REVIEW ARTICLE

Clinical biomarkers for detection of ovarian cancer

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ABSTRACT: Between all of the gynecological cancers, ovarian cancer (OvCa) shows high clinical challenge because it is difficult to be detected in early stage and it has the highest mortality proportional to the other gynecologic malignancies. Despite medical advances and the development of diagnostic tools such as biomarkers and detection techniques, OvCa remains a fatal cancer with high progression. There are different types of OvCa based on histological classification; Epithelial Ovarian Cancer (EOC) is the most common, whereas, stromal and germ cell tumors are of lower abundance. EOC is identified in over 80% of women at late-stage with complications include the spread of tumor implants throughout the peritoneal cavity. Early diagnosis of OvCa is helpful in the treatment and promotion of survival rate. Such diagnostic medical methods and biomarkers include vaginal and pelvic examination, diagnostic imaging, serum CA125, and screening tests or a combination used in medical centers, however, it is necessary to find new biomarkers with long-term stability and high specificity and sensitivity to detect OvCa in early stages of disease. This review presents novel and robust biomarkers and methods for detecting OvCa.

Key Words: *Ovarian Cancer; Serum and genetic Biomarkers; Early diagnosis*

ABBREVIATION

OvCa: Ovarian Cancer; EOC: Epithelial Ovarian Cancer; BRCA: Breast Cancer Gene; MAPK: Mitogen Activated Protein Kinase; PI3K: Phosphatidylinositide 3-kinase; CA125: Carbohydrate Antigen 125; ERK: Extracellular Signal Regulated Kinase; MMPs: Matrix Metallopeptidases; HE4: Human Epididymis Protein 4; FDA: Food and Drug Administration; EGFR: Epidermal Growth Factor Receptor; ROMA: Risk of Malignancy Algorithm; ApoA1: Apolipoprotein A1; KLKs: Kallikrein related Peptidases; VCAM-1: Vascular Cell Adhesion Molecules 1; miRNAs: MicroRNAs.

Ovarian cancer is the fifth frequency occurring cancer among women and the leading cause of death among gynecological cancers. The risk factors that are specific to OvCa are positive familial history (transfer of mutant genes can increase the chance of OvCa in succeeding generations) [1], age and menopause [2], breast cancer; women with breast cancer are likely to develop to OvCa because mutant genes such as breast cancer genes 1 and 2 (*BRCA1* and *BRCA2*) are common in this type of cancer [3,4]. Other OvCa risk factors include obesity [5], diet (mainly meats and saturated fats), hormone therapy and some inflammatory diseases such as endometrioses [6]. On the basis of histological classification there are different types of OvCa, EOC is the most common whereas stromal and germ cell tumors are of lower abundance [7]. Epithelial tumors in OvCa classified to two types: type 1, with mutations in genes such as *PTEN, KRAS* and *BRAF* [8] which ultimately increase the expression of Mitogen-Activated Protein Kinase (MAPK) and phosphatidylinositide 3-kinase (PI3K) signaling pathways which lead to proliferation and metastasis of OvCa cells, and type 2 which involves mutations in *P53* [9]. EOC is diagnosed in more than 80% of women at a late-stage, during which spread of tumor implants throughout the peritoneal cavity is observed [10]. In 2012 approximately 15.500 deaths occurred because of OvCa in the US [11]. The metastasis of OvCa cells through the vasculature is unusual and more metastases occur in serosal such as the peritoneum [12]. In this review, we introduce screening algorithms for serum and genetic biomarkers that can be used to diagnose OvCa in its early stages. Some of the biomarkers are still under investigation and have not been implemented for clinical use. Information about OvCa in the human within the past 10 years was obtained from the PubMed and Scopus databases, and the gynecological text books.

APPROACHES FOR DIAGNOSING OVCA

The early diagnosis of OvCa and corresponding efforts from the medical community in this regard are important in increasing patient survival and reducing treatment costs. Diagnostic imaging technologies, such as ultrasonography, and the use of biomarkers and screening tests are the available methods for detecting OvCa.

Vaginal and pelvic examinations

Patients with vaginal or pelvic masses are tested by one or two doctors of registrar grade. An abnormal examination is defined as a palpable pelvic mass of any size that can be clinically distinct from the uterus and gastrointestinal tract. The problem with pelvic examinations is that they are not useful in the distinction of early or premalignant lesions from a normal ovary. Evidence showed that the sensitivity and specificity of detecting a pelvic mass on the basis of a pelvic exam alone are about 40% and 90%, respectivelym [13,14].

Diagnostic imaging

Transvaginal ultrasonography, sonography and color Doppler are the most frequently adopted approach techniques for detecting and assessing OvCa and tumor vascularity. Given the limited sensitivity and specificity of these methods, however, they are usually implemented in combination, along with tumor markers and screening tests [15,16].

Serum biomarkers

CA125

CA125 (carbohydrate antigen 125 or MUC16) is a high-molecular-weight glycoprotein found on the surface of epithelial cells. It is overexpressed in EOC and is a widely used serum biomarker for the monitoring of patients with OvCa. The expression of CA125 is usually low in normal ovaries, but a proteolytic site presented in the structure of CA125 is believed to cause the formation of high invasive characteristics of OvCa cells [17]. The interaction of CA125 with mesothelin on the surface of mesothelial cells mediates cell adhesion. Therefore, it is proposed that CA125 may contribute to the metastasis of ovarian cancer [18]. Two of the most important features of OvCa are invasion and metastasis. Expression of PI3K/AKT and Extracellular Signal-Regulated Kinase (ERK) signaling pathways are stimulated by overexpression of CA125 in OvCa. Consequently cause enhances the expression of Matrix Metallopeptidases (MMPs). MMPs, a family of more than 20 zinc-dependent enzymes, are known to degrade the extracellular matrix and basement membrane components [19]. MMPs are not only a critical component of cancer development but also play important roles in cancer cell invasion and metastasis. They are correlated with OvCa, with patients exhibiting elevated levels of MMP-2, MMP-7, and MMP-9 [20,21]. CA125 is measured in serum, values higher than 35 U/mL is significant levels that indicate the need for follow-up; an important consideration, is that serum CA125 levels also increase in some physiological conditions such as pregnancy, and certain diseases such as uterine fibroids, endometriosis, and pelvic inflammation [22]. The use of CA125 as a biomarker results in a 47% likelihood of detecting OvCa in its early stage and a likelihood of 80%-90% in late stages. The sensitivity of CA125 is about 50%-60%, and its specificity is about 90% [23]. Because of the low sensitivity of a CA125 test, physicians usually request its combination with other biomarkers. The

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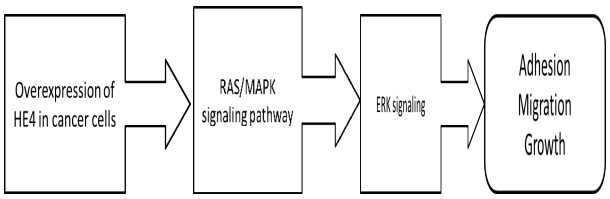

Figure 1) *Signaling pathways which affected by overexpression of HE4 in cells of OvC.*

most commonly used supplement to CA125 is human epididymis protein 4 (HE4) [24].

HE4

HE4 is coded by the *WFD2* gene, with a molecular weight of 25 kDa. It belongs to a "four-disulfide core" family that contains various groups of small acid- and heat-stable proteins with different functions. It circulates in the bloodstream and can be detected via enzyme immunoassay [25]. Research suggested that HE4 functions as a protease inhibitor similar to other whey proteins, such as elafin and secretory leukocyte protease inhibitor [26,27]. In 2009, the Food and Drug Administration (FDA) approved the use of HE4 for monitoring of women to diagnose EOC. Scientists demonstrated that HE4 is overexpressed in EOC but not in other types of OvCa [28]. The detection of serum HE4 levels is always requested by physicians to be combined with the use of CA125 for the early detection of OvCa, because CA125 alone might be elevated in certain benign lesions and other diseases. HE4-based early OvCa detection presents a sensitivity of about 90% and a specificity of 72.9%; the combination of HE4 and CA125 can distinguish between benign and malignant conditions and improve the early detection of cancer [29]. The overexpression of HE4 in OvCa cells can irritate the Epidermal Growth Factor Receptor (EGFR) and MAPK signaling pathway and induce tumor cell adhesion, migration, and growth [30] (Figure 1). The reference range of serum HE4 in normal conditions is less than 140 pmol/L. Serum HE4 levels also increase with pregnancy, aging, and menopausal status.

Risk of Ovarian Malignancy Algorithm (ROMA)

The Risk Of Malignancy Algorithm (ROMA), which was approved by the FDA in 2011, is used to measure CA125 and HE4 simultaneously (with menopausal status) for the detection of epithelial OvCa in women presenting with pelvic masses. The sensitivity and specificity of ROMA are higher than those of CA125 alone (i.e., 90.7% and 93.1%, respectively). In premenopausal women, a ROMA score ≥1.31 reflects a high risk of ovarian malignancy, but in postmenopausal women, such risk is reflected by a ROMA score ≥2.71 [31,32].

OVA1 screening test

In OVA1 screening, which was approved by the FDA in 2009, a woman presenting with a pelvic mass is tested for the presence of several biomarkers in the blood, including CA125, apolipoprotein A1 (ApoA1), β2 microglobulin, and transferrin and transtertin. The patient obtains a score ranging from 0 to 10. The sensitivity of OVA1 screening is 93% [33,34]. An OVA1 test is intended as an aid to the further assessment of the probability that malignancy is present when a physician's independent clinical and radiological evaluation does not show malignancy. It can be used by a patient's primary physician to decide on whether the patient should be referred to a gynecologist or a gynecologic oncologist.

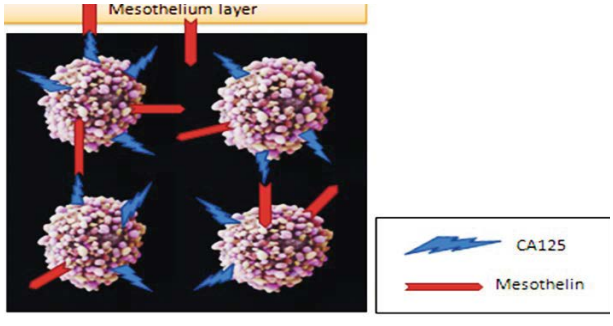

Figure 2) *Attachment between CA125 and mesothelin mediate peritoneal metastasis of OvCa.*

Mesothelin

Mesothelin is a glycoprotein with a molecular weight of 40 kDa and is expressed on the surface of mesothelial cells. Serum and urine levels of mesothelin can be elevated in some cancers, such as mesothelioma, ovarian cancer, and pancreatic cancer [35,36]. Studies demonstrated that CA125 and mesothelin on the surface of cancer cells can interact with each other and mediate cell attachment. Such interaction facilitates peritoneal metastasis in OvCa [6,19,20] (Figure 2). The overexpression of mesothelin in mesothelioma n OvCa influences the MAPK, PI3K, and NF-ΔB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathways, in which changes can inhibit apoptosis and cause cell proliferation in cancer cells [37] (Figure 3). Mesothelin alone has a 60% sensitivity and a 98% specificity in cancer detection, but in combination with CA125, improved OvCa detection is achieved [38]. The measurement of mesothelin in urine is more effective than performing a serum assay; urine assays exhibit 95% specificity in early-stage detection for OvCa patients [39]. Some factors, such as age, smoking, and body mass index, affect mesothelin expression levels [40]. Mesothelin in serum and urine can be measured by Enzyme Linked Immunosorbent Assay (ELISA).

Kallikrein-related peptidases (KLKs)

Kallikreins are a group of serine proteases with proteolytic functions (molecular weight=30 kDa) that play different roles in the human body. A cluster of genes located on chromosome 19q13 encode 15 related serine proteases. This is the largest contiguous cluster of proteases within the human genome. The cleavage of propeptide is needed to activate mature proteins [41,42]. Research on SKOV3 EOC cell lines indicated that some kallikreins, such as KLK4, KLK7, and KLK6, are overexpressed and play certain roles in OvCa, including extracellular matrix cleavage, peritoneal invasion, cell shedding, spheroid formation, and chemoresistance [43,44] (Figure 4). KLKs exhibit low sensitivity in the early detection of OvCa, but a 90% specificity and a 72% sensitivity have been reported for KLK combination with CA125 [42]. Serum levels more than 4.4 mg/L indicate poor prognosis in patients [45]. KLKs are measured in serum through ELISA.

Osteopontin (OPN)

OPN is an adhesive glycoprotein synthesized by vascular endothelial cells

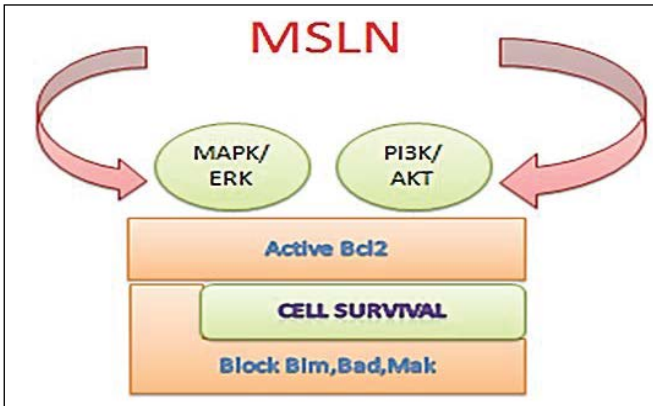

Figure 3) *Effects of overexpression mesothelin in cell survival and proliferation of cancer cells.*

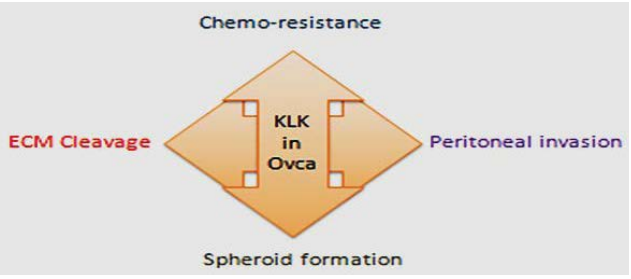

Figure 4) *Different functions of overexpressed kallikreins in extension of OvCa.*

and osteoblasts. Its function is related to bone remodeling and immunity. OPN was first identified in bones, but immune cells can also express this protein [46]. Through the Arg-Gly-Asp (RGD) domain, OPN can bind to αvβ3 integrin and fibronectin and advance cell survival and the migration of cancerous cells. OPN has a sensitivity of 83.3% in the detection of OvCa, and this sensitivity improves when the protein is combined with CA125 [47]. Its specificity is low, but Mor et al. demonstrated that blood tests based on four analytes, namely, leptin, prolactin, OPN, and insulin-like growth factor-II, can distinguish between disease-free and cancerous patients, including those diagnosed with stages I and II disease [48].

ApoA1

ApoA1 is part of the family of high-density lipoproteins. Reduced ApoA1 concentrations are related to OvCa. Decreased ApoA1 levels were previously reported in the serum of patients with OvCa, but the mechanism through which this effect occurs is unclear. Researchers suggested that such reduction is linked to damage in cellular biomembranes, thus resulting in lipid peroxidation. ApoA1 can serve as a biomarker for the detection of early-stage OvCa; combined with CA125, it presents a sensitivity of 93.9% and a specificity of 95% [49,50].

Vascular cell adhesion molecules 1 (VCAM-1)

VCAM-1 is a receptor found on the surface of endothelial and mesothelial cells. It regulates attachments in leukocyte and extravasations of immune cells at inflammation sites and is overexpressed in the mesothelium layer of the ovaries in women with OvCa (in comparison with healthy women). VCAM-1 can stimulate cancerous OvCa cells to move to the peritoneal cavity. When VCAM-1 is combined with other biomarkers, it has a sensitivity of 86% in the detection of early-stage cancers and a sensitivity of 93% and a specificity of 98% in the detection of late stages of OvCa [12,51].

Genetic markers

The accumulation of mutations in tumor suppressor genes and genes related to the cell cycle, DNA repair, growth factor receptor genes, or cell adhesion leads to the uncontrolled proliferation, survival, and metastasis of cancerous cells in different types of cancers, such as OvCa. Genetic biomarkers aid early detection; disease subtyping, staging, and prognosis; and the selection of effective therapies. Some of the most important genes that mutate in OvCa are as follows:

BRCA1

BRCA1 has an important role in the familial transition of OvCa. The gene is located in chromosome 17q12-21, and its preserved function is genome repair. Studies demonstrated that the massive hyper-methylation of *BRCA1* occurs in ovarian tumors. Hyper-methylation causes a 12% to 16% decrease in expression and is strongly associated with diminishing RNA and *BRCA1* protein concentrations, especially in EOC. Gene hyper-methylation is also related to poor OvCa prognosis [3,52-54].

P53

P53 is a tumor suppressor gene that is implicated in the cell cycle and apoptosis. Studies reported that mutations in P53 exist in about 50% of OvCa cases [55]. It serves as a good marker for assessing the metastatic potential of OvCa and can distinguish between EOC ovarian tumors from other types of OvCa. Mutations of *P53* increase in all stages of ovarian tumors [56].

KRAS

KRAS, which is a membrane of the RAS protein, is a GTPase and an early player in many signal transduction pathways. The normal *KRAS* protein performs a necessary role in normal tissue signaling, and the mutation of a *KRAS* gene is a necessary step in the development of many cancers [57]. Proto-oncogene *KRAS* is mutated in about 25% of human cancers. *KRAS* mutations are found in approximately 40% of patients with type I EOC tumors. In most cases, these mutations are missense mutations at position 12, 13, or 61, and they result in the constitutive activation of the *KRAS* signaling pathway. In one study, *KRAS* mutations were related to poor prognosis for metastatic type I tumors [58]. By contrast, another study reported favorable outcomes for low-grade tumors with *KRAS* mutations [59].

EGFR

EGFR is a kind of tyrosine receptor kinase for an epidermal growth factor that is substantially important for normal cell function. Mutation in this receptor may contribute to the transformation of cellular phenotypes into tumor cells with high proliferation and may increase survival rate [60]. EGFR mutates in 70% of ovarian carcinoma cases. Mutations cause the overexpression of EGFR and the overexpression of downstream factors, such as the AKT signaling pathway. An EGFR/AKT mutant can cause tumor infiltration, metastasis, and angiogenesis. The overexpression of EGFR and AKT signaling is related to aggressive forms of OvCa and poor prognosis [61,62]. In OvCa, mutations in other genes, such as RAS-association domain family 1 [63], opioid binding protein/cell adhesion molecule-like gene [64], aplysia RAS homology member I [65], ARID1a [66], and *BRAF* [67], occur. The percentage of mutations in these genes is low and needs further research.

MicroRNAs (miRNAs)

MicroRNAs are a type of non-coding RNA of about 21 to 24 nucleotides in length and act in the post-transcriptional regulation of gene expression. Typically, miRNAs interact with specific mRNAs through complementary base pairing, thereby influencing the translation or stability of a target mRNA molecule [68,69]. Some investigated microRNAs in OvCa that can be used as biomarkers for the detection of OvCa include miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214 [70]. Studies compared the exosomes isolated from benign and malignant tumors of OvCa and found that miRNA profiles in these groups differed [70,71]. In research on the OVCAR3 cell line, higher levels of miR-203 and miR-205 were found in malignant OvCa than in normal tissue [68], suggesting that that miRNAs can be used as biomarkers for detecting early stages of OvCa and determining prognosis.

CONCLUSION

Ovarian carcinoma is a progressive tumor with low survival rates; it's fifth common cancer in women. Thus, early diagnosis and good screening tests is important in treatment. An ideal screening test characterize with high sensitivity to diagnose correctly of patients and good specificity in order to prevent false positive results. CA125 a tumor marker with ultrasonography are selective diagnostic test for detection of ovarian cancer but these tests detect only 30% to 45% of women in early stage of OvCa; however CA125 increase in some non-malignancy conditions for examples benign ovarian diseases and endometriosis, so it has low sensitivity for detection in early stage and not enough potential as a single biomarker for screening of OvCa, therefore the majority of patients detect in advance stages. Hence variety of techniques includes proteomics and gene-expression assay and different biomarkers use in laboratories to improve the positive predictive value and better diagnosis.

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