Chapter 7

The Role of Circulating Biomarkers in the Early Diagnosis of Ovarian Cancer

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75484

Abstract

Ovarian cancer is the leading cause of gynecologic-related cancer death and epithelial ovarian cancer (EOC) is the most lethal sub-type. EOC is usually asymptomatic, and few screening tests are available. Diagnosis of ovarian cancer can be difficult because of the nonspecific symptoms. Despite the various diagnostic methods used, there is no reliable early diagnostic test and it needs to be developed. Specific biomarkers may have potential with the least possible invasive procedure. Biomarkers with a high sensitivity to ovarian cancer should be identified. Circulating biomarkers that are significant tools for non-invasive early diagnosis can be analyzed using circulating tumor cells, exosomes, and circulating nucleic acids. Protein, gene, metabolite, and miRNA-based biomarkers can be used for ovarian cancer diagnosis. As non-coding RNAs, MiRNAs may have an important role in ovarian cancer diagnosis due to their effects on mRNA expression levels. The most recent developments regarding the potential of circulating biomarkers to detect early ovarian cancer is presented in this chapter.

Keywords: ovarian cancer, biomarker, cell-free nucleic acids, early diagnosis, miRNA

1. Introduction

Ovarian cancer is a heterogeneous disease and the most important cause of gynecological cancer-induced deaths [1]. It is the fifth most important cause of cancer-related deaths among women in the world [2]. Different types of tumors may develop from each cell type. These tumors are epithelial tumors, germ cell tumors (originating from the ovary cell and follicular), and stromal tumors [3].

Molecular and cellular analyses of these tumor types may lead to earlier diagnosis of ovarian cancer and it is hoped better survival rates. Many factors play a role in the development of cancer, while genomic mutations and epigenetic changes are very important. For this reason, studies on mutations and epigenetic alterations may provide information about features such as early diagnosis, surveillance, and response to treatment.

2. Biomarkers used in the diagnosis of ovarian cancer

Tumor biomarkers are molecules that are produced by cancer cells or cells around them, which can be measured in body fluids or in the blood during the diagnosis, screening or treatment of cancer. Molecules that can be used as tumor biomarkers can be counted as cytoplasmic proteins, enzymes, hormones, surface antigens, receptors, oncofetal antigens (re-emerging proteins in cancer that is normally lost after birth), oncogenes or their products. An ideal tumor biomarker should be sensitive enough for early detection of small tumors while retaining the specificity of the identified cancer type. Unfortunately, however, today there is no known tumor biomarker carrying these features [4].

The features that should be found in an ideal tumor biomarker are given below [5]:

- It should have high specificity; it should be specific to only one type of tumor.
- Must have high sensitivity, should not be detected in cases of physiological or benign tumors.
- Levels should be proportional to tumor characteristics and size.
- The predictive and prognostic benefit of tumor biomarkers should be known.
- Half-life should be short, frequent and serial monitoring is possible.
- It should be cheap and easy to apply.
- Can be used as a screening test.
- Sample taking should be easy.

Potential biomarkers used in ovarian cancer are grouped as gene, protein, metabolite, and miRNA-based biomarkers according to their type [5].

The vast majority of ovarian tumors arise from the accumulation of genetic damage, but the specific genetic pathways that are involved in the development of epithelial, borderline, and malignant tumors are largely unknown. Considering the important relationship between genetic alterations and ovarian tumors, potential ovarian-cancer biomarkers can be found at gene-level (hereditary gene mutations, epigenetic changes, and gene expression) studies. The most common genes associated with epithelial ovarian cancer are shown in **Table 1** [6].

BRCA1, BRCA2, and Lynch syndrome genes show high penetrance and offer lifetime risks of 7–40% for ovarian cancer. Nowadays, the multigene panels used for clinical genetic testing

Gene	Gene full name	Protein class	Score[a]	No. of PMIDs[b]	No. of SNPs[c]
TP53	Tumor protein p53	Transcription factor	0.245958	144	2
CLDN7	Claudin 7	Cell junction protein	0.201099	5	0
ABO	ABO, alpha 1–3-N-acetylgalactosaminyltransferase and alpha 1–3-galactosyltransferase	Transferase	0.200549	3	0
SYNPO2	Synaptopodin 2	Cytoskeletal protein	0.200275	1	0
GPX6	Glutathione peroxidase 6	Oxidoreductase	0.200275	1	0
RSPO1	R-spondin 1		0.200275	1	0
WNT4	Wnt family member 4	Signaling molecule	0.200275	1	0
ATAD5	ATPase family, AAA domain containing 5	Nucleic acid binding	0.200275	1	0
EHMT2	Euchromatic histone lysine methyltransferase 2	Transferase; nucleic acid binding	0.2	1	0
MIR376C	MicroRNA 376c		0.2	1	0
BRCA1	BRCA1, DNA repair associated		0.02933	99	5
ERBB2	erb-b2 receptor tyrosine kinase 2		0.017792	57	0
BRCA2	BRCA2, DNA repair associated	Nucleic acid binding	0.01422	44	4
VEGFA	Vascular endothelial growth factor A	Signaling molecule	0.012847	39	0
MUC16	Mucin 16, cell-surface associated		0.009	25	0
EGFR	Epidermal growth factor receptor		0.008176	22	0
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	Transferase; kinase	0.007627	20	3
PGR	Progesterone receptor	Transcription factor; receptor; nucleic acid binding	0.007287	10	0
ERCC1	ERCC excision repair 1, endonuclease non-catalytic subunit	Nucleic acid binding	0.007077	18	0
EGF	Epidermal growth factor	Extracellular matrix protein; receptor	0.006528	16	0
ESR1	Estrogen receptor 1	Transcription factor; receptor; nucleic acid binding	0.006528	16	0
IGF2	Insulin like growth factor 2		0.006253	15	1
NBR1	NBR1, autophagy cargo receptor		0.006044	22	0
CDKN1A	Cyclin dependent kinase inhibitor 1A	Enzyme modulator	0.005704	13	0
TNF	Tumor necrosis factor	Signaling molecule	0.005704	13	0
ABCB1	ATP binding cassette subfamily B member 1		0.005495	20	3

Gene	Gene full name	Protein class	Score[a]	No. of PMIDs[b]	No. of SNPs[c]
MLH1	mutL homolog 1	Nucleic acid binding	0.005154	11	0
PTGS2	Prostaglandin-endoperoxide synthase 2	Oxidoreductase	0.005154	11	1
BRAF	B-Raf proto-oncogene, serine/threonine kinase	Transferase; kinase	0.00488	10	1
CDKN2A	Cyclin dependent kinase inhibitor 2A	Enzyme modulator	0.004605	9	0

[a]Gene-Disease Score.

[b]Total Number of PubMed ID (PMIDs) Supporting the Association.

[c]The Number of Associated Single Nucleotide Polymorphism (SNPs).

Table 1. The most common genes associated with epithelial ovarian cancer.

include the mild-penetrance genes (lifetime risks of 6–13%) such as BRIP1, RAD51C, and RAD51D. The common low-penetrance susceptibility genes make up the rest of the genetic risk. Besides, SNPs have approximately 1% risk which is shown by population-based genome-wide association studies (GWASs) [7]. Expression analyses of quantitative or semi-quantitatively specific genes in serum or tumor tissue can potentially contribute to tumor recognition. In the last decade, analysis of gene expression has gained momentum due to improvements in microarray technology. This is because microarray technology enables analysis of tens or hundreds of gene expressions in a single piece of tissue. Gene expression profiling has focused on three main topics: the separation of tumor tissue by normal ovarian tissue, the identification of different subtypes of ovarian cancer, and the determination of cancer according to possible responses to treatment.

DNA methylation and histone modification are epigenetic mechanisms that play important roles in gene regulation, tumor formation, and progression. Measuring the rate of methylation in specific genes in the promoter region helps early detection of cancer, detection of disease progression, and prediction of therapeutic response. Identification of specific genes that change with epigenetic regulation is one of the areas that are actively studied in ovarian cancer. In this chapter, we want to focus on circulating biomarkers and other types of biomarkers will not be discussed.

3. Tumor materials in circulation: liquid biopsy and their biomarker potentials

Non-invasive tumor diagnosis and screening has become an important area of study. Contrary to tissue biopsy, through detection of circulating tumor cells (CTCs), tumor nucleic acids ("circulating tumor DNA/RNA"), and exosomes, predictive and prognostic markers may potentially be developed which is far less invasive. Hence early and multiple evaluations of the disease can be made, including retrospective follow-up, identification of treatment effects and investigation of clonal development. Isolation and characterization of CTCs, exosomes,

and circulating tumor DNA (ctDNA) will improve cancer diagnosis, treatment, and imaging. Liquid biopsy can be performed "real-time" and at every stage of cancer. Although, it has some potential disadvantages such as; still is not certain to use in cancer diagnosis, difficulties in analysis of data obtaining from high-throughput screening and lack of data verification through clinical trials; it has significant potential for clinical cancer diagnosis in future [8].

3.1. Circulating tumor cells (CTCs)

Some cancer derived cells are detected in peripheral blood, and appear as solid tumor cells that have broken away into the circulation [9]. There are two main types of CTCs to explain this phenomenon. The majority are "Accidental CTCs", and these are CTCs that are passively pushed by external forces, such as tumor growth, mechanical forces during surgical operation or friction. The rest are CTCs which gain more plasticity and metastatic potential via the epithelial-mesenchymal transition (EMT) process [8]. These CTCs can stay in the non-divided form in the vein, can spread together, or settle into a new tissue to compose the metastatic deposit. Regardless of the CTC pathway, these cells carry important information about tumor composition, metastasis, drug sensitivity, and treatment.

CTCs have been demonstrated to have prognostic value among patients with breast, colorectal, gastric, lung, and pancreatic cancers in previous meta-analyses. However, the value of CTCs in ovarian cancer still remains controversial. Some studies did not observe any correlation between CTC status and prognosis. In contrast, other studies demonstrated an association Zhou et al. has shown that the prognostic value of CTCs was not associated with disease stage but with an elevated CA-125, both of which are known to correlate with prognosis either directly or indirectly. It has also been known that the CTC status was significant in respect to the overall survival (OS), progression-free survival (PFS), and disease-free survival (DFS) in ovarian cancer [10].

CTCs can be detected in both metastatic patients and patients with early, localized tumors. There is a significant potential for CTCs in the clinical management of cancers such as ovarian cancer. CTCs may enable real-time monitoring of treatment efficacy, identification of new therapy targets, and detecting and understanding drug resistance mechanisms [11]. CTC imaging and separation from leukocytes is dependent on reliable cell-surface markers. Based on the precipitation of CTCs in the low-speed centrifuge, the leukocyte fractions can be distinguished via physical features as well. Lee et al. used a nanoroughened microfluidic platform and detected CTCs in the sera of nearly all female participants (53/54, 98.1%) with ovarian cancer [12]. They also showed that although there is no relationship between CTC count and PFS in patients with newly diagnosed epithelial ovarian cancer (EOC), in patients with recurrent disease and chemoresistance; a relationship was found between CTC-cluster positivity and diminished OS [12]. It has been postulated that CTCs could result in metastatic progression and recurrence by way of epithelial-mesenchymal-transition (EMT) or development of stem-like features and hence a reduced OS. Therefore, researchers have tried to identify therapy-resistant tumor cells and to overcome treatment failure by analyzing CTCs transcriptional profiles [13]. In this study, the authors analyzed 15 single CTCs from 3 ovarian cancer patients and found them to be positive for stem cell (CD44, ALDH1A1, Nanog, Oct4) and EMT markers (N-cadherin, vimentin, Snai2, CD117, CD146) [13].

3.2. Circulating cell-free tumor DNA

Chang et al. were the first to examine the amount of cell-free DNA (cfDNA) in a patient's serum as a marker of disease presence in gynecologic malignancies [14, 15]. Cell free tumor DNAs (ctDNAs) circulate in the bloodstream and are derived from tumor cells. The presence of ctDNAs has been proven by detection of tumor-specific anomalies such as the presence of mutation in circulating tumor DNA (ctDNA), loss of heterozygosity of microsatellite, and methylation of CpG islands [16–18]. Similar to CTCs source; ctDNAs are released into the bloodstream in two ways: passively whereby ctDNAs from dead tumor cells and actively whereby ctDNAs are derived from live tumor cells spontaneously [8, 19]. ctDNA and apoptotic cell levels are lower in healthy individuals compared to cancer patients because chronic inflammation and excessive cell death cause accumulation of cell residues. cfDNA (cell-free DNA) is believed to originate from apoptotic cells content and found in elevated levels in cancer patients and related to higher tumor stage [20, 21].

The level of ctDNA is higher in the bloodstream of patients with solid tumors and metastatic disease compared to those without metastases [20, 21]. In patients with metastatic disease, the serum ctDNA level is higher (prevalence 86–100%) when compared to early-staged cancer types and patients with no radiographic evidence of disease (prevalence 49–78%) [20, 22]. Olsen et al. showed that in 86% of patients, ctDNA can be detected approximately 1 year before metastases while they are not observed in those clear of recurrence [23, 24] The anticipated short half-life of ctDNA of around 2 hours allows for an almost continuous analysis of tumor features including development, metastatic progression, and treatment efficacy. Thus, the identification of ctDNA has extraordinary potential as a potential biomarker for observing tumor load in the patient both prior and during treatment and in follow up [23].

Earlier studies in gynecological malignancies evaluated the presence of ctDNA at one time point using pelvic washings, ascites, serum, and plasma. Pereira et al. has demonstrated that serial estimation of ctDNA is a surveillance biomarker in gynecologic malignancies that is as sensitive and specific as the FDA-approved serum biomarker CA-125 [25]. Additionally, disease recurrence can be detected months earlier with ctDNA than CT checking [25]. Furthermore, the survival profiles of patients can be predicted with ctDNA level during the start of primary treatment, debulking surgery, and combined platinum/taxane doublet chemotherapy [25]. Both improved progression free and overall survival appear to be associated with undetectable levels of ctDNA [25] Additionally, ctDNA level maybe a stronger predictor than CA-125 of tumor size because of the longer half-life of CA-125 (9–44 days). It is also shown that in some patients, relapse of disease can be detected with ctDNA approximately 7 months before any CT scan changes [25]. Pereira et al. detected occult ovarian cancer cases by continuously monitoring the ctDNA even during apparent clinical remission [25]. These studies demonstrate that ctDNA could be used in early detection, it can act as a marker of disease stage as well as disease progression for gynecological cancers especially ovarian cancer.

Early diagnosis seems to be the best solution to reduce rates of ovarian cancer deaths unless highly effective drugs are developed with fewer side effects. Bettegowda et al. showed that for ctDNA detection in solid tumors, patients are treated at an earlier stage resulting in improved

survival [21]. Moreover, even in stage I patients (usually curable with surgery alone), detection of ctDNA level can be observed in around 47% of all patients [21]. Using ctDNA-level analysis, ovarian cancer can be detected in around 70% of all stage III patients [21].

3.3. Circulating cell-free tumor RNA (ctRNA)

Cancer cells have a very specific gene expression profile which differs from normal tissues. These tumor-specific gene transcripts can be detected in the circulation of cancer patients [26]. Despite the high amount of RNase present in the blood, circulating RNAs have been found to be surprisingly stable. This can be explained by the possibility that RNA is destructively protected by exosomes (such as microparticles, microvesicles, multivesiculas) that pass through the cell membrane into the bloodstream [26]. In addition, these mRNAs that are present in blood can be used as prognostic and predictive biomarkers [27]. Similar to ctDNA, ctRNA requires further study to assess the exact value as a biomarker in ovarian cancer.

3.3.1. Circulating microRNAs

MicroRNAs (miRNAs) are RNAs that do not encode proteins, at about 22 nucleotides in length, but they are involved with translation suppression, mRNA degradation, or sequencing specific gene regulation. Thus these molecules regulate various biological processes such as development, cell proliferation, differentiation, and apoptosis [28]. Approximately 3% of human genes encode miRNAs, while about 30% of genes encoding protein are regulated by miRNAs. These miRNAs vary according to the type of each cell, the stage of development, and differentiation of the cell. The release and biological functions of extracellular miRNAs are still not fully understood [29].

It has been shown that blood miRNAs in cancer patients have the similar importance as the miRNAs in tissues, and the relationship between solid tumors and miRNA expression profiles in the blood have been investigated [30, 31]. Circulating miRNAs are not bonded to the cell but are protected against endogenous RNase breakdown by binding to microvesicles, exosomes, microparticles, apoptotic bodies, and protein-miRNA complexes [32]. MiRNAs are resistant to severe conditions such as high temperature, low/high pH, long-term storage, and over-applied freezing/thawing [29]. Measurement of circulating miRNA level is difficult because it can be contaminated with cellular miRNAs of different hematopoietic origin [29]. The isolation and stabilization protocols of circulating miRNAs should be standardized and the cancer patient's plasma should be selectively distinguishable at the single molecule level [33]. MiRNA expression varies in tumor tissue with respect to normal tissue, and these changes can be detected in serum/plasma samples of cancer patients when compared to healthy individuals [34]. Further work is needed because of the low level of difference detected [29]; however miRNA has been shown to play an important role in cancer development as a new oncogene or tumor-suppressor gene class that varies according to the target gene [35].

In eukaryotic cells, there are several stages in miRNA biogenesis stages (transcription, pri-miRNA clipping, pre-miRNA transport, and pre-miRNA cloning) [36, 37]. MiRNA expression

levels vary from normal to ovarian cancer, with epigenetic changes, genetic changes (such as copy number changes), or differentiated expression of transcriptional factors, targeting miRNA genes. Transcriptional gene silencing in cancer cells is often associated with epigenetic defects [38, 39]. Studies have suggested that dysfunction or irregularity may occur in key proteins that are effective in miRNA biogenesis and may lead to tumor formation [39].

In recent years, many studies have been performed on the miRNA expression profile in EOC and it has been shown that there are significant differences in the miRNA expression profile compared to normal [35]. Iorio et al. compared 59 EOC operation samples with 15 normal ovarian species using a "custom" microarray and found 29 differently expressed miRNAs [35]. In EOC patients, miRNA expression profiles obtained from circulating tumor exosomes were compared with benign tumors and normal individuals and separated by different expression profiles. In this study, exosomes were separated by magnetic beads and anti-EPCAM antibodies, and miRNAs were analyzed by isolated microarray. As a result, there are several differentially expressed miRNAs in ovarian cancer samples [40]. In a study by Resnick et al., real-time PCR analysis of miRNA expression was performed on the serum collected from ovarian cancer patients and normal subjects, with different miRNAs expression found [41]. Patients with the three up-regulated miRNAs (miR-21, miR-92, and miR-93) were found to have a normal level of CA-125. Therefore, miRNA analysis may be complementary to other diagnostic methods [41].

It is clear that miRNAs play a crucial role in both normal and pathological processes due to their ability to regulate the expression of specific genes. However, no consensus has been reached as to the exact role/potential in diagnosis, metastasis, and prediction of response to treatment in EOC [28]. In addition, ovarian cancer is a heterogeneous disease, treatment and diagnostic options may vary from individual to individual; in this context, the tissue and origin specificity of miRNAs may be exploited and individualized treatment methods may be applied [42].

3.3.2. Circulating long non-coding RNAs

The Long Non-Coding RNAs (lncRNAs) are defined as >200 nucleotides in length and divided into five subclasses, which are intergenic, intronic, sense overlapping, *anti*-sense, and bidirectional lncRNAs [43]. LncRNAs are involved in various regulation processes which include protein-coding genes, functions at the level of splicing, chromatin remodeling, transcriptional control, and post-transcriptional processing after binding to DNA, RNA, or proteins [44]. These differ from tissue to tissue [45, 46] and lncRNAs play a role in growth, metabolism, and cancer metastasis [20, 47]. In several human cancer types, differentially expressed lncRNAs have been identified [48] which can be related to cancer metastasis and prognosis [49–51]. In addition, lncRNAs are specific for certain tumor origins such as the lymphatics, the cardiovascular or nervous system, circulating peripheral blood cells, or hematologic stem cells. Therefore, circulating lncRNAs may be informative about the tumor microenvironment [20, 52].

In ovarian cancer, lncRNAs have been shown to regulate several cancer processes such as development, metastasis, and relapse. Gao et al. [53] showed that a lncRNA named *HOST1*

(human ovarian cancer-specific transcript 1) plays a role in key biological pathways of EOC through the stimulation of tumor cell migration, invasion, and proliferation by inhibiting let-7b which is one of the most important miRNA involved in EOC [54]. In another study, Tong et al. showed that a lncRNA named RP11-190D6.2 regulates the WW domain-containing oxidoreductase (*WWOX*) expression by acting like an antisense transcript of this gene [55]. WWOX is linked with poor prognosis in several cancers, including EOC [56]. In addition, RP11-190D6.2 appears to play a role in the regulation of tumor metastasis, thus it can be counted as a potential biomarker and therapeutic target for EOC [55]. Zhou et al. compared several lncRNA expression profiles in a large number of OvCa patients from TCGA and found an eight-lncRNA signature predictive of overall survival [57]. Moreover, using lncRNA expression profiles, they could separate similarly aged patient into high-risk and low-risk groups, identify good or poor survival potential of patients, the eight-lncRNA signature maintained independent prognostic value, and was significantly correlated with the response to chemotherapy [57]. In a separate study [51], examining the expression profiles of lncRNAs and mRNAs in the high-throughput molecular profiles of OV patients; they found a correlation between lncRNA and malignant OV progression. Therefore; they suggest that two specific lncRNAs (RP11-284 N8.3.1 and AC104699.1.1) as may be candidate biomarkers for prognosis [51]. Clearly further study is required to understand their clinical application as a biomarker in EOC.

3.3.3. Circulating Piwi RNAs(piRNA)

Piwi RNAs (PiRNAs) are single-stranded, 26–31 nucleotide long RNAs which may inhibit transposons and target mRNAs through the formation of the miRNA silencer complex (RISC). Post-transcriptional regulation of piRNA (piRISC) happens in the cytoplasm [58]. The piRISC protects the integrity of the genome from alterations made by transposable elements (TE)—by silencing them; mRNA and lncRNA are other targets of piRNA complexes [58, 59]. piRNAs pathways play an important role to regulate some cancer-related pathways such as DNA hypomethylation and transposable element (TE) derepression. *L1* is a piRNA pathway gene that regulates these pathways, also overexpression of these genes (PIWIL1 and 2), have been shown in several tumor tissues [60]. Lim et al. showed that overexpression piRNA pathway genes and L1 elements may have a role in EOC [60]. They compared the EOC tissues and cell lines to benign and normal ovaries and found overexpression of PIWIL1 and MAEL, known as a cancer/testis gene [61] which are two genes of piRNA pathway which is a germ-line-specific RNA silencing mechanism. In situ analysis indicated that L1, PIWIL1, PIWIL2, and MAEL are up-regulated in cancerous cells, while MAEL and PIWIL2 genes are expressed in the stromal cells lining tumor tissues as well. PIWI, MAEL genes are essential for Drosophila and other vertebrates' germ-line stem-cell differentiation [60, 62]. These gene changes may promote a change in cell composition or identity in the tissue surrounding the cancer cells [60]. Also cancer stem cells may have potential as a biomarker for stem-cell definition [60, 63].

In addition, synthetic piRNAs may offer a new therapeutic approach through their use in silencing the expression of cancer-related genes. This approach has an advantage over other miRNA-based blocking methods because it does not require extra components for processing such as Dicer [59].

3.4. Exosomes and circulating microvesicles

Exosomes are multivesicular endosomal-derived extracellular vesicles (EVs) which are 30–120 nm size [64–67]. Exosomes can be distinguished from microvesicles which are heterogeneous in size (50–1500 nm) and result from the plasma membrane directly via a budding mechanism [68, 69]. Exosomes include several molecules such as proteins, metabolites, RNAs (mRNA, miRNA, long non-coding RNA), DNAs (mtDNA, ssDNA, dsDNA), and lipids and are used in cell communication [64, 70, 71]. Similar to circulating microvesicles, exosomes have also been shown to have specific functions and play an important role in coagulation, intercellular signaling, and the management of debris. Both circulating parts of the cell are found in different body and interstitial fluids [72, 73].

Tumor-derived exosomes are different from circulating healthy exosomes in terms of number of exosomes, content, and also cell-surface proteins [74]. Exosomes can be detected and isolated with several markers especially cell-surface proteins including those found only in the primary tissue. TGF β1, MAGE 3/6 proteins have a cell-surface biomarker feature special for ovarian cancer. These markers can be detected by filtration and ultracentrifugation methods in ovarian cancer plasma samples and can be used for prognosis/therapy monitoring of disease [74, 77]. Exosome contents are variable for cancer types as well. Taylor et al. indicated that several ovarian cancer specific exosomal miRNAs, (miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, miR-214), have been differentiated in serum samples by magnetic-activated cell-sorting (MACs) using anti-EpCAM array for diagnosis and screening of stage [40]. Exosomes are informative about tumor-specific features such as metastatic or benign form, stage, response to chemotherapies, and other drugs at that point in time via a possible blood sample [64].

Microvesicles have several common features with the primary cell such as membrane lipids, receptors, and diverse types of nucleic acids and proteins [75]. As in exosomes, microvesicles also have a potential to be biomarkers in several malignancies. Galindo-Hernandez et al. demonstrated that there were an increased number of microvesicles in breast cancer serum compared to healthy control samples [76]. It is also revealed that microvesicles derived from renal cancer stem cells include different miRNAs and mRNAs and these appear to play a function in tumor vascularization [75, 77, 78]. Microvesicles originated from tumor cells have been found in biological fluids in ovarian cancer. It has been shown that the number of microvesicles in malignant ovarian tumors is higher when compared to benign and nonmalignant pathologies (e.g., ovarian serous cysts, mucinous cystoadenomas, and fibromas) [79]. Ovarian cancer-induced ascites contains high levels of proteolytic enzymes such as matrix metalloproteinase (MMP-2, MMP-9) and urokinase-type plasminogen activator (uPA), which are the enzymes carried inside microvesicles [80–82]. Microvesicles may represent an ideal biomarker for ovarian cancer diagnosis and prognosis.

4. Biomarker detection technologies for ovarian cancer

High-throughput techniques of cellular transcriptome analysis mean that gene expression can be correlated with various aspects of disease in a variety of cancer types. This technology

used today in ovarian cancer research, such as expression microarrays and CGH, Real-time PCR, and Next-Generation Sequencing (NGS) allow genome-wide scanning and the discovery of altered genes involved in cancer.

4.1. Real-time PCR

Cell-free nucleic acids reflect both normal and tumor-derived nucleic acids released into the circulation through cellular necrosis and apoptosis. Stroun et al. have demonstrated with Reverse Transcription Quantitative PCR (RT-qPCR) that there is a consistent correlation between tumor load and quantity of cell-free DNA detected in a wide range of malignancies including ovarian cancer [83]. Several studies in OC with free DNA have also shown that miRNAs are abnormally expressed. Initial studies identifying tumor-derived miRNAs in the circulation of OC patients was published by Taylor et al. [40]. Zou et al. identified nine differentially expressed microRNAs (microRNA199a-5p, microRNA199a-3p, microRNA199-b3p, microRNA-645, microRNA-335, microR-NA-18b, and microRNA-141) through qRT-PCR expression analysis in SKOV3/DDP and A2780/DDP cells and these agreed with microRNA chip results [84].

4.2. Microarray

Microarrays together with clustering analysis have allowed genome-wide expression patterns in a lot of cancer types to be deciphered and compared. Wong et al. studied a group of genes (CLDN7, EPHA1, FOXM1, and FGF7), for the validation of the microarray findings; these were selected as these genes were associated with the alteration of crucial pathways involved in the regulation of cell cycle and cell proliferation [85]. Liu et al. [86] using the bioinformatics analyses of mRNA expression profiles retrieved from the Oncomine and Gene Expression Omnibus (GEO) Profiles online databases, they enriched two biological processes (cell cycle- and microtubule-related) and identified six genes (ALDH1A2, ADH1B, NELL2, HBB, ABCA8, and HBA1) that all were associated with ovarian cancer progression.

4.3. Next-generation sequencing

Clinical cancer next-generation sequencing (NGS) assays are dependent on many software subsystems and databases to deliver their results. The building of software systems for clinical use is a mandatory requirement of reliability and reproducibility imposed by diagnostic laboratory accreditation bodies such as Clinical Laboratory Improvement Amendments (CLIA), National Association of Testing Authorities (NATA), and the International Organization for Standardization (ISO 15189).

Pinto et al. [87] validated the use of next-generation sequencing (NGS) for the detection of BRCA1/BRCA2 point mutations in a diagnostic setting and also investigated the role of other genes associated with hereditary breast and ovarian cancer in Portuguese families. They obtained 100% sensitivity and specificity (total of 506 variants) for the detection of BRCA1/BRCA2 point mutations with their bioinformatics pipeline using a targeted enrichment approach when compared to the gold standard Sanger sequencing.

5. Conclusion

Ovarian cancer is one of the most significant and fatal gynecological cancer types worldwide. The earlier this disease can be detected, the better the success of treating it. There are several detection methods for ovarian cancer, but molecular diagnosis methods are more accurate, faster, and suitable for early detection. Recent developments have focused on identifying biological material with newer technological devices and these have become more precise, reliable, and more widely available over a short period of time. Although molecular markers, which are specific for ovarian cancer, have been extensively studied, they are still not used in a clinical setting. Clearly a greater understanding of their mechanisms and specificities are needed before they can be applied to early detection of OC.

Liquid biopsy using body fluids (e.g. blood, urine, saliva, and ascites) to isolate and characterize CTCs, exosomes, circulating tumor DNA, RNAs, and circulating free small RNAs is a new technique used in the detection and treatment of several diseases. Clearly further investigation is required but it is hoped that this may become a very important tool for early detection of ovarian cancer. In addition, these biomarkers may become an important part of the clinical strategies used in cancer diagnosis, treatment, and imaging. In this chapter, their roles in the early detection and management of ovarian cancer have been discussed. It is hoped that as our understanding of these markers increases, we will see an improvement in the rate of early cancer detection and ultimately increased survival.

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