to 12 months, it is clinically challenging to predict chemotherapy response. Using clinico-pathologic data and the top DhMGs identified in chemo-naïve and -treated samples, a model was built to stratify intermediate-sensitive women into platinum sensitive- and resistant-like groups (Fig. 5A and D). The chemo-naïve model was built using a combination of the largest residual implant, pre-operative CA-125, and the DhMGs "*OSEGPL1*", a tRNA-modifying gene found in mitochondria [31]. The resulting model was trained on chemo-naïve, therapy-sensitive and -resistant patient serum (i.e., training set), and the area under the curve (AUC) calculated to assess predictive performance. The model achieved an AUC of 0.91 (95% CI, 0.82–0.99.85) in the training set (Fig. 5B). The model was then validated on intermediate sensitive samples and successfully stratified intermediate-sensitive women into resistant- and sensitive-like groups (Fig. 5C; $p = 0.033$). Based on the models stratification, Kaplan-Meier plots were generated showing a significant difference in OS. TCGA analysis revealed that approximately 25% of OSGEPL1-profiled HGSOC showed genetic alterations and changes in transcription levels (Fig. S5A). Further analysis revealed that OSGEPL1 is downregulated in HGSOC, and that expression decreases with higher stage (Fig. S5B–C). OSGEPL1 was favorably prognostic for overall survival (Fig. S5D) in the TCGA dataset. Similarly, a model for chemo-treated samples was built using a combination of CRS, the largest residual implant, and the DhMGs "*ALG1L2*" (Fig. 5D), a glycosylation modifying enzyme [32], achieving an AUC of 0.927 (95% CI, 0.84–1.00) (Fig. 5E). Though the model for chemo-treated samples achieved a slightly higher AUC than the model for chemo-naïve samples in their respective testing sets, there was no significant difference in the survival curves for intermediate-sensitive women if the sample was taken after chemotherapy, but before IDS (Fig. 5F; $p = 0.34$). Taken together, these data demonstrate that 5hmC-modified genes combined with clinico-pathologic features are capable of predicting survival outcomes in chemo-naïve samples of AC and NACT-treated women with HGSOC.

4. Discussion

Most women (70–80%) with HGSOC will suffer from disease progression due to chemotherapy resistance, and no single mechanism explains how resistance develops [33]. In addition, tumor heterogeneity of HGSOC metastasis makes it difficult to understand drug resistance, determine patient response, and requires invasive biopsies. Non-invasive methods, such as liquid biopsies, can help overcome some of these issues since they contain diverse tumor-derived analytes from primary and distant tumor sites [34]. The contribution of the methylome to tumor development and progression has revealed that methylation changes occur early in tumorigenesis, are highly pervasive across tumor types, and can be easily detected in cfDNA from liquid biopsies [5,15]. Therefore, methylation changes and liquid biopsies have been the focus of biomarker development. Platinum-free intervals are

used clinically to determine if women with recurrence will benefit from further platinum-based therapy. While this measure may be helpful in women with platinum-sensitive recurrence, its helpfulness for women with refractory-, intermediate-sensitive or platinum-resistant disease is less established. We therefore sought to identify methylation-based biomarkers predictive of chemoresistance and to identify their underlying epigenetically regulated genes and pathways.

In our study, DhMGs identified in the chemo-naïve, treatment-resistant group were enriched in xenobiotic (CYP2B6, CYP3A7, GSTA2), lipid (ACOT12, AGPAT1, PLIN2) and fatty acid metabolism (SLC27A5, PTGR1, GCDH). The cytochrome P450 (CYP) superfamily of enzymes catalyzes xenobiotic oxidations (and lipid hydroxylations), which can create reactive oxygen species [35], leading to the upregulation of antioxidant genes (GSTA2, GSS, PTGR1) [36]. Chemo-treated, serum samples from therapy-resistant women showed enrichment in DhMGs associated with various metabolic and catabolic processes suggesting that changes in metabolism and stress response provide a selection advantage for therapy resistant HGSOC. Metabolic reprogramming inducing fatty acid uptake and β-oxidation has been observed in platinum-resistant OvCa [37].

Half of all recurrent patients will suffer from platinum-resistant (25%) or -intermediate-sensitive HGSOC (25%). While <10% of women with resistant HGSOC will respond to further platinum treatment, most uncertainty arises when selecting treatments for women with intermediate-sensitive HGSOC, who have an approximately 30% response rate to additional platinum therapy [38]. Using the top DhMG for chemo-naïve samples O-sialoglycoprotein endopeptidase like 1 (OSGEPL1), the pre-operative CA-125, and largest residual implant at the end of surgery, we build a model that can stratify intermediate-sensitive patients as sensitive (into a more sensitive group), who could benefit from further platinum treatment, and a more resistant group, likely to benefit from non-platinum therapies, regardless of treatment approach. An independent analysis of TCGA data confirmed that high OSGEPL1 expression is a favorable prognostic marker for OvCa. OSGEPL1 is a protein located in mitochondria, and is involved in tRNA threonylcarbamoyladenosine modifications and mitochondrial genome maintenance [31]. Knock down of OSGEPL1 in HEK293T cells causes mitochondrial dysfunction [39], lower oxygen consumption rates and ATP levels, indicative of damage to oxidative phosphorylation (“electron transfer chain”), and reduced mitochondrial translation. Therefore, OSGEPL1 may promote platinum sensitivity by maintaining normal mitochondrial functions (e.g., activation of intrinsic apoptosis etc.) [40]. In chemo-treated samples, ALG1L2 was the top DhMG. Though the resultant chemo-treated model had a high AUC, it was not able to efficiently stratify intermediate-sensitive women. The number of upfront chemotherapy cycles and inherent patient heterogeneity might have influenced model performance. Like OSGEPL1, the role of ALG1L2 in chemoresistance and OvCa is currently unknown. If validated, our predictor may help identify women at risk of developing therapy resistance prior to intervention and identify women with intermediate-sensitive disease that may profit from further platinum-based chemotherapy.

4.1. Limitations

Limitations of our study include low samples numbers, especially in the validation cohorts (chemo-naïve- and -treated, intermediate-sensitive women). This, in addition to patient heterogeneity, may explain why the model for chemo-treated samples could not stratify the intermediate-sensitive group. Women undergoing NACT often have widespread metastatic disease that can not be completely surgically removed without prior chemotherapy. The number of neoadjuvant cycles can rage from 1 to 8 with a general median of four cycles, further increasing heterogeneity. Another limitation of this study was the use of a few archived serum samples that were up to ten years old. This was not a prospective clinical trial, therefore follow-up (CA-125 and imaging) was variable making the precise time of progression and recurrence a less accurate than in a prospective trial. While we showed no correlations between storage age, hemolysis, and cfDNA content, we had limited records on how older serum samples were processed. Furthermore, while there are published studies that show 5hmC in gene bodies, promoters, and enhancers is correlated with gene expression (Cui et al., 2020), we did not perform RNA-sequencing on matched tumor samples to validate these findings, due to a lack of frozen tissue availability. Therefore, conclusions drawn from our data rely on literature references. Of note clinical practices have changed in the last years with increased use of PARP inhibitors and universal BRCA testing. While we did not observe a significant number of BRCA carriers and usage of PARP inhibitors in our dataset, their use impacts treatment response and prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of competing interest

Ernst Lengyel reported receiving research funding to perform experimental pre-clinical research on ovarian cancer from Arsenal Bioscience, AbbVie, outside the submitted work. Chuan He is a scientific founder, a member of the scientific advisory board and equity holder of Aferna Bio, Inc., and AccuraDX Inc., a scientific cofounder and equity holder of Accent Therapeutics, Inc., and a member of the scientific advisory board of Rona Therapeutics. Wei Zhang is a consultant for the biomarker discovery program of Tempus Labs, Inc. All other authors declare no relationships that may present a conflict of interest.

Data availability

Requests for resources, reagents, and additional information to reanalyze the data should be directed to the lead contact, Ernst Lengyel (elengyel@bsd.uchicago.edu). De-identified human DNA sequencing data have been deposited at NCBI GEO (GSE240535).

Abbreviations:

5hmC	5-hydroxymethylcytosine
AC	Adjuvant chemotherapy
cfDNA	Cell-free DNA
CRS	Chemotherapy response score
DFI	Disease-free intervals
DhMGs	Differentially hydroxymethylated genes
HGSOC	High-grade serous ovarian cancer
IDS	Interval debulking surgery
LINE	Long interspersed nuclear element
LTR	Long terminal repeat
NACT	Neoadjuvant chemotherapy
SINE	Short interspersed nuclear element
TSS	Transcription start site
TTS	Transcription termination site
UTR	Untranslated region

References

[1]. Kurnit KC, Fleming GF, Lengyel E, Updates and newoptions in advanced epithelial ovarian cancer treatment, Obstet. Gynecol 137 (1) (2021) 108–121. [PubMed: 33278287]

[2]. Markmann M, et al. , Second-line platinum therapy in patients with ovarian cancer previously treated with cisplatin, J. Clin. Oncol 9 (3) (1991) 389–393. [PubMed: 1999708]

[3]. Gore ME, et al. , Treatment of relapsed carcinoma of the ovary with cisplatin or carboplatin following initial treatment with these compounds, Gynecol. Oncol 36 (2) (1990) 207–211. [PubMed: 2404837]

[4]. Khan MA, et al. , Platinum-resistant ovarian cancer: from drug resistance mechanisms to liquid biopsy-based biomarkers for disease management, Semin. Cancer Biol 77 (2021) 99–109. [PubMed: 34418576]

[5]. Nishiyama A, Nakanishi M, Navigating the DNA methylation landscape of cancer, Trends Genet. 37 (11) (2021) 1012–1027. [PubMed: 34120771]

[6]. Vasanthakumar A, Godley LA, 5-hydroxymethylcytosine in cancer: significance in diagnosis and therapy, Cancer Gene Ther. 208 (5) (2015) 167–177.

[7]. Tahiliani M, et al. , Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1, Science 324 (5929) (2009) 930–935. [PubMed: 19372391]

[8]. Hackett JA, et al. , Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine, Science 339 (6118) (2013) 448–452. [PubMed: 23223451]

[9]. Song CX, et al. , Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine, Nat. Biotechnol 29 (1) (2011) 68–72. [PubMed: 21151123]

[10]. Han D, et al. , A highly sensitive and robustmethod for genome-wide 5hmC profiling of rare cell populations, Mol. Cell 63 (4) (2016) 711–719. [PubMed: 27477909]

[11]. Li W, et al. , 5-Hydroxymethylcytosine signatures in circulating cell-free DNA as diagnostic biomarkers for human cancers, Cell Res. 27 (10) (2017) 1243–1257. [PubMed: 28925386]

[12]. Song CX, et al. , 5-Hydroxymethylcytosine signatures in cell-free DNA provide information about tumor types and stages, Cell Res. 27 (10) (2017) 1231–1242. [PubMed: 28820176]

[13]. Cai J, et al. , An integrative analysis of genome-wide 5-hydroxymethylcytosines in circulating cell-free DNA detects noninvasive diagnostic markers for gliomas, Neurooncol. Adv 3 (1) (2021) p. vdab049. [PubMed: 34151267]

[14]. Chennakesavalu M, et al. , 5-Hydroxymethylcytosine Profiling of Cell-Free DNA Identifies Bivalent Genes that Are Prognostic of Survival in High-Risk Neuroblastoma, bioRxiv 2023.

[15]. Chiu BC, et al. , Alterations of 5-hydroxymethylation in circulating cell-free DNA reflect molecular distinctions of subtypes of non-Hodgkin lymphoma, NPJ Genom. Med 6 (1) (2021) 11. [PubMed: 33574286]

[16]. Sawada K, et al. , Loss of E-cadherin promotes ovarian cancer metastasis via alpha 5-integrin, which is a therapeutic target, Cancer Res. 68 (7) (2008) 2329–2339. [PubMed: 18381440]

[17]. McCluggage WG, et al. , Data set for reporting of ovary, fallopian tube and primary peritoneal carcinoma: recommendations from the international collaboration on cancer reporting (ICCR), Mod. Pathol 28 (8) (2015) 1101–1122. [PubMed: 26089092]

[18]. Bohm S, et al. , Chemotherapy response score: development and validation of a system to quantify histopathologic response to neoadjuvant chemotherapy in Tuboovarian high-grade serous carcinoma, J. Clin. Oncol 33 (22) (2015) 2457–2463. [PubMed: 26124480]

[19]. Pastor WA, et al. , Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells, Nature 473 (7347) (2011) 394–397. [PubMed: 21552279]

[20]. B. Bioinformatics, Fastqc: A Quality Control Tool for High Throughput Sequence Data, Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ 2011.

[21]. Bioinformatics, B, Trim Galore, 11/19/19; Available from: http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/; 2019.

[22]. Li H, et al. , The sequence alignment/map format and SAMtools, Bioinformatics 25 (16) (2009) 2078–2079. [PubMed: 19505943]

[23]. Fishilevich S, et al. , GeneHancer: genome-wide integration of enhancers and target genes in GeneCards, Database (Oxford) (2017) bax028. [PubMed: 28605766]

[24]. Liao Y, Smyth GK,Shi W, featureCounts: an efficient general purpose program for assigning sequence reads to genomic features, Bioinformatics 30 (7) (2014) 923–930. [PubMed: 24227677]

[25]. Varet H, et al. , SARTools: a DESeq2- and EdgeR-based R pipeline for comprehensive differential analysis of RNA-Seq data, PLoS One 11 (6) (2016), e0157022. [PubMed: 27280887]

[26]. Zhou Y, et al. , Metascape provides a biologist-oriented resource for the analysis of systems-level datasets, Nat. Commun 10 (1) (2019) 1523. [PubMed: 30944313]

[27]. Kuhn W, et al. , Neoadjuvant chemotherapy followed by tumor debulking prolongs survival for patients with poor prognosis in International Federation of Gynecology and Obstetrics stage IIIC ovarian carcinoma, Cancer 92 (2001) 2585–2591. [PubMed: 11745193]

[28]. Vergote I, et al. , Neoadjuvant chemotherapy is the better treatment option in some patients with stage IIIC to IV ovarian cancer, J. Clin. Oncol 29 (31) (2011) 4076–4078. [PubMed: 21931032]

[29]. Vergote I, et al. , Neoadjuvant chemotherapy versus debulking surgery in advanced tubo-ovarian cancers: pooled analysis of individual patient data from the EORTC 55971 and CHORUS trials, Lancet Oncol. 19 (12) (2018) 1680–1687 (%! Neoadjuvant chemotherapy versus debulking surgery in advanced tubo-ovarian cancers: pooled analysis of individual patient data from the EORTC 55971 and CHORUS trials %@ 1474-5488 (Electronic). [PubMed: 30413383]

[30]. Nagaraj AB, et al. , Critical role of Wnt/beta-catenin signaling in driving epithelial ovarian cancer platinum resistance, Oncotarget 6 (27) (2015) 23720–23734. [PubMed: 26125441]

[31]. Zhou JB, et al. , Molecular basis for t6A modification in human mitochondria, Nucleic Acids Res. 48 (6) (2020) 3181–3194. [PubMed: 32047918]

[32]. Qiu P, et al. , Emerging glyco-risk prediction model to forecast response to immune checkpoint inhibitors in colorectal cancer, J. Cancer Res. Clin. Oncol 149 (9) (2023) 6411–6434. [PubMed: 36757621]

[33]. Marchetti C, et al. , Chemotherapy resistance in epithelial ovarian cancer: mechanisms and emerging treatments, Semin. Cancer Biol 77 (2021) 144–166. [PubMed: 34464704]

[34]. Ignatiadis M, Sledge GW, Jeffrey SS, Liquid biopsy enters the clinic - implementation issues and future challenges, Nat. Rev. Clin. Oncol 18 (5) (2021) 297–312. [PubMed: 33473219]

[35]. Klotz LO, Steinbrenner H, Cellular adaptation to xenobiotics: interplay between xenosensors, reactive oxygen species and FOXO transcription factors, Redox Biol. 13 (2017) 646–654. [PubMed: 28818793]

[36]. Silva MM, et al. , The balance between NRF2/GSH antioxidant mediated pathway and DNA repair modulates cisplatin resistance in lung cancer cells, Sci. Rep 9 (1) (2019) 17639. [PubMed: 31776385]

[37]. Tan Y, et al. , Metabolic reprogramming from glycolysis to fatty acid uptake and beta-oxidation in platinum-resistant cancer cells, Nat. Commun 13 (1) (2022) 4554. [PubMed: 35931676]

[38]. Oronsky B, et al. , A brief review of the management of platinum-resistant-platinum-refractory ovarian cancer, Med. Oncol 34 (6) (2017) 103. [PubMed: 28444622]

[39]. Lin H, et al. , CO(2)-sensitive tRNA modification associated with human mitochondrial disease, Nat. Commun 9 (1) (2018) 1875. [PubMed: 29760464]

[40]. Porporato PE, et al. , Mitochondrial metabolism and cancer, Cell Res. 28 (3) (2018) 265–280. [PubMed: 29219147]

HIGHLIGHTS

- Chemotherapy does not alter global 5hmC deposition patterns on genomic features.
- Neoadjuvant-treated platinum resistant disease is enriched in pathways associated with cellular responses to stress.
- Adjuvant-treated platinum resistant disease is enriched in pathways associated with drug- and metabolism pathways.
- An adjuvant chemotherapy-specific model stratifies intermediate sensitive disease into resistant- and sensitive-like groups.

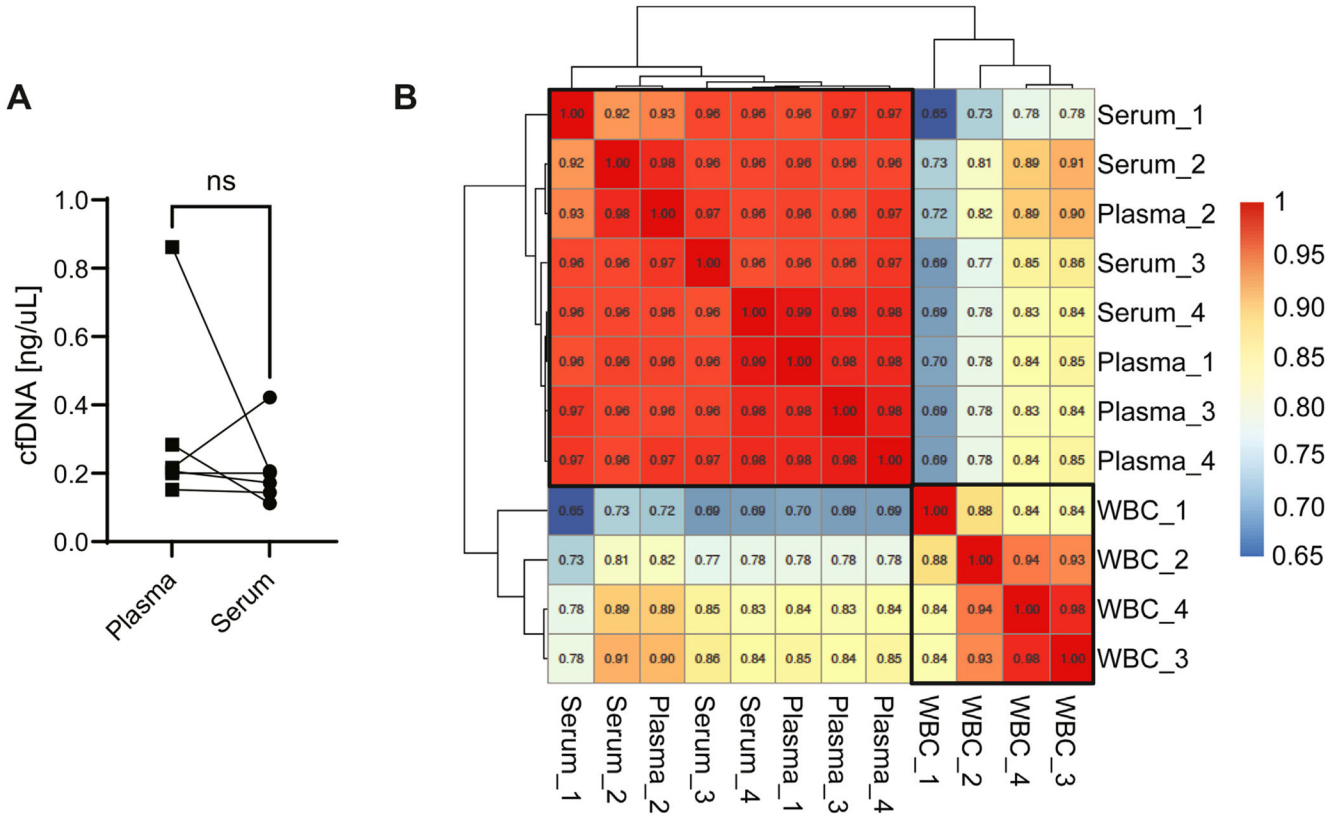

Fig. 1.

Serum-derived cfDNA as an analyte for nano-hmC-Seal. (A) Normalized cfDNA yield from patient-matched serum and plasma samples. (B) Spearman correlation coefficient matrix of serum-, plasma- and gDNA (WBC)-derived 5hmC annotated reads using a 10 kb sliding window.

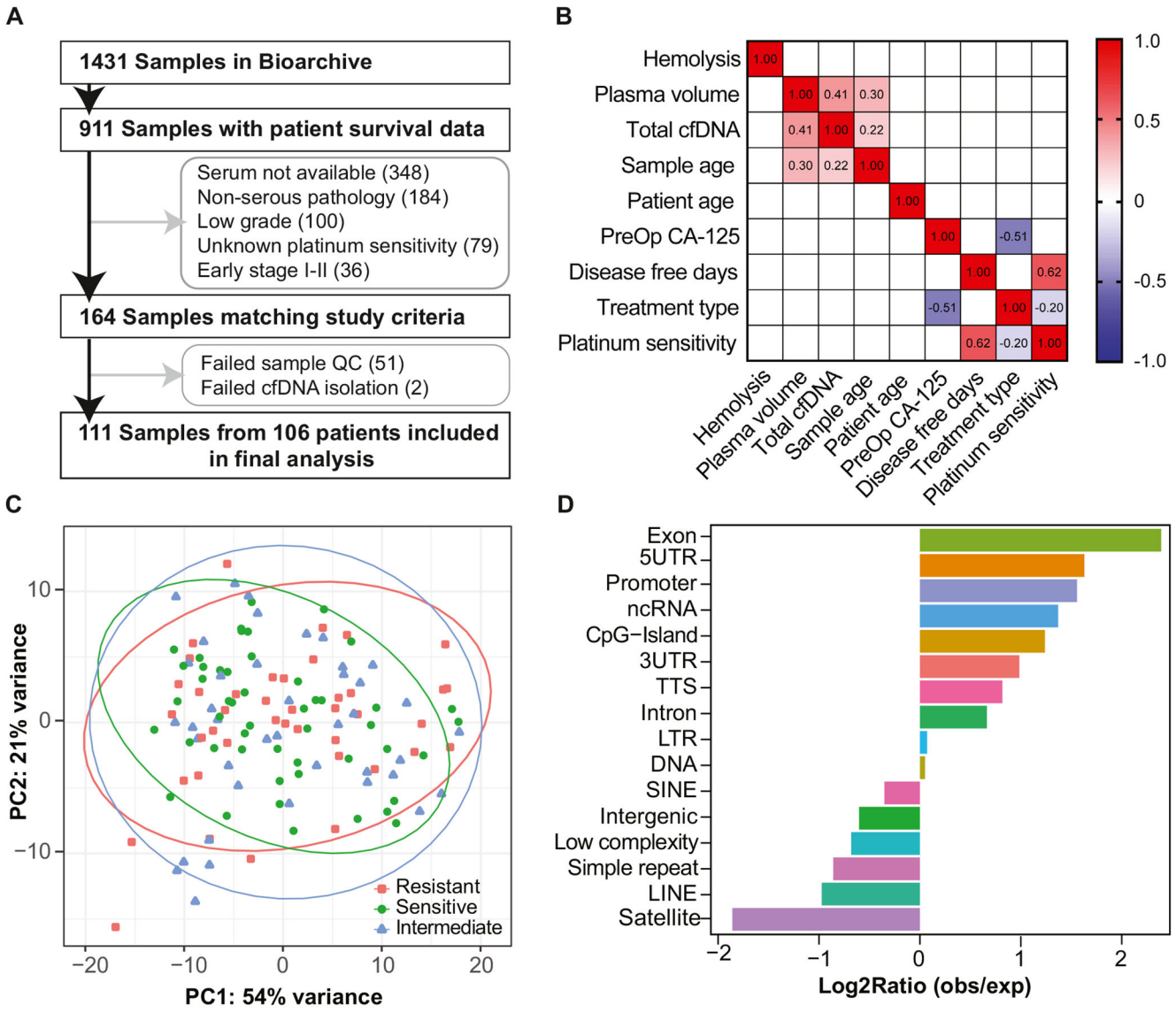

Fig. 2.

Clinical cohort characteristics and serum-based 5hmC profiling in women with HGSOC. (A) Flowchart illustrating the sample selection and inclusion criteria for the final cohort and (B) Heatmap of spearman correlation matrix for factors influencing cfDNA concentration. Spearman rank correlation coefficient (r) for each comparison is shown in individual cells that have a *p*-value of <0.05. (C) Principal component analysis (PCA) plot of global differentially hydroxymethylated genes (DhMGs) by platinum sensitivity. (D) 5hmC distribution enrichment by genomic features for all samples. LINE: Long-interspersed nuclear element, SINE: Short-interspersed nuclear element, LTR: Long terminal repeat, TTS: Transcription termination site, 3'UTR: 3' prime untranslated region, CpG-Island: palindromic cytosine-phosphate-linked-guanine islands, ncRNA: Non-coding RNA and 5'UTR: 5' prime untranslated regions.

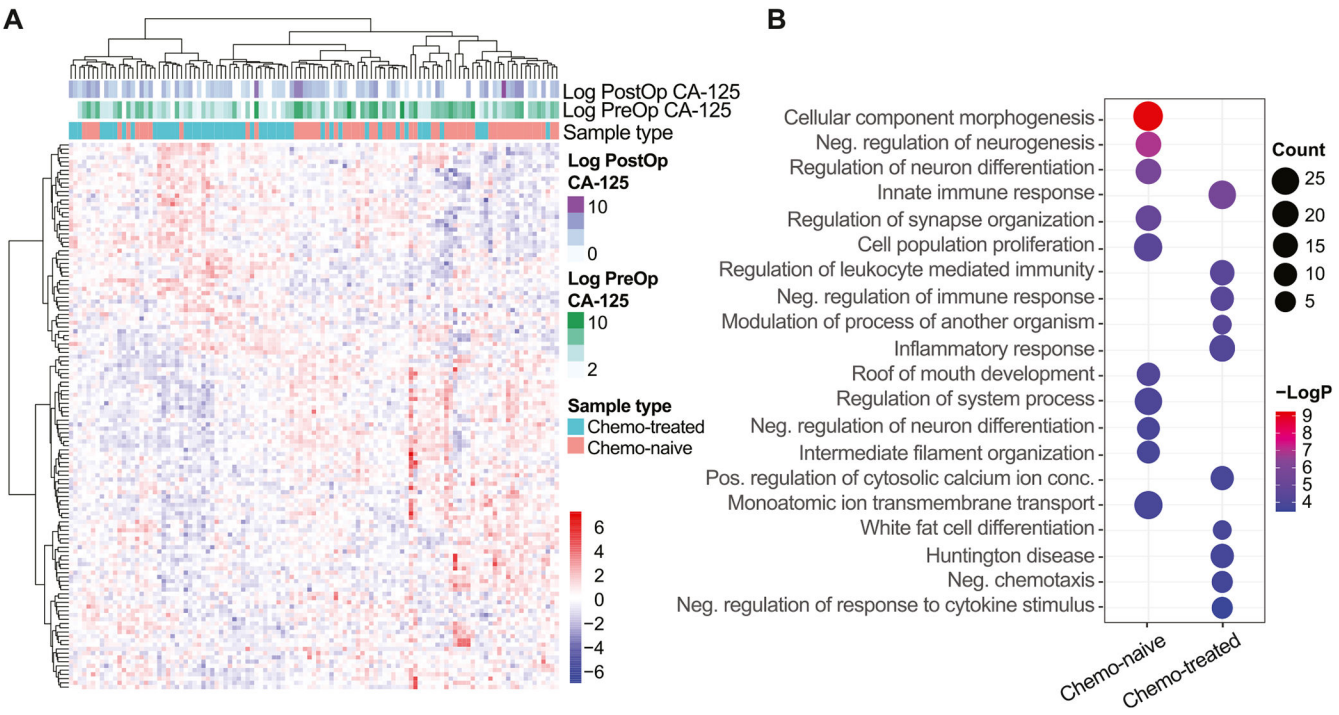

Fig. 3.

5hmC patterns during treatment in HGSOC.A, B) Hierarchical clustering based on 129 DhMGs (A) and functional enrichment analysis (B) of all samples by chemotherapy-treatment.

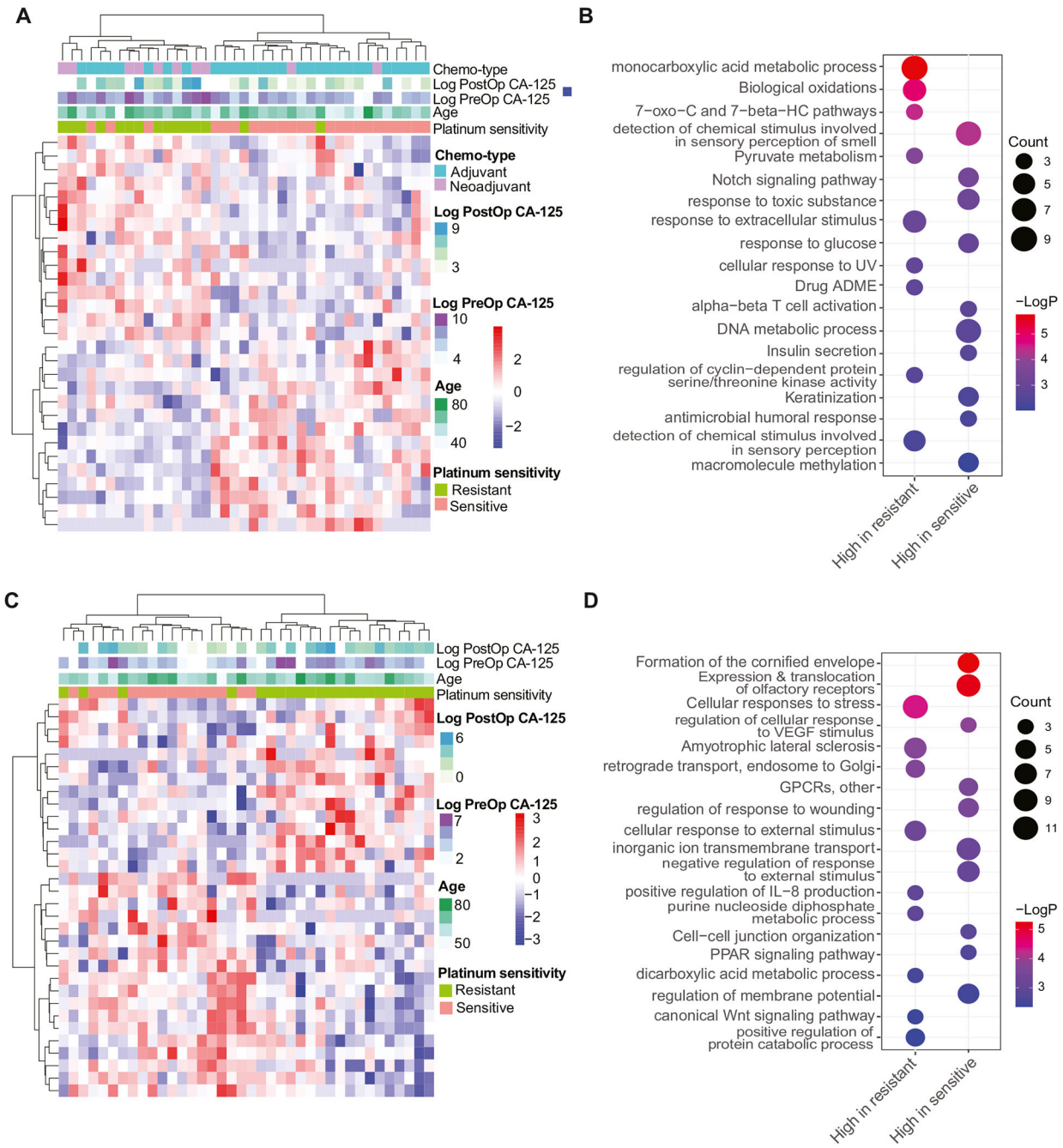

Fig. 4.

DhMGs can separate platinum sensitive from -resistant women. A, B) Hierarchical clustering based on 29 DhMGs (A) and functional enrichment analysis of chemo-naïve samples from women with platinum-sensitive and –resistant HGSOC. C, D) Hierarchical clustering based on 32 DhMGs (C) and functional enrichment analysis of 5hmC modified genes (D) in women with prior chemotherapy treatment. A, C) Rows indicate DhMGs.

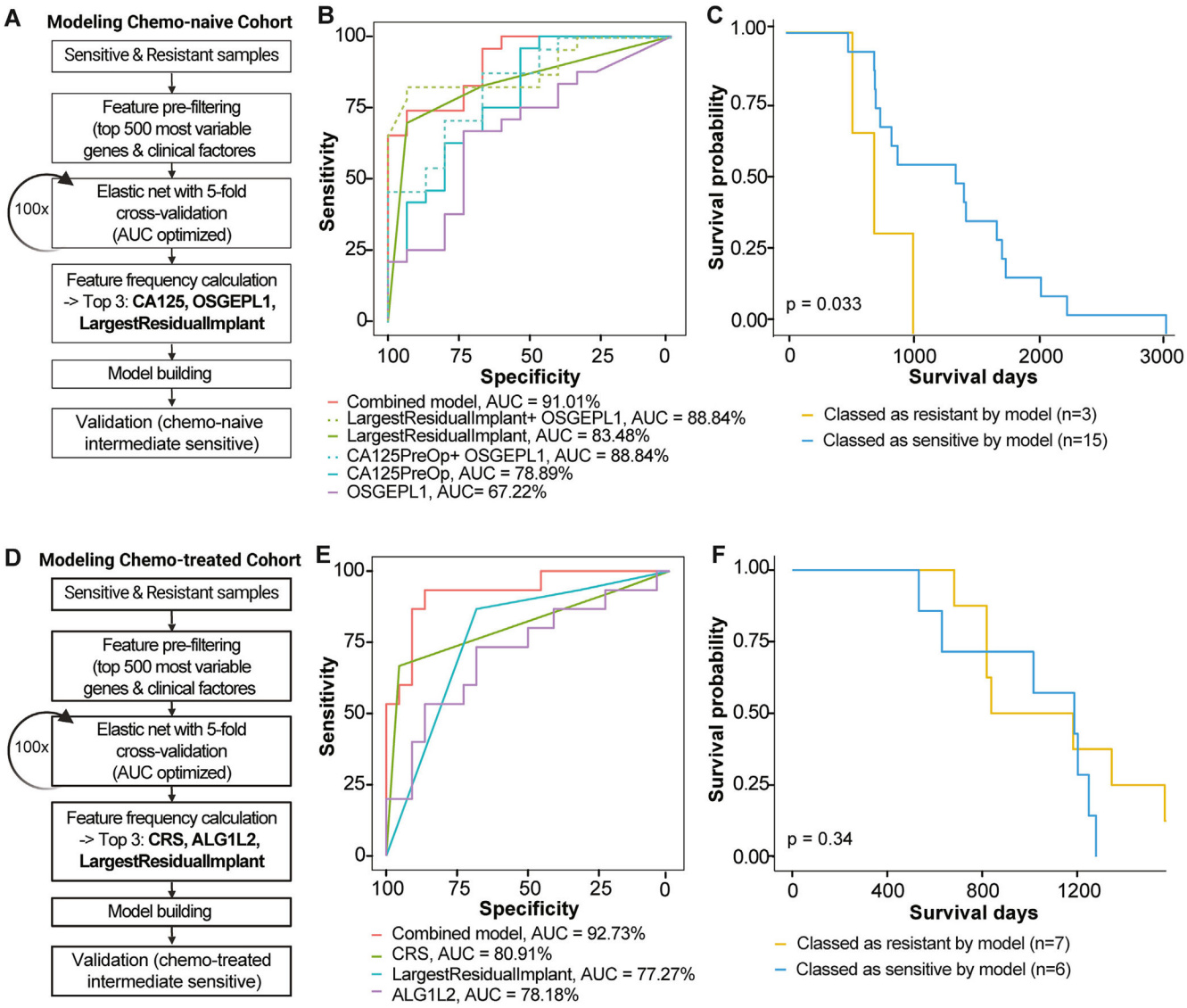

Fig. 5.

DhMGs can be used as biomarkers to predict survival. A, D) Overview of the steps involved in model building and validation. B, E) Receiver operating curves optimized for the area under the curve for the AC- (B) and NACT-model (E). C, F) Survival curve analysis of AC-treated intermediate sensitive (C) and NACT-treated intermediate sensitive women (F) as classified by the model.