Review Article

A Review on Biomarkers accessible for the life threatening Ovarian Cancer

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A B S T R A C T

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Ovarian cancer has the highest fatal outcome ratio of all gynecologic malignancies. This is ascribed to the lack of early warning signs and sharpened early detection techniques. Another problem impeding the successful management of the disease is the lack of prognostics. One of the most promising ways towards the enhancement in diagnosis and surveillance of ovarian cancer is the identification of serum markers. A major complication in finding a diagnostic biomarker is the terrific molecular heterogeneity that exists for nearly all human cancer, signifying that simultaneous screening of a patient specimen for multiple biomarkers. DNA chip technologies deal with this problem at the genomic level, and provide accessibility to gene expression profiles. On the other hand, since proteins are, the mediators of a cell's function, the study of the changes in proteins result from cancer, would appear to be a valuable source of potential cancer biomarkers. So this communication is ultimately intended to discuss about the biomarkers on hand for the earlier diagnosis of ovarian cancer.

Introduction

Ovarian cancer is a key cause of morbidity and mortality, especially in the middle aged women. It is particularly deadly gynecologic malignancy. It ranks among the top ten diagnosed cancers and top five deadliest cancers in most countries (1) (Ferlay *et al.,* 2010). During the year 2002, it was ranked third in frequency (4.1%) among all cancers in women, with an estimated 2, 04, 499 new cases occurring in the world (2) (Parkin *et al.,* 2005). In India, during the period 2004-2005, proportion of ovarian cancer varied from 1.7% to 8.7% of all female cancers in various urban and rural population based registries operating under the net- work of the National Cancer Registry programme

(NCRP) of Indian Council Medical Research. The proportion of this cancer was 6.0% and 7.7% of all cancers among females in rural Barshi and Ahmedabad registry areas (National Cancer Registry Programme, 2008).

The prognosis for ovarian cancer is poor, particularly when the disease is diagnosed in its later stages. Symptoms are unsure and often misdiagnosed. So the majority of patients are only identified in the later stages of the disease. Ovarian cancer is therefore often referred to as "The Silent Killer". A methodical cancer trend analysis helps to

understand the changing cancer risk and how it is in future. It gives clues as to understand the causes of disease and the variation in frequency around different geographical areas. The cancer analysis is very essential for public health and health care planning for prevention and control. In the present communication, an effort has been made to analyze the trends of diagnosis procedures involved in ovarian cancer through various biomarkers.

What is Ovarian Cancer?

Ovarian cancer begins in the ovaries. Ovaries are reproductive glands found only in females (women). The ovaries produce eggs (ova) for reproduction (Fig.1). The eggs travel through the fallopian tubes into the uterus where the fertilized egg implants and develops into a fetus. The ovaries are also the main source of the female hormones estrogen and progesterone. One ovary is on each side of the uterus in the pelvis.

The ovaries are made up of 3 main kinds of cells. Each type of cell can develop into a different type of tumor:

Epithelial tumors start from the cells that cover the outer surface of the ovary. Most ovarian tumors are epithelial cell tumors.

Germ cell tumors start from the cells that produce the eggs (ova). Stromal tumors start from structural tissue cells that hold the ovary together and produce the female hormones estrogen and progesterone.
Most of these tumors are benign and never spread beyond the ovary. Benign tumors can be treated by removing either the ovary or the part of the ovary that contains the tumor. Malignant (cancerous) or low malignant potential ovarian tumors can spread (metastasize) to other parts of the body and can be fatal.

The Risk Factors and Causes for Ovarian Cancer

Risk factors are differing for different types of cancers. For example, strong exposure to tough sunlight is a risk factor for skin cancer. Risk factors do not always mean disease. So there is a need to identify and sharpen methodology to diagnose it earlier.

Pregnancy and taking birth control pills both lower the risk of ovarian cancer. Since both of these things reduce the number of times the ovary releases an egg (ovulation).

There may be some relationship between ovulation and the risk of developing ovarian cancer.

Tubal ligation and hysterectomy lower the risk of ovarian cancer.

Mutagens may enter the body through the vagina; pass through the uterus and fallopian tubes to reach the ovaries. This would explain how removing the uterus or blocking the fallopian tubes affects ovarian cancer risk.

Male hormones (androgens) can cause ovarian cancer.

Some genes contain instructions for controlling when our cells grow and divide. DNA mutations in these genes can lead to the development of cancer.

A small portion of ovarian cancers occur in women with inherited gene mutations linked to an increased risk of ovarian cancer. These include mutations in the *BRCA1* and *BRCA2* genes, as well as the genes related to other family cancer syndromes linked to an increased risk of ovarian cancer, such as *PTEN* (PTEN tumor hamartoma syndrome), *STK11* (Peutz-Jeghers syndrome), *MUTYH* (MUTYH-asociated polyposis, and the

many genes that can cause hereditary nonpolyposis colon cancer (*MLH1*, *MLH3*, *MSH2*, *MSH6*, *TGFBR2*, *PMS1*, and *PMS2)*.Most ovarian cancers have several acquired gene mutations. Research has suggested that tests to identify acquired changes of certain genes in ovarian cancers, like the *TP53* tumor suppressor gene or the *HER2* oncogene, can help to predict a woman's prognosis. The role of these tests is still not certain, and more research is needed. Several studies are in progress to learn the best ways to find ovarian cancer in its earliest stage.

Biomarkers: The Life-Saving Screening Tool to Predict the Ovarian Cancer

Ovarian cancer is not normally diagnosed until it has destroyed the ovaries and rotted other parts of the body. Because the symptoms of ovarian cancer are not obvious. When it diagnosed before the cancer has spread beyond the ovaries, chances of a woman's survival are very good, with about 93% of women surviving at least 5 years. There are some promising biomarkers (Fig.2) available for the life threatening ovarian cancer to do early diagnosis procedures.

Diagnostic Imaging

Transvaginal ultrasonography is the initial diagnostic modality of choice for the evaluation of the adnexa. However, the sensitivity and specificity of transvaginal ultrasonography for the definitive diagnosis of ovarian cancer is limited.

In a screening study from the National Ovarian Cancer Early Detection Program, 4526 women at high risk for ovarian cancer were screened with transvaginal ultrasonography, which demonstrated the limited value of this modality when all ovarian, primary peritoneal and fallopian tube cancers detected in asymptomatic women were stage III (3). In order to improve the efficacy of sonography, the technique that combines grayscale morphologic assessment with tumor vascularity in a diagnostic system is significantly better in ovarian lesion characterization than Doppler arterial resistance measurements, color Doppler flow imaging, or grayscale morphologic information (4). There is a evidence that the use of contrast agents with 3D power Doppler sonography is superior to that of non enhanced sonography (95 vs 86.7%) (5).

CA-125

To date, the CA-125 glycoprotein antigen is the most commonly measured tumor marker for epithelial ovarian tumors, which account for 85–90% of ovarian cancers. CA-125 was first detected using the OC125 murine monoclonal antibody (6). CA-125 was originally developed to monitor patients previously diagnosed with ovarian cancer and not for screening. Alone, CA-125 is only elevated in 47% of women with early-stage ovarian cancer, while CA-125 levels are elevated in 80–90% of advanced-stage ovarian cancers (7). As CA-125 levels are elevated in many benign conditions in premenopausal women, its utility as a tumor marker is more effective in postmenopausal women.

Clinically, CA-125 has been used to follow women diagnosed with ovarian cancer for prognosis, surveillance and optimization of care. However, as CA-125 has been the oldest and one of the best performing biomarkers, a biomarker panel used to detect ovarian cancer in its early stage will include CA-125 (8). Together with the use of CA-125, the focus has incorporated other biomarkers with and without combined imaging techniques and simultaneous evaluation of multiple markers may achieve the required sensitivity and specificity.

Serum Biomarkers are under Evaluation Beside CA-125

Multiple publications have identified potential biomarkers for the detection of ovarian cancer beside CA-125.

HE4

This protein has a WAP-type four disulfide-core and is encoded by the *WFDC2* gene on chromosome 20q1213.1 (9). It is elevated in ovarian cancer and there is increased HE4 mRNA expression in different types of EOC's (10, 11). HE4 is over expressed in specific subtypes of ovarian cancer, 100% in endometrioid and 93% in serous ovarian cancer, possibly enabling one to distinguish among several tumor types. HE4 is also noted as a potential marker for adenocarcinoma of the endometrium (12). Recently, two meta-analysis studies independently published a similar conclusion that HE4 is a valuable marker in the diagnosis of ovarian cancer (13). HE4 has recently obtained US FDA approval for monitoring of disease recurrence or progression but not for screening. HE4 was advanced to CA-125 in separating benign, borderline ovarian tumors, cancers of the fallopian tubes, as well as early-stage EOC.

Mesothelin

Mesothelin is a glycosyl phosphatidylinositol-linked cell surface molecule expressed by mesothelial cells. Mesothelin level can be measured in urine and elevated in mesothelioma, pancreatic and ovarian cancers. Elevated serum mesothelin was detected in 60% of ovarian cancers with a specificity of 98% (14). Mesothelin may aid in the peritoneal implantation and metastasis of tumors through its interaction with mucin MUC16 (CA-125). Combination of mesothelin and

CA-125 is being detected more in ovarian cancers than each marker alone. Mesothelin was elevated in 42% of urine assays in comparison with 12% of serum assays of early-stage ovarian cancer patients at 95% specificity (15).

Transthyretin

Transthyretin (TTR) is prealbumin indicator of nutritional status, and an acute phase reactant involved in tumor development (16). TTR is the major carrier for serum thyroxine and facilitates the transport of retinol via retinol binding protein. The serum level of full-length TTR was down regulated among patients with later stage ovarian cancer relative to that in healthy controls and patients with colorectal, breast or prostate cancer. It was identified that a truncated form of TTR showed a lack of the N-terminal ten amino acids. It has been reported that TTR is decreased in EOC. When combined with CA-125, ApoA1 and transferrin in a study of 358 serum samples, it yielded a sensitivity and specificity of 96% and 98%, respectively, for detection of early-stage ovarian cancer (17).

ApoA1

ApoA1 is constituent of high-density lipoproteins. Exogenous ApoA1 prevents tumor development in mice while lowered ApoA1 concentrations are associated with ovarian cancer. Decreased ApoA1 levels were previously reported in the serum of patients with ovarian cancer. The mechanism of this association remains unclear at this time; however, it has been proposed to be associated with free radical-mediated damage to cellular biomembranes resulting in lipid peroxidation (18). ApoA1 can be a biomarker for the detection of early-stage ovarian cancer, and may also be a promising therapeutic agent for the

treatment of ovarian cancer (19). When ApoA1 was combined with CA-125 and TTR, the overall sensitivity and specificity were significantly improved in the receiver operating characteristic curve. For stage I and II detection, the sensitivity was 93.9% at 95% specificity (20).

VCAM

VCAM-1 is a cell surface receptor expressed on activated endothelial and mesothelial cells, which functions to regulate leukocyte attachment and extravasation at sites of inflammation. VCAM-1 protein was found to be preferentially expressed on the mesothelium of ovarian cancer patients compared with the mesothelium of women without cancer. Ovarian cancer cell invasion of the mesothelium was quantified using a coculture assay system. Inhibition of VCAM-1 function in the coculture system decreased ovarian cancer transmigration of the mesothelium. When VCAM-1 was combined with CA-125 and other biomarkers, the panel yielded sensitivity of 86% for early-stage and 93% sensitivity for late-stage ovarian cancer at 98% specificity (21).

IL-6 & IL-8

IL-6 is an acute phase reactant and promotes inflammation. High levels of IL-6 were found in 50% of 114 patients with primary ovarian cancer. IL-6 is also correlated with poor prognosis and clinical outcome. A multimarker test which combines CA-125 with C-reactive protein, serum amyloid A (SAA), IL-6 and IL-8, was reported to have 94.1% sensitivity and 93.1% specificity for detection of ovarian cancer (22). IL-8 is an acute phase reactant recruiting leukocytes. IL-6 and IL-8 are pleomorphic cytokines that have been implicated in aspects of tumor growth, disease progression and/or treatment. Analysis of concentrations of

anti-IL-8 IgG assisted for the prediction of early ovarian cancer (stages I and II) with 98% specificity and 65.5% sensitivity (22).

B7-H4

B7-H4 is a 282 amino acid protein, expressed on the surface of a variety of immune cells and functions as a negative regulator of T-cell responses. It may promote malignant transformation. B7-H4 expression was consistently higher in serous, endometrioid and clear cell ovarian carcinomas compared with mucinous subtypes or normal somatic tissues. These findings indicated that B7-H4 should be further investigated as a potential serum biomarker for ovarian cancer . In early-stage patients, the sensitivity at 97% specificity increased from 52% for CA-125 alone to 65% when used in combination with B7-H4 (23).

Serum Amyloid A

SAA is an acute phase reactant, which is expressed primarily in liver as a modulator of inflammation and metabolism, and transport of cholesterol. Expression of SAA was increased as epithelial cells progressed through benign and borderline adenomas to primary and metastatic adenocarcinomas. Real-time PCR analysis confirmed the overexpression of the *SAA1* and *SAA4* genes in ovarian carcinomas compared with normal ovarian tissues . When combined with CA-125 the panel yielded an accuracy rate of 95.2% for ovarian cancer screening (24). Combination of CA-125, transferrin, TTR and ApoA1 using proteomic analysis yielded a sensitivity of 89% at specificity of 92% for early detection screening (25).

Osteopontin

Osteopontin (OPN) is an adhesive glycoprotein related to bone remodeling as

well as immune function. It is synthesized by vascular endothelial cells and osteoblasts. Using osteopontin in combination with leptin, prolactin and IGF, 96% of sensitivity and a 94% of specificity was reported (26). Similar to mesothelin, a fragment of osteopontin can be detected in the urine of ovarian cancer patients.

Kallikreins

Kallikreins (KLK) are a family of serine proteases that regulate proteolytic cascades. KLK promote or inhibit cancer cell growth, angiogenesis, invasion and metastasis by proteolytic processing of growth factors, angiogenic factors and extracellular matrix components. KLK6 and KLK10 were elevated in ovarian cancer tissues that had low levels of CA-125 (27), elevated KLK11 was found in 70% of ovarian cancer sera at a specificity of 95%.

OVX1

OVX1 is an epitope of high molecular weight mucin-like glycoprotein, also an ovarian or breast cancer related glycoprotein antigen. OVX1 is increased in 70% of ovarian cancers; also increased in 59% of ovarian cancers with normal CA-125 level (27).

VEGF

VEGF is a glycosylated angiogenesis mediator with serum levels significantly higher in patients with ovarian or gastrointestinal carcinoma than in healthy individuals, and the VEGF concentrations in sera from patients with metastatic disease were higher than those in sera from patients with localized tumors (28). When combined with CA-125 the sensitivity is 77% and specificity is 87% (29). VEGF inhibition has been shown to inhibit tumor growth and ascites production, and to suppress tumor invasion and metastasis.
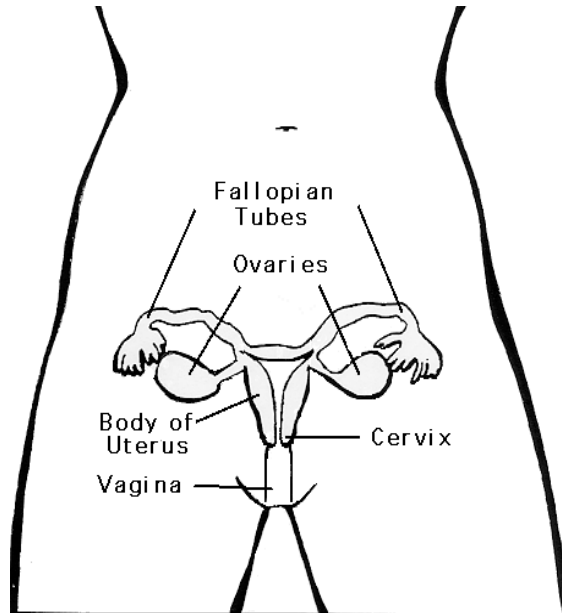
Other Biomarkers

The following are other markers that have been evaluated as potential biomarkers for detecting EOC: (30-37)

AGR-2
Inhibin
M-CSF
uPAR
EGF receptor
Matrix metalloproteinases
Lysophosphatidyl acid

miRNAs

An attention has been recently focused on miRNAs. miRNAs consist of approximately 22 nucleotides of noncoding RNAs that post-transcriptionally regulate mRNA translation into the protein of a large number of target genes (38). miRNAs globally influence gene expression, which determines cellular behavior by targeting com-plementary gene transcripts for translational repression or degradation of the mRNA tran-script . In addition, miRNA signatures of ovarian cancer could be helpful to distinguish the tumors based on their histological subtype, miR200b, miR-141, miR- 21, miR-203 and miR-205 were upregulated in endometrioid and serous subtypes, whereas miR-145 was downregulated in both serous and clear cell carcinomas and miR-222 in both endometrioid and clear cell carcinomas (39). furthermore, miRNAs are promising biomarkers as they are remarkably stable to allow isolation and analysis from tissues and from blood in which they can be found as free circulating nucleic acids and in mononuclear cells.

Fig.1 Reproductive System of Female

Fig.2 Biomarkers in Ovarian Cancer
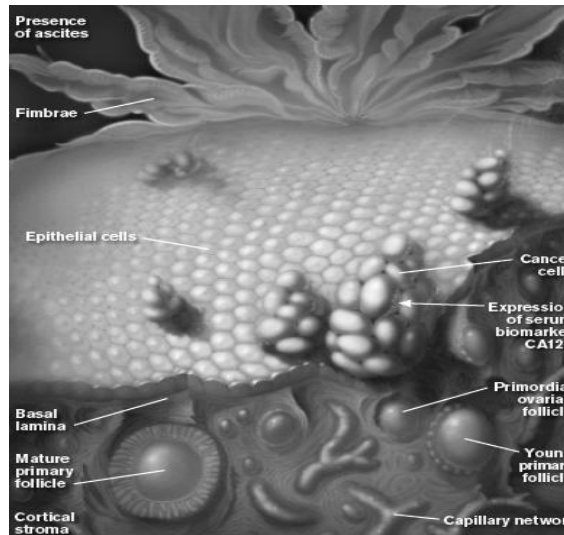
Ovarian cancer is the most lethal gynecological malignancy due to the lack of highly sensitive and specific screening tools for detection of early-stage disease. Recent developments in identification of potential biomarkers and application of technologies especially bioinformatics tools may improve the positive predictive value. It is anticipated that the detection of early-stage EOC will be achieved using a combination of serum biomarkers in conjunction with imaging technologies to improve women's healthcare.

References

1. Cragun JM. Screening for ovarian cancer. *Cancer Control* 18(1), 16–21 (2011).

2. Myers ER, Bastian LA, Havrilesky LJ *et al.* Management of adnexal mass. *Evid. Rep. Technol. Assess. (Full Rep.)* (130), 1–145 (2006).

3. Fishman DA, Cohen L, Blank SV *et al.* The role of ultrasound evaluation in the detection of early-stage epithelial ovarian cancer. *Am. J. Obstet. Gynecol.* 192(4), 1214–1221; discussion 1221–1212 (2005).

4. Kupesic S, Kurjak A. Contrast-enhanced, three-dimensional power Doppler sonography for differentiation of adnexal masses. *Obstet. Gynecol.* 96(3), 452–458 (2000).

5. Marret H, Sauget S, Giraudeau B *et al.* Contrast-enhanced sonography helps in discrimination of benign from malignant adnexal masses. *J. Ultrasound Med.* 23(12), 1629–1639; quiz 1641–1642 (2004).

6. Bast RC Jr, Klug TL, St John E *et al.* A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N. Engl. J. Med.* 309(15), 883–887 (1983).

7. American College of Obstetricians and Gynecologists. *PROLOG Gynecology and Surgery (6th Edition)*. American College of Obstetricians and Gynecologists, Washington, DC, USA (2009).

8. Drapkin R, Von Horsten HH, Lin Y *et al.* Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res.* 65(6), 2162–2169 (2005).

9. Moore RG, Brown AK, Miller MC *et al.* The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol. Oncol.* 108(2), 402–408 (2008).

10. Fishman DA, Liu Y, Ellerbroek SM, Stack MS. Lysophosphatidic acid promotes matrix metalloproteinase (MMP) activation and MMP-dependent invasion in ovarian cancer cells. *Cancer Res.* 61(7), 3194–3199 (2001).

11. Jeong KJ, Cho KH, Panupinthu N *et al.* EGFR mediates LPA-induced proteolytic enzyme expression and ovarian cancer invasion: inhibition by resveratrol. *Mol. Oncol.* (2012).

12. Huhtinen K, Suvitie P, Hiissa J *et al.* Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. *Br. J. Cancer* 100(8), 1315–1319 (2009).

13. Wu L, Dai ZY, Qian YH, Shi Y, Liu FJ, Yang C. Diagnostic value of serum human epididymis protein 4 (HE4) in ovarian carcinoma: a systematic review and meta-analysis. *Int. J. Gynecol. Cancer* 22(7), 1106–1112 (2012).

14. Mcintosh MW, Drescher C, Karlan B *et al.* Combining CA 125 and SMR serum markers for diagnosis and early detection of ovarian carcinoma. *Gynecol. Oncol.* 95(1), 9–15 (2004).

15. Badgwell D, Lu Z, Cole L *et al.* Urinary mesothelin provides greater sensitivity for early stage ovarian cancer than serum mesothelin, urinary hCG free beta subunit and urinary hCG beta core fragment. *Gynecol. Oncol.* 106(3), 490–497 (2007).

16. Schweigert FJ, Sehouli J. Transthyretin, a biomarker for nutritional status and ovarian cancer. *Cancer Res.* 65(3), 1114; author reply 1114 (2005).

17. Nosov V, Su F, Amneus M *et al.* Validation of serum biomarkers for detection of early-stage ovarian cancer. *Am. J. Obstet. Gynecol.* 200(6), 639 e1–e5 (2009).

18. Gadomska H, Grzechocinska B, Janecki J, Nowicka G, Powolny M, Marianowski L. Serum lipids concentration in women with benign and malignant ovarian tumours. *Eur. J.*

Obstet. Gynecol. Reprod. Biol. 120(1), 87–90 (2005).

19. Su F, Kozak KR, Imaizumi S *et al.* Apolipoprotein A-I (apoA-I) and apoA-I mimetic peptides inhibit tumor development in a mouse model of ovarian cancer. *Proc. Natl Acad. Sci. USA* 107(46), 19997–20002 (2010).

20. Kim YW, Bae SM, Lim H, Kim YJ, Ahn WS. Development of multiplexed bead-based immunoassays for the detection of early stage ovarian cancer using a combination of serum biomarkers. *PloS ONE* 7(9), e44960 (2012).

21. Yurkovetsky Z, Skates S, Lomakin A *et al.* Development of a multimarker assay for early detection of ovarian cancer. *J. Clin. Oncol.* 28(13), 2159–2166 (2010).

22. Edgell T, Martin-Roussety G, Barker G *et al.* Phase II biomarker trial of a multimarker diagnostic for ovarian cancer. *J. Cancer Res. Clin. Oncol.* 136(7), 1079–1088 (2010).

23. Ahmed N, Oliva KT, Barker G *et al.* Proteomic tracking of serum protein isoforms as screening biomarkers of ovarian cancer. *Proteomics* 5(17), 4625–4636 (2005).

24. Slack-Davis JK, Atkins KA, Harrer C, Hershey ED, Conaway M. Vascular cell adhesion molecule-1 is a regulator of ovarian cancer peritoneal metastasis. *Cancer Res.* 69(4), 1469–1476 (2009).

25. Su F, Lang J, Kumar A *et al.* Validation of candidate serum ovarian cancer biomarkers for early detection. *Biomark. Insights* 2, 369–375 (2007).

26. Mor G, Visintin I, Lai Y *et al.* Serum protein markers for early detection of ovarian cancer. *Proc. Natl Acad. Sci. USA* 102(21), 7677–7682 (2005).

27. Rosen DG, Wang L, Atkinson JN *et al.* Potential markers that complement expression of CA125 in epithelial ovarian cancer. *Gynecol. Oncol.* 99(2), 267–277 (2005).

28. Kraft A, Weindel K, Ochs A *et al.* Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. *Cancer* 85(1), 178–187 (2009).

29. Li L, Wang L, Zhang W *et al.* Correlation of serum VEGF levels with clinical stage, therapy efficacy, tumor metastasis and patient survival in ovarian cancer. *Anticancer Res.* 24(3b), 1973–1979 (2004).

30. Rice GE, Edgell TA, Autelitano DJ. Evaluation of midkine and anterior gradient 2 in a multimarker panel for the detection of ovarian cancer. *J. Exp. Clin. Cancer Res.* 29, 62 (2010).

31. Edgell TA, Barraclough DL, Rajic A *et al.* Increased plasma concentrations of anterior gradient 2 protein are positively associated with ovarian cancer. *Clin. Sci. (Lond.)* 118(12), 717–725 (2010).

32. Robertson DM, Pruysers E, Jobling T. Inhibin as a diagnostic marker for ovarian cancer. *Cancer Lett.* 249(1), 14–17 (2007).

33. Kawamura K, Komohara Y, Takaishi K, Katabuchi H, Takeya M. Detection of M2 macrophages and colony-stimulating factor 1 expression in serous and mucinous ovarian epithelial tumors. *Pathol. Int.* 59(5), 300–305 (2009).

34. Woo HH, Laszlo CF, Greco S, Chambers SK. Regulation of colony stimulating factor-1 expression and ovarian cancer cell behavior *in vitro* by miR-128 and miR-152. *Mol. Cancer* 11(1), 58 (2012).

35. Hildenbrand R, Gandhari M, Stroebel P, Marx A, Allgayer H, Arens N. The urokinase-system – role of cell proliferation and apoptosis. *Histol. Histopathol.* 23(2), 227–236 (2008).

36. Henic E, Borgfeldt C, Christensen IJ, Casslen B, Hoyer-Hansen G. Cleaved

forms of the urokinase plasminogen activator receptor in plasma have diagnostic potential and predict postoperative survival in patients with ovarian cancer. *Clin. Cancer Res.* 14(18), 5785–5793 (2008).

37. Zeineldin R, Muller CY, Stack MS, Hudson LG. Targeting the EGF receptor for ovarian cancer therapy. *J. Oncol.* 2010, 414676 (2010).

38. Siwak DR, Carey M, Hennessy BT *et al.* Targeting the epidermal growth factor receptor in epithelial ovarian cancer: current knowledge and future challenges. *J. Oncol.* 2010, 568938 (2010).

39. Lopez J, Percharde M, Coley HM, Webb A, Crook T. The context and potential of epigenetics in oncology. *Br. J. Cancer* 100(4), 571–577 (2009).