Selected markers of ovarian cancer and their relation to targeted therapy (Review)

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Abstract. Despite advances in surgical treatment techniques and chemotherapy-including anti-angiogenic and immune poly (ADP-ribose) polymerase inhibitors, the 5-year survival rate in ovarian cancer (OC) remains low. The reasons for this are the diagnosis of cancer in advanced clinical stages, chemoresistance and cancer recurrence. New therapeutic approaches are being developed, including the search for new biomarkers that are also targets for targeted therapy. The present review describes new molecular markers with relevance to targeted therapy, which to date have been studied only in experimental research. These include the angiogenic protein angiopoietin-2, the transmembrane glycoprotein ectonucleotide pyrophosphatase/phosphodiesterase 1, the adhesion protein E-cadherin, the TIMP metallopeptidase inhibitor 1 and Kruppel-like factor 7. Drugs affecting cancer stem cells (CSCs) in OC, such as metformin and salinomycin, as well as inhibitors of CSCs markers aldehyde dehydrogenase 1 (with the drug ATRA) and the transcription factor Nanog homeobox (microRNA) are also discussed. A new approach to prevention and possible therapies under investigation such as development of vaccines containing a subpopulation of CD117(+) and CD44(+) stem cells with a promising option for use in women with OC was described.

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Contents

1. Introduction

Ovarian cancer (OC) is the gynecological malignancy with the worst prognosis. According to GLOBOCAN, in 2020, 314,000 women were diagnosed with OC, while a total of 207,000 deaths due to OC were recorded. In 2040, a total of 428,000 new OC cases and 307,000 deaths from OC are predicted. In early stage of OC, overall survival (OS) is ~92% (1). However, >70% of the cases are diagnosed at an advanced clinical stage, which is associated with an unsuccessful disease course. After successful primary therapy, ~80% of patients are found to have recurrence while OS is only 29%. OC does not only show clinical diversity, but is also histologically and molecularly heterogeneous which influences chemoresistance in therapy. The most aggressive type of OC is type II high-grade serous ovarian carcinoma (HGSOC).

Although anti-angiogenic treatment with bevacizumab and poly (ADP-ribose) polymerase inhibitors have been introduced to standard surgery and platinum- and taxane-based chemotherapy in recent years, the recurrence rate, which is the main cause of death, is still high. There are no effective markers for early detection of HGSOC. Transvaginal ultrasound and determination of serum cancer antigen 125 (CA125) levels are used in practice. CA125 and risk of ovarian malignancy algorithm assay incorporating the CA125 serum level and human epididymis protein 4 (according to menopausal status) are not

sufficiently reliable in diagnosis to screen for early-stage OC. It is therefore necessary to introduce new biomarkers that are also effective for targeted therapies (2-5).

Potential molecular markers and their relationship to therapeutic benefit in patients with OC, most of which are undergoing *in vitro* and *in vivo* testing, are described below. Estimation of new molecular markers is essential in the development and monitoring of novel treatment options. For the present review, the following electronic databases were used: Medline (https://www.ebsco.com/products/research-databases/medline), Scopus (https://www.elsevier.com/products/scopus) and Web of Science (https://webofscience.help.clarivate.com/en-us/Content/home.htm/). Selected markers of OC are described in Table I.

2. Angiopoietin 2 (Ang-2)

Ang-2 is an angiogenic protein expressed in endothelial cells at the site of vascular remodeling. It is a ligand of the TEK receptor tyrosine kinase. Regulation of its activity is mediated by hypoxia-inducible factor-1α and VEGF. It shares 60% identical amino acid sequence with Ang-1, but the functions of the two angiopoietins are opposite; Ang-2 is a Tie-2 receptor antagonist while Ang-1 is a Tie-2 receptor agonist. In addition, Ang-2 is not bound to the extracellular matrix, therefore it can be active at different sites in the body (6).

A previous study examining the cancer tissue and serum of 138 patients with OC detected the higher expression of Ang-2 in patients with retroperitoneal spread compared with patients with intraperitoneal disease (P=0.039). High Ang-2 expression levels were significantly correlated with longer OS (P=0.017) and OS in patients receiving bevacizumab (P=0.013). Ang-2 may serve as a molecular marker for patients with OC with early spread to lymph nodes and for patients who receive maintenance targeted therapy with bevacizumab (7).

3. Sodium-dependent phosphate transport protein 2B (NaPi2b)

NaPi2b is located at the cell surface and regulates phosphate homeostasis under physiological conditions. It is encoded by the SLC34A2 gene and expressed in ~80-90% of OC cases. Additionally, it is detected in OC tissues via biopsy. Expression of the SLC34A2 gene was determined at the transcriptional and translational level in 41 OC samples considering different clinicopathological features (clinical grade, neoadjuvant chemotherapy and presence of ascites). The expression of this gene was found to be downregulated in patients receiving neoadjuvant chemotherapy. Thus, expression of this gene may be a marker for predicting response to such therapy (8).

A different study described for the first time the results of treatment in patients with platinum-resistant OC with a humanized anti-NaPi2b antibody conjugated to the antimitotic drug monomethylauristatin E-lifastuzumab vedotin (LIFA). A total of 47 patients received LIFA and 48 patients (representing the second arm of platinum-resistant patients) received pegylated doxorubicin (PLD). Progression-free survival (PFS) was found to be prolonged in patients receiving LIFA compared with the PLD group (5.3 vs. 3.1 months, respectively); however, these values were not statistically significant (9).

A subsequent multicenter study involving 41 patients with recurrent platinum-sensitive OC assessed the safety and tolerability of LIFA. All patients were administered LIFA with carboplatin, and 12 of them were additionally treated with bevacizumab. All patients experienced ≥1 side effects (adverse events); the most common was neutropenia. Side effects, according to the investigators, were acceptable. A complete or partial response to the treatment was observed in 59% of the patients. Thus, this study was encouraging regarding the use of LIFA (10). A careful analysis of the literature review established that NaPi2b fulfils the conditions of an OC biomarker and may serve as a target for therapy in this cancer (3).

4. Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1)

ENPP1 is a type II trans-membrane glycoprotein with pyrophosphatase and phosphodiesterase capabilities. It is a regulator of extracellular ATP and GTP signalling. It plays a key role in phosphate balance and bone mineralization but is also frequently overexpressed in various cancers (breast cancer, glioma), including OC (11).

A study by Wang *et al* (12) of ENPP1 in 241 patients with OC revealed that its expression was significantly increased (P<0.05) in HGSOC (85.4%) and was low in serous adenocarcinoma (1.03%); however, it was not detected in normal ovarian epithelium. Similar expression of ENPP1 was found in established OC cell lines. After downregulation of ENPP1 expression (RNA interference), disease progression exponents such as proliferation, migration and invasion decreased significantly, and expression of proapoptotic caspase 3 increased significantly; epithelial-mesenchymal transition (EMT) was also inhibited, as expressed by decreased expression of the EMT-E-cadherin marker (12).

Subsequent findings (13) confirmed the value of ENPP1 as a molecular marker and target for targeted therapy. In addition to expression in cancer, ENPP1 was found to induce strong immune remodeling (it is expressed in neutrophils and macrophages) promoting cancer progression using the STING pathway associated with antitumor defense mechanisms.

In their review of preclinical studies with ENPP1, Ruiz -Fernández de Córdoba *et al* (13) mentioned several of its inhibitors such as: i) STF-1623, which acts through Zn^{2+} chelation (CM-3163; Angarus Therapeutics), ii) AVA-NP-695 (Avammune Therapeutics), iii) ZX-8177, which can be administered with anti-programmed death-ligand 1 and iv) RBS2418 (Riboscience LLC). Thus, increased expression of ENPP1 may, according to the researchers, be associated with the occurrence of HGSOC and indicate a poor prognosis, and at the same time, may serve as a therapeutic target (13).

5. E-cadherin

E-cadherin belongs to a family of transmembrane proteins that maintain cell adhesion. It participates in signal transduction affecting cell proliferation, survival and differentiation. As aforementioned, E-cadherin is downregulated during EMT, which is related to the mesenchymal phenotype. The EMT process in tumors can be initiated and promoted by multiple signaling pathways, including those originating from hypoxia

Table I. Selected OC markers.

Markers	Characteristics	(Refs.)
Ang-2	Ligand of tyrosine kinase 2 receptor	(6,7)
NaPi2b	1. Regulates phosphate homeostasis under physiological conditions 2. Downregulated expression in patients receiving neoadjuvant chemotherapy	(3,8-10)
ENPP1	Increased expression associated with the occurrence of HGSOC	(11-13)
E-cadherin	Low concentration associated with poor prognosis	(14-16)
TIMP-1	1. High levels in patients with advanced OC associated with reduced OS 2. Associated with platinum resistance	(17-19)
ESM1	1. Participates in the progression of OC 2. Expression correlated with progression-free survival and OS	(20,21)
CSCs	Play a role in progression, multidrug resistance and the formation of metastases and recurrences	(22-28)
ALDH1	High expression associated with platinum resistance in serous carcinomas	(35-38)
NANOG-CSCs transcription factor	Marker of CSCs pluripotency (it promotes tumor growth, metastasis, invasion and chemoresistance)	(39-43)
KLF7	Regulator of the CSCs pluripotency (regulation of the transcription factors OCT4 and NANOG)	(47,48)

Ang-2, angiopoietin-2; NaPi2b, sodium-dependent phosphate transport protein 2B; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; HGSOC, high-grade serous ovarian carcinoma; TIMP-1, TIMP metallopeptidase inhibitor 1; OC, ovarian cancer; OS, overall survival; ESM1, endothelial cell-specific molecule 1; CSCs, cancer stem cells; ALDH1, aldehyde dehydrogenase 1 family member A1; NANOG, Nanog homeobox; KLF7, Kruppel-like factor 7; OCT4, octamer-binding transcription factor 4.

and the microenvironment of the developing cancer. A number of messengers have been shown to be disrupted, including the E-cadherin binding partner, β-catenin and phosphatidylinositol 3 kinase (PI3K) (14).

Based on the results of 18 cell lines and the evaluation of E-cadherin expression using microarrays and immunofluorescence in 101 patients with OC, it was revealed that low levels of E-cadherin and high levels of keratin 7 predict poor response to treatment in HGOC. E-cadherin together with keratin 7 (a component of the cytoskeleton fibres) assay can be an independent prognostic marker of response for HGOC treatment and also for OS (P=0.031 and P=0.041 for E-cadherin and keratin 7, respectively) (15).

Investigation on cell lines and E-cadherin mRNA expression in 20 cases of existing ascites in patients with OC patients revealed that loss of E-cadherin expression is associated with OC progression and aggressiveness, and that E-cadherin levels are associated with CA125 levels.

Options were provided for targeted therapies that stimulate E-cadherin expression. These include: i) α-Solanine (a glycoalcaloid extract of *Solanum nigrum*), which lowers the expression of matrix metalloproteinases (MMPs), ii) simvastatin and metformin which increase E-cadherin values and iii) numerous other synthetic peptides used in cancer therapy, such as ADH-1 (antidiuretic hormone) (16).

6. TIMP metallopeptidase inhibitor 1 (TIMP-1)

TIMP-1 is a tissue inhibitor of MMP-9, a member of the gelatinases. MMPs are a group of >20 zinc-dependent proteolytic enzymes. They are involved in matrix remodeling and degradation processes. TIMP-1 has been found to model matrix metalloproteinase activity and play a role in OC progression by mediating metastasis through colonization, migration and invasion of cancer cells. It is present in both ascites and plasma. It was found from comparative studies (70 ascites and 20 plasma samples) that its concentration is higher in ascites and correlates with its volume. Serum levels in patients with OC (stage I-IV) are the highest after cytoreductive surgery and the lowest after treatment (17). Sonego *et al* (18) observed that high TIMP-1 levels in patients with OC at stage III and IV were associated with reduced OS especially if patients were treated with platinum or bevacizumab. This indicated that TIMP-1 is associated with platinum resistance regulation and progression and there is potential for its use as a novel biomarker of platinum resistance. According to the researchers, this demonstrates the possibility of using TIMP-1 as a target for targeted therapy. This was confirmed by a study of TIMP-1 in circulating tumor cells (CTCs) in 38 patients with HGSOC (depending on the method used, CTCs are detected in 18-88% of patients with OC). It was identified that, in addition to the stem cell markers CD24 and CD44, TIMP-1 promotes cancer promotion, suggesting its therapeutic target (19).

7. Endothelial cell-specific molecule 1 (ESM1)

ESM1 gene is located on chromosome 5q11.2 and is involved in migration, proliferation, invasion, angiogenesis and escape from apoptosis. It regulates signaling of the conservative, carcinogenesis-related Wnt/β catenin pathway and is associated with the AKT/mTOR pathway promoting cancer cell proliferation and inhibiting apoptosis (20,21).

A molecular study of 379 OC samples demonstrated that ESM1 expression was higher in cancer tissues compared with

peritumor tissues and healthy ovarian tissues. ESM1 expression was closely correlated with clinical parameters such as lymph node metastasis and cancer recurrence. The authors concluded that ESM1 plays an important role in OC development and progression; it was positively correlated with PFS, but negatively with OS. It may be considered as a novel OC biomarker and therapeutic target (21).

8. Cancer stem cells (CSCs)

CSCs are a subpopulation of cancer cells usually representing <2-5% of the tumour mass, involved in cancer growth, multidrug chemoresistance, metastasis formation and recurrence formation (22-24).

CSCs have been shown to possess properties that allow them to survive under adverse conditions. These include the ability of self-renewal, asymmetric division, staying in an inactive state (as 'dormant cells' in the G0/G1 phase), the ability to repair DNA, overexpression of ATP-binding cassette family genes associated with chemoresistance, lack of apoptosis and the ability to utilize different signaling pathways such as Wnt/β catenin, Notch and Hedgehog which are associated with survival and self-renewal (22,23).

Molecular markers of CSCs have been identified, both superficial, such as CD44, CD133 (Promin), CD111 (c-Kit), CD24, CD117, and intracellular, such as aldehyde dehydrogenase 1 family member A1 (ALDH1), octamer-binding transcription factor 4 (OCT4), SRY-Box transcription factor 2 (SOX2) and Nanog homeobox (NANOG). Determining their expression is necessary to understand the efficacy and monitoring of targeted therapy (22,24-27). In 2005, Bapat *et al* (28) detected CSCs markers in OC.

Most applied trials of therapies against CSCs *in vitro* and *in vivo* and also in women with OC affect the expression of not only one CSCs marker, but also the signaling pathway. Such known drugs affecting CSCs include metformin and salinomycin.

Metformin, used in the treatment of type 2 diabetes, exerts beneficial effects in OC. An electronic database study on >700 patients with OC revealed an association of metformin use with longer survival (P=0.03) and synergistic activity with carboplatin. Metformin has an inhibitory effect on the AKT/mTOR pathway (29). Furthermore, in a phase I clinical trial, no adverse effects were reported using metformin in combination with carboplatin and paclitaxel (30).

A previous *in vitro* and *in vivo* study detected that metformin selectively inhibits the CD44(+) and CD117(+) populations, also glycoproteins of CSCs affecting cancer progression, including: Cell adhesion, cell growth, migration and resistance to chemotherapy (31).

Salinomycin, an ionophore antibiotic isolated from *Streptomyces albus,* selectively eradicates CSCs (32-34). Previous studies on OC cell lines (OVCAR3) have revealed an inhibitory effect of salinomycin in combination with paclitaxel on CD44(+) and CD117(+) expression (32). This was confirmed by a study by Lee *et al* (33) on isolated CD44(+) and CD117(+) cells from ascites of women with OC. Salinomycin in combination with paclitaxel decreased their viability and promoted apoptosis.

Constructing a carrier mimicking high-density lipoprotein (HDL) in combination with salinomycin (S-HDL) has been demonstrated to have potent anticancer effects in OC as it inhibits translation of the CSCs proteins c-Myc, NANOG, OCT4 and SOX2 which are associated with chemoresistance and OC recurrence (34). The authors of the investigation on the effects of metformin and salinomycin consider that these two drugs will be an effective future therapy in improving survival and preventing recurrence of OC.

ALDH1. High expression of ALDH1 has been demonstrated to be associated with platinum resistance in serous carcinomas. This was based on a study of 124 patients with stage III and IV serous OC with high malignancy (35). A different study on OC cell lines found that high expression of ALDH1A1 was associated with resistance to paclitaxel and topotecan. At the same time, it was associated with overexpression of P-glycoprotein (P-gp), known as the multidrug resistance protein responsible for removing the 'pumping out' of the cytostatic from the cell. The use of all-trans retinoic acid (ATRA) decreased ALDH1A1 expression and reduced P-gp expression (36).

Kim *et al* (37), who investigated A2780 cells isolated from chemotherapy-resistant OC of female patients, confirmed that high ALDH1 expression is associated with drug resistance and facilitated growth of OC. Furthermore, high expression of ALDH1 was associated with increased expression of nuclear factor erythroid 2-related factor 2 (NRF2). Furthermore, ATRA treatment decreased ALDH1 expression and inhibited NRF2 activation. The effect was to attenuate the malignancy of CSCs in OC.

A different study on cells isolated from ascites of untreated or receiving neoadjuvant therapy patients with OC also showed their resistance to platinum and taxanes, accompanied by high ALDH1 expression. It was revealed that several compounds including EGFR/mTOR-PI3K inhibitors are candidates for targeting the ALDH1 cell population (38).

NANOG (CSCs transcription factor). NANOG is a marker of CSCs pluripotency. It promotes tumour growth, metastasis, invasion and chemoresistance. Using different signaling pathways such as JAK/STAT, Notch, Hedgehog and canonical Wnt/β-catenin, it participates in the reciprocal regulation of other transcription factors such as SOX2 and OCT4, and its expression is related to several microRNAs such as miR-214, whose downregulation reduces NANOG expression and induces cancer cell apoptosis. High NANOG expression is present in 69.7% of OC cases, particularly HGSOC. It is recognized as a key marker of progression. Although gene therapy with microRNA is in the experimental phase, NANOG is a diagnostic marker and a future target for personalized therapy (39-43).

Vaccines containing CSCs. In 2015, the efficacy of a vaccine containing CSCs was described for the first time in a mouse model. Cell lines containing the CD117(+) and CD44(+) subpopulations were isolated from the SKOV3 human OC line. When administered in mice [SKOV3 containing CSCs, CD117(+) and CD44(+) subpopulations], it induced the desired effect: Inhibition of xenograft tumor growth and reduction of CD117(+) and CD44(+) cell subpopulations (44). The same group of researchers produced a vaccine from a patient-derived cell line (HO8910) that also contained CD117,

CD44 and receptor tyrosine kinase-like orphan receptor 1 (ROR1). It was identified that high expression of ROR1 was strongly correlated with vaccine efficacy, while downregulation of ROR1 expression by small interfering RNA (siRNA) reduced vaccine efficacy. It was concluded that in OC immuno-prophylaxis, ROR1 expression should be high (45). A subsequent study confirmed the efficacy of both H08910 and D8 (a cell line isolated in mice) regarding the inhibition of tumor growth and prolongation of survival. According to the authors, it is possible to convert a CSC-containing vaccine into an immunotherapeutic approach (46).

9. Kruppel-like factor 7 (KLF7)

KLF7 is one of 17 KLFs identified in humans and animals and is encoded on chromosome 2q33.3. It is a transcription factor with a broad spectrum of regulatory functions. It is involved in physiological and pathological processes such as: Cardiovascular disease, hematological disease and metabolic disease (type 2 diabetes), and is also a regulator of the maintenance of CSCs pluripotency that involves transcription factors OCT4 and NANOG which in turn regulate KLF7 expression (47). Mao *et al* (47) studied the expression of KLF7 on cell lines and animal models. They discovered chemical substances that downregulate KLF7 expression, including catechin (polyphenol, antioxidant), pitavastatin (lipid-lowering drug) and trametinib (MEK inhibitor).

KLF7 is involved in the development and progression of numerous cancers in various localizations, including OC. A novel bioinformatics meta-analysis of transcriptome data in 2 cohorts of patients with advanced stages of HGSOC (III/IV) including 185 patients from the Gene Expression Omnibus (GEO) database and 266 patients from the National Cancer Institute Genomic Data Commons (NCI-GDC) Data Portal detected that KLF7 is significantly associated with this cancer. A high expression of KLF7 is significantly related to shorter OS (for GEO multivariate analysis, $P<0.0001$; for NCI-GDC, $P=0.03$). *In vitro* studies (OV-90 and PEO1 cells) have determined that KLF7 may play a role as an oncogene to stimulate tumor growth and invasion, and is involved in maintaining the pluripotency and the self-renewal characteristics of stem cells.

Previous results have suggested that KLF7 is a promising prognostic marker and therapeutic target in HGSOC (48). The study by De Donato *et al* (48) revealed the first evidence that high expression of KLF7 in HGSOC is connected to shorter OS. The authors attempted to silence KLF7 expression in OV-90 and PEO1 cells by using TransFectin Lipid Reagent and specific siRNAs, but it may be possible to apply the repositioning drugs mentioned by Mao *et al* (47) in *in vivo* studies and clinical trials in an attempt to lower KLF7 expression in HGSOC.

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AM: Originator of the study and writing of the abstract, introduction and chapter on Ang-2. ZK: Writing of chapters on NaPi2b and ENPP1. DT: E-cadherin, TIMP-1. JP: Writing of chapters on ESM1 and KLF7. JM: Writing of chapter on CSCs. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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