

Ovarian cancer biomarkers for molecular biosensors and translational medicine

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Multiple omics researches in the past two decades have identified over 200 potential biomarkers for ovarian cancer. Discoveries during the 1990s were more focused on clinicopathology-based biomarkers that were targeted to support diagnosis, but the emphasis has shifted to the identification of prognostic biomarkers in the past 10 years. The post-genomic era has opened the door for personalized cancer treatments and the trend of discovery is moving forward to identify more stratified biomarkers to accurately predict the progression of disease, as well as efficacy biomarkers to precisely determine drug response. To better meet future challenges, biomedical research needs the reformed and standardized infrastructure of tissue banks/biorepositories, with national and international initiatives. Of the hundreds of biomarker candidates for ovarian cancer, only a small number are actively being validated with clinical samples, owing to the lack of biomaterials that are linked with accurate clinical data. The purpose of this article is to present selected biomarkers from the past 20 years of ovarian cancer research, placing special emphasis on biomarkers that are strongly associated with positive or negative clinical outcomes. The article also presents a global view of all known potential biomarkers and mutations for ovarian cancer from NCI's Cancer Gene Index developed by Sophic, and Sanger's Catalogue of Somatic Mutations in Cancer database.

KEYWORDS: biomarker • biosensor • ovarian cancer • personalized medicine • tissue bank

Ovarian cancer kills over 125,000 women worldwide each year and kills more women than all other gynecologic cancers combined. In the USA alone, over 15,000 women die every year as a result of ovarian cancer, ranking the disease as the second deadliest cancer for women and the fifth leading cause of cancer death in women [201]. Early stage (I/II) detection has a survival rate of over 90%, but only approximately 20% of all reported cases are caught in the early stages; the 5-year survival rate is approximately 11% when detected in the advanced stages (III/IV) [202]. Symptoms of ovarian cancer are complex and often misdiagnosed as other diseases, but recent developments prescribe more well-defined clinical symptoms for a better diagnosis. Current treatment options, including surgical resection methods and various chemotherapies, have improved for late-stage ovarian tumors, but recent statistics demonstrate that less than a 10% improvement has been made for the 5-year survival rate during the past 35 years [1].

These statistics suggest that more research efforts related to the discovery of ovarian cancer

biomarkers are required. Identification of robust and accurate biomarkers for early detection and diagnosis will prevent major misdiagnosis and provide better cancer patient care. A robust detection method based on molecular profiles for ovarian cancer has not yet been established because the disease exhibits a wide range of morphological, clinical and genetic variations during the course of tumor progression. Owing to these cellular and molecular characteristics, identifying appropriate treatments for the disease is a major clinical challenge. Several recently published review articles provide a good overview of ovarian cancer from a molecular and clinical perspective [2–4], and discussions of these perspectives will not be repeated in this article.

Another major challenge for identifying biomarkers is the lack of availability of high-quality normal and cancerous tissue and biofluid biospecimens that are associated with accurately documented clinical data [5]. This is the result of the workflow of the typical clinical setting. Frequently, most of the tumor specimens from surgical resection are used by the pathology

department to complete the diagnosis of the disease, and the remaining portions are normally archived for up to 20 years, in case they are needed to aid in future patient diagnosis. For larger tumor masses, the leftover tissues are discarded without patient consent, and without the establishment of pre-approved institutional study protocols to salvage and make use of the remaining tissue. Thus, the typical clinical workflow in most regional/community hospitals generates inadvertent situations where only a less than adequate amount of tissue becomes available for biomarker and molecular profiling studies. Efficient tissue banking initiatives can certainly correct these problems.

Multinational efforts in tissue banking (such as those of the Office of Biorepositories and Biospecimen Research and the national biobank initiatives of the NIH/NCI of the USA), close collaboration between academic and community/regional medical centers, and standardization of protocols (spanning from the surgical procedure to the procurement method used by the pathology department) would definitely provide an infrastructure more conducive to biospecimen availability and quality. This kind of reformed infrastructure will enhance biomarker discovery and lay the ground for the construction of the most appropriate assay platforms for ovarian cancer. This article will focus on the molecular diagnostic and prognostic perspective of ovarian cancer by use of potential biomarkers that are already in the literature and in bioinformatics databases, and will discuss how these biomarker candidates can be translated into clinical use.

Clinical methods & molecular profiling

A quantitative and systematic review showed that ultrasonography with color Doppler is a useful preoperative test for predicting the diagnosis of pelvic masses, with a pooled sensitivity of 0.87 and a specificity of 0.92 [6]. Transvaginal sonography is another commonly used detection method designed to provide the size of ovaries via medical imaging technologies. However, the method lacks specificity, is insensitive for early detection and often misses late-stage ovarian tumors [7]. The sensitivity and quantitative design of these technologies tend to depend only on the physical size of the ovary and not on the tumorigenicity of the enlarged mass [8]. The diagnosis of ovarian cancer is complicated due to differences in tumorigenesis origin, including stromal cells (5–10%), germ cells (10–15%) and epithelium-surface cells (>80%) [2]. In addition, many genetic alterations and chromosomal aberrations contribute to the existence of over 100 subtypes for histologic classification of ovarian tumors [9–11]. For this reason, an effective diagnosis would be arrived at more effectively via a combination of robust biomarkers specific for ovarian cancer and current clinical methods.

Over a decade of omics studies have identified many potential biomarkers for ovarian cancer but only a few are currently used in the clinic. Most of this omics research has been carried out at the levels of cancer cell lines and retrospective formalin-fixed, paraffin-embedded biospecimens. Moreover, the newly discovered biomarkers have not yet convinced pathologists, who are the decision makers in the clinic. Many of the molecular signatures/biomarkers from previous reports are related to the differentiation of tumors from normal tissues or the identification of subtypes/

clinical phenotypes of cancer. However, most clinicians believe that such clinical phenotypes can be systematically determined by experienced pathologists using established methods, and that only a few of the previously reported biomarkers make suitable adjuncts to help extrapolate on the already established practices of the pathology department. Furthermore, clinicians argue that these limited molecular profiles provide less clinical impact as classifications and nomenclatures continue to evolve on complex clinical phenotypes for ovarian tumors. To counter such arguments, researchers should focus more on identifying stratified and predictive biomarkers for the future. Indeed, there is a need for molecular tools that are based on robust biomarkers specific to clinical outcomes of ovarian cancer subtypes, and for assay platforms that can be easily adapted for routine clinical use. This type of molecular diagnostics can suggest appropriate treatment options based on patients' genomic or proteomic profiling, either from cancer cells or biofluids, including blood and urine. However, developing such diagnostics is a difficult task for researchers that do not have patient biospecimens associated with clinical outcome data. It is thus crucial to raise awareness of the fact that national and international efforts are needed to promote the following: tissue banking; patient and general population education about tissue/blood donations; active participation from patients for donations; and the use of electronic medical records (EMR) to associate scientific data with accurate clinical outcomes.

The task of incorporating molecular profiles into the routine clinical workflow and the diagnostic process may come naturally in the future. To translate molecular signatures into the clinical setting, researchers must identify a set of robust biomarkers that are indisputably accepted by the pathologists in medical centers. These biomarkers can be a combination of genes, proteins, miRNAs, SNPs and mutations that are derived from tissues and biofluids. Biomarkers from biofluids (i.e., blood) would provide more benefits to patients because they are routinely collected in the clinic, are minimally invasive and can be procured and stabilized immediately for future molecular studies. Furthermore, biofluid biomarkers are particularly important for early screening at community events because ovarian cancer is normally asymptomatic until the tumors reach an advanced stage (III/IV). The clinic expects many more potential biomarkers for ovarian cancer to be discovered as we move forward from the postgenomic era to meet the age of the proteome and metabolome. However, simply discovering hundreds of more potential biomarkers will not persuade the clinic; it is not about the quantity but the quality of the clinically relevant biomarkers. The clinic may not accept a profile of hundreds of biomarkers but a handful of the most robust and specific biomarkers that will contribute to accurate diagnosis. This may be the only route to make sure molecular profiling or biomarker assays become an integrative part of the clinical workflow.

Ovarian cancer biomarkers that were discovered in the 1990s are listed in TABLE 1 and only a couple of these are mainly used in the clinic. In fact, the cancer antigen 125 (CA125/ MUC16) assay is the most used clinical biomarker for ovarian cancer. It has become a semi-mandatory test in the clinic because a rise in

Table 1. Ovarian tumor markers discovered during the 1990s[†].

Marker	Full name	Diagnostic/ prognostic	Expression	Localization	Sensitivity	Specificity	Description	Ref.
B2M	β2-microglobulin	Diagnostic	High	Serum	87%	48%	Suitable tool to monitor course of disease when used in combination with CA125	[47]
CA54/61	Mucin-type glycoprotein antigen	Diagnostic	High	Serum	~50–76%	91%	In case of mucinous cystadenocarcinoma Sensitivity 65% (compared with 36% of CA125)	[51]
CA72-4	Cancer antigen 72-4/ TAG-72	Monitoring/ diagnostic	High	Serum/tissue	High	Fair	Discriminates negative serous adenomas from positive serous carcinomas	[108,109]
CA125 II	Cancer antigen 125 II	Diagnostic	High	Serum	Fair	Low	More precise than CA125	[110]
CA602	Cancer antigen 602	Diagnostic	High	Serum	92%	Low	100% sensitivity in serous adenocarcinoma 67% of sensitivity in mucinous adenocarcinoma	[111]
caGT	Cancer-associated galactotransferase antigen	Diagnostic	High	Serum	75%	90%	8/9 in clear cell carcinoma	[112]
Cathepsin B	Cathepsin member B	Preoperative differential diagnosis	High	Serum	100%	Low	Serum level is fairly proportional to FIGO stage; serous > endometroid tumors (p < 0.001)	[113]
CD34	CD34 molecule	Prognostic	High	Tissue	Low	Low	Blood vessel count related to lower overall survival (p = 0.022)	[73]
COX-1	Cyclooxygenase-1	Diagnostic	High	Serum	68%	Fair	Serum level is proportional to tumor progression	[50]
GAT	Glyphosate N-acetyltransferase	Diagnostic	High	Serum	~47.9–52.9%	94%	Differential diagnosis from endometriosis	[114–116]
IAP	Immunosuppressive acidic protein	Diagnostic	High	Serum	89.5%	91.9%	Early detection of recurrence	[117]
M-CSF	Macrophage colony-stimulating factor	Diagnostic	High	Serum	61%	92.7%	Serum level is useful in detecting ovarian cancer	[37]
nm23-H1	Non-metastatic cells 1, protein (NM23A)	Prognostic	Low	Tissue	Fair	Fair	Inverse association with metastatic potential	[74]

[†]Most of these potential biomarkers can be used for diagnostic purposes but are not currently used in the clinic. FIGO: International Federation of Gynecology and Obstetrics.

