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Biological markers of prognosis, response to therapy and outcome in ovarian carcinoma

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Abstract

Introduction—Ovarian cancer (OvCa) is among the most common types of cancer and is the leading cause of death from gynecological malignancies in western countries. Cancer biomarkers have a potential for improving the management of OvCa patients at every point from screening and detection, diagnosis, prognosis, follow up, response to therapy and outcome.

Areas covered—The literature search has indicated a number of candidate biomarkers have recently emerged that could facilitate the molecular definition of OvCa, providing information about prognosis and predicting response to therapy. These potentially promising biomarkers include immune cells and their products, tumor-derived exosomes, nucleic acids and epigenetic biomarkers.

Expert commentary—Although most of the biomarkers available today require prospective validation, the development of noninvasive liquid biopsy-based monitoring promises to improve their utility for evaluations of prognosis, response to therapy and outcome in OvCa.

Keywords

Ovarian carcinoma; serum; tissue; immune biomarkers; exosomes; microRNAs

1. INTRODUCTION

Malignant ovarian neoplasms are a heterogeneous group of tumors that include primary epithelial and non-epithelial ovarian cancers, with the former having the highest mortality rates of all gynecological malignancies with a median age at diagnosis of 65 years [1].

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Declaration of interest

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According to International Federation of Gynecology and Obstetrics (FIGO), most patients with ovarian carcinoma (OvCa) present with advanced stage, due to the lack of specific symptoms and adequate diagnostic tools [2]. The etiology of OvCa is unknown, but multiple genetic and environmental factors have been identified as potential contributors to its pathogenesis. Based on recently obtained morphological and molecular data, a new paradigm of the OvCa pathogenesis has emerged, which divides the epithelial tumor into two groups. Type I tumors are linked to ovarian precursor lesions such as cystadenofibroma, borderline tumors and endometriosis and include low-grade serous carcinoma, endometrioid, mucinous and clear cell carcinomas. These tumors are characterized by slow growth, presentation as low stage tumors and contain multiple genetic mutations. In contrast, type II OvCa are linked to precursors arising from the fallopian tube epithelium and consist of high-grade serous and endometrioid carcinomas, carcinosarcomas and undifferentiated carcinomas. Type II tumors are highly aggressive, are in advanced stage at diagnosis and have poor prognosis. Type I and type II tumors are also genomically distinct. Type I tumors are frequently associated with specific mutations in oncogenes such as KRAS, BRAF, PTEN and ARID1A. In the vast majority of type II tumors, TP53 is mutated, and a high level of chromosomal disruption is evident [3,4]. Standard treatment for advanced stage OvCa is tumor-debulking surgery and adjuvant platinum-based chemotherapy. However, after complete surgery and chemotherapy, the risk of relapse is approximately 85%, with substantial variations based on the well-known clinicopathological features. The five-year survival of patients with advanced disease is only 10–30% [5]. In the past two decades, several new drugs have become available for patients with OvCa. However, in individual patients, it is difficult to determine the effectiveness of a given treatment due to a lack of objective measures that define efficacy. These clinical findings have emphasized the need to identify novel biomarkers for OvCa that can be used to measure responses to therapy, prognosis and overall outcome. Recently, a number of candidate biomarkers have been evaluated, but the lack of the understanding of the OvCa etiology as well as the disease heterogeneity, make it unlikely that a single biomarker will satisfy criteria formulated for acceptance of cancer biomarkers [6].

2. DEFINITION OF A CANCER BIOMARKER

The so called *REMARK* criteria have been formulated and are listed in the literature as guidelines for biomarker studies [7,8]. Briefly, a new biomarker has to be validated in prospective (and not retrospective) studies of adequate size and statistical power. These studies should include a unique cohort of patients in whom the biomarker correlates with disease activity and the known (if any) molecular factors predictive of survival. The biomarker should be able to discriminate between pathologic and physiologic conditions even if they are similar. The biomarker should have a defined molecular mechanism of biological activity, and the data in support of its validity have to be based on thorough specimen collection, assay results confirming specificity, sensitivity, reproducibility, robustness as well as statistical rigor and on stringent patient follow-up. Cancer biomarkers may be discovered using molecular, cellular, and imaging methodologies. They should be detectable in biological samples that are easily obtainable, for example; serum, plasma, whole blood, ascites, urine and tissues accessible for sampling. Biomarkers can be normal

endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained inactive in normal cells. Biomarkers may include intracellular molecules or proteins in tissues or may be released into the circulation and body fluids. In addition, the assays for biomarkers to be used clinically should be simple, inexpensive and lend themselves readily to high through-put technologies. These are by no means trivial requirements, and they emphasize the difficulties that are associated with the field of biomarker discovery.

3. CA125 AND HE4 BIOMARKERS FOR OVCA

Today, the most frequently used biomarker for OvCa is CA 125 (MUC 16). CA125 is useful for monitoring responses to chemotherapy, detecting disease recurrence and for differentiation of malignant from non-malignant pelvic masses. In the past decade, major efforts have been made to improve the performance of CA125 in differential diagnosis of pelvic masses and in screening for OvCa [9]. However, the positive predictive value is low for CA125, and it is only effective when used in combination with other diagnostic tests (Table 1). Moreover, CA125 can be elevated in a number of conditions unrelated to OvCa [9], and 20% of OvCa have little or no expression of CA125. Nevertheless, according to the current guidelines, CA125 remains the only serum marker accepted for diagnosis and follow up of OvCa.

To improve the diagnostic value of CA125 in OvCa, especially in the presence of a suspicious pelvic mass or symptoms, the risk malignancy index (RMI) has been developed [10]. The RMI combines CA125 results with ultrasound findings and the menopausal status to calculate the index score that helps in predicting the risk of OvCa in women with adnexal mass (Table 1). The RMI remains the most accurate tool for stratifying patients into high and low risk groups.

Another commonly used serum biomarker for OvCa is human epididymis protein 4 (HE4), recently approved by the FDA for monitoring disease progression or recurrence in OvCa. This protein was initially identified in the epithelium of the distal epididymis and then discovered to be a protease inhibitor involved in sperm maturation [11]. It appears to be useful in differential diagnosis of adnexal masses and in early diagnosis of OvCa (Table 1). Various factors aside from malignancy including age, smoking and chronic renal disease may influence serum HE4 levels and, thus, the interpretation of the results of HE4 assays is not straightforward [12,13]. On the other hand, the HE4 serum level, in contrast to that for CA125, is not affected by menstrual cycle, endometriosis and oral contraceptives [14,15]. Therefore, HE4 seems to be a potentially more specific detection marker than CA125 for early diagnosis and has been cleared by the FDA for monitoring disease progression and recurrence [16].

Promising HE4 results in OvCa diagnosis, especially in combination with CA125, have led to the development of the Risk of Ovarian Malignancy Algorithm (ROMA) [17]. It combines HE4, CA 125 and the menopausal status to enhance sensitivity and specificity of OvCa detection (Table 1). As neither CA125 nor HE4 measurements alone meet the requirements for sensitivity or specificity that should be attributes of the biomarkers used for

screening or monitoring responses to therapy in OvCa, ROMA provides a greater power for diagnosis of OvCa than either of the two markers alone (Table 1) [18]. Whether ROMA is better than the RMI in distinguishing OvCa from benign diseases remains unclear [18]. Furthermore, a survival advantage of early detection of OvCa or OvCa recurrence using HE4 or CA125 markers is yet to be established.

In 2009, the FDA approved another test for clinical testing of OvCa named OVA1 [19]. It evaluates serum concentrations of five different markers (Table 1), and appears to have not only high levels of sensitivity but also high negative predictive value [19].

In view of the paucity of reliable biomarkers in OvCa and inadequate diagnostic or prognostic performance of those that are available, the identification of novel biomarkers for early detection, prognosis, prediction of treatment efficacy and overall outcome in OvCa is an unmet need. This review considers some of the most promising recently described biomarkers and their potential role in the detection and prognosis of OvCa.

4. EMERGING SERUM BIOMARKERS

Blood contains a wide range of proteins that reflect the physiological conditions in tissues and thus can be used as a biological marker of impending or ongoing disease. Blood collection is a minimally invasive procedure, and sampling can be carried out in large cohorts of patients. Recently, rapid advances in high-throughput technologies has allowed for new and exciting opportunities for serum biomarkers discovery. More than 30 serum markers have been evaluated alone and in combination with CA125 by different investigators. Some of the most promising include: osteopontin, mesothelin, kallikrein(s), macrophage colony-stimulating factor (M-CSF), and soluble epidermal growth factor receptor (EGFR). Much of this effort has utilized proteomics analyses to monitor and identify proteins that regulate functions and cellular interactions responsible for cancer cell growth. For example, in 2002, Petricoin et al. used an independent set of 116 serum samples: 50 from women with OvCa and 66 from normal controls or those with benign tumors to search for markers that would best distinguish the two cohorts. The results showed that the used algorithm identified a cluster of proteins which completely segregated OvCa sera from non-cancer specimens [20]. In 2014, Cheng et al. analyzed serum samples from patients with OvCa and from healthy controls. Proteomic analyses identified 1200 serum proteins, among which 57 were upregulated in the sera collected from cancer patients. Retinol binding protein 4 (RBP4), which is an adipokine secreted by adipose tissue and liver, was found to be highly elevated in sera of OvCa patients compared to healthy individuals [21]. These examples suggest that proteomics profiles of OvCa patients' sera are distinct from those of sera of healthy controls.

Among proteins found to be consistently elevated in OvCa sera is osteopontin, a multifunctional cytokine that plays an important role in many physiological and pathological processes, including cell proliferation, survival, drug resistance, invasion, inflammation, immune response, stem cell behavior and tumorigenesis [22,23]. Osteopontin is overexpressed in many different tumor types, including OvCa [24]. Preoperative plasma levels of osteopontin were significantly higher in patients with OvCa compared to those with

benign ovarian tumors and to healthy women's plasma. Thus, preoperative osteopontin levels could be a potentially useful biomarker for detecting OvCa, especially when used together with CA125 [25].

Mesothelin, a cell surface protein on OvCa cells, is a potential target for antibody-based therapy due to its high expression levels in OvCa. It is also a new target for chimeric antigen receptor (CAR) T cell adoptive therapy [26]. Mesothelin plays a role in cancer progression; it may aid in the peritoneal implantation and metastasis of tumor through its interaction with CA125; it promotes cancer cell proliferation and survival via the NF-kB signaling pathway; it also promotes resistance to chemotherapy, including paclitaxel, platinum and cyclophosphamide. In patients with OvCa, high expression of mesothelin in both tissues and serum indicate poor prognosis [27]. Ibrahim et al evaluated diagnostic accuracy of serum mesothelin levels in 110 patients with ovarian masses relative to serum CA125 levels (Table 2). Mesothelin seemed to have the same sensitivity, but higher specificity than CA125 and was a significant predictor of early stage OvCa [28].

The epidermal growth factor receptor (EGFR) is overexpressed in 30–98% of EOC, and signaling cascades it activates impact on tumor cell proliferation, migration, invasion and resistance to chemotherapy [29]. While EGFR emerges as an attractive therapeutic target in OAC, soluble EGFR (sEGFR) has been of interest as a potential biomarker for EOC diagnosis and/or resistance to platinum therapy [30]. Circulating sEGFR is a 90–110kDisoform of EGFR readily detectable by immunoassays. Its concentrations are lower in OvCa patients compared to healthy women or women with benign ovarian tumors or other gynecological conditions [31,32]. This suggests that sEGFR alone or in combination with CA125 may be a useful biomarker for risk assessment, early detection and diagnosis of OvCa (Table 2). Also, sEGFR in combination with CA125 can differentiate benign from malignant OvCa [32]. A recent study of sEGFR levels in late-stage patients suggested that OvCa patients with increased sEGFR levels had poor PFS [33].

The macrophage colony-stimulating factor (M-CSF), a cytokine also known as colony stimulating factor-1 (CSF-1), regulates growth and differentiation of myeloid cells. I t is a chemoattractant for monocytes, and is strongly expressed in OvCa specimens of patients with advanced disease. Detectable in sera by immunoassays, it has been used alone or in combinations with HE4 or CA125 as a screening assay for OvCa [34]. In a recent study of 110 OvCa patients, detection sensitivity of M-CSF was somewhat greater than that of HE4 or CA125 and improved upon its combination with these two markers [35]. Its specificity was very high at 94% (Table 2). Thus, M-CSF emerges as a potentially useful screening test for OvCa.

Another promising serum biomarker for OvCa diagnosis is the soluble form of the folate receptor alpha (sFRA). It is a GPI-anchored glycoprotein encoded by the FOLR1 gene, which can be shed from the cell surface into the circulation [36]. Kurosaki et al. investigated serum levels of sFRA in 231 patients and found high sFRA in sera of EOC patients, but not in patients with benign or borderline gynecological disease or with OvCa metastasizing from colorectal cancers. Serum levels of sFRA were highly correlated to the clinical stage, tumor grade and histological tumor type and demonstrated greater accuracy for the detection of

OvCa than did serum CA125 (Table 2). In both early and advanced stage patients, high sFRA levels were significantly associated with shorter PFS [36].

A number of other potentially promising diagnostic markers for EOC are currently under investigation, including vascular endothelial growth factor (VEGF), interleukin-10 (IL-10), metalloproteinases (MMP-2, MMP-7, MMP-9) or the tissue inhibitor of metalloproteinase-1 (TIMP-1).

5. TISSUE BIOMARKERS

Immunohistochemistry (IHC) with antibodies specific for OVCa antigens helped to identify those that are overexpressed or aberrantly expressed in tumor tissues. Human leukocyte antigen-G (HLA-G) is a non-classical MHC class I molecule. Its major biological role is suppression of ongoing immune responses. Measurements of HLA-G protein or mRNA levels have shown promise in detection and prognosis of OvCa [37,38]. HLA-G expression can be associated with either favorable or unfavorable clinical outcome. On the one hand, high expression levels of HLA-G such as seen in aggressive types of OvCa, i.e., high-grade serous carcinomas, tend to suppress anti-tumor immune responses promoting tumor escape. On the other hand, HLA-G expression emerges as potential marker of tumor susceptibility to chemotherapy and associates with longer progression-free survival [38]. In high-grade epithelial OvCa, HLA-G expression is an independent prognostic factor and a predictor of platinum sensitivity [38], which outweighs its tumor immunoprotective role.

A growing body of evidence suggests that reproductive hormones can affect tumor progression, proliferation and metastasis through paracrine, autocrine or intracrine mechanisms [39]. Hydroxysteroid (17B) dehydrogenase type 12 (HSD17B12) is a multifunctional isoenzyme functional in the conversion of estron to estradiol and elongation of long-chain fatty acids, in particular the conversion of palmitic to archadonic acid, the precursor of sterols and the inflammatory mediator, prostaglandin E_2 (PGE_2). In the normal ovary HSD17B is detected in granulosa cells of developing follicles, but not in the surface epithelium. In contrast, epithelial OvCa is positive for HSD17B, and its expression in OvCa is associated with poor prognosis [40]. The clinical implications attributed to HSD17B12 overexpression in OvCa suggest that it could serve not only as biomarker but also as candidate for T-cell based immunotherapy [41].

Proteomics have introduced another powerful approach to the detection of proteins that have promise as biomarkers in OvCa such as kallikrein-related peptidase (KLK). Tissue kallikrein (KLK1) and kallikrein-related peptidases (KLK2-15) are aberrantly expressed in OvCa tissues, in particular, in the more metastatic type II-tumors. KLKs play a role in many aspects of pathophysiology including hydrolysis of growth factors, proteases, cell membrane bound receptors, adhesion proteins, and cytokines, regulating intracellular signaling pathways and their downstream events. High KLK levels are associated with poor prognosis of OvCa patients, suggesting that KLK might be a useful as a marker of disease progression [42]. Two kallikrein serine protease family members (KLK6 and KLK7) were found to be significantly overexpressed relative to normal tissue controls in most of the OvCa cell lines. Overexpression of KLK6 and KLK7 mRNA was specific for ovarian cancer, in particular for

serous and papillary serous cancer subtypes. In situ hybridization and IHC further confirmed significantly elevated levels of KLK6 and KLK7 mRNA and proteins, respectively, in tissue epithelia relative to a lack of expression in the neighboring stroma [43].

6. IMMUNOLOGICAL BIOMARKERS

The idea of the immune system as a valuable parameter in the development and prognosis of ovarian cancer has been neglected for a long time [44]. Newer data indicate that the host immune system strongly influences outcome in patients with OvCa. Studies of immune cells infiltrating OvCa have indicated that accumulations of lymphocytes, regulatory T cells, macrophages, dendritic cells (DC), myeloid derived suppressor cells (MDSC) and natural killer (NK) cells might determine biologic behavior of the tumor as well as cancer therapy and prognosis. Interestingly, the absolute lymphocyte count (ALC) in peripheral blood was found to be associated with survival in OvCa independent of tumor-infiltrating lymphocytes (TILs) in OvCa [45]. T lymphocytes represent a major population of TILs. Their diversity makes it difficult to evaluate the significance of these cells in the tumor development, since individual subtypes of T cells could have opposite effects on tumor progression. High density of tumor-infiltrating $CD3^+$ T cells has been associated with favorable prognosis in various types of cancer, including OvCa [46–48]. Among specific subtypes of TILs, cytotoxic T cells ($CD8^+$) have also been associated with better survival in OvCa patients [49]. TIL infiltration patterns in OvCa can range from $CD8^+$T cells alone to complex lymphoid follicle-like structures that include $CD8^+$T, $CD4^+$T and $CD20^+$ B cells [50]. Cooperation between tumor-infiltrating B and T cells leads to increased patient survival, presumably because B cells, which serve as APC and secrete polarizing cytokines, enhance antitumor immunity [51]. Analyses of TILs in high-grade serous OvCa further showed that expression by intraepithelial $CD8^+$ T cells of CD103 (an αE integrin) strongly correlated with increased disease-specific survival [52]. In aggregate, new insights into anti-tumor immunity in OvCa indicate that patient survival rates increase as the complexity and size of the TIL compartment increases, suggesting that cooperative interactions between various infiltrating immune cells are important for survival.

Another subset of $CD4^+$ T cells variably present within the tumor environment are the $CD4^+CD25^+$regulatory T cells (Treg). They usually represent a smaller fraction of tumor infiltrating lymphocytes, but may have a significant influence on the tumor development [53,54]. It has been shown that the frequency of Treg is higher in tumors than in normal tissues due to an active recruitment of Treg by factors present in the tumor microenvironment [55]. Increased percentages of Treg are also seen in the peripheral circulation of patients with cancer [56,57]. Their potential to inhibit Th1 responses by interfering with anti-tumor functions of effector T cells could contribute to poor outcome, as seems to be the case in some human solid tumors, including OvCa [55]. Moreover, high percentages of Treg in OvCa contribute to an immunosuppressive microenvironment that curbs anti- tumor immunity [55].

Macrophages represent a major population of tumor–infiltrating leukocytes. Their number is often increased in tumors compared to healthy tissues [44]. It is well known that M1 macrophages, predominantly situated within tumor epithelial islets, have anti-tumor effects,

whereas M2 cells, located in the stroma, promote tumor growth. Emerging evidence suggests that tumor-associated macrophages (TAM) display a unique activation profile in OvCa and are able to create an immunosuppressive microenvironment, allowing tumors to evade immune detection and promoting tumor progression. Studies investigating the presence of TAMs in OvCa demonstrate a significant increase in their number in malignant ovarian tumors compared to benign and borderline tumors [58,59]. However, the quantity of $CD68^+$ tumor–infiltrating macrophages does not appear to influence outcome [60,61]. More detailed studies using specific M2-associated markers in OvCa indicated that TAM can predict the patient's prognosis in certain subsets of OvCa. In addition to $CD68^+$ cells, Lan et al. also studied expression of CD163, an M2 marker, in 110 advanced stage OvCa and demonstrated that both PFS and overall survival (OS) were significantly reduced in patients whose tumors contained high numbers of $CD163^+$ cells [62]. Serum levels of CD163 have also been shown to predict poor prognosis in patients with OvCa [63].

Dendritic cells (DC) are professional antigen presenting cells that regulate immune system. The two major DC subset are the classic DC and the plasmacytoid DC, and both have been studied in OvCa.[64]. Unlike other TILs, the density of DC at the tumor site is lower than that in the corresponding normal tissues. The results of several studies indicated that DC from OvCa patients are functionally defective or immunosuppressive and that prognosis of OvCa is inversely related to the presence of tumor infiltrating DC [65,66].

Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of immature myeloid cells with profound immunosuppressive abilities that contribute to immune dysfunction and tumor progression. MDSC accumulate in OvCa, inhibit T cells responses and correlate with more rapid recurrence in OvCa patients [67,68]. Obermajer et al showed that MDSC accumulated in the ascites of patients with advanced OvCa, and that they suppressed T cell proliferation *ex vivo* [69]. These findings show that MDSC, representing a specific innate immune population, may serve as a potential prognostic marker with the potential to predict time to relapse in OvCa.

Extensive research of the last two decades suggest that tumors are inflammatory organs, in which the tumor microenvironment (TME) has been co-opted to support tumor growth [70]. In this context, inflammatory chemokines assume major roles in cancer. OvCas are known to produce a variety of chemokines which impact on the TME, including cancer cells and immune cells. These factors that in principle could have protected the individual against the developing tumor are being used by the tumor cells for their own propagation, motility and spread. A recent study of OvCa progression has shown that two phenotypically distinct monocyte subsets were present in the peritoneum at different stages of tumor progression. These two monocytes population suppressed activities of naïve CD8+ and CD4+ T cells. CCR2, a chemokine which specifically mediates monocyte chemotaxis, was a critical factor in recruiting these suppressive cells to the ovarian tumor microenvironment [71]. Moreover, CCR2-expressing MDSC limited the efficacy of immune therapy by down regulating the migration of CD8+ T cells to the tumor site [72]. Another chemokine produced by OvCa cells as well as associated macrophages is CCL22, which mediates trafficking of Treg to the tumor. This specific recruitment of Treg represents a mechanism by which tumors may foster immune privilege [55].

7. TUMOR-DERIVED EXOSOMES (TEX)

One of the biggest challenges in identification of biomarkers of pathological mechanisms operating in the ovary during OvCa progression is the inaccessibility of the diseased tissue. Many secreted molecules and factors, such as cytokines or chemokines, are readily detectable in body fluids, including ascites, and can serve as important biomarkers of the inflammatory processes. However, secreted biomolecules originating from non-circulating OvCa cells are often present in very low concentrations and thus are difficult to detect. In recent years, a better understanding of cell-to-cell signaling through secreted extracellular vesicles (EVs) has indicated that EVs may be an important source of biomarkers in many different diseases [73]. All cells secrete EVs, which vary in size from large apoptotic bodies (1,000–5,000 nm), smaller microvesicles (MVs, 200–1,000nm) and the smallest, exosomes (30–150nm). Tumor cells produce masses of EVs, which are found in all body fluids. Exosomes represent information-containing packages, which transfer their content to target cells, inducing changes in the phenotype and functions of the target cells [74]. The molecular profile of exosomes found in body fluids resembles that seen on the surface membrane of cells from which they originate (Figure 1). Exosome cargos include proteins, such as enzymes, cytokines, growth factors, lipids and nucleic acids, including mRNA or microRNA and ncRNA [75–77]. It has been shown that tumor cells are avid producers of exosomes, which play a role in many aspects of tumor progression including: degradation of the extracellular matrix [78]; angiogenesis [79]; suppression of immune cell functions [56,80,81] and drug resistance [82].

OvCa cells release large quantities of exosomes which have a molecular composition in part resembling that of cell surface membranes in the parent tumor cells. The OvCa patients' plasma contains higher levels of exosomal proteins compared to those found in plasma of patients with benign tumors or normal controls (NC) [83]. Exosomes isolated from OvCa patients' plasma carry TGFβ-B1 and other OvCa-associated antigens, e.g., melanoma-associated antigen MAGE 3/6, previously shown to be present in OvCas [84] which distinguish OvCa patients from benign tumors or NCs [83]. High protein levels of exosome fractions are seen in newly diagnosed patients [83]. Moreover, in advanced stages of OvCa, the protein content of isolated exosomes is significantly higher than that in early stages [83]. Thus, the protein levels in isolated exosome fractions could differentiate OvCa patients with early from those with advanced disease. The exosome protein levels variably changed during/after chemotherapy [83]. Preliminary correlations between the changes in exosomal protein levels and clinical data suggested that the protein content of exosomes in plasma might be useful in predicting responses to therapy and prognosis in OvCa patients [83]. A study by Luciani et al. suggested that tumor-derived exosomes could be responsible for removing cytotoxic drugs from tumor cells thus reducing the anti-tumor potential of chemotherapy [85].

Also, Safaei et al. showed that cisplatin-resistant OvCa patients secreted higher quantities of microvesicles which carried the cisplatin export transporters, MRP2, ATP7A, and ATP7B [86]. Moreover, enhanced microvesicles production and resistance to cisplatin were associated with higher expression of genes whose products are known to be responsible for membrane fusion, vesicle trafficking and export of drugs by microvesicles [86]. In recent

years, it has become increasingly clear that exosomes obtained from plasma of OvCa patients contained significant levels of mRNA and miRNA, providing an explanation for the ability of TEX to transfer genetic material between the cells [87]. miRNA and other molecular features of MV represent a unique combination representative of the cancer cells from which they were derived [88]. Thus, their presence in cancer-derived exosomes may serve as a novel source of disease-related information and possibly as unique and specific cancer biomarkers that may prove useful for screening and diagnosis [75].

8. CANCER STEM CELL BIOMARKERS

Cancer stem cells (CSC) are a small population of malignant cells within a tumor (typically 0,01–1% of cells) that have increased tumorigenicity and differentiating capacity and unlimited self-renewal. Thus, CSC are capable of recreating the original tumor. The concept of OvCa as a stem-cell disease has been gaining credence in recent years. It profiles CSC as important drivers of carcinogenesis, metastasis, tumor recurrence and drug resistance. In this context, CSC are seen as predictors of poor prognosis. [89,90]. Tumors identified as being stem cell-like generally have a high tumor grade and significantly worse prognosis [91,92]. However, there exists a significant controversy in regard to markers that define CSC in human OvCa and prognostic significance of these markers [93]. For example, a number of cell surface markers including CD133 (prominin), CD44 (hyaluronic acid and receptor), CD24 (mucin-like cell surface glycoprotein marker), ALDH (aldehyde dehydrogenase), CD117 (mast/stem cell growth factor receptor), EpCAM (tumor associated calcium signal transducer 1) and ABCG2 (ATP-binding cassette sub-family G member 2), are used singly or in combination to identify and isolate CSC from human cancers. Among these markers, the membrane glycoprotein prominin (CD133) is most widely used but the least understood in terms of its functions [94]. Nevertheless, it has been shown that increased expression of CD133 is a negative prognostic factor in OvCa [95]. OvCa patients with combined expression of CD133 and ALDH within the tumor had significantly poorer outcome. Single ALDH expression in OvCa correlated with poor prognosis in some studies but not others [96,97] [98]. Steffensen et al reported that early stage OvCa patients whose tumor contained >20% of $CD44^+$ CSC, had a shorter PFS compared to patients with tumors with <20% of these cells [99]. Similarly, CD24 expression of has been correlated with poor prognosis [100]. Zhang et al found that CSC express a type I receptor tyrosine kinase-like orphan receptor (ROR1) and that patients with high ROR1 expression levels had high rates of relapse and short median survival. [101]. Recently, an expression profile analysis of 145 serous OvCas identified a stem-like subtype characterized by the 51-gene signature. This signature was significantly enriched in type-II tumors and it was prognostic within high-stage serous OvCa, identifying a small subset of high stage tumors with better prognosis. This signature remained a significant independent predictor of relapse even after adjusting for common clinical factors [102].

9. EPIGENETIC BIOMARKERS

Aberrant DNA methylation occurs frequently in human tumors and is considered to be one of the earliest molecular changes in carcinogenesis [103–105]. Thus, epigenetic candidate gene studies promise to reveal new biomarkers specially for early cancer diagnosis. Indeed,

already numerous studies of differential methylation have identified potential biomarkers not only for diagnosis, but also for progression and response to therapy [106]. In OvCa, two opposite epigenetic phenomena take place; namely, a global decrease in DNA methylation of heterochromatin leading to demethylation of several oncogenes and a specific CpG island hypermethylation associated with the promoters of tumor suppressor genes (TSG) [107]. The epigenetic silencing of TSGs in OvCa is best described for *BRCA1*. Studies have demonstrated that hypermethylation of the *BRCA1* promoter occurs in 10–15% of sporadic cases of OvCa leading to a loss of *BRCA 1*expression [108,109]. It is associated with the serous histotype [108,110] and aggressive malignancy [111]. Accordingly, patients with hypermethylation of the *BRCA1* promoter have a significantly shorter PFS and OS compared with patients harboring mutated or wild-type *BRCA1* [112]. Additionally, *BRCA1* methylation has been shown to be associated with sensitivity to platinum treatment in a pilot study of 35 OvCa cases [113]. Another protein responsible for DNA repair, MutL homolog 1 (MLH1) is encoded by the *MLH1* gene which is also hypermethylated in OvCa, leading to resistance to platinum based therapy in patients. This platinum resistance is reversed upon restoration of *MLH1* expression [114]. Gifford et al. compared the DNA methylation patterns of *MLH1* in plasma DNA from OvCa patients prior to and following treatment (at relapse) with platinum-based therapy [115]. They found that promoter *MLH1* methylation during treatment was significantly correlated with poor outcome. However, expression of *MLH1* prior to treatment was not able to predict outcome [116]. These results suggest that *MLH1* methylation may serve as a marker for enrichment of inherently resistant cells under therapy and could help in determining response to platinum treatment.

Montavon et al. found that the gene encoding homeobox protein A9 (*HOXA9)* is methylated in 75/79 (97.5%) of high-grade serous ovarian carcinoma and only in 1/12 (8%) of normal ovarian surface epithelium [117]. Furthermore, methylation of both *HOXA9* and *EN1* in combination with CA-125 levels was able to discriminate high-grade serous OvCa from normal ovarian epithelium with a sensitivity of 100% and a specificity of 91.7%, suggesting that *HOXA9* methylation could serve as a potential biomarker for the detection of OvCa. Tam et al. investigated methylation of *HIC1* in a large number of OvCas, including borderline and benign ovarian tumors, as well as normal ovarian tissues, and found that *HIC1* was methylated in >50% of OvCa cancer tissues, >40% of borderline tumors, >20% of benign tumors and 10% of normal ovarian tissues [118], suggesting that *HIC1* methylation correlates with the presence of invasive OvCa. Another TSG in OvCa, secreted protein/acidic/cysteine-rich (SPARC), is involved in cell adhesion, motility and ECM interactions, and is downregulated in OvCa through hypermethylation of its promoter. Socha et al. recently demonstrated that the *SPARC* promoter is hypermethylated full in 68.2% OvCa and partially in 22.7% OvCa, respectively. In addition, *SPARC* expression was inversely correlated with tumor grade [119]. It has been shown that genes involved in the TGF-β and Wnt pathways are often dysregulated through methylation in numerous cancers including melanoma, gastric and prostate carcinomas. Kang et al. observed *TGFBI* methylation in 23/38 (60.5%) of OvCa cases, 5/18 (27.8%) borderline ovarian tumors and 0/38 normal ovarian tissues [120]. However in this cohort, *TGFBI* methylation was not associated with any clinical outcome. Matsumura *et al.* identified depressed TGF-beta pathway activity by methylation of several genes involved in 39 ovarian cancer cell lines and OvCa tissues [121].

In a recent study Chou et al showed that *FBXO32*, the gene encoding F-box-only protein 32, is a TGF-beta/SMAD4 target gene and a regulator of apoptosis. This gene was downregulated through methylation in 96 OvCa cases but remained unmethylated and at normal levels in healthy ovarian surface epithelium [122]. Methylation was significantly associated with tumor stage and poor PFS. Re-expression of *FBXO32* restored sensitivity to cisplatin *in vitro*, suggesting that methylation of *FBXO32* may serve as a prognostic marker for OvCa.

In contrast to the downregulated TGF-β pathway, the Wnt pathway is often activated in cancers – but again through the hypermethylation of specific regulators. For example, the gene encoding secreted frizzled-related protein, *SFRP5,* a Wnt antagonist, was found to be hypermethylated in 44.4% of examined OvCa and only in 1.3% of benign cases [123]. Also, expression of *SFRP5* sensitized OvCa cells to cisplatin and taxol *in vitro*, and patients with methylated *SFRP5* had worse clinical responses [124]. Dai et al. demonstrated that methylation at 7 loci (*FZD4, DVL1, NFATC3, ROCK1, LRP5, AXIN1*, and *NKD1*) in a panel of 137 Wnt pathway genes in a screening cohort of 111 and a validation cohort of 48 serous and endometrioid OvCa was associated with poor progression-free survival. Hypermethylation of *DVL1* and *NFATC3* significantly correlated with poor response, with patients with progressive or stable disease harboring increased methylation versus patients with partial or complete response [125]. These studies demonstrate that epigenetic regulation of the Wnt pathway may serve as a biomarker for prognosis and/or treatment response in OvCa.

A novel biomarker candidate could be opioid-binding protein/cell adhesion molecule (OPCML), an immunoglobulin domain-containing GPI-anchored cell adhesion molecule encoded by the gene OPCML which behaves like a tumor suppressor. It is methylated and inactivated in a high proportion (33–83%) of ovarian tumors [126,127]. These studies also indicate that methylation of *OPCML* may be an early event, as higher methylation frequencies were present in borderline and early stage tumors compared with late-stage tumors, showing its potential to serve as a specific biomarker of surgically curable ovarian tumors.

Taken together, since aberrant methylation is one of the earliest molecular alterations during tumorigenesis it represents a promising strategy for the early detection of OvCa. However, the studies so far suggest that methylation in a panel of biomarkers, rather than individual gene methylation, will be necessary to achieve a suitable diagnostic assay with high specificity [128]. Indeed, studies that have examined combinations of gene methylation as OvCa biomarkers have achieved the greatest degree of sensitivity and specificity [125,129].

10. NUCLEIC ACIDS AS BIOMARKERS

Since most ovarian-cancer patients are diagnosed with advanced-stage disease, identification of an early tumor is essential to improve prognosis and therapy. Micro-RNAs as well as cell-free DNA promise to be good candidates for early diagnostic biomarkers because they are abundant in blood and can be easily detected. Since the first discovery of microRNA (miR) dysregulation in cancer in 2005 (i.e., deletion of miR-15a and miR-16-1 in B-CLL),

extensive research has demonstrated that miRs are involved in the initiation and progression of human cancers, including OvCa [130]. MicroRNAs are small (19–25 nucleotides) non-coding RNAs that suppress the translation of target mRNAs by binding to their 3' untranslated regions. Thereby, they act as critical regulators of cellular processes such as proliferation, differentiation, apoptosis and development [131]. Numerous studies have shown that expression of individual miRs or specific miR signatures can be linked to the diagnosis and prognosis of many cancer types [132].

Interestingly, circulatory miRs are resistant to degradation and remain stable, despite high levels of RNAses in the blood. This startling stability has been explained by the association of miRs with protein complexes such as Argo2 or high-density lipoprotein (HDL) [133,134]. Around 10% of circulating miRs are packed in circulating extracellular microvesicles such as exosomes [135]. In this context, Gercel-Taylor showed that miR signatures of exosomes released from tumors in the bloodstream were distinct from signatures of patients with benign disease and could be strongly correlated with the tumor stage [87]. Twelve miRNAs were present at a higher proportion in malignant cells (e.g., mir-155, -29), while 31 were present at elevated levels exclusively in exosomes (e.g., miR-203, -205). A recent miRNA profiling performed by Vaksman et al identified 99 miRNAs with high expression levels in exosomes from OvCa effusion supernatants [136]. Some of these miRNAs showed significant associations with effusion sites (peritoneum versus pleura) and the FIGO stage. In univariate survival analysis, high levels of miRNAs 21, 23b and 29a were associated with poor PFS whereas high expression of miRNA 21 correlated with poor OS. In experimental mouse models, OvCa exosomes affect both tumor cells and cells in the tumor microenvironment and induce more aggressive disease. Table 3 lists the circulating miRNAs that could be potentially useful for early diagnosis of OvCa.

Additionally, some miR signatures can be used to differentiate the histological subtypes of OvCa [137–139]. The Cancer Genome Atlas (TCGA) study, identified two clinically relevant subtypes, one mesenchymal and one epithelial, based on the specific miR signatures [140]. The mesenchymal subtype was associated with poor prognosis and included the miRNA regulatory network consisting of miR-29c, miR-101, miR-128, miR-141, miR-200, and miR-506 [141]. Various miRs promise a potential utility also as predictive markers of prognosis and clinical response to chemotherapy (Table 4). Several miRs were identified as oncogenes upregulated in tumors and were associated with poor overall survival. In contrast, other miRs were shown to act as tumor suppressors, being downregulated in ovarian cancers. Table 4 summarizes potential prognostic miRNAs which were significant in multivariate analysis.

Recent data show that microRNAs could also be used to predict the clinical response to chemotherapy. For example Leskelä et al [142] demonstrated that the miR-200 family showed a significant association with response to paclitaxel–carboplatin. Patients who achieved complete response had tumors with significantly higher miR-200c expression levels than patients unresponsive to paclitaxel–carboplatin. In addition, higher expression of miR-200c was associated with lower relapse/progression rates. Lower expression of miR-31 significantly correlated with chemoresistance and poor prognosis in OvCa patients [143]. Vecchione *et al* analyzed miR signatures in 198 serous OvCa samples and identified that

expression of miR-217, miR-484 and miR-617 predicted chemoresistance of these tumors [144].

Similar to miRNAs, circulating cell-free DNA (cfDNA) could be used for the early detection and monitoring of OvCa. Circulating cfDNAs are short (70–200 bp) or long (up to 21 kb) fragments of double-stranded DNA, which are released mostly by apoptotic or necrotic cells. Genome-wide sequencing of plasma DNA showed that circulating tumor DNA represents the tumor genome and reflects the clonality of tumor cells [145]. The total cfDNA level in blood samples of a patient is higher than in healthy controls, particularly in advanced stages of disease. OvCa cfDNA can be detected already in early stages [146] and high pre-operative cfDNA plasma levels were significantly associated with decreased patient survival and constituted an independent predictor of death from OvCa [147–149]. Interestingly, OvCa patients have significantly different levels of circulating cell-free mitochondrial DNA, but not of circulating nuclear DNA [150]. The concentration of tumor-specific DNA in plasma increases, as one can suspect, with the tumor burden and decreases following chemotherapy. Kamat *et al* showed that in mice treated with a combination of cytotoxic chemotherapy and anti-angiogenic agents plasma DNA levels were significantly lower than in untreated mice [151]. Thus, tumor-specific cfDNA might serve as useful biomarker of therapeutic response. Indeed, recent papers showed that the exome-wide analysis of circulating tumor DNA could represent a non-invasive liquid biopsy, complementing or even in future replacing the current invasive biopsy approaches.

Overall, the above studies indicate that cell-free nucleic acids, such as miRNAs and cfDNAs, are likely to play an increasingly important role as non-invasive markers of OvCa detection/prognosis. However, the broad heterogeneity of results, based on differences in the samples analyzed and technologies used, suggests that additional studies are needed to define predictive and reliable nucleic acids signatures that can find a clinico-pathological application.

11. BIOMARKERS PREDICTING RESPONSES TO BIOTHERAPIES IN OVCA

An improved understanding of the molecular basis of OvCa has encouraged testing of newer therapies, including pharmacological or biological agents, such as poly (ADP-ribose) polymerase inhibitors (PARPi) or an anti-angiogenic antibody (Ab), bevacizumab, respectively [152,153]. More recently, clinical trials with immune checkpoint inhibitors, ipilimumab (anti-CTLA-4 Ab), nivolumab (anti-PD-1 Ab) or avelumab (anti-PD-L1 Ab) have been offered to patients with cancer, including patients with OvCa either as monotherapies or in combination with ParPi or with chemotherapy [154]. While biological therapies are promising, only a proportion of patients achieve long-term responses, and there exists an urgent need for the establishment of biomarkers that would predict effectiveness of these therapies.

PARP inhibition (PARPi) has offered new opportunities for treatment of BRCA1- and BRCA2-related breast and ovarian cancers. One of the PARP inhibitors, olaparib, was recently approved by the FDA to treat BRCA1 and BRCA2 germline-mutated OvCa patients, and other PARPi drugs are undergoing active phase I and II trials [155]. However,

BRCA1 and 2 germline mutations only account for approximately 15% of the OvCa patients. Recent studies showed that PARPi sensitivity is not restricted to familial BRCA-mutated cancers and that all tumors incapable of the error-free DSB repair, i.e., tumors with a defect in the HR repair pathway, can benefit from PARPi treatment [152]. Zhang et al. identified the copy number deletion of RAD50 as a candidate marker for survival and response to PARPi in BRCA-wildtype OvCa tumors [156]. Lee et al. developed a multiparameter flow cytometry assay to measure γH2AX and MRE11, indicators of double-strand breaks and DBS-repair, in PBMCs [157]. Other studies identified gene BRCA-like signatures [158] or LOH patterns [159] or mutations in eight ADAMTS genes [160] as potential markers. None of these potentially useful assays for surrogate biomarkers has been validated, and prospective studies will be required to confirm their role in predicting responses to PARPi-based therapy.

Bevacizumab (BV) is a humanized monoclonal Ab targeting vascular endothelial growth factor (VEGF). BV therapy has been initially reported to be effective for OvCa. However, more recent data show that the BV treatment does not prolong OS of many patients with either primary or recurrent OvCa. Given the high cost associated with the treatment, patients who receive BV treatment should be carefully selected. To this end, Backen et al, suggested that circulating Ang1 and Tie2 proteins (in combination) could serve as potential predictive biomarkers of improved PFS in BV-treated patients [161]. Other potential candidates for predictive biomarkers are VEGFR-1 and neuropilin-1 found in the plasma or cancer tissue [153] as well as plasma cell-free DNA [162].

Numerous clinical trials have reported an outstanding anti-tumor efficacy of immune checkpoint inhibitors in non-small lung cancer, melanoma or renal cell cancer [163]. However, in OvCa response rates to these inhibitors were relatively low, ranging from 6–20% [154]. Thus, predictive biomarkers of anti-tumor responses are urgently needed. Several studies have described that expression levels of PD-L1 on tumor cells serves as a correlate of response to anti-PD-1 Ab therapy [164]. However, the predictive validity of PD-L1 expression has not been confirmed in more recent clinical trials in patients with solid tumors. The numbers of TILS and the proportions of T cells positive for PD-L1 and/or PD-1 expression were reported to correlate to therapeutic responses. In OvCa, two clinical trials, one with nivolumab, the other with avelumab, showed no correlation between PD-L1 expression levels and anti-tumor response. The high frequency of somatic gene mutations was linked to therapeutic responses in various solid tumors [165]. In OvCa, mutations of BRCA1/2 genes were reported to be closely related to tumor-infiltrating lymphocyte (TIL) frequency and PD-L1 expression levels on tumor cells [166]. Thus, OvCa patients with BRCA-mutated tumors might be good candidates for PD-1/PD-L1 inhibitors.

12. EXPERT COMMENTARY

Recent technological advances have made it possible to in part define molecular, epigenetic or immunologic signatures of human OvCas. These signatures of tumor cells or the tumor microenvironment (TME) derived from genomic, proteomic or immune-based analyses have provided a complex map of factors with potential diagnostic or prognostic significance. However, few of these factors have been validated and so far, their correlations to disease, its

progression or response to therapy remain incomplete. Perhaps the most important conclusion to date is that no single biomarker or a single signature emerges as the universally informative adjunct to clinical evaluations. Rather, the definition of a profile of markers obtained for each OvCa at the time of diagnosis seem to offer the most promising approach. Subsequent linking of the biomarker profile to outcome will be necessary to establish its prognostic value. Qualitative or quantitative changes taking place in the composition of this profile during therapy and disease progression or regression monitored over time are expected to inform about responses to therapy. It remains to be determined which of the biomarkers discussed above should be included in this profile. The availability of non-invasive biomarkers, i.e., serum markers or "liquid biopsies," would make serial monitoring easy and less costly. In the era of personalized medicine, it is expected that individual profiles of biomarkers will be compiled for each patient and used in combination with clinicopathologic endpoints to predict prognosis, select appropriate treatment and monitor responses to therapy.

13. FIVE YEAR VIEW

The large number of potentially useful biomarkers for OvCa will be subject to intense evaluations in the near future. The development and validation of blood tests that can accurately detect OvCa at an early stage when the disease is asymptomatic are of utmost importance. Today, there are no screening tests that are sufficiently sensitive and reliable to meet the challenge. Given the large variety of serum and tissue candidate biomarkers, including "liquid biopsy" sampling, the next five years are likely to see optimization and validation of a new crop of screening assays for OvCa detection. One such candidate biomarker might be a combination of osteopontin and CA125. Assays that require minimal quantities of biological fluids and are noninvasive will be of special interest. Among a wide variety of molecular, genetic or immunological assays already available, those that best meet requirements for accurate early diagnosis of OvCa and its differentiation from benign diseases will be selected and validated. These biomarkers will make it possible to screen individuals at risk of OvCa, e.g., OvCa patients' first-degree relatives, close relatives diagnosed with OvCa at an early age or those with the history of early-age breast cancer. A rapid development of prognostic biomarkers for evaluating OvCa responses to therapy or outcome will result in an improved selection of therapies for OvCa patients. In this respect, biomarkers of sensitivity/resistance to chemotherapies as well as biomarkers for responses to immunotherapies will become available and will be validated in prospective clinical trials. The developmental efforts will focus on high throughput technologies performed in real time at a minimal cost. Because monitoring using a single serum or tissue biomarker is not likely to be applicable to different clinical situations, panels of biomarkers will be used for following responses to therapies or predicting outcome.

At the time when immunotherapy is increasingly frequently used alone or in combination with conventional therapies to treat OvCa, immunologic biomarkers of response are likely to gain in importance. The next five years will see rapid growth in the development and prospective validation of new biomarkers that include OvCa-reactive immune cells in situ or in the peripheral circulation. In conjunction with other molecular and genetic markers,

immunologic profiling will provide much needed metrics of the role the host immune system plays in OvCa.

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14. KEY ISSUES

- There exists an unmet need for OvCa biomarkers that could serve as surrogates of response to therapy, prognosis or outcome.
- A search for novel, reliable biomarkers for early OvCa detection, prognosis, treatment efficacy or outcome is an immediate and urgent current goal.
- Currently, emerging serum or tissue biomarkers that measure anti-tumor immunological responses of the host offer special promise and deserve attention.
- Tumor-derived exosomes potentially serving as a “liquid tumor biopsy” are being considered as future OvCa biomarkers.
- Profiling of OvCa stem cells and quantitation of circulating tumor cells (CTC) are potentially promising future biomarkers.
- Numerous epigenetic biomarkers are available and await validation.
- Nucleic acid, including circulating DNAs and miRNAs, are in the limelight, with considerable progress made in establishing their clinical significance.
- None of these potential OvCa biomarkers have been validated in prospective clinical trials so far.
- The next five years will determine which of these biomarkers will be included in a panel of potentially non-invasive biomarkers for evaluating patients with OvCa.

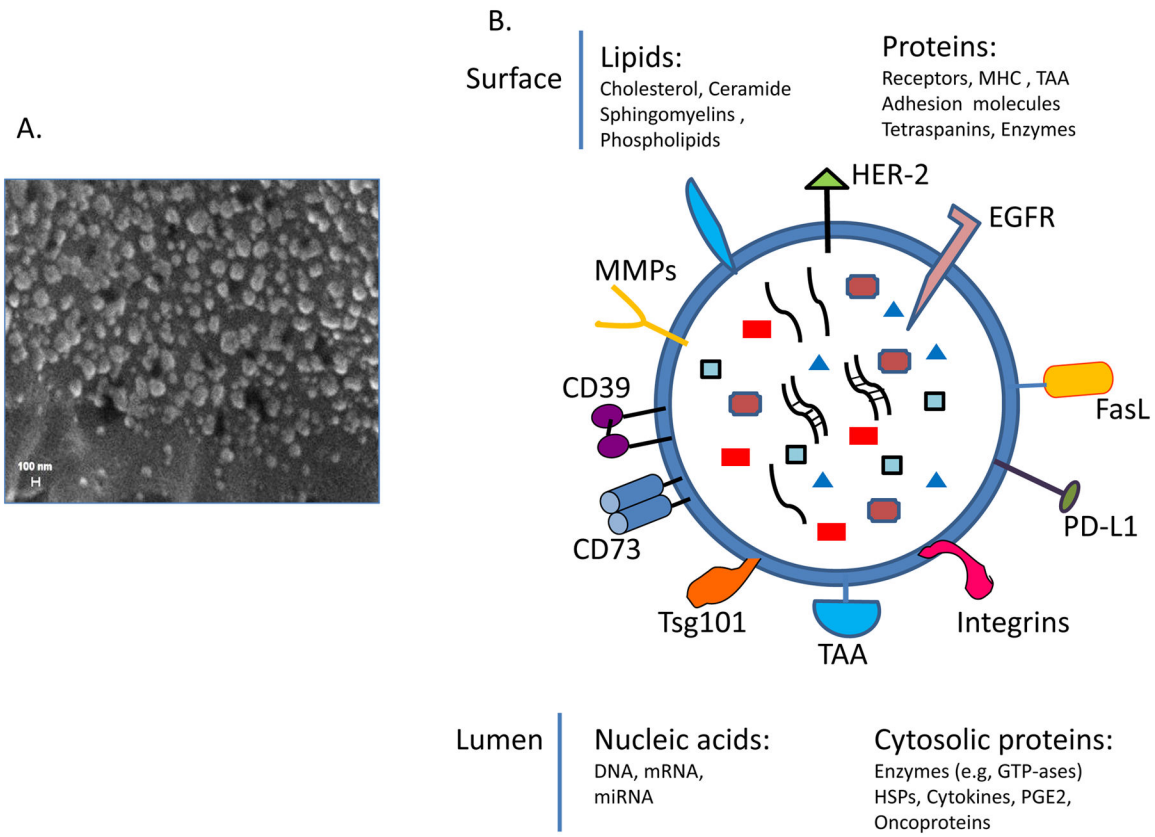

Figure 1.

Exosomes in plasma of OvCa patients. **A**. A scanning electron micrograph image of gold-coated exosomes isolated from plasma of a patient with OvCa.. Reproduced with permission from Szajnik M, Derbis M, Lach M et al. Exosomes in Plasma of Patients with Ovarian Carcinoma: Potential Biomarkers of Tumor Progression and Response to Therapy. Gynecol Obstet (Sunnyvale) 2013 Suppl 4:3. doi:10.4172/2161-0932.S4-003 **B.** A diagram of a tumor-derived exosome illustrating the variety of molecules in the exosome cargo. Reproduced from Whiteside TL. Tumor-derived exosomes and their role in tumor progression. Adv Clin Chem. 2016;74:103-41. doi: 10.1016/bs.acc.2015.12.005. Epub 2016 Apr 7. with permission from Elsevier.

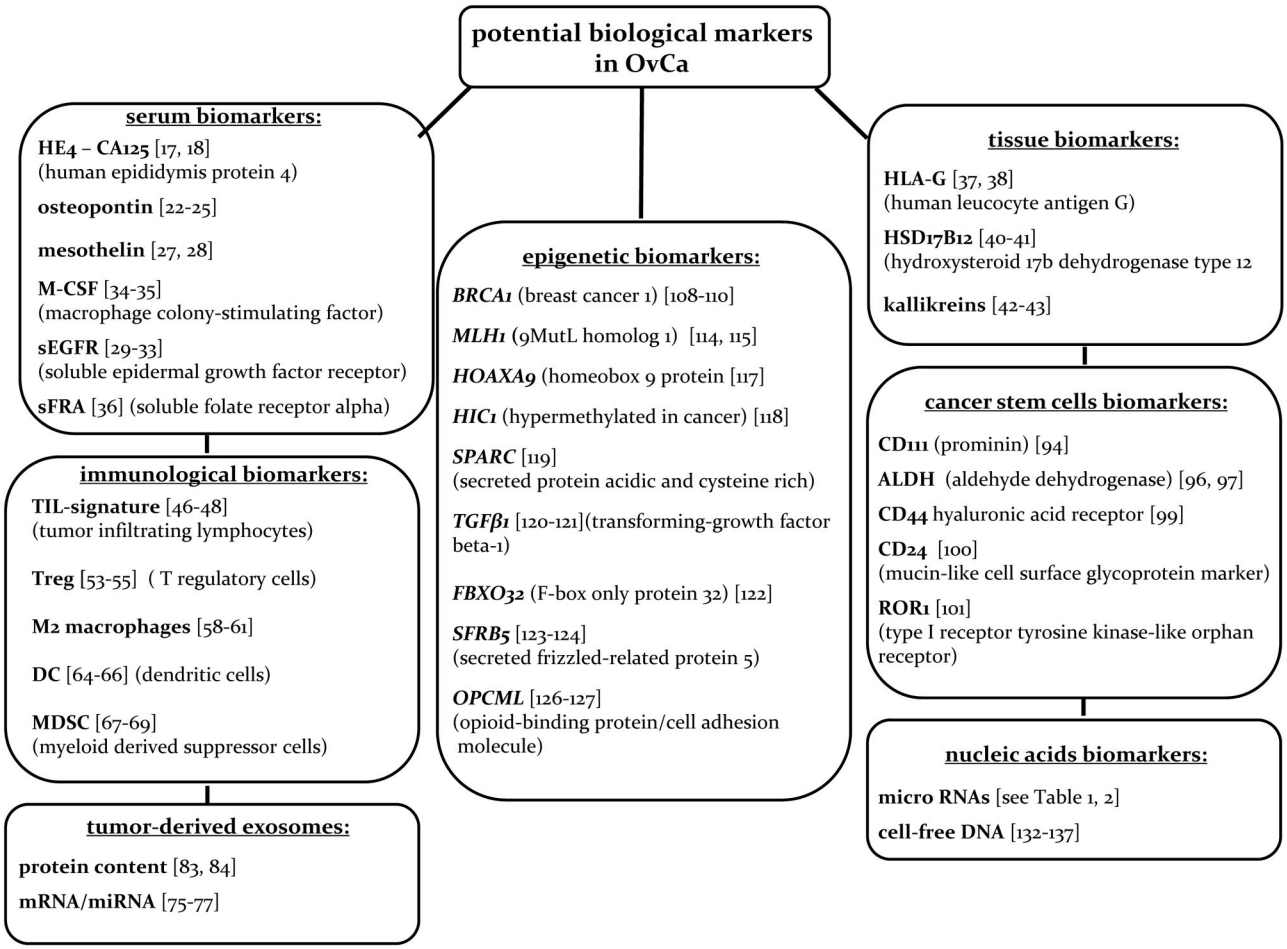

Figure 2.

A diagram listing various categories of potential biological markers that are various categories of being developed for OvCa diagnosis, prognosis and response to therapies.

Table 1

Clinically-used serum biomarkers for detection of ovarian carcinoma[a]

Biomarkers	Sensitivity	Specificity	References
CA125	92%	62%	[17]
RM1 (risk of malignancy index) CA125 + ultrasound[b]	96%	82%	[10,17]
HE4	91%	63%	[18]
ROMA (risk of ovarian malignancy algorithm) CA125 + HE4[b]	95%	77%	[17, 18]
OVA1	96%	40% (pre)[c]	[19]
5 different markers: (↑CA125; ↑β macroglobulin ↓apolipoprotein A1; ↓prealbumin ↓transferrin)	85%	28% (post)[c]	

[a]The sensitivity and specificity values are for differentiating benign diseases from OvCa.

[b]The menopausal status influences risk. The values shown are for all patients without consideration of the menopausal status.

[c]Pre vs. post refer to pre-menopause and post-menopause

Table 2

Emerging serum biomarkers in ovarian carcinoma[a]

Marker	Sensitivity	Specificity	References
Osteopontin	77%	90%	[22, 24]
Osteopontin & CA125	87%	88%	[25]
Mesothelin	97%	98%	[28]
Mesothelin & CA125	97%	98%	[28]
sEGFR[b]	64%	65%	[31]
sEGFR & CA125	68%	100%	[32]
M-CSF[b]	70%	94%	[35]
M-CSF & CA125	86%	86%	[35]
sFRA[b]	59%	98%	[36]

[a]The sensitivity and specificity values are for differentiating benign disease for OvCa

[b]

sEGFR: soluble epidermal growth factor receptor;

M-CSF: macrophage colony stimulating factor;

s-FRA: soluble folate receptor alpha

Table 3

Potential diagnostic micro RNAs for OvCa

Elevated miR	Source	Reference	Decreased miR	Source	Reference
miR-21,-29a, -92, -93, -126	serum	[135]	let-7b, miR-26a, -132, -145	serum	[167]
miR-16, -21, -191	plasma	[168]	mi-99b, -127, -155	serum	[135]
miR-205	plasma	[169]	let-7f	serum	[169]
miR-30c-1-p	whole blood	[170]	miR-181a-3p, -342-3p, -450-5p	whole blood	[170]
miR-21,-141, -200a	serum exosomes	[87]	miR-145	serum	[171]
miR200a/b/c	serum	[172]			
miR-92	serum	[173]			
miR-205, -483-5p	serum	[169]			
miR-221	serum	[174]			

Table 4

Potential prognostic micro RNAs for OvCa

Elevated miR	Prognosis	Reference	Decreased miR	Prognosis	Reference
miR-21	poor	[175]	miR-31	good	[143]
miR-25	poor	[176]	miR-100	good	[177]
miR-29b	poor	[178]	miR-150	good	[179]
miR-203	poor	[180]	miR-187	good	[181]
miR-221	poor	[174]	miR-200a	good	[182]
miR-484	good	[143]	miR-200c	good	[183]
miR-520d-3p	good	[184]	miR-221/222 ratio	good	[185]
			miR-335	good	[186]
			miR-410, miR-645	good	[187]