Ovarian Cancer Biomarkers: Current Options and Future Promise

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stage III and IV disease, highlighting a significant need for reliable and accurate biomarkers of ovarian cancer. This article focuses on secreted, shed, and circulating ovarian biomarkers, either currently used or in development, that are present in human body fluids, such as plasma, serum, and urine. This discussion is complemented by a review of the state-of-the-art molecular approaches currently being used in the discovery of new ovarian cancer biomarkers.

Current Diagnostic and Prognostic Approaches

Current diagnosis of ovarian cancer relies on pelvic examination, transvaginal ultrasonography, abdominal ultrasonography, and exploratory or diagnostic laparoscopy when evaluating a pelvic mass. One tumor biomarker, the cancer antigen 125 (CA125), is commonly used preoperatively to help predict potential for malignancy. Disease stage at diagnosis is a powerful prognostic variable for predicting patient outcome in ovarian cancer. Patients with International Federation of Gynecology and Obstetrics (FIGO) stage III ovarian cancer, indicating tumor dissemination and seeding of the peritoneal lining outside the pelvis, have a 5-year survival rate of approximately 35%. This rate drops to less than 10% in patients diagnosed with stage IV ovarian cancer, in whom disease has spread to distant metastasis. In contrast, patients with FIGO stage I disease, classified as ovarian carcinoma confined to the ovary, and having well to moderately differentiated tumors, have a greater than 95% of 5-year survival. Development of new biomarkers of early-stage ovarian cancer has the potential to significantly improve these bleak survival statistics.

Histologic subtype is also considered when evaluating patient prognosis. The 5 common histologic subtypes of epithelial ovarian carcinoma are serous, mucinous,
clear cell, transitional cell, and endometrioid. Evidence suggests that clear cell carcinoma may predict a worse outcome for patients with early-stage disease.15,16

Currently, for patients with advanced stage III and IV ovarian tumors, the most important prognostic factor for predicting favorable outcome is success of complete cytoreductive surgery and minimal residual tumor volume.7

Biomarkers

CA125

Preoperative detection of elevated levels of serum CA125 has been useful to predict malignant potential;1 increased preoperative serum CA125 is correlated with poor overall survival.4 In combination with transvaginal sonography, CA125 is also used to screen for ovarian cancer in patients with BRCA1 mutations and other high-risk populations.9 CA125 has been used to evaluate how ovarian tumors respond to chemotherapy and to monitor disease recurrence, because the levels correlate with progression or regression of established disease.10 Postsurgery, the CA125 level is correlated with the volume of remaining disease. Although the standard use of this marker is primarily for monitoring therapeutic response, increased CA125 levels are not completely consistent with a lack of therapeutic efficacy, because early fluctuations in CA125 patterns are often seen during chemotherapy treatment involving certain drugs. Therefore, treatment decisions should not be based solely on CA125 levels.11

Elevated levels of circulating CA125 are occasionally detected months before late-stage ovarian cancer is diagnosed.12 Up to 80% of women diagnosed with late-stage epithelial ovarian cancer have elevated CA125 levels in their serum. However, as many as 20% of advanced epithelial ovarian tumors have been shown to not express CA125 at all.13

Unfortunately, CA125 has limited usefulness in detecting early ovarian carcinoma when the disease is confined to the ovary; one study showed that only 50% of early-stage I and II cases had elevated CA125 levels.14 Many benign ovarian and nongynecologic conditions are also associated with increased circulating CA125 levels, suggesting that this antigen lacks the specificity and sensitivity to be a reliable biomarker for early detection of ovarian cancer.14 Researchers are now developing biomarkers complimentary to CA125 to aid in the early detection and prognostic evaluation of this disease.15,16 More than 30 potential serum biomarkers have been evaluated alone or with CA125.

Osteopontin

One protein biomarker evaluated alone and with CA125 is osteopontin, a glycoprophosphoprotein secreted by activated T lymphocytes, macrophages, and leukocytes, found in extracellular matrix, sites of inflammation, and body fluids. Osteopontin was initially found to be differentially expressed during a cDNA microarray study of RNA isolated from ovarian cancer cell lines and human ovarian surface epithelial cells.17 Subsequently, osteopontin plasma levels were significantly higher in 51 patients with ovarian cancer compared with 107 healthy controls, 46 patients with benign ovarian disease, and 47 women with other gynecologic malignancies.18

A study to evaluate osteopontin levels in serum collected from 234 patients with ovarian cancer after debulking primary surgery and longitudinally throughout chemotherapy showed that mean osteopontin levels after surgery in patients with cancer were significantly lower than those in normal controls, suggesting that serum osteopontin might be secreted by the primary tumor.19 In postsurgery patients, osteopontin levels in the serum significantly correlated with recurrent and bulky disease.19

Additionally, the level of osteopontin staining in metastatic lesions of 40 women with stage III ovarian cancer was shown to be significantly increased compared with the corresponding primary ovarian tumor, and this increase was reported to be an independent prognostic indicator of metastasis.20 Osteopontin staining in metastatic tissue correlated with extremely poor outcome in these patients (3-year survival, < 5%) compared with patients with stage III ovarian carcinoma who did not display increased osteopontin in metastatic tissue compared with primary tumor (3-year survival rate, 75%).20

Recently, a proteomic-based method identified a C-terminal fragment of osteopontin in presurgical urine samples from patients with ovarian cancer and those with early-stage disease, suggesting that this protein may be a useful noninvasive biomarker for early detection and prognosis of ovarian cancer.21

Kallikreins

A specific human gene locus containing 15 kallikrein genes was identified on chromosome 19q13.4 as being the largest uninterrupted gene cluster of serine proteases
in the human genome. Research has focused on kallikreins as potential serum biomarkers in ovarian, breast, and prostate cancers because they are expressed in epithelial and endocrine tissues, regulated by hormones in cancer, and are shed and detectable in human body fluids.21 Many kallikreins have been shown to have differential expression in ovarian carcinomas compared with normal ovaries,22–24 and are present in circulation. In one study, most of the 20 screened patients with ovarian cancer had elevated human kallikrein 14 in their serum, which was undetectable with enzyme-linked immunosorbent assay (ELISA) in the serum of 28 normal controls.25

In a larger study comparing 146 patients with ovarian cancer with 97 healthy women and 141 women with benign gynecologic diseases, mean human kallikrein 10 (hK10) serum level was significantly elevated in 141 of the patients with ovarian cancer before surgery but was not elevated in the serum of patients with benign gynecologic diseases.26 This serum elevation of hK10 significantly correlated with unfavorable prognosis, serous subtype, late-stage disease, and poor response to chemotherapy.26 Interestingly, hK10 was found in the serum of 35% of patients with ovarian cancer who were negative for CA125. When hK10 was combined with CA125 in patients with early stage I and II disease, sensitivity increased 21% (with 90% specificity) compared with analysis of CA125 alone.26 Detection of other human kallikreins, including kallikrein 5, 6, 8, and 11, in the serum of patients with breast and ovarian cancer is also being investigated for its prognostic potential when combined with CA125.

Bikunin
Bikunin is a glycosylated Kunitz-type protease inhibitor that inhibits invasion and metastasis. A study of 41 patients with ovarian cancer found that reduced gene expression of bikunin in 17 of the 41 ovarian cancer tissues predicted poor prognosis in patients with ovarian cancer.27 Further analysis using immunohistochemistry of surgically removed ovarian tumor samples showed high bikunin expression in 40 of 89 samples, where it predicted a favorable outcome and longer disease-free survival.28 A larger study reported the development of an immunoassay to detect bikunin in the circulation of 200 normal healthy women, 200 patients with benign gynecologic diseases, and 327 patients with ovarian cancer presurgery,29 and showed that low plasma levels of bikunin were associated with late-stage disease, presence of residual tumor after surgery, poor response to chemotherapy, and reduced survival time. High preoperative plasma bikunin levels have been reported to be a strong favorable prognostic marker for ovarian cancer (median survival, 60 months) compared with low serum bikunin levels (median survival, 26 months).30

Human Epididymis Protein 4
The gene encoding human epididymis protein 4 (HE4) is commonly amplified in ovarian tumors. Although its exact function remains uncharacterized, HE4 is a secreted protein that is absent in normal ovarian surface epithelium but is expressed specifically in 100% of the 16 human endometrioid epithelial ovarian cancers and 93% of the 60 serous ovarian carcinomas stained for HE4.31 An ELISA analysis of serum HE4 levels in 37 patients with ovarian cancer compared with 65 healthy controls showed that HE4 had the same specificity and sensitivity as CA125 and detected fewer false-positives in patients without malignant disease.32 Additionally, analysis of a series of biomarkers, including CA125, osteopontin, HE4, epidermal growth factor receptor, ErbB2, evaluated alone and in combination, in the preoperative urine and serum samples from 67 patients with invasive ovarian carcinomas and 166 controls with benign ovarian neoplasms, showed that, as a single tumor marker, HE4 had the highest sensitivity for detecting stage I ovarian cancer.15 When multiplexed, CA125 and HE4 showed the highest sensitivity (with a specificity of 95%) in detecting disease compared with either protein alone.15

Vascular Endothelial Growth Factor
Multiple angiogenic factors and cytokines in circulation have been analyzed for their potential role in evaluating ovarian cancer detection and prognosis. The most studied of these is vascular endothelial growth factor (VEGF) or vascular permeability factor. VEGF levels are known to be elevated in patients with ovarian cancer and contribute to the accumulation of ascites.33 Several groups have since evaluated the prognostic potential of serum VEGF levels in women with ovarian cancer.34 A study correlating clinical outcome with VEGF levels in the preoperative serum of 314 patients with both early- and advanced-stage ovarian cancer reported that higher serum VEGF levels independently correlated with shorter survival...
In a multivariate Cox analysis regression model, high serum VEGF expression in patients with stage I disease correlated with an 8-fold increase in cancer-related death.\(^3\)\(^4\)

Other studies have reported that, although elevated VEGF in ascites is an important prognostic factor of outcome in ovarian cancer, no discernable difference in serum VEGF levels were noted among patients with ovarian cancer, controls, and patients with benign disease.\(^1\)\(^5\)\(^6\)

Given the complexity and limitations of circulating biomarkers, it is not surprising that studies reporting the multiplexing of a small panel of biomarkers present in urine or serum yielded the most powerful sensitivity and specificity in detecting ovarian carcinoma.\(^1\)\(^5\)\(^7\)\(^8\)

The authors and others are examining biomarkers originally discovered and validated in other cancers for their ability to detect and monitor ovarian cancer.\(^9\)\(^-\)\(^4\(^5\)\(^6\) Some reported urinary biomarkers include a panel of matrix metalloproteinases (MMPs)\(^4\(^4\)\(^-\)\(^4\(^8\) and a disintegrin and metalloproteinase 12.\(^4\)\(^1\)\(^4\)\(^3\)\(^4\) Given that these biomarkers are involved in basic processes common to most human cancers, they may also be useful in ovarian cancer.

**Molecular Approaches to the Discovery of New Ovarian Cancer Biomarkers**

The discovery of new biomarkers relies on state-of-the-art methods for detecting genes and proteins in human body fluid and tissues. This section discusses some approaches currently used to identify new biomarkers of ovarian cancer.

**Whole-Genome Analysis**

Comparative genomic hybridization (CGH) is a whole-genome assay that detects gains or losses of gene copy number. This assay has identified several chromosome regions with an abnormal gene copy number in ovarian cancer.\(^9\)\(^5\)\(^0\) Some genes in these regions have been further evaluated as potential prognostic markers.

One study in advanced serous epithelial ovarian cancers determined that chromosome 1q22, harboring the RAB25 gene encoding a small GTPase, was amplified in 28 of 52 ovarian cancers.\(^1\)\(^3\)\(^4\) Gene expression analysis confirmed that RAB25 mRNA was increased in advanced-stage ovarian tumor samples. Furthermore, increased RAB25 mRNA levels were associated with decreased disease-free survival.

CGH analysis from another study suggested that fibroblast growth factor 1 (FGF1) may also be a potential prognostic marker for ovarian cancer.\(^5\)\(^2\) In the 42 late-stage, high-grade serous ovarian cancer cases analyzed, amplification in the chromosome region 5q31–35 was significantly associated with poor survival. Amplification of the FGF1 gene, which is located in this region, was further confirmed to associate with survival. This study also found a significant correlation between FGF1 expression and tumor angiogenesis, suggesting a possible mechanism underlying the association between FGF1 and patient survival.

Clear cell ovarian cancers generally have poor prognosis and are more chemoresistant than other ovarian cancer histologic subtypes. Tsuda et al.\(^5\)\(^1\) applied CGH on DNA from 30 patients with clear cell ovarian cancer and 19 with the serous subtype and found that ABCF2, a member of the ATP-binding cassette (ABC) family, was amplified in clear cell cancers. ABCF2 protein levels were also shown to correlate with chemoresistance in 20 patients with ovarian cancer.

**Transcription Profiling**

Because different ovarian cancer histologic subtypes are associated with different prognoses, many transcription profiling studies have focused on the discovery of markers that can discriminate among subtype. Schwartz et al.\(^4\)\(^4\) found a gene expression pattern that distinguished clear cell cancer from other subtypes; 7 of 8 clear cell cancers were accurately identified using a panel of 158 genes. The uniqueness of clear cell ovarian cancer was later confirmed in another study.\(^5\)\(^5\) However, both studies showed that a certain overlap of gene expression signatures still existed between the subtypes, suggesting some shared mechanisms underlying ovarian carcinogenesis.

Transcription profiling studies have also identified markers that may predict patient survival. Spentzos et al.\(^5\)\(^6\) reported a 115-gene signature identified from 34 samples that could distinguish between unfavorable (median survival, 30 months) and favorable overall survival (median survival, not yet reached). Another study, which profiled 54 late-stage serous ovarian cancers, identified a panel of genes that could discriminate between short-term (< 3 years) and long-term (> 7 years) survival.\(^5\)\(^7\) This gene expression signature was 100% accurate in classifying 11 patients with early-stage ovarian cancers as long-term survivors in an independent test set. The prognostic value of
this signature was confirmed using the independent data set previously published.\textsuperscript{16}

Several other studies have attempted to identify gene expression patterns that predict response to chemotherapy. These findings could potentially influence treatment decisions and make individually tailored therapy possible. A 93-gene signature predictive of pathologic complete response to chemotherapy was identified in a training set of 24 patients with ovarian cancer.\textsuperscript{58} In a separate validation set, this signature distinguished between unfavorable (median, 41 months) and favorable (median, not yet reached) overall survival.

Interestingly, the 93-gene signature shared no genetic overlap with the 115-gene survival signature described earlier. However, the combination of these signatures provided more powerful prognostic discrimination than either one alone. Jazaeri et al.\textsuperscript{59} found another 85 genes to be differentially regulated between primary chemosensitive and chemoresistant tumors. Dressman et al.\textsuperscript{60} further evaluated gene expression signatures that defined the status of oncogenic signaling pathways. Gene signatures consistent with activated Src and Rb/E2F pathways were identified in chemoresistant patients. This finding could potentially lead to patient-tailored therapy that specifically targets these implicated pathways.

**MicroRNA Profiling**

MicroRNA was first discovered in Caenorhabditis elegans in 1993.\textsuperscript{61} These RNAs are 19 to 24 nucleotides long and do not encode proteins. They interact with the 3’ untranslated region of target mRNAs, leading to target mRNA degradation and inhibition of translation.\textsuperscript{62}

MicroRNAs have been found to be differentially expressed in tumor versus normal tissues in a range of solid and hematopoietic tumors. In some cases, distinct microRNA signatures can accurately distinguish tumor from normal tissues and are correlated with disease outcome.\textsuperscript{63} Furthermore, a study analyzing microRNA signatures in a range of tumor types suggested that the expression pattern of a relatively small number of microRNAs (approximately 200) was more accurate than cDNA arrays in classifying human cancers.\textsuperscript{64} These studies strongly suggest that microRNA profiling may have significant potential in cancer diagnosis and prognosis.

Iorio et al.\textsuperscript{65} compared the microRNA profiles of 69 malignant ovarian tumors with 15 normal ovarian samples, and identified 39 microRNAs that were differentially expressed in tumor and normal tissues. This study also identified microRNAs associated with specific ovarian carcinoma subtypes. Some of the most regulated microRNAs have either tumor suppressors or other cancer-associated molecules as their known or potential targets. For example, all 4 of the most upregulated microRNAs in tumors have the tumor suppressor BRCA-1 associated protein-1 as their putative target. miR-140, which is among the most downregulated microRNAs, is predicted to target MMP13 and FGF2.

The differentially expressed microRNAs identified in this study were mostly consistent with an earlier study that focused on the alterations in the microRNA genes.\textsuperscript{66} The 4 most upregulated microRNAs were also amplified at the DNA level, and 12 of the 29 downregulated microRNAs were deleted.

In these studies, let-7 family members were found to be either downregulated at the transcript level or deleted at the DNA level in ovarian cancers. Consistently, miRNA let-7d was identified as a marker for less-advanced ovarian cancer.\textsuperscript{67} The combination of let-7d and its target high-mobility group A2 (HMGA2) was shown to be a superior prognosis predictor compared with other classical markers, such as E-cadherin and vimentin. The HMGA2/let-7d expression ratio inversely correlated with progression-free survival.

**Proteomic Profiling**

One significant limitation of transcription profiling studies is that changes at the mRNA level do not always translate into changes at the protein level. Therefore, proteomic profiling is the most direct approach to search for diagnostic and prognostic biomarkers of ovarian cancer, and mass spectrometry is one of the principle methods used in proteomic profiling.

Proteomic profiling in ovarian cancer has been performed using 2 strategies. One is to identify the distinct proteomic patterns of peptides in cancer samples. For example, mass spectra from a training set of serum samples from 50 women without cancer and 50 women with ovarian cancer were obtained, and a cancer-specific proteomic pattern was identified. Another study identified 3 panels, each containing 4 to 5 protein peaks, as differentially expressed in ovarian cancer versus normal serum samples.\textsuperscript{68} These protein peaks were different from those reported previously.\textsuperscript{69}
An alternative strategy is to identify individual peptides that are differentially expressed between cancer and normal samples. Three of these proteins—transferrin, haptoglobin precursor fragment, and immunoglobulin heavy chain—were identified by comparing the proteomic profiles of plasma samples from 43 women with cancer and 38 without.20 The combination of these proteins and CA125 significantly improved predictive performance compared with CA125 alone. Haptoglobin-alpha was also identified in a later serum profiling study.21

Three other proteins—apolipoprotein A1, a truncated form of transferrin, and a cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4—were identified in a 5-center case-control study.22 When the sensitivity was fixed at 97%, a combination of these 3 biomarkers and CA125 showed increased specificity for detecting early-stage invasive epithelial ovarian cancer compared with CA125 alone (74% vs. 65%). These findings were later replicated in an independent blinded study.23

In addition to the proteomic profiling of serum and plasma samples, profiling has also been performed on other body fluids. For example, glycosylated eosinophil-derived neurotoxin and COOH-terminal osteopontin fragments were identified as potential urinary markers for detecting early-stage ovarian cancer.24 Gortzak-Uzan et al.25 studied the ascites proteome of ovarian cancer and reported 80 potential biomarkers. These genomic and proteomic profiling studies have yielded important mechanistic information on the development and progression of ovarian cancer and, once validated in large studies, may identify new diagnostic and prognostic biomarkers. However, many profiling studies are performed on small numbers of samples, results of different studies show limited overlap, and they are not always reproducible.26 Large numbers of samples and multiple and independent sample sets will be required to validate the discriminatory power of these candidate biomarkers.27

References
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