Biomarkers for screening, diagnosis and monitoring of ovarian cancer

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Abstract

Serum tumor markers have a major role in the screening, diagnosis and monitoring of most gynecologic cancers. Ovarian cancer is one of the deadliest of the group because it is so frequently asymptomatic until it has advanced to an untreatable stage. Even CA 125, clinically one of the most reliable serum markers for ovarian cancer, is elevated in only half of early-stage still-treatable tumors. Because of the very low prevalence of ovarian cancer in the general population, at present there is no cost-effective imaging or simple microscopic screening test for ovarian cancer, as there is for breast and cervical cancers. However, recent proteomics and nucleic acid -based analyses have shown great promise for the discovery of new and more useful serum biomarkers, that cumulatively might provide such a screening tool. In this review we will
discuss both the currently utilized serum tumor markers for screening, diagnosis, monitoring of ovarian cancer and the novel biomarkers that are now under investigation and validation.

Introduction

Endometrial, cervical and ovarian cancers are three of the most common malignancies of the female reproductive tract. Of the three, ovarian cancer, although rare in occurrence, is the deadliest; in 2008 alone, 224,747 women were diagnosed with ovarian cancer worldwide, and a heartbreaking 62% of these women died from the disease (1). This is primarily because roughly three-quarters of ovarian cancer cases present at an advanced stage, with the disease spread well beyond the ovaries (2). The cancer is insidious, patients usually have their first symptoms only in the advanced-stage of the disease, and these are often related to the presence of a grossly enlarging tumor and extensive ascites fluid; in the early- and mid-stage disease, most patients are largely asymptomatic (3). Serum cancer antigen-125 (CA 125) levels and transvaginal ultrasonography (TV-USG) screening have contributed to an earlier detection of ovarian cancer; however, the value of tumor markers and ultrasonography to screen for epithelial ovarian cancer has yet to be clearly established by prospective studies (3, 4).

For any hope of curing ovarian, endometrial and cervical cancers, it is critical to detect these diseases at the earliest possible stage. These tumors are phenotypically and genetically heterogeneous, so no single tumor marker will detect all variations; therefore, the discovery of additional useful serum biomarkers for the early detection of gynecologic cancers has thus been highly sought after. Such tumor markers will be molecules arising from the presence of a tumor which can appear in the surrounding tissues, blood and excretions because they are secreted or shed by the tumor in excess of the normal tissue or cell phenotype. Sometimes the marker will be uniquely specific to a tumor subtype, for example, as embryonic, fetal, undifferentiated, or stem-cell phenotypes. Tumor markers
can occur as re-expression of genes silenced during differentiation or as anomalous alternative mRNA splicing products of a currently expressed gene. Glycoproteins produced by cancer cells can have detectably altered glycan structures, although the core proteins themselves are ubiquitous (5). Tumor markers might be unique extracellular matrix or cell adhesion molecules, or they can be receptors, growth factors, cytokines, or products of abnormal metabolism. Rarely, the marker molecules can be released by other tissues and organs in response to signals from the tumor. Even the body’s auto-antibodies against tumor antigens can be markers.

Tumor markers can be associated with patient diagnosis, prognosis, clinical management and follow-up. In an ideal world, tumor markers would be highly tumor-specific, would always be produced in sufficient amounts to allow fast, easy, cheap and non-invasive detection of minimal disease and would quantitatively reflect tumor burden. These idealistic tumor markers would enable their use in screening, diagnosis, monitoring response to therapy, and detecting earlier recurrence during follow up.

Recent advances in clinical proteomics and serum microRNA analysis have propelled us into an exciting period of discovery of new cancer biomarkers, although the available technologies still have their limitations. The principles of serum marker technology require stringent guidelines for the collection of clinical material, the application of analytical techniques and for our interpretation of the data.

In this review, we will present an overview of the currently used serum tumor markers for the screening of ovarian cancer. Also, we will discuss novel biomarkers that have given us great hope for the future of better detection and management of ovarian cancers.

**Serum Markers for Ovarian Cancer**

Roughly three quarters of all cases of ovarian cancers are diagnosed only after the disease has
progressed to stage III or IV, and have involved the peritoneal cavity or other organs. The ultra poor prognosis for this cancer results directly from the lack of reliable, sensitive screening tests and our limited understanding of the mechanisms of its chemoresistance and relapse. Thus, establishment of an appropriate earlier stage screening test for ovarian cancer has long been sought.

The symptoms that are commonly associated with early to mid-stage ovarian cancer are typically nonspecific and the association is often not clinically recognized until the disease is irretrievably advanced (6). Previous studies have shown that ultrasonography (USG) can provide some degree of high sensitivity; however, its specificity and positive predictive values were found to be unsatisfactory (7, 8).

Given the low prevalence of ovarian cancer in the general population, an effective and acceptable screening strategy must have not only a high sensitivity for early-stage disease (>75%), but must also have a very high specificity (99.6%), so as to prompt no more than ten exploratory operations for each actual case of ovarian cancer diagnosed; i.e., it must have a positive predictive value [PPV] of 10%, even in postmenopausal women over 50 years of age, who are at a significantly higher risk than younger women (9). Even though, at present, there is no effective screening test for ovarian cancer (like there is breast and cervical cancer), the serum markers and novel biomarkers of ovarian cancer that are being currently used, and those that are under investigation, are discussed below.

Usefulness of CA 125 for screening and surveillance of ovarian cancer

Early detection of ovarian cancer

To date, CA 125 is the serum marker that has received the most use, and is the most trusted, as an identifying method for ovarian cancer early detection (Table 1). CA 125 was originally developed to monitor patients previously diagnosed with an ovarian cancer, but not for its screening. When used
as an individual marker on a single occasion, CA 125 is not sufficiently sensitive to detect most cases of early-stage ovarian cancer. Serum CA 125 levels do become more frequently elevated in patients as the disease progresses; elevations are detected in 50% and 92% of ovarian cancers in early and late stages, respectively (10). Nossov et al. (11) found the positive predictive value of CA 125 assay for early detection of ovarian cancer was 57%. Unfortunately for identifying the source of this tumor marker, elevated CA 125 occurs in other cancers as well, such as endometrial, breast, pancreatic, gastrointestinal, and lung cancers. Elevated CA 125 levels can also be found in patients with benign gynecologic conditions, such as during menstruation, pregnancy, endometriosis and pelvic inflammatory disease, and even in non-gynecologic conditions, such as hepatitis and pancreatitis (12). The physician, therefore, has to always consider the possibility that this tumor marker is creating a false positive case due to another pathological condition. A one-time determination of CA 125 is thus not sufficiently sensitive nor specific enough to be used as a biomarker for screening the general population.

To augment its usefulness for screening, CA 125 has been combined with transvaginal ultrasonography. Various combinations of CA 125 and imaging screening, both concurrent testing as well as sequentially, are being tested. There are currently 4 major ovarian cancer screening trials, 2 of which are still ongoing and 2 that have been completed (Table 2). The PLCO trial in the United States was a randomized control trial of 78,216 women, aged 55 to 74 years, assigned either to annual screening (N=39,105) or usual-care (N=39,111) (13). The ‘intervention group’ received annual screening with CA 125 for 6 years and transvaginal ultrasound for 4 years, at 10 medical centers throughout the country. The control ‘usual-care’ group was not offered this advanced screening for 6 years, but did receive their usual medical care. 22% of patients with screening-detected cancers had stage I or II disease, versus 22% in the control group, and there was no evidence of a shift to early-stage disease associated with screening. There was equivalent ovarian cancer mortality in both groups.
The second completed study, a multicenter screening trial in Japan, was a prospective randomized trial conducted between 1985 and 1999, in which asymptomatic postmenopausal women were assigned either to a screening group (N=41,688) or a control group (N=40,799) (14). Women in the screening arm received an annual pelvic examination, a serum CA 125 test, and an ultrasound examination. Ovarian cancers were detected by screening in 27 women, of which 67% had a stage I or stage II disease. Thirty-two women in the control group developed ovarian cancer, 44% of whom had stage I or II disease. Analysis of site-specific ovarian cancer mortality in the screening and control groups has not yet been reported.

The largest ongoing screening trial is the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) (15). From 2001 to 2005, 202,638 postmenopausal women, aged 50 to 74 years, were randomly assigned to annual transvaginal ultrasound screening (N=50,639), multimodal screening with sequential serum CA 125 testing, and ultrasound (N=50,640), or no treatment (N=101,359). Fifty-eight invasive ovarian cancers were detected by screening, 28 patients (48%) had stage I or II disease, versus 26% in the control population, and 22% in the prevalence screen of the PLCO trial. This trial is ongoing; therefore the effect of the screening program on ovarian cancer mortality awaits further analysis.

The University of Kentucky Ovarian Cancer Screening Trial has been in progress from 1987 to the present time, and 37,293 women have been screened (16). To date, 47 epithelial ovarian cancers have been detected, with 70% of patients having stage I or II disease. 12 women developed detectable ovarian cancers within 12 months of a negative screen. The stage at detection and the site-specific ovarian cancer mortality in women with screen-detected cancers have been compared with women from the same geographic area whose cancers were detected clinically during the same time period. Screening produced a stage shift, in that 70% of women with screening-detected ovarian cancers had stage I or II disease, versus 27% in the unscreened control group (p <0.01). The 5-year survival of all women whose epithelial ovarian cancers were detected by this screening study, including the interval cancers, was 74.8 ±6.6%, as compared with 53.7 ±2.3% for women with routine clinically detected
ovarian cancers treated at the same institution, with the same surgical and chemotherapy protocols ($p < 0.01$).

Although, in several of the trials described above, screening seems to have allowed for detection of the tumor at an average of an earlier stage, the effects of screening on ovarian cancer mortality has varied significantly, and disappointingly, in the different trials, and that itself is the subject of further investigations. In addition, these tests (combined transvaginal ultrasonography and CA 125) are not cost-effective as currently conducted, and are thus still not used routinely to screen for ovarian cancer.

**Differentiation from other malignancies**

The differentiation of a primary ovarian cancer from a tumor metastatic to the ovary is still tremendously challenging. In a previous study, Yedema et al. (17) described the preoperative discrimination of ovarian cancer from colorectal cancer. They reported that the specificity increased significantly when using a combination of a CA 125 positive score (>35 U/ml) and a simultaneous negative tumor marker CEA score ($\leq 5$ ng/ml) (specificity 100%, sensitivity 81%). A CA 125/CEA serum ratio >25 resulted in the highest discriminative power, with a specificity of 100% and a sensitivity of 91%, resulting in an overall test accuracy of 94%. They concluded that a combination of CA 125 and CEA are helpful in the preoperative differential diagnosis between a primary ovarian cancer and a colorectal origin.

Sørensen et al. (18) also reported the ability of CEA in combination with CA 125 to differentiate between malignant ovarian and malignant non-ovarian disease. They reported that, among the patients with CEA levels >5 ng/ml, 68% had non-ovarian malignancies. In patients with a CA 125/CEA ratio >25, an ovarian cancer was found in 82%. The specificity increased to around 85% when the cut-off value of the CA 125/CEA ratio was increased from 25 to 100 (18). From these results, a combination of CA 125 and CEA may be helpful in the preoperative differential diagnosis between
ovarian cancer and another originated cancer.

**Prediction of prognosis and surveillance of recurrence**

The predictive value of pre-treatment CA 125 levels for prognosis is controversial. While some studies did not find preoperative CA 125 levels to be an independent prognostic factor (19-21), others reported that it could identify poor prognostic subgroups, independent of stage (22, 23). However, changes in CA 125 levels can also correlate with regression, stability and progression of the disease in 87-94% of instances (12).

Elevation levels in CA 125 can be used to document progressive disease in patients who achieve a normal CA 125 after primary treatment. Rustin et al. (24) found that a doubling of CA 125 level from the upper limit of normal had a sensitivity of 86% and a specificity of 91% for detecting progression. A second confirmatory value reduces the false-negative rate to less than 2%. Similarly, a doubling of CA 125 from baseline in patients with persistently elevated CA 125 following primary treatment accurately predicts progression (25). Rises in CA 125 tend to precede symptomatic relapse by a median of 4.5 months (range 0.5-29.5 months) and there is considerable debate about whether additional treatment should be commenced on the basis of rising CA 125 alone. In the recent MRC/EORTC trial, Rustin et al. demonstrated no difference in overall survival (HR 1.00) between patients who received chemotherapy based on a rising CA 125 and those who did not receive chemotherapy until they were symptomatic (26). Thus, whether or not early reintroduction of treatment produces a survival advantage remains unclear.

Although a high probability exists that some tumor response can be achieved with chemotherapy, a complete cure of these patients is rarely possible. Potential advantages of early treatment of relapse include delaying cancer-related symptoms, providing psychological reassurance and possibly improved survival. Potential disadvantages include loss of time without treatment and associated toxic effects. Patients should be counseled on these advantages and disadvantages before deciding whether to have their CA 125 concentrations routinely measured during follow-up.
Other tumor markers

Serum levels of CA 19-9 (a monosialoganglioside antigen widely used in GI adenocarcinoma diagnostics) are elevated in 68-83% of mucinous ovarian cancers but in only 28-29% of non-mucinous types; whereas CA 125 is elevated in 80% of non-mucinous ovarian tumors (27-30), providing a differential diagnostic tool for non-mucinous versus mucinous subtypes. Other markers, alone or in combination, have also been used; serum CA 15-3, CA 72-4 and CEA levels are elevated, respectively, in 50-56%, 63-71% and 25-50% of ovarian cancer patients (27, 31-38) (Table 3). According to Gadducci et al., the levels of the markers CA 19-9, CA 15-3 and CA 72-4 were poorly correlated with the clinical course of the disease, when compared with CA 125, and thus these markers did not offer additional clinical benefit for monitoring ovarian cancer. However, the serial measurement of these markers may still play an important role in the management of the relatively large group of patients with a CA 125 negative tumor (12). This would be similar to monitoring Her-2-negative/estrogen receptor-negative breast tumors with other breast tumor markers.

There are additional serum markers for ovarian cancer that are under active investigation (Table 3). For example, HE 4 has recently been accepted by the United States Food and Drug Administration (FDA) as a monitoring method for patient management with EOC. In a review by Li et al., they found that HE4 displayed the highest sensitivity (72.9%) among all single markers, including CA 125, in the detection of ovarian cancer, in both the early (62-83%) and late (75-93%) stages (39). In addition, serum levels of HE4 are elevated in at least a third of the EOC patients who do not have tumors that overexpress CA 125, suggesting a complementary application of the two tests would be useful (40, 41).

Elevated serum lysophosphatidic acid (LPA) levels, another potentially useful marker, were found in 90% and 98% of ovarian cancer in early and late stages, respectively; however, serum levels of LPA do not correlate well with the stage of the disease, and nonspecific elevation of LPA was detected in healthy and benign gynecologic conditions (11, 42, 43).
Significantly elevated sFas levels are detected in some ovarian cancer patients compared to healthy women, and serum sFas level was demonstrated to be a statistically significant indication factor for survival, as well as histological grade, in ovarian carcinomas (44). Another antigen marker, mesothelin (41), is a protein of unknown biological function which is present in normal mesothelial and has been detected at elevated levels in the serum of patients with mesothelioma, ovarian cancer and some squamous cell carcinomas. Through transcriptional profiling, MES was found to be elevated in the serum of 76% of ovarian cancer patients and was also found to be informatively complementary to CA 125 in early detection of ovarian cancer (45).

Haptoglobin-\(\alpha\) (HP-\(\alpha\)) is a liver glycoprotein (with alpha-electrophoretic mobility on a gel) that binds to free hemoglobin released from red cells. Using Surface Enhanced Laser Desorption and Ionization (SELDI) and Mass Spectrometric (MS) protein profiling, HP-\(\alpha\) has been identified as being a potential tumor marker having a 64% sensitivity and a 90% specificity (46).

Bikunin is a glycosylated protease (glycoprotein) that inhibits tumor cell invasion and metastasis. Preoperative plasma bikunin levels have been reported to be a strong prognostic marker for ovarian cancer. A large study showed that low plasma level of bikunin were associated with late-stage disease, probable sub-optimal debulking with a large residual tumor (>2 cm) outcome, low response to chemotherapy and reduced survival time (47).

OVX1 is an epitope of a high molecular weight mucin-like glycoprotein which can be detected by radioimmunoassay. OVX was found to be elevated in 67% of patients with ovarian cancer who were CA 125 negative (48, 49).

Other novel biomarker panels have been also investigated for early detection of ovarian cancers. Zhang et al. identified a panel of markers that consisted of three proteins, including apolipoprotein A-I (apoA-I), a truncated form of transthyretin (TTR) and a cleavage fragment of H4 (inter-of H4 (intinhibitor heavy chain) to detect early-stage ovarian cancer with a sensitivity of 83% and a
specificity of 94% (50). Su et al. utilized a multiple logistic regression model (MLRM), with values for CA 125, ApoA-I, transferrin TF and TTR, for early detection of ovarian cancer (51). This model provided a sensitivity of 89% and a specificity of 97% for detection of early stage ovarian cancer. The sensitivity and the specificity in distinguishing normal and mucinous ovarian cancer samples were 95% and 92%, respectively. Nosov et al. applied this same MLRM model and marker panel to analyze serous and endometrioid histological types of ovarian carcinomas; they demonstrated a sensitivity of 94% and a specificity of 94% for serous ovarian carcinoma in its early stage, and a sensitivity of 98% and a specificity of 98% for endometrioid ovarian carcinoma in its early stage (52).

Visintin et al. proposed a panel of serum biomarkers that consisted of leptin, prolactin, osteopontin, insulin-like growth factor II (IGFII), macrophage inhibitory factor (MIF) and CA 125 to discriminate between ovarian cancer patients and healthy women. The panel had a sensitivity of 95% and a specificity of 99% (53). Not surprisingly, this panel provided a significant improvement over CA 125 alone. However, these studies had similar methodological limitations of excessive numbers of tumor cases versus small numbers of matched population controls.

Still, with all this said, novel proteomics-based investigations and bioinformatics analysis provide great promise for finding ever more accurate and useable biomarkers for these gynecological cancers.

**MicroRNAs**

MicroRNAs (miRNA or miRs) are a class of small (18-25 nt) non-protein-coding gene-regulatory RNA molecules that are emerging as immensely important diagnostic and potentially therapeutic tools. MiRNAs play important roles in a variety of human biological processes, including development, organogenesis, metabolism, and homeostasis. MiRNAs negatively regulate messenger RNA (mRNA) translation into protein of a large number of important target genes, either by translational repression or by degradation of the messenger RNA transcript after targeting, by sequence
complementarity, the 3′-UTR of the mRNA.

Similar to other cancers, the initiation and development of ovarian cancer is characterized by disruption of oncogenes and tumor suppressor genes by both genetic and epigenetic mechanisms (54). It is now well known that altered or deregulated miRNA expression can also be a determinant of disease development and/or progression in a host of pathologic conditions. Importantly, for the purposes of this review, miRNA are functionally involved in the pathogenesis of many tumors (including our subject, ovarian cancer), where miRNAs can have important roles as regulatory molecules, acting as oncogenes (oncomirs) or tumor suppressors. A variety of miRNA candidates are differently or aberrantly expressed in ovarian carcinomas, or by adjoining stromal tissues, and even by other tissue in the host body in response to the tumor.

Changes in tumor miRNA expression patterns occur through a variety of mechanisms, such as genetic alterations, epigenetic regulation, or altered expression of transcription factors which target the miRNA genes. For example, in cancer cells, transcriptional gene silencing has frequently been associated with epigenetic defects. miR-125b1 has been suggested to be an miRNA with tumor suppressor activity, and it has been shown to be deregulated in various human cancers. DNA methylation at its regulatory-region-associated CpG island can reduce miR-125b1 expression, and these effects have been observed in several gynecological cancers, including ovarian and cervical tumors (55).

RNases are abundant in the bloodstream. Therefore, to be stable, some secretory miRNAs are contained in apoptotic bodies, microvesicles, or bound to the RNA-binding proteins (56). However, the vast bulk of the miRNA in serum and saliva is found in tiny membrane vesicles known as exosomes (57), which are cell-derived extracellular vesicles of endosomal origin. In addition to miRNAs, exosomes can contain proteins and mRNAs, and thus exosomes have been shown to constitute a mode of intercellular communication, selectively transmitting several types of information between cells. These "bioactive shuttle vesicles" are known to transfer these various molecules, including the miRNAs, to recipient cells, and to promote cell-cell communication and immunoregulatory functions.
Cancer cells can secrete excessive amounts of exosomes compared to normal cells (60). A new aspect of cancer research is being revealed by the emergence of these "secretory miRNA". The molecular composition and functional role of tumor cell-derived exosomes in tumorigenesis, metastasis and response to therapy are slowly being decrypted (60). Inappropriate release of miRNAs via exosomes may cause significant alterations in biological pathways that affect disease development. Their active secretion has functional implications, albeit it is often still unknown whether they are tumor promoting or suppressing. Notably, the interplay via the exchange of exosomes between cancer cells and between cancer cells and the tumor stroma may promote the transfer or expression of oncogenes (e.g., β-catenin, CEA, HER2, Melan-A/Mart-1 and LMP-1) and onco-miRs (e.g. let7, miR1, miR15, miR16 and miR375) from one cell to another, leading to the reprogramming of the recipient cells (60).

Some miRs exert negative control over the expression of numerous oncoproteins in normal cells and consequently their deregulation is believed to be an important mechanism underlying cancer development and progression (61). MiRs have distinct patterns of expression associated with specific cancer types, and once secreted by the cancer cells, they have remarkable stability in blood and other body fluids (61).

Because of the amount of signal amplification possible with nucleic acid serum markers, the identification of "miR signatures" associating cancer cell phenotypes with disease outcome and specific risk factor exposures will open new avenues for early diagnosis of cancer, as well as for the development of novel strategies for cancer prevention and therapy (61). Since these miR signatures can appear in the body fluids in exosomes, they can serve as relatively stable circulating diagnostic biomarkers, and have been shown to do so for ovarian cancer (62). Isolation of an exosome fraction also improves the sensitivity of miRNA amplification from human biologic fluids and reduces the probability of false negative results involving low abundance miRNAs that may be missed by using unfractionated serum or saliva (57).
Moving from merely being biomarkers for ovarian cancer to being targets for therapy, the development of strategies that might block the expression or mimic the functions of miRNAs could represent new therapeutic strategies for any of the aforementioned gynecological disorders. Exosome vesicles can also be used as gene therapy vehicles for delivery of miRNAs and small interfering RNA (siRNA) with therapeutic effects. The ability to do so has already been shown in mice (59). It thus appears that exosomal RNA has the potential to play important roles in the diagnosis, prognosis, and treatment of such diseases in the future.

Using well characterized examples from other tumors, clinicians can begin to understand some of the functions of tumor miRs. Some miRNAs, such as let-7 in lung cancer and mirs-15/16 in leukemia, normally act as tumor suppressor genes, in these cases suppressing the expression of the oncogenes Ras and BCL2, respectively (63, 64). When they are under-expressed, tumor growth is permitted. Tumor-over-expressed miRNAs, such as mir-21 and the cluster mir-17–92, can act as oncogenes (oncomirs), targeting tumor suppressors PTEN and E2F1 in solid and hematologic malignancies, respectively (65, 66).

MiRNA research in the gynecologic malignancies is now progressing quite rapidly, since the miRNA signature profiles of ovarian cancer was first published (67-69). The use of miRNA signatures of tumor-derived serum exosomes as a diagnostic biomarker for ovarian cancer was first convincingly demonstrated by Taylor and Gercel-Taylor (70) (Table 4). The authors showed that the level of tumor-derived miRNA-containing exosomes in serum is strongly increased in women with invasive ovarian cancer, compared to women with benign ovarian tumors or healthy controls. In addition, the levels of circulating, tumor-derived exosomes increased in parallel to the stage of disease. Further, they demonstrated, by miRNA microarray profiling, that the 218 miRNAs that were identified in tumor samples were also identified in circulating exosomes and that some miRNAs are even more overexpressed in the circulating exosomes than in the original tumor samples.

Differences in serum miRNAs between healthy controls and ovarian cancer patients were also reported by Resnick et al. (71) (Table 5). They were seeking an alternative or complementary diagnostic approach to trans-vaginal ultrasound and serum CA-125 levels for women at high risk for
ovarian cancer, knowing that this would be of great importance because CA 125 remains such a poor marker for early stage disease, with a documented sensitivity of only 40%. Thus, it was hoped that miRNAs might serve as early detection biomarkers in patients with normal CA 125 levels. They identified 21 miRNAs that were differentially expressed between normal and ovarian cancer patient sera. Analyzing these miRNAs in more detail, five miRNAs were found to be overexpressed and three miRNAs were decreased in the serum of ovarian cancer patients, compared to controls, establishing a possible set of miRNAs as biomarkers for ovarian cancer.

The Cancer Genome Atlas (TCGA) Network has recently catalogued the most extensive set to date of molecular aberrations in ovarian cancers. Patterns of miRNA expression in 487 high-grade serous tumors revealed multiple tumor subtypes and a set of 34 miRNAs predictive of overall patient survival (72). The miR-29 family and predicted target genes were among the most strongly anti-correlated miR:mRNA pairs, meaning the mRNA targets were suppressed when the miRs were active. In the standard test for miR functionality, over-expression of miR-29a in vitro repressed several anti-correlated genes (including DNMT3A and DNMT3B) and substantially decreased ovarian cancer cell viability. Mining the TCGA microarray database has also shown that the expression level of RAD51AP1 was found to be strongly anti-correlated with the expression of hsa-miR-140-3p, which was significantly down-regulated in the tumor samples (73). Other pairs of potentially biological relevance included: hsa-miR-145/E2F3, hsa-miR-139-5p/TOP2A, and hsa-miR-133a/GCLC (73).

The interplay between various families of miRs is quite complex, resulting in researchers finding “signatures” of expression where no single component is essential, but overall patterns are consistent. For example, Bentink et al. (74) identified a previously undescribed patient stratification based on an "angiogenesis signature" of miRNA-expression profiles. These pathways are probably determined early on in tumorigenesis. Recent recognition of HG-SOC precursor lesions, defined as serous tubal intraepithelial carcinoma (STIC) in fimbria, provides a new venue for the study of early genetic changes in HG-SOC. Using miRNA profiling analysis, Liu et al. (75) found that miR-182 expression was significantly higher in STIC than in matched normal Fallopian tube. Further study revealed that miR-182 was significantly overexpressed in most HG-SOC cases.
overexpression resulted in increased tumour transformation in vitro, and enhanced tumour invasiveness in vitro and metastasis in vivo. Mechanistically, they demonstrated that the oncogenic properties of miR-182 in ovarian cancer were mediated in part by its impaired repair of DNA double-strand breaks and negative regulation of breast cancer 1 (BRCA1) and metastasis suppressor 1 (MTSS1) expression, as well as its positive regulation of the oncogene high-mobility group AT-hook 2 (HMGA2).

Chang et al. (76) have suggested that miR-148b may be one of the dysregulated miRs involved in the early stage of ovarian carcinogenesis. They found that miR-148b was overexpressed in 92.21% (71/77) of the ovarian cancer samples they examined, and the overexpression was not associated with any of the clinicopathological features of patients with ovarian cancer (meaning it correlated with the causation and not the symptoms of the disease).

The human kallikreins are a cluster of 15 kallikrein-related peptidases (KLKs). Evidence shows the involvement of KLKs in a wide range of pathological processes and their potential contribution to cancer. Recently, epigenetic changes (including methylation and miRNA regulation) were shown to control KLK expression. Target prediction showed that KLK mRNAs are potential targets of miRNAs that are dysregulated in tumors, including ovarian cancers, with downstream effect on tumor proliferation (77).

Malignant ovarian disease is characterized by high rates of mortality arising from high rates of recurrent chemoresistant disease due to the chemoresistant properties of cancer stem cells (CSCs). Microarray analysis demonstrated a 90% difference between gene expression events involved in early regulation of differentiation in murine EC (mEC) and embryonic stem (41) cells. Genelist comparisons identified a cancer stemness signature set of genes in primary versus recurrent data, a subset of which are known p53-p21 regulators. The regulation of p53-p21 in ovarian cancer involves, at least partially, a cancer stemness component (78) have presented a p53-p21 cancer stemness signature model for ovarian cancer. They propose that this tumor signature of miRNA expression may, at least partially, differentially regulate the p53-p21 mechanism in ovarian disease. Targeting CSCs within ovarian cancer via miR expression targeting, represents another potential therapeutic avenue.
In ovarian cancer, unique CD44+/CD117+ stem cells, also known as cancer-initiating cells (CIC), are highly proliferative, have a low degree of differentiation and are resistant to chemotherapeutics. Therefore, the CD44+/CD117+ subpopulation is thought to be an important target for novel therapeutic strategies. CD44+/CD117+ ovarian CICs were enriched from human primary ovarian tumor tissues and studied for miR expression and responses to miRs. When MiR-199a was cloned and transfected into ovarian CICs it significantly increased the chemosensitivity of the ovarian CICs to cisplatin, paclitaxel and Adriamycin, and reduced mRNA expression of the multi-drug resistance gene ABCG2, compared to miR-199a mutant transfected and untransfected cells (79). The expression of “stemness markers” was also significantly reduced. Furthermore, xenograft experiments confirmed that miR-199a suppressed the growth of xenograft tumors formed by ovarian CICs in vivo. Thus, expression of an endogenous mature miR-199a may prevent tumorigenesis in human ovarian cancer, via regulating expression of its target gene, CD44.

Mesothelin, the aforementioned differentiation antigen present in a series of malignancies such as ovarian, mesothelioma, lung and pancreatic cancer, has been studied as a marker for diagnosis and a target for immunotherapy. Wang et al. (80) have been evaluating the effects of direct targeting of mesothelin on the viability of cancer cells as the first step towards developing a novel therapeutic strategy. They have shown that the gene-specific silencing for mesothelin by distinct methods (siRNA and miRNA) decreased viability of ovarian cancer Skov3 and Ovcar-5 cell lines. Additionally, the invasiveness of these cancer cells in vivo was also significantly decreased upon such treatment. Mesothelin-silencing revealed a significant decrease in phospho-ERK1 and PI3K/AKT activity. The molecular mechanism of reduced invasiveness was connected to the reduced expression of β-Catenin, an important marker of EMT (epithelial-mesenchymal transition). Ero1, a protein involved in clearing unfolded proteins and a member of the ER-Stress (endoplasmic reticulum-stress) pathway, was also markedly reduced (80).

Tiam1 has been implicated in the aggressive invasive phenotype of ovarian cancer, as Tiam1 expression was remarkably increased in both primary and metastatic ovarian cancer tissues. Li et al.
(81) showed that miR-22, miR-183 and miR-31 expression had negative regulatory effects on Tiam1 expression and that down-regulation of Tiam1 in SKOV-3ip and HO-8910PM ovarian cancer cells lead to reduced cell migration and invasion, and to growth inhibition, without significantly affecting cell apoptosis, suggesting that the differential expression profiles of these miRs may contribute to the dysregulation of Tiam1 abundance, which contributes to the invasive, migratory and viability properties of ovarian cancer cells.

**MiRNA in Prognosis**

Recently, miR-100 was reported to be significantly down-regulated in human ovarian carcinoma, however, the clinical significance and functional roles of miR-100 expression in human epithelial ovarian cancer (EOC) were unclear. Peng et al. (82) now report that low miR-100 expression was found to be closely correlated with advanced FIGO stage, higher serum CA 125 expression levels and lymph node involvement. Also, low miR-100 expression is correlated with shorter overall survival of EOC patients, and multivariate analysis showed that the status of miR-100 expression was an independent predictor of overall survival in EOC. Additionally, they show that miR-100 could affect the growth of EOC cells by post-transcriptionally regulating polo-like kinase 1 (PLK1) expression. Together, these results suggest that low miR-100 expression may be an independent poor prognostic factor and miR-100 can function as a tumor suppressor by targeting PLK1 in human EOCs.

Bagnoli et al. (83) delineated a miRNA signature associated with early relapse in advanced-stage EOC patients. Thirty-two differentially expressed miRNAs in early vs. late relapsing patients were identified; 8 of these, belonging to a cluster located on chrXq27.3, were down-modulated in early relapsing patients. Forced expression of the chrXq27.3-cluster selected miRNAs in human EOC cellular models was associated to reduction of cell proliferation and increased sensitivity to cisplatin.

**Drug Resistance**

miR-93 is significantly up-regulated in cisplatin-resistant ovarian cancer cells and inversely correlates with PTEN expression in cis-platin-resistant and -sensitive human ovarian cancer tissues.
They used *in vitro* assays to show that over-expression and knock-down of miR-93 regulates apoptotic activity, and thereby cisplatin chemosensitivity, in ovarian cells. Furthermore, they found that miR-93 could directly target PTEN, and participated in the regulation of the AKT signaling pathway.

The miR-34 family has a strong role in regulating the genotoxic-response p53 pathway in ovarian cancer. Zhang et al. (85) have shown that the miR-449a, miR-449b and miR-192 family of miRNAs may play the same role. They have shown that the expressions of miR-449a/b, miR-34b and miR-34c were 19-fold to 21-fold elevated after p53 activation by genotoxic agent. Ectopic expression of miR-449b, as well as miR-34c, resulted in cell-cycle arrest in SKOV3.ipl cells. Thus, as tumor-suppressor miRNAs, miR-449a/b, miR-34b and miR-34c cooperate and play important roles in p53 pathway. Their inactivation may contribute to the carcinogenesis and progression of serous ovarian carcinomas.

**Conclusions and future directions**

For gynecologic cancers, only a small handful of tumor-associated antigens, such as SCC and CA 125, have been routinely used as tumor markers. Some markers are useful not only as a diagnostic tool but also as a predictive marker for the prognosis and clinical course after treatment. Some newer serum markers being recently investigated seem to be clinically useful, such as HE 4 for endometrial and ovarian cancers. The future of tumor marker research is being rapidly expanded due to recent technological advances in genomics and proteomics. While a large amount of information has been gained regarding the roles and possible therapeutic use of miRs in ovarian carcinoma, much remain to be done. In particular, more thorough miR expression profiling will be necessary to understand the intricacies of their expression in ovarian carcinoma of various grades, stages, or drug resistance status. The next step, the identification of relevant therapeutic miRNA targets, will likely be a tedious task, complicated by the fact that miRs can have multiple functional targets and that these targets may be dependent on several factors, including the expression of other miRs. Once relevant
miRs and their functional targets are identified, the investigation of possible clinical use for these molecules will represent the next frontier in cancer research, and may, ultimately, lead to novel strategies for ovarian cancer detection and therapy.
References


Table 1
Clinical significance of CA125 level for ovarian cancer

<table>
<thead>
<tr>
<th>Screening of ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential diagnosis between primary ovarian cancer and metastatic ovarian cancer*</td>
</tr>
<tr>
<td>Prediction of prognosis</td>
</tr>
<tr>
<td>Surveillance of recurrence</td>
</tr>
</tbody>
</table>

* in combination with CEA
Table 2
Results from major ovarian cancer screening trials

<table>
<thead>
<tr>
<th>Screening trial</th>
<th>Years</th>
<th>Study design</th>
<th>Screening test</th>
<th>Non screened</th>
<th>Cancers detected</th>
<th>Stage I, II</th>
<th>Stage III, IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLCO (USA)</td>
<td>1993-2001</td>
<td>Randomized Control</td>
<td>Ultrasound C125 vs usual care</td>
<td>34,253</td>
<td>212</td>
<td>22%</td>
<td>77%</td>
</tr>
<tr>
<td>UKCTOCS (UK)</td>
<td>2001-2005</td>
<td>Randomized Control</td>
<td>Ultrasound C125 or ultrasound vs usual care</td>
<td>101,279</td>
<td>58</td>
<td>48%</td>
<td>52%</td>
</tr>
<tr>
<td>SCSOCS (Japan)</td>
<td>1985-1999</td>
<td>Randomized Control</td>
<td>Ultrasound C125 vs usual care</td>
<td>41,688</td>
<td>27</td>
<td>67%</td>
<td>33%</td>
</tr>
<tr>
<td>University of Kentucky</td>
<td>1987-2011</td>
<td>Population Control</td>
<td>Ultrasound</td>
<td>37,293</td>
<td>47</td>
<td>70%</td>
<td>30%</td>
</tr>
</tbody>
</table>

*; not reported until present
| Survival benefit |  
|------------------|---
| (-)              |  
| analysis pending* |  
| (+)              |  
| analysis pending* |  

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cut off</th>
<th>Ref No.</th>
<th>SE (%)</th>
<th>SP (%)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA125</td>
<td>&gt;35U/ml</td>
<td>26</td>
<td>82.2</td>
<td>67.3</td>
<td>47.1</td>
<td>91.4</td>
</tr>
<tr>
<td>CA125</td>
<td>&gt;65U/ml</td>
<td>26</td>
<td>75.6</td>
<td>86.6</td>
<td>66.7</td>
<td>90.9</td>
</tr>
<tr>
<td>CA19-9</td>
<td>&gt;40U/ml</td>
<td>26</td>
<td>35.6</td>
<td>81.1</td>
<td>40</td>
<td>78</td>
</tr>
<tr>
<td>CA15-3</td>
<td>&gt;32U/ml</td>
<td>26</td>
<td>57.1</td>
<td>93.9</td>
<td>75.9</td>
<td>86.7</td>
</tr>
<tr>
<td>CA72-4</td>
<td>&gt;3.8U/ml</td>
<td>26</td>
<td>70.7</td>
<td>91.8</td>
<td>75.7</td>
<td>89.6</td>
</tr>
<tr>
<td>CEA</td>
<td>&gt;3ng/ml non smo</td>
<td>37</td>
<td>16</td>
<td>93</td>
<td>37</td>
<td>83</td>
</tr>
<tr>
<td>HE4</td>
<td>&gt;70pM</td>
<td>41</td>
<td>72.9</td>
<td>95</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>LPA</td>
<td>1.3μmol/L</td>
<td>41</td>
<td>98</td>
<td>90</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IAP</td>
<td>482μg/ml</td>
<td>34</td>
<td>93.3</td>
<td>91</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>HP-α</td>
<td>65μg/ml</td>
<td>44</td>
<td>64</td>
<td>90</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>OVX-1</td>
<td>7.2μ/ml</td>
<td>49</td>
<td>70</td>
<td>95</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Methothelin</td>
<td>−</td>
<td>43</td>
<td>60</td>
<td>98</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Ref No.; reference number, Se; sensitivity, Spec; specificity, PPV; positive predictive value, NPV; negative predictive value, −; not shown NA; not assed.
<table>
<thead>
<tr>
<th>Stage</th>
<th>Number of patients</th>
<th>Level of circulating tumor-derived exosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>0.320 ± 0.056 mg/ml</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>0.640 ± 0.053 mg/ml</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>0.995 ± 0.084 mg/ml</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>1.42 ± 0.228 mg/ml</td>
</tr>
</tbody>
</table>

Table modified 71.
<table>
<thead>
<tr>
<th>Differently expressed microRNAs in the serum</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over-expressed</td>
<td></td>
</tr>
<tr>
<td>MiRNAs-21</td>
<td>0.0002</td>
</tr>
<tr>
<td>MiRNAs-29a</td>
<td>0.0003</td>
</tr>
<tr>
<td>MiRNAs-92</td>
<td>0.0001</td>
</tr>
<tr>
<td>MiRNAs-93</td>
<td>0.0003</td>
</tr>
<tr>
<td>MiRNAs-126</td>
<td>0.007</td>
</tr>
<tr>
<td>Under-expressed</td>
<td></td>
</tr>
<tr>
<td>MiRNAs-127</td>
<td>0.0001</td>
</tr>
<tr>
<td>MiRNAs-155</td>
<td>0.0003</td>
</tr>
<tr>
<td>MiRNAs-99b</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table modified 72.

Table 5
Biomarkers for screening, diagnosis and monitoring of ovarian cancer

Eiji Kobayashi, Yutaka Ueda, Shinya Matsuzaki, et al.

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