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The Clinical Use of Genomic Profiling for Prognosis Prediction in High Grade Serous Ovarian Cancer

I Nyoman Bayu Mahendra[1]*, Fransiskus Rendy[2], Pande Prayudi[2]

[1]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Faculty of Medicine, Udayana University, Prof. dr. I Gusti Ngoerah Gde Ngoerah General Hospital, Denpasar, Bali, Indonesia; [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Udayana University, Prof. dr. I Gusti Ngoerah Gde Ngoerah General Hospital, Denpasar, Bali, Indonesia

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***Correspondence:** I Nyoman Bayu Mahendra, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Faculty of Medicine Udayana University, Prof. dr. I Gusti Ngoerah Gde Ngoerah General Hospital, Denpasar, Bali, Indonesia. E-mail: bayu@unud.ac.id
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Abstract

BACKGROUND: The majority of ovarian cancer cases are high-grade serous ovarian cancers (HGSOC). HGOSC harbors several genomic alterations that play crucial roles in carcinogenesis. Studies on the molecular characterization of HGSOC have suggested that HGSOC is a heterogenous disease, rather than a singular disease entity. Genomic profiling using gene expressions, methylation patterns, and non-coding RNA expression patterns have all been used as the basis for the molecular categorization of HGSOC.

AIM: This study aims to get an understanding on HGSOC classifications in relation to the prognosis, such as overall survival (OS), progression-free survival (PFS), and response to chemotherapy.

METHODS: The literature review study model was adopted in this essay. PubMed, EMBASE, Web of Science, Medline, Cochrane, Science Direct, and Google Scholar were the data bases used in the source search, which attempted to gather topics about the debate of genomic profiling for prediction prognosis of HGSOC.

RESULTS: Gene expressions, methylation patterns, and non-coding RNA expression patterns have all been used as bases for the molecular classification of HGSOC. Understanding prognostic classifications such as OS, PFS, and chemotherapeutic response are crucial in the era of precision medicine to optimize the prognosis and direct focused or particular treatment.

CONCLUSION: HGSOC is a heterogenous disease. Research in the future should concentrate on creating therapies targeted at certain molecular subtypes of HGSOC to optimize the prognosis.

Introduction

High-grade serous ovarian cancer (HGSOC) is the most common type of ovarian cancer, making up about 75% of all ovarian cancers. According to the WHO, there are 225,500 people diagnosed with this cancer annually. Moreover, 140,200 people die every year from this disease. This causes ovarian cancer to rank as the 7th most commonly experienced type of cancer as well as ranked 8th in the cause of death from cancer in women [1]. Primary debulking surgery and adjuvant chemotherapy remain the mainstay for the treatment of advance stage HGSOC. In the era of precision medicine, maintenance treatment such as PARP-inhibitor or targeted therapy with VEGF inhibitor has been studied extensively.

Studies based on genomic, proteomic, and metabolomic profiling of cancer have demonstrated that HGSOC is a heterogenous disease, rather than a single disease entity [2]. For example, findings from the cancer genome atlas (TCGA) research network confirms the distinct molecular subtypes of HGSOC and termed them “Mesenchymal (C1), Immunoreactive (C2), Differentiated (C4), and Proliferative (C5)” [3]. Mesenchymal subtype was characterized by some gene signatures of increased stromal components, angiogenesis, and epithelial-to-mesenchymal transition (EMT). Immunoreactive subtype was characterized by expression of T-cell chemokine ligands (*CXCL11* and *CXCL10*) and receptor (CXCR3). High expression of *MUC16/MUC1* and the secretory fallopian tube maker *SLPI* suggesting a more mature stage of development defined the Differentiated subtype. Proliferative subtype was characterized by high expression of transcription factors (TFs) *HMGA2* and *SOX11*, low expression of ovarian tumor markers (*MUC1, MUC16*), and high expression of proliferation markers (*1MCM2* and *PCNA*). Furthermore, these group of subtypes is associated with different response to treatment and different prognosis of the disease. Recent studies have suggested other alternatives to HGSOC subtyping with the aim of knowing the prognosis and furthermore, find other therapeutic alternatives to improve the prognosis, such as the overall survival (OS), progression-free survival (PFS), chemotherapy response (CR), and disease recurrence.

In this review, we will discuss in detail the genomic alterations in HGSOC and the basis for classifications or subtyping of HGSOC. We will also

discuss the implications of specific gene expression and subtypes in regard to the prognosis of HGSOC.

Methods

The aim of this publication's method a literature review is to investigate the genomic profiling of HGSOC and their relation to the prognosis of HGSOC. The first step in the review process is to find journal articles that are relevant to the study's subject. PubMed, EMBASE, Web of Science, Medline, Cochrane, Science Direct, and Google Scholar are the databases used during the source search. The inclusion criteria for the publication under consideration were full-text qualitative research written in English and released between 2008 and 2022. Genomic profiling of HGSOC was documented in examined papers must also have had prognosis and/or survival rate. The criteria for the articles included in the review are as follows: Cohort study, case study, review, and observational study. The keywords for the search were high grade serous ovarian cancer, genomic or genetic profiling, gene expression, methylation patterns, non-coding RNA expression, prognosis, OS, progression free survival, response to chemotherapy. The overall process of finding, screening, deleting, and selecting the articles which were applied at the present study is presented in Figure 1.

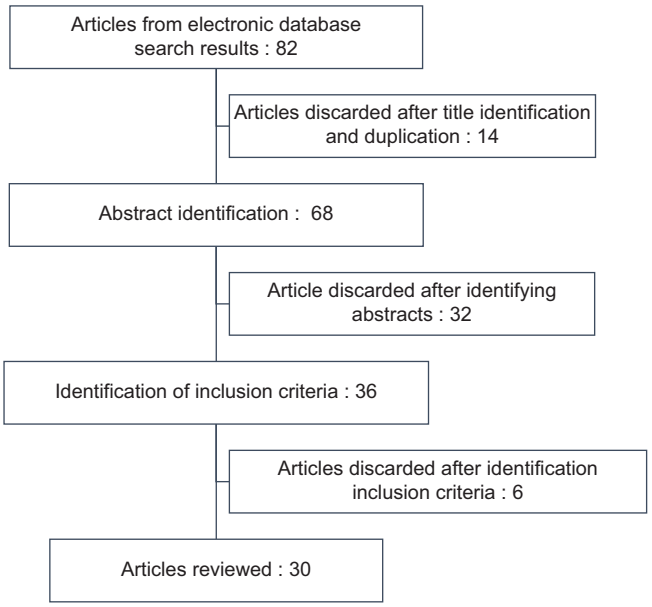

Figure 1: Search results through the selected database

A total of 82 articles were found in the literature search results from the selected databases. The initial author performed the process of article selection on their own. Reading the possible publication's titles and abstracts was the first step in the process. Fourteen duplicate articles and 38 items that did not match the inclusion criteria were eliminated. Thirty final papers were taken in full-text for evaluation.

Results

Genomic alterations in HGSOC

HGOSC harbors genomic alterations that play a crucial role in carcinogenesis. The predominant mutation in HGSOC is TP53 mutation, occurring in at least 96% of HGSOC while BRCA1/2 is mutated in 22% of cases due to a combination of germline and somatic mutations [4]. Other recurrently mutated genes include RB1, NF1, FAT3, CSMD3, GABRA6, and CDK12. Some other rare mutations have been identified and might serve as important drivers in HGSOC carcinogenesis, including BRAF (N581S), PIK3CA (E545K and H1047R), KRAS (G12D), and NRAS (Q61R). With regard to epigenetic changes, 168 genes exhibit elevated Deoxyribonucleic acid (DNA) methylation, and thus are epigenetically silenced in HGSOC. The BRCA1 promoter was hypermethylated and silenced in more than 10% HGSOC. Other noteworthy hypermethylation is observed in the promoter regions of AMT, CCL21, SPARCL1, and RAB25 [4]. Both local and regional copy number alterations are common aberrations in HGSOC. A study by TCGA research network identified 8 regional CN gains and 22 losses. TCGA also identified the most common focal amplification involving CCNE1, MYC, and MECOM, with each highly amplified in more than 20% cases. Other localized amplifications involve the receptor for activated C-kinase (ZMYND8), the p53 target gene (IRF2BP2), the DNA-binding protein inhibitor (ID4), the embryonic development gene (PAX8), and the telomerase catalytic subunit. Several cancer-associated pathways are also deregulated in HGSOC, including RB1 (67%), PI3K/RAS (45%), FOXM1 (84%), and Notch signaling pathways (22%). More than 50% of HGSOC cases exhibit homologous recombination deficiency. With regard to stage, early and late-stage HGSOC have highly similar patterns of mutation and focal somatic CAN [5]. However, there is a significant difference in both ploidy and CN signature between early and late-stage HGSOC, with higher ploidy and CN signature 4 exposures in late-stage cases.

The basis for the molecular classification of HGSOC

The first classification of HGSOC molecular subtypes was introduced by Tothill *et al*. in 2008 [6]. Based on the predominant gene expression patterns, HGSOC was classified into four subtypes: C1 (high stromal response), C2 (high immune signature), C4 (low stromal response), and C5 (mesenchymal/ dedifferentiated, low immune signature) subtypes. C1 subtype displayed significant differential expression of stromal gene clusters, such as markers of activated myofibroblasts (*ACTA2, FAP*), vascular endothelial cells (PECAM1, CD31 antigen), and pericytes (*PDGFRB*), as well as extracellular matrix production and remodeling,

cell adhesion, cell signaling, and angiogenesis. C1 also displayed higher degree of desmoplasia, that is, a non- rarified fibrotic reaction involving abundant collagen deposition and a high density of myofibroblasts that are distinct from resident nonactivated fibroblast, as compared to other subtypes. C2 was characterized by overexpression of genes associated with immune cells, including markers of T-cell activation (*CD8A,* Granzyme B) and T-cell trafficking (*CXCL9/MIG*). Along with C4 subtype, C2 showed a high number of both intra-tumoral and stromal associated CD3+ cells. C5 subtype was defined by genes expressed in mesenchymal development, such as overexpression of developmental TFs including homeobox genes (*HOXA7, HOXA9, HOXA10, HOXD10,* and *SOX11*), as well as high-mobility group members (*HMGA2, TOX*, and *TCF7L1*). WNT/ß-catenin and cadherin signaling pathway members were highly enriched in C5 tumors, including N-cadherin and P-cadherin (*CDH2* and *CDH3*). Furthermore, C5 subtype displayed overexpressed proliferative and extracellular matrix-related genes (*COL4A5, COL9A1,* and *CLDN6*) and very low expression of immune cell markers (*CD45, PTPRC*; lymphocyte markers, *CD2, CD3D,* and *CD8A*) and differentiation markers (*CA125* and *MUC1*). C5 subtype tumors had strikingly low CD3+ and CD45+ cell infiltration in tumor and stroma.

Later on, findings from TCGA research network confirms the molecular subtype and termed them “Mesenchymal (C1), Immunoreactive (C2), Differentiated (C4), and Proliferative (C5)” [4]. Immunoreactive subtype was characterized by expression of T-cell chemokine ligands (*CXCL11* and *CXCL10*) and receptor (*CXCR3*). Proliferative subtype was characterized by high expression of TFs *HMGA2* and *SOX11*, low expression of ovarian tumor markers (*MUC1* and *MUC16*) and high expression of proliferation markers (*1MCM2* and *PCNA*). High expression of *MUC16/MUC1* and the secretory fallopian tube maker *SLPI* which suggests a more mature stage of development defined the Differentiated subtype. High expression of HOX genes and markers suggestive of increased stromal components such as for myofibroblasts (*FAP*) and microvascular pericytes (*ANGPTL2* and *ANGPTL1*) defined the Mesenchymal subtype. Those four subtypes were also associated with specific genomic aberrations as follows: *LAD1* for Differentiated subtype; *NOTCH3* and *HMGA2* for Proliferative subtype; and *MYC* for Differentiated and Immunoreactive subtype. Immunoreactive subtype displayed higher *BRCA1/2* mutation frequency, whereas proliferative subtype has the highest *CCNE1* amplification rate [7]. HGSOC molecular subtype also correlate to specific histopathological feature: Differentiated subtype is well-differentiated Grade 1 carcinoma with papillo-glandular pattern of growth; proliferative subtype is poorly differentiated Grade 3 carcinoma with solid growth; mesenchymal subtype is associated with dense desmoplastic stroma; and immunoreactive subtype is characterized by many lymphocytes surrounding the cancer nest [8].

With regard to survival, Tothill *et al*. reported that C1 was associated with the poorest survival as compared to other subtypes. C2 and C4 subtype, characterized by higher numbers of intra-tumoral CD3+ cells and lower expression of stromal response genes, had better survival than C1 tumors. The C5 subtype displayed a trend for reduced OS compared with subtypes C2 and C4. In a multivariate analysis incorporating other known predictors (stage, grade, residual disease, patient age, and primary site of origin), C1 subtype remained a significant predictor for survival [5]. However, result from TCGA study reported that different molecular subgroups did not have prognostic significance [4]. Later on, it was reported that survival differed significantly between subtypes and was the best for the immunoreactive subtype, but significantly worse for the mesenchymal and proliferative subtypes [9]. Molecular subtypes were also significantly associated with rate of optimal surgical debulking (≤1 cm). Mesenchymal subtype was associated with the lowest RD0 rates, while the immunoreactive subtype consistently had the highest RD0 debulking rate [10]. Using isoform level expression analysis, one study demonstrated that the median OS for each subtype was as follows: Mesenchymal, 3.2 years (95% CI, 2.6–4 years); Differentiated, 4.2 years (95% CI, 3.8–4.8 years); Immunoreactive, 4 years (95% CI, 3.0–6.8 years); and Proliferative, 3.6 years (95% CI, 3.2–4.1 years) [11].

Besides the four transcriptional subtypes, TCGA study also demonstrated three miRNA subtypes, four promoter methylation subtypes and a transcriptional signature associated with survival duration [4]. DNA methylation subtypes were significantly associated with differences in age, BRCA inactivation events, and survival. A prognostic gene signature for OS was defined which comprised 108 genes correlated with poor prognosis and 85 genes correlated with good prognosis. One study developed a combined prognostic model of HGSOC classification that incorporated TCGA subtype and survival gene expression signatures and termed it “Classification of Ovarian Cancer” (CLOVAR) [12]. CLOVAR survival classifier, that is, good and poor prognosis, predicted outcome with high significance within each of the 4 CLOVAR subtype groups. CLOVAR Mesenchymal and CLOVAR poor prognosis group had the worst prognosis and showed a median OS of only 23 months. Sixty-three percent of CLOVAR Mesenchymal/Poor Prognosis group were found to be resistant to platinum therapy. Interestingly, the CLOVAR study demonstrated the presence of overlap in gene signature scores between subtype, suggesting that HGSOC does not consist of mutually exclusive subtypes, but each tumor sample is rather represented by multiple gene signatures at different levels of activation. This phenomenon may reflect a

higher level of homogeneity of HGSOC than is seen in other tumor types, such as glioblastoma and breast cancer.

A large number of studies have been conducted in recent years to characterize the molecular heterogeneity of HGSOC using several classifiers such as DNA methylation [3], copy number aberrations [13], genomic rearrangements [14], homologous recombination pathway [15], expression of long noncoding RNA (lncRNA) [16], lipid metabolism gene signatures [17], EMT [15], immune cells profiling [18], and tumor microenvironment [19], [20], [21], [22].

The relation among subtypes or specific gene expression and the prognosis of HGSOC

EMT index

EMT is a molecular process that results in phenotypic changes of epithelial cells into mesenchymal cells, which are more motile and invasive. EMT is believed to be involved in metastasis and chemoresistance of cancer cells. A study by Sohn *et al*. in 2021 performed EMT index calculations and high-dimensional RNA sequence analysis to divide HGSOC into two groups, that is, homologous recombination repair (HRR)-activated type and mesenchymal type. The activation of HRR pathway could be explained by the genetic compensation for the dysfunction of *BRCA1* or *BRCA2* in *gBRCA1/2mut* group. Then, the researchers conducted a TFs analysis to search for enriched TFs. According to the analysis, there are some TFs associated with EMT, e.g., *TCF21, TWIST2, MEOX32, OSR1, PRRX1, PRXX*2, and *TWIST1*. These genes are more likely to be upregulated in mesenchymal type [15]. This study demonstrated that patients with mesenchymal type HGSOC had a significantly worse OS than HRR-activated type HGSOC. Patients with mesenchymal-type HGSOC demonstrate activation of EMT transcription programs, low genomic alterations, and diverse cell compositions. Furthermore, this study also used samples from the cancer genomic atlas (TCGA) to validate the prediction of OS using EMT index. The EMT index is divided into high and low based on its median. The results of this calculation show that the OS among those with high EMT index is significantly worse than the low EMT index (median survival: 44 months vs. 47.4 months, respectively, p = 0.03) [15].

CLOVAR

A study by Verhaak *et al*. in 2013 expanded the description of the four molecular subtypes using the genomic catalog TCGA and clinical data. This study integrated subtypes and prognosis classifications on 489 profiles of genetic expression into one large frame called CLOVAR. In addition, Verhaak *et al*. divided the subtypes based on the risk scores into good, intermediate, and poor prognosis. Furthermore, Verhaak *et al*. also tested their accuracy in 879 publicly available HGSOC expression profiles. However, there is an overlap of genetic expression among the four known subtypes, namely, in immunoreactive and proliferative as well as differentiated and mesenchymal. Therefore, this study concludes that these subtypes are not completely exclusive to each other, but rather have several signatures in different levels of activation. The worst outcome was found in the tumors classified as CLOVAR mesenchymal and CLOVAR poor prognosis, with the median OS of 23 months. The best outcome was found in the tumors classified as CLOVAR Immunoreactive and CLOVAR good prognosis. All CLOVAR good prognosis samples have higher OS compared to all CLOVAR poor prognosis samples [12].

GAB2, BMP8B, and ATP13A4 genes expressions

A study conducted by Davis *et al*. looked for associations between amplified and overexpressed genes in HGSOC genome and the OS. The study used 499 gene expression data on HGSOC, and identified the presence of 11 amplified genes. Then, these genes were analyzed by univariate methods to determine the relationship of mRNA expression with OS and PFS. From the analysis, *GAB2*, *CACNA1C*, and *PAK4* gene are associated with the OS. The study also modeled a multivariate analysis using cancer stages, age, and disease course. *CACNA1C* and *PAK4* are inversely proportional with the OS, although the associations are not statistically significant (*CACNA1C*: Hazard ratio [HR] 1.07, p = 0.184, *PAK4*: HR 1.06, p = 0.428) [23].

However, *GAB2* gene expression is significantly associated with the OS (HR 0.79, p = 0.006). *GAB2* has been implicated as an oncogene in other cancer types including breast cancer, ovarian cancer, leukemia, and melanoma. Furthermore, *GAB2* gene expression remains significantly associated with the OS when residual disease is incorporated into a multivariate analysis model (HR 0.8, p = 0.02). HR<1 indicates that *GAB2* expressions are directly proportional to OS. In *in vitro* models, high *GAB2* expression is associated with dual phosphoinositide-3-kinase (PI3K)/mTOR inhibitors (bimiralisib and gedatolisib) and can be used as a genomic marker to identify patients can respond to therapies that inhibit *PI3K* signals. *GAB2* binds to the p85 regulatory subunit of *PI3K* to stimulate *PI3K* signaling and overexpression of *GAB2* demonstrated to potentiate ovarium tumorigenesis through *PI3K* signaling activation dependent to *mTOR* [23].

Other study associating *GAB2* with higher EMT, cell migration and invasion and response to follicle stimulating hormone in ovarian granulose cells through *PI3K* pathway as well. In addition to being associated with OS, *GAB2* is also associated with PFS according to a multivariate analysis (HR 0.84, p = 0.02). In addition

to *GAB2*, two other genes are associated with PFS in both univariate and multivariate analyses with cancer stage, age, and disease course, that is, *BMP8B* and *ATP13A4* (HR 0.91, p = 0.02 and HR 0.84, p = 0.004). *BMP8B* (*bone morphogenic protein 8B*) encodes molecules that are important in embryonic development and can regulate adipogenesis. In ovarian cancer, this can be related to increased adipocytes distribution within the peritoneum, which are a source of energy for cancer cells. In gastric cancer, bone marrow-related gene expression is associated with metastases and a poor prognosis, as opposed to this study [4]. The study by Davis *et al*. shows that the higher the expression of *BMP8B*, the higher the PFS. Meanwhile, *ATP13A4* is a transporter of calcium ions in the endoplasmic reticulum and when overexpressed, intracellular calcium levels will rise. This gene has never been associated with cancer, but calcium regulation issues are one of the important tumorigenic pathways [23].

FAP gene expression

Research conducted by Li *et al*. showed that of the 57,331 genes analyzed in HGSOC, *FAP* gene became the only new gene that contributed to the OS. The group with low *FAP* expression showed a significant protective effect against OS on HGSOC (p = 0.005). This group had a survival rate of 91.1% in 12 months, compared to 84.4% in the low *FAP* expression group. In 50 months, the group with low *FAP* had a survival rate of 31.9%, compared to the high *FAP* group which had a survival rate of 21.4%. FAP itself is a plasma membrane-bound serine protease, which is related to matrix digestion and cancer invasion. The overly expressed *FAP* is thought to be related to the prognosis of various diseases, especially cancer. *FAP* also belongs to the cancer-associated fibroblasts (CAFs) group, which typically plays a role in remodeling the structure of the extracellular matrix and reconstruction of tumor microarrays. In addition to being associated with the OS, *FAP* is also associated with PFS (p = 0.008). The higher the expression *FAP*, the lower its PFS. It is likely related to the nature of *FAP* as CAF [24].

USP19 dan RPL23 genes expression

Studies conducted by Kang *et al*. showed a relationship between *USP19* and *RPL23* levels with the prognosis of HGSOC. First, the study screened all RNA in 43 HGSOC patients based on the top 30% median absolute deviation. This first stage gained 6,123 genes from this first screening. Second, the study screened the RNA with an area under the curve (AUC) of >0.85 and gained 51 genes. The same RNA was then filtered with a PFS p < 0.1 and obtained 28 genes. However, none of the 51 genes filtered with AUC were the same as the 28 genes filtered with p-values of PFS. After that, the 28 genes obtained were processed with random forest modeling. Of the 28 genes, there are two genes that have the highest scores for prognosis predictions, namely, *USP19* and *RPL23. USP19* is an ubiquitin-specific protease known to have suppressive properties against tumors because it regulates DNA repair, chromosome stabilization, and tumorigenesis. Meanwhile, *RPL23* is a ribosomal protein known to be associated with multi-medication resistance and cancer progression due to its properties that negatively regulate apoptosis (lowering apoptosis). Of the 43 patients, 41 patients had PFS data. The patients were then grouped into three based on *USP19* expression levels: low (≤25%, quartile 1), middle (25–75%, quartile 1–quartile 3), and high (>75%, quartile 3). The low and middle groups showed significantly worse PFS than the high group (n = 41, the p value of the log test rank was 8.3×10^{-3}). After that, the 41 patients were grouped by *RPL23* expression level, in the same division way as *USP19*. In contrast to *USP19*, the high group had a much worse PFS than the low and middle groups (n = 41, the p value of the log rank test was 0.062) [25].

Valosine containing protein (VCP) gene expression

Univariate analysis or differential gene expression was performed to test the association of 11,107 probes with CR in HGSOC patients from TCGA. The analysis carried out by Choi *et al*. showed that the most powerfully associated genes were those expressing *VCP* (p = 3. 91E-06). The study also identified that probes showing VCP were associated with chemotherapy resistance (FDR's adjusted p < 0.05). FDR is a false discovery rate method used as a measure for multiple test corrections to control type I errors. Low *VCP* expressions are associated with PFS (p = 0.015) and a shorter median survival time. *VCP* has an important role in breaking down the cell structure of large polypeptides to be decomposed by proteolytic enzymes. In addition, *VCP* has the function of regulating important pathways of DNA repair, DNA replication, and cell cycle progress by removing defective polypeptide structures from chromatin, ribosomes, endoplasmic reticulum, and mitochondria [26].

HDAC4 and STAT1 genes expression

Studies conducted by Stronach *et al*., state that resistance to chemotherapy is the result of selection, not formed by therapy. The study analyzed transcription in the cells of three people with HGSOC and identified 91 overexpressed genes and 126 less expressed genes. Incorrect expression of these genes is often encountered in chemotherapy resistance. Increased apoptosis in response to platinum therapy in resistant cells was found after knockdown or decreased histone deacetylase (*HDAC4*), *FOLR2, PIK3R1*, or *STAT1* (p < 0.05). *HDAC4* and *STAT1* have physical

interactions. *Acetyl-STAT1* is found in platinum-sensitive cells but not in platinum-resistant cells with too high *HDAC4.* In resistant cells, small phosphorylation/ translocation at *STAT1* was found after exposure to platinum. If *HDAC4* is lowered in number, acetyl *STAT4* increases. In addition, a decrease in *HDAC4* prevents the activation of *STAT1* induced by platinum, as well as restores sensitivity to cisplatin. In addition, the study analyzed 16 results of paired tumor biopsies taken before and after therapy with platinum. In resistant cells, *HDAC4* expression is significantly higher (44%; p = 0.04). This means that clinical selection leaving tumor cells that produce *HDAC4* excessively increases the deacetylation of *STAT1* and the survival of cancer cells [27].

PD-L1 gene expression

The study conducted by Weberpals *et al*., grouped HGSOC patients by their response to platinum-based chemotherapy into "Good Response" (GR) and "Poor Response" (PR). Then, the study looked for genes that could be related to the therapeutic response. One of the genes discussed is *programmed death ligand* 1 (*PD-L1*), an inhibitory immune receptor ligand commonly used as an immune inhibition checkpoint. According to transcriptomic analysis, more *PD-L1* gene expression was found in the GR group (p = 0.014). The expression of the *PD-L1* protein in tumor immune cells was also much more prevalent in the group of patients with the *BRCA2* mutation (p = 0.029). Based on the positive association between *PD-L1* gene expression and good CR, researchers conducted immunohistochemistry examinations to confirm protein expression and describe patterns of tissue expression. The number of immune cells expressing *PD-L1* was more prevalent in the GR group while in PR it was less based on the number of positive cells (p < 0.001 Mann–Whitney) [28].

NACC1 gene expression

The study conducted by Shih *et al*. began by digitally identifying the karyotype on HGSOC. On the identification of the karyotype, amplified *ch19p13.2* areas were found. Then, an analysis of 341 HGSOC samples from TCGA was carried out to find features from the area. All amplified genes in the TCGA data were correlated with amplified genes at *ch19p13.2*. There were seven amplified genes found. One of the loci of such genes, *NACC1*, which encodes *NAC1*, is related to the development of tumor recurrence in HGSOC and has a causality relationship with the development of paclitaxel drug resistance. This gene is then further studied. Cases with amplified *NACC1* loci are associated with recurrence of the disease within 6 months (p = 0.013). Furthermore, the expression of the *NAC1* protein is also very high statistically in the amplified tumor group versus the unamplified tumor (p < 0.005). This suggests that the amplification of the locus of *NACC1* at *ch19p13.2*, which triggers excessive expression of *NAC1*, is one of the molecular genetic alterations that have associations with tumor recurrence in ovarian cancer [29].

Gain of function p53

The research conducted by Kang *et al*. is based on the inactivation of *TP53* which is one of the mutations commonly found in people with cancer. Gain-of-function (GOF) mutations also have potential for oncogenic activity. GOF mutations are mutations that make genes have new functions or have new patterns of expression. Patients with the mutant protein *p53 (mutp53)* with GOF mutations showed mRNA (p = 0.03) and higher *p53* expression (p = 0.01) than patients with *p53* mutations without evidence of GOF (no evidence of gain-of function, [NE-GOF]). Statistically, GOF is more associated with distant metastases (36/55, 65.5%) compared to local recurrence (19/55, 34.5%). Meanwhile, patients with NE-GOF mutations had a possibility of locoregional recurrence (26/47, 55.3%) than distant metastases (21/47, 44.7%) with a p = 0.035. However, there are no significant OS and PFS differences between these two groups. The level of expression of mRNA *p53* and proteins in GOF *mutp53* may indicate the presence of additional mechanisms that make *mutp53* stable and produce more GOF effects. There is also a lot of evidence to suggest an oncogenic role in GOF *p53* in tumorigenesis, cancer invasion, and metastasis. However, this evidence has not been found in clinical samples. Until now, with existing evidence, GOF *mutp53* has a significant role in HGSOC patients through platinum resistance and metastasis [30].

Collagen type II alpha 1 (COL2A1) and solute carrier family 6 member (10 SLC6A10P) gene expression

The study conducted by Ganapathi *et al*. began with primary and recurrent HGSOC analysis to find different and uniquely expressed genes. From the dataset, researchers selected 21 coding genes and 1 noncoding RNA based on significant differences in tumor expression profiles. Then, these genes and RNA were used for validation with quantitative polymerase chain reaction (PCR) in 110 ovarian tumors (71 primary tumors and 39 recurrent tumors). The Kaplan–Meier test on 64 primary tumors showed that higher expressed COL2A1 was associated with a slower time-to-recurrence (TTR) (HR = 0.47.95% CI: 0.27–0.82, p = 0.008). Meanwhile, low pseudogene expression of SLC6A10P is associated with an older TTR (HR = 0.53.95% CI: 0.30–0.93, p = 0.027) [31].

DNA methylation pattern

A study conducted by Wang *et al*. in 2021 used 233 samples for training and 232 samples for validation. The training samples then analyzed to decide an ideal cluster amount for these samples. The area under curve showed an appreciable increase at k = 2 to k = 4 and insignificant increase when k > 4. Therefore, these samples were divided into four subgroups. These subgroups are C1 (28.8%; 67 samples), C2 (22.7%; 35 samples), C3 (38.6%; 90 samples), and C4 (9.9%; 23 samples). DNA methylation was then identified to compare the subgroups. The methylation levels between the subgroups were significantly associated with different molecular features. Among all subgroups, C4 had the worst prognosis and showed hypermethylation in 54 methylation loci, which corresponded to 51 genes. In contrast, C2 had the best prognosis and featured hypomethylation of *cg13791131, cg25574024, cg24673765,* and *cg27239157*, as known as *IGF2* (cg13791131, cg25574024), *HSPB6* (*cg24673765*), dan *MCF2L2* (*cg27239157*). *IGF2* plays a key role in glucose metabolism, HSBP6 is associated with insulin resistance, and *MCF2L2* is associated with type I diabetes and polycystic ovary syndrome. All genes have associations with metabolism; therefore, these subgroups based on methylation level may reflect some changes in genetic molecular features. The difference of median OS between these subgroups is significant (p = 0.0001). C2 has a median OS of 64 months, whereas C1, C3, and C4 have the median OS of 35 months, 48 months, and 24 months, respectively [3].

lncRNA expression

lncRNAs are mRNA-like transcripts with more than 200 base pairs in length without the coding capacity. A study conducted by Fang *et al*., in 2018 correlated lncRNA-mRNA with platinum-sensitive and platinum-resistant. lncRNA or mRNA in platinum-resistant samples contain all lncRNA or mRNA in platinum-sensitive samples. Therefore, the analysis was continued on platinum-resistant samples [32]. This analysis showed a significantly higher level of lncRNA than mRNA (coding gene) ($p = 1.46 \times 10^{-20}$, t-test), so we can conclude that lncRNAs play a key role in a platinum-resistant network. Then, a log rank test was conducted on each lncRNA to examine whether HGSOC patients with high and low expression showed differences in OS. These high and low expressions were divided based on the median of expression level. This study showed a low expression of *RP5-1120P11.1* lncRNA and a significantly shorter OS median than those of high expression group in TCGA ($p = 2.74 \times 10^{-5}$, log rank test) and in *GSE63885* (p = 0.0242, log rank test) datasets. Furthermore, *RP5-1120P11.1* is correlated with *NCAM1* and *ABCC10* genes. *NCMA1* gene (CD56) encodes a cell adhesion protein. Deregulated *NCAM1* was reported in some cancers such as acute myeloid leukemia, neuroblastoma and ganglioneuroma. Meanwhile, *ABCC10* gene is related to the resistance of docetaxel and paclitaxel treatment. Based on former studies, the downregulation of *ABCC10* may be related to platinum resistance in HGSOC patients, which mediated by *RP5-1120P11.1.* In TCGA dataset, *RP5-1120P11.1* was significantly downregulated in platinum-resistant group compared with platinum-sensitive group (p = 0.038, t-test) [32].

Conclusion

Molecular classification of HGSOC has been develop on several basis such as gene expressions, methylation pattern and non-coding RNA expression pattern. In the era of precision medicine, understanding those classification in relation to the prognosis such as OS, PFS and response to chemotherapy become important to guide specific or targeted treatment and to optimize the prognosis. Future studies will focus on development of treatment specific for certain molecular subtype.

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