Update on Epithelial Ovarian Cancer (EOC). Types, origin, molecular pathogenesis, and biomarkers. (Review Article)

Salina Yahya Saddick

Faculty of Science, Department of Reproductive Biology, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia, Jeddah 21551 P O Box 42671.
sysaddick@yahoo.co.uk

Abstract: Ovarian cancer remains the most lethal gynecological malignancy due to the lack of highly sensitive and specific screening tools for detection of early-stage disease. The OSE provides the progenitor cells for 90% of human ovarian cancers. Recent morphologic, immunohistochemical and molecular genetic studies have led to the development of a new paradigm for the pathogenesis and origin of epithelial ovarian cancer (EOC) based on a dualistic model of carcinogenesis that divides EOC into two broad categories designated Types I and II which are characterized by specific mutations, including *KRAS, BRAF, ERBB2, CTNNB1, PTEN PIK3CA, ARID1A,* and *PPPR1A*, which target specific cell signaling pathways. Type 1 tumors rarely harbor *TP53*. type I tumors are relatively genetically stable and typically display a variety of somatic sequence mutations that include *KRAS*, *BRAF*, *PTEN*, *PIK3CA CTNNB1* (the gene encoding beta catenin), *ARID1A* and *PPP2R1A* but very rarely *TP53* . The cancer stem cell (CSC) hypothesis postulates that the tumorigenic potential of CSCs is confined to a very small subset of tumor cells and is defined by their ability to self-renew and differentiate leading to the formation of a tumor mass. Potential protein biomarker *miRNA,* 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. Recently, genomic anaylsis have identified biomarkers and potential therapeutic targets for ovarian cancer namely, FGF18 which plays an active role in controlling migration, invasion, and tumorigenicity of ovarian cancer cells through NF-κB activation, which increased the production of oncogenic cytokines and chemokines. This review summarizes update information on epithelial ovarian cancers and point out to the most recent ongoing research.

[Salina Yahya Saddick. **Update on Epithelial Ovarian Cancer (EOC). Types, origin, molecular pathogenesis, and biomarkers. (Review Article).** *Life Sci J* 2014;11(4):1-16]. (ISSN:1097-8135). http://www.lifesciencesite.com. 1

Keyword: Epithelial Ovarian Cancers, Somatic Sequence Mutations, Cancer Stem Cell (CSC), Potential protein biomarker, genomic analysis and FGF18 biomarker.

Introduction

Ovarian Surface Epithelium (OSE) is a complete layer of epithelial cells covered the mammalian ovary and adapt to the cyclical changes that occur within the ovary before and after ovulation [1]. The OSE layer is separated from the underlying structure by a basement membrane and underneath is the tunica albugenea. Abnormal changes in the OSE can result in ovarian cancer (OC) which is the fifth most common cancer in women [2]. Despite its clinical relevance there is still little known about the features of OSE cells and how they respond to local ovarian factors and cyclical changes. OSE cells are generated from the mesodermally derived epithelial lining of the intra-embryonic coelom. The OSE has characteristic differences from other epithelia, for example expression of Cancer Antigen 125 (CA125), a surface glycoprotein of unknown function, in the adult is localized in the oviductal, endometrial and endocervical epithelium and some extraovarian epithelia, but not in the OSE [3 ;4]. Therefore, either OSE has never acquired this differentiation marker or it is lost early in development [5]. CA125 is, however, expressed in tumorigenic OSE suggesting that the original coelomic characteristic has been retained by OSE but is only expressed under pathological conditions [6]. OSE is believed to be the source of at least some of the ovarian granulosa cells [7; 8].

The OSE is important in maintaining the health and structure of the ovary, it occupies the entire ovarian lining, and varies in morphology from simple squamous to cuboidal to low pseudo stratified columnar [9]. OSE cells are held together by zona occludens along their lateral surfaces. Several typical proteins are produced by OSE cells which are different from the other extraovarian epithelia [9]. E-cadherin (epithelial) is a typical calcium-dependent adhesion protein produced in resting surface epithelia of oviduct, endometrium, endocervix and the ovary of mouse and porcine species [10;11] while, it is N-cadherin (neural) that is found in human OSE cells and the granulosa cell lining of the growing follicles [12;13]. It is believed that when surface epithelial cells undergo transformation into a columnar shape due to

metaplastic or neoplastic differentiation, particularly in inclusion cysts and crypts, the E-cadherin starts to co-express along with N-cadherin [14; 15]. Another form of adhesion protein, P-cadherin (placental) is also typical of Müllerian origin epithelia, but is absent in resting OSE, and occasionally expresses in adenocarcinoma cell lines derived from cancerous OSE cells. Thus, it appears that E-/P-cadherins are induced during OSE neoplasia [16; 17].

The dynamic nature of the OSE morphology and its lack of tissue-specific markers make it almost inconspicuous [18]. Nonetheless, the OSE is responsible for nutrient transport and postovulatory epithelial wound repair [19]. Despite performing its important endocrine and reproductive functions, the OSE provides the progenitor cells for 90% of human ovarian cancers [20]. Ovarian cancer ranks first among the cause of death from a gynecological malignancy and accounts for more than 3% of cancer related deaths in women.

Despite their inconspicuous appearance, OSE participates in transporting and exchanging nutrients and other bioactive metabolites from the peritoneal cavity and ovary. At pre-ovulation, OSE in proximity to rupture site undergoes apoptotic cell death and the wound caused by ovulation is repaired by highly proliferating OSE cells from surroundings of the ruptured follicle [21]. OSE cell proliferation also occurs at post-ovulatory phase especially in post-menopausal woman when due to ageing of ovary; epithelial line invaginates, producing crypts and glands, which eventually develop into cysts within the stromal compartment [22]. Although mostly benign, these cysts can turn malignant and initiate epithelial cancerous growth. It has been hypothesized that repeated cycles of ovulation-induced trauma and repair of the OSE at the site of ovulation contributes to malignancy, it makes the OSE susceptible to mitogenic factors and other genotoxic radicals. There are several studies that suggested that menstrual cycle can affect tumor growth through the high levels of reproductive hormones [23]. Chronic repeated ovulation without pregnancy-induced rest periods contributes to neoplasia of the ovarian epithelium [24]. The ovarian surface epithelium—a single-cell layer surrounding the ovary and derived from the same mesodermal celomic epithelium as that lining the peritoneal cavity and other Müllerian structures undergoes rapid proliferation during 24 hours after ovulation, and that invaginations of the epithelium to form clefts and inclusion cysts within the ovarian stroma are most pronounced just after ovulation [24].

The OSE revealed estrogen receptor α (ERα) and progesterone receptor (PR) during pregnancy and estrous cycle in rat [25]. Immunohistochemistry of the ovary showed low levels of OSE cells staining positive for ERα expression. ERα positive cells were absent on day 7 and 14 of pregnancy, only day 21 recorded a very low percentage of immunostaining (0.5%) within the nuclei of OSE cells. On the contrary, immunostaining of PR receptors was not observed within the nuclei of OSE cells in all groups of study. The study suggested that understanding the factors affecting OSE proliferation may help elucidating the mechanism(s) of assisted diseases such as ovarian cancer [25]. Several studies revealed that gonadotropins treatment may increase OSE proliferation in different animal models [26; 27].

The effects of follicular and luteal products on the proliferation of sheep OSE cells in culture, and to analyse the influences of large antral follicles and corpora lutea (CL) on the expression of gonadotrophin receptors (FSHR and LHR) in the OSE was investigated. [28].The study showed that follicular fluids from medium and large follicles, and extracts of corpora lutea stimulated the growth of OSE cells. OSE proliferation in cycling sheep is associated with underlying developing follicles and CL, mediated by, at least in part, the up-regulation of gonadotrophin receptors, and facilitated by the action of mitogenic glycopeptides and growth factors, but not steroids [28].

The relationship between progesterone (also oestrogen)-mediated OSE apoptosis and expression of p53, a cell-cycle arresting protein and potential tumor suppressor was described [29]. Immunohistochemical staining with cytokeratin confirmed epithelial nature of the cells in the OSE layer and inclusion cysts that invaginate inside stroma after ovulation takes place. The *in situ* apoptosis index was determined during estrus, and at mid and late-pregnancy stages in heifers. Epithelia of both tissues exhibited significantly high nuclear staining, suggesting that these cells are aiming to apoptotic destruction. The study concluded that progesterone during cycling and pregnancy may reduce the risk of developing ovarian cancer by ceasing cell cycle and diverting damaged and mutagenized OSE cells for apoptosis, and the process may be mediated through elevated p53 synthesis. However, it is also possible that progesterone and p53-induced apoptosis may be entirely different cancer suppressive actions but coincidently happening together [29].

Epithelial Ovarian Cancer (EOC)

Recent morphologic, immunohistochemical and molecular genetic studies have led to the development of a new paradigm for the pathogenesis and origin of epithelial ovarian cancer (EOC) based on a dualistic model of carcinogenesis that divides EOC into two broad categories designated Types I and II. Type I tumors are comprised of low-grade serous, low-grade endometrioid, clear cell and mucinous carcinomas and Brenner tumors. They are generally indolent, present in stage I (tumor confined to the

ovary) and are characterized by specific mutations, including *KRAS, BRAF, ERBB2, CTNNB1, PTEN PIK3CA, ARID1A,* and *PPPR1A*, which target specific cell signaling pathways. Type I tumors rarely harbor *TP53* and are relatively stable genetically. Type II tumors are comprised of high-grade serous, high-grade endometrioid, malignant mixed mesodermal tumors (carcinosarcomas) and undifferentiated carcinomas. They are aggressive, present in advanced stage, and have a very high frequency of *TP53* mutations but rarely harbor the mutations detected in type I tumors [30].

Recent studies strongly suggest that fallopian tube epithelium (benign or malignant) that implants on the ovary is the source of low-grade and high-grade serous carcinoma rather than the ovarian surface epithelium as previously believed. Similarly, it is widely accepted that endometriosis is the precursor of endometrioid and clear cell carcinomas and as endometriosis is thought to develop from retrograde menstruation these tumors can also be regarded as involving the ovary secondarily. Type I and type II ovarian tumors develop independently along different molecular pathways, and that both types develop outside the ovary and involve it secondarily. If this concept is confirmed it leads to the conclusion that the only true primary ovarian neoplasms are gonadal stromal and germ cell tumors analogous to testicular tumors. This new paradigm of ovarian carcinogenesis has important clinical implications. By shifting the early events of ovarian carcinogenesis to the Fallopian tube and endometrium instead of the ovary, prevention approaches, for example, salpingectomy with ovarian conservation may play an important role in reducing the burden of ovarian cancer while preserving hormonal function and fertility [30].

Molecular Pathogenesis of Epithelial Ovarian Carcinoma

The introduction of the "borderline (low malignant potential)" category was an important step in refining the morphologic classification of EOC by identifying a group of tumors, defined as lacking destructive invasive growth that had a significantly better outcome than the invasive carcinomas. Since it was rare to find a borderline tumor coexisting with an invasive carcinoma it was generally believed that they were unrelated. In 1996 a relationship between serous borderline tumor (SBT) and invasive serous carcinoma was described based on the subdivision of SBT into two groups. One group designated "atypical proliferative serous tumor (APST)" behaved in a benign fashion and a second, smaller group, designated "micropapillary serous carcinoma (MPSC)" also termed "noninvasive low-grade serous carcinoma" behaved like a low-grade malignant tumor **[**29**]**. Moreover, this latter subset was closely associated with invasive low-grade serous carcinoma (LGSC) and the investigators proposed that MPSC was the immediate precursor of LGSC. The key element leading to this conclusion was the recognition that LGSC was a distinct entity that differed from HGSC in several ways (see below). Prior to this, serous carcinoma was graded well, moderately and poorly differentiated with the implication that serous carcinoma was a spectrum of disease in which well differentiated carcinoma (LGSC) progressed to poorly differentiated carcinoma (HGSC). Following this morphologic study linking APST to MPSC and LGSC, a series of molecular genetic studies was performed, which culminated in the proposal of a dualistic model to explain the pathogenesis of EOC **[**29**]**.

The dualistic model accommodates and confirms the heterogeneous nature of EOC and places the major histologic types into two groups (type I and type II) based on their distinctive clinicopathologic and molecular genetic features. Type I tumors are comprised of low-grade serous carcinomas, low-grade endometrioid, clear cell and mucinous carcinomas which develop in a stepwise fashion from well-established precursor lesions, such as borderline tumors and endometriosis. They typically present as large masses that are confined to one ovary (stage Ia), are indolent and have a good prognosis. The type I tumors are relatively genetically stable and typically display a variety of somatic sequence mutations that include *KRAS*, *BRAF*, *PTEN*, *PIK3CA CTNNB1* (the gene encoding beta catenin), *ARID1A* and *PPP2R1A* but very rarely *TP53* [30;31]. In contrast, type II tumors are comprised of HGSC (usual type of serous carcinoma), high-grade endometrioid carcinoma, malignant mixed mesodermal tumors (carcinosarcomas) and undifferentiated carcinomas, which present in advanced stage (stages II-IV) in over 75% of cases; they grow rapidly and are highly aggressive. Type II tumors, of which HGSC is the prototypic type, are chromosomally highly unstable and harbor *TP53* mutations in >95% of cases. They rarely display the mutations found in the type I tumors. BRCA inactivation, either by mutation or inactivation of expression of BRCA and its downstream genes via promoter methylation occurs in up to 40-50% of HGSC **[**32**]**. BRCA inactivation has not been reported in the type I tumors.

Serous Tumors

The relationship of APST and MPSC to LGSC based on morphologic studies was supported by mutational analysis, gene expression studies and methylation profiling demonstrating that these three tumor types shared molecular alterations that differed dramatically from HGSC **[**33-35**]**. Initial molecular genetic studies focused on individual genes but more recent studies have highlighted the importance of

molecular signaling pathways (Fig. 1). For example, the MAPK signaling pathway is important for the cellular response to a variety of growth and differentiation factors and activating mutations in *KRAS* or one of its downstream effectors, *BRAF*, (mutations of *KRAS* and *BRAF* are mutually exclusive) results in constitutive activation of MAPK-mediated signaling in more than half of APSTs, MPSCs and LGSCs [36, 39]. In addition, a 12-bp insertion mutation of *ERBB2* (encoding HER-2/neu), which activates an upstream regulator of K-Ras, has been detected in 9% of these tumors. Interestingly, tumors with *ERBB2* mutations lack *KRAS* and *BRAF* mutations [35, 36]. Accordingly, 60-70% of APSTs, MPSCs and LGSCs express active MAPK [37]; they rarely harbor *TP53* mutations. Recent studies have further clarified the molecular pathogenesis of APST, MPSC and LGSC. First, *KRAS* and *BRAF* mutations have not been detected in serous cystadenomas, the putative precursor of SBTs, but laser capture microdissection (LCM) studies have detected these mutations in the adenoma epithelium and APST epithelium in serous cystadenomas containing small APSTs suggesting that these mutations occur early in the development of APST [38].

In an attempt to elucidate the relationship of APST to LGSC a recent study compared the gene expression profiles of APST, MPSC and LGSC and found that MPSC is closer molecularly to invasive LGSC than to APST and that the genes involved in MAPK signaling showed higher expression in MPSC than in APST. In addition, a previous study reporting that MPSC harbors a pattern of chromosomal imbalance distinct from that of APST [39] confirms the proposal that LGSC develops in a stepwise fashion from cystadeno-fibroma to APST and MPSC, supporting the biological role of the *KRAS-BRAF*-MEK-MAPK pathway in the development of LGSC. By globally profiling the epigenetic landscape, it has recently reported that the methylation profiles in low-grade serous carcinoma are closer to APST and serous cystadenoma than high-grade serous carcinoma [30]. This finding lends further support to the dualistic model of ovarian serous carcinogenesis.

Clear Cell and Endometrioid Tumors

After serous carcinoma, endometrioid and clear cell carcinomas are the most frequent types of EOC accounting for approximately 15-20% of EOC in Western countries. The molecular genetic alterations that underlie the development of these tumors are now beginning to emerge. Based on genome-wide mutational analysis, the most common molecular genetic changes in clear cell carcinoma are a somatic inactivating mutation of *ARID1A* [40,41], a tumor suppressor gene detected in about 50% of cases, an activating mutation of *PIK3CA* in about 50% of tumors [42] and deletion of *PTEN*, a tumor suppressor gene involved in the PI3K/PTEN signaling pathway, in about 20% (43), supporting the role of an aberrant PI3K/ PTEN pathway in the development of clear cell carcinoma. In addition, SNP array analysis has identified frequent amplification of the *ZNF217* (zinc finger protein 217) locus and deletion of the *CDKN2A/2B* locus in clear cell carcinomas, suggesting that the pathways involving these two genes are also important in their development.

Morphologic studies over the past two to three decades have repeatedly shown an association of endometrioid and clear cell carcinoma with endometriosis and early molecular genetic studies demonstrated LOH in the same chromosomal regions in endometrioid carcinoma and adjacent endometriosis [43] confirming a clonal relationship between endometriosis and endometrioid carcinoma. In addition, a recent study reported mutation of *ARID1A* in atypical endometriosis adjacent to clear cell carcinoma but not in distant sites of endometriosis further linking endometriosis to clear cell carcinoma and thereby providing further evidence that endometriosis is the likely precursor of endometrioid and clear cell carcinoma. Although both clear cell and endometrioid carcinomas are derived from endometriosis and share some molecular genetic features, such as mutation of *ARID1A* and deletion of *PTEN*, they clearly adopt different molecular programs for their development, as is evident by their distinctly different morphologic phenotype and clinical behavior. For example, canonical Wnt signaling pathway defects and microsatellite instability, which occur frequently in low-grade endometrioid carcinoma have only rarely been detected in clear cell carcinoma. Also it has been recently demonstrated that unlike all the other types of EOC, clear cell carcinoma has significantly longer telomeres and this finding correlates with poor outcome [44].

Mucinous Tumors

These tumors have been the least studied histologic types probably due to their relative rarity (approximately 3% of EOC). *KRAS* mutations occur in up to 75% of primary mucinous carcinomas and using *KRAS* as a molecular marker, LCM studies have shown the identical *KRAS* mutation in mucinous carcinomas and adjacent mucinous cystadenomas and borderline tumors supporting the morphological continuum and tumor progression in ovarian mucinous neoplasms. In summary, each of the major histologic types of EOC is associated with a different set of cell signaling pathways abnormalities, which for the type I tumors are shared with their respective precursor lesions (borderline tumors and endometriosis) supporting their stepwise progression (Fig.1). In contrast, the type II tumors, aside from a very high frequency of *TP53*

mutations and molecular alterations of BRCA1/2, are characterized by marked genetic instability and lack other mutations. The identification and characterization of their precursor lesions have only recently been recognized [28].

Origin of Epithelial Ovarian Carcinoma

Serous Tumors

The conventional view of the origin of serous tumor has been that they were derived from the ovarian surface epithelium or cortical inclusion cysts. Therefore, there was surprise and skepticism when a group of Dutch investigators in 2001 first described tubal intraepithelial carcinomas (TICs), later designated "serous tubal intraepithelial carcinomas (STICs) and occult invasive HGSCs in the Fallopian tube that closely resembled ovarian HGSC, in women with a genetic predisposition to ovarian cancer [45]. Similar lesions were not found in the ovaries of the same women. In hindsight, the failure to identify the tubal carcinomas in the past was because it was assumed that precursors of ovarian carcinoma would logically be in the ovaries, and therefore the Fallopian tubes were not carefully examined. It was subsequently proposed that implantation of malignant cells from the tubal carcinoma to the ovary develop into a tumor mass that gives the impression that the tumor originated in the ovary [46; 47] (Fig. 2). A gene profiling study showing that the gene expression profile of HGSC is more closely related to Fallopian tube epithelium than to ovarian surface epithelium and immunohistochemical studies showing that HGSC expresses PAX8, a Müllerian marker, but not calretinin, a mesothelial marker (ovarian surface epithelium has a mesothelial not a Müllerian morphologic phenotype) lends further support to the proposal that the tubal lesions are precursors of HGSC and not the ovarian surface epithelium [48].

The carcinoma developed from ovarian cortical inclusion cysts. Although it is generally stated that these cysts develop by invagination of ovarian surface epithelium, there is reason to believe that during ovulation, as the fimbria come into close contact with the ovary, tubal epithelial cells implant on the disrupted ovarian surface to form a cortical inclusion cyst [48] (Fig. 3). In addition, ovulation itself with the release of follicular fluid, which has been shown to contain reactive oxygen species (free radicals), and possibly associated changes in the microenvironment, such as inflammation, may play a role in early ovarian carcinogenesis. This is consistent with epidemiologic evidence linking decreased ovulation (either as a result of oral contraceptive usage or multiple pregnancies) with a decreased risk of ovarian cancer [49, 50]. Therefore, some HGSCs may develop from ovarian cortical inclusion cysts [51] but these cysts could be derived, not from the ovarian surface epithelium but from implanted fimbrial tubal epithelium [48] (Fig. 4).

Clear Cell and Endometrioid Tumors

As previously noted it is well established by morphologic and molecular genetic studies that low-grade endometrioid and clear cell carcinomas develop from endometriotic cysts (endometriomas) and are frequently associated with implants of endometriosis elsewhere in the pelvis [52]. Although the precise origin of endometriosis has not been completely established, specifically, whether it develops *in situ* in the peritoneum through a process of metaplasia or from retrograde menstrual flow, the preponderanc of data favor the latter mechanism [53]. Admittedly, the former theory is more difficult to prove experimentally. Thus, if retrograde menstruation accounts for most cases of endometriosis, it is logical to assume that endometrioid and clear cell tumors develop from endometrial tissue that implanted on the ovary and therefore the ovary is involved secondarily [54] (Fig. 5).

Mucinous Tumors

Studies over the last decade have shown that the majority of the gastrointestinal-type tumors involving the ovary are secondary [55;56] and that, in fact, primary mucinous carcinomas of the ovary are one of the least common types of EOC comprising about 3% of EOC. Malignant Brenner tumors are the least common type of EOC. The origin of these mucinous tumors and Brenner tumors tumors is puzzling, as unlike serous, endometrioid, and clear cell tumors, they do not display a Müllerian phenotype. Although it has been argued that mucinous tumors bear some relationship to the endocervix, the mucinous epithelium that characterizes them more closely resembles gastrointestinal mucosa. It seems unlikely that they develop from cortical inclusion cysts, as mucinous metaplasia involving cortical inclusion cysts is a very rare finding. On the other hand, the association of Brenner tumors and mucinous tumors has been recognized for many years. In a provocative study of mucinous cystadenomas and Brenner tumors, it was reported that after extensive sectioning, mucinous cystadenomas contained foci of Brenner tumor in 18% of cases [57].

Role of genetic factors in ovarian cancer

Though occurrence of ovarian cancer is recognized as sporadic, about 5-10% of incidences have familial history and risk among first-degree relatives (mother, sister and daughter) increases by 50% [58]. In many cases people carrying germline mutations in one of the alleles of the tumor suppressor genes *BRCA1* (Breast cancer antigen 1) or *BRCA2* are at significantly higher risk of acquiring breast or ovarian cancers. The *BRCA1* and *BRCA2* genes which are found on chromosome 17q code for proteins that are responsible for DNA double stranded breaks by

homologous recombination [59].Consequently, several chromosomal abnormalities and genetic instability lead to onset of cancerous transformation of mammary and ovarian epithelia. Tumour suppression of a normal cell occurs when the BRCA1 gene controls remodeling processes of the concerned chromosomes. The gene also works with the retinoblastoma (Rb) gene during this remodeling process. When a mutation has occurred, then steps in the cell cycle will not include it. This causes tumorigenesis or unchecked growth inside the cell.

If BRCA1 and BRCA2 mutate and a person's offspring inherits them, then that progeny has a high susceptibility to ovarian cancer even if the disease has not manifested itself in the parent. BRCA2 and BRCA1 mutations compounded by the family history of the patient, age or menopausal status, and other risk factors. Therefore, a harmful mutation may not always translate into ovarian cancer. It should be noted that the above genes are not the only ones responsible for genetic ovarian cancer risk. Other genes such as Phosphatase and tensin homolog (PTEN), MutL homolog 1 (MLH1), MutS homolog and p53 also account for this disease [60]. While BRCA protein is widely expressed in all kinds of cells, the prevalence of *BRCA* mutations in some tissues and not others, relates to the microenvironment of a particular tissue which becomes crucial for pathogenesis. Women inheriting mutations of *BRCA1* or *BRCA2* genes have a ~40% or ~10% risk, respectively, of developing ovarian cancer by the age of 70. In the case of the *BRCA1 and 2 genes*, certain criteria ought to be examined in order to understand the likelihood of developing the disease. Women with first degree relatives, like mothers and sisters, who had been diagnosed with breast cancer at an early age might develop the disease. Another criterion is the existence of more than two second degree or first degree relatives, like aunts and grandmothers, with breast cancer. Additionally, the combination of ovarian cancer and breast cancer among the second or first degree relatives also accounts for an increased level of harmful *BRCA1 and BRCA2* mutations **(Fig.6)**. Bilateral breast cancer or cancer in both breasts among first degree relatives is another criterion. Breast cancer in a male relative is also cause for alarm. Genetic testing may be done in order to determine whether one has a high risk. If the tests are negative, this does not imply that a person will never have ovarian cancer, it simply demonstrates that their likelihood of contracting the disease is minimal. Females with lynch syndrome or hereditary non-polyposis colorectal cancer (HNPCC) also have an increased chance of developing ovarian cancer [61]. This is an autosomal syndrome that increases a woman's risk of acquiring ovarian, gastric or endometrial cancers. It originates from mutations in the mismatch repair (MMR) gene, which is located in chromosomes 7p, 3p, 2q, and 2p. The purpose of these genes is to ensure that DNA transcription takes place normally by repairing mistakes in the process. Ovarian cancer that comes from this mutation accounts for all histopathologic tumors. They are also heritable and will increase an offspring's chances of developing ovarian cancer.

Cancer stem cells constitute a small proportion of OSE stem cells

The cancer stem cell (CSC) hypothesis postulates that the tumorigenic potential of CSCs is confined to a very small subset of tumor cells and is defined by their ability to self-renew and differentiate leading to the formation of a tumor mass .The observation that cancers can arise long after initial exposure to carcinogens. The carcinogenic event may have occurred in the long-lived slowly proliferating stem cell population which in many cases may have been triggered by unknown mechanism(s) (e.g., DNA damage, exposure to inflammatory cytokines and reactive oxygen species, etc) after being dormant for an indefinite length of time ranging from months to years. These early cancer cells or CSCs would then give rise to generations of cells resulting in tumor masses [61].

CSCs are not necessarily transformed adult stem cells but they may be progenitor cells or differentiated cells that have acquired stem cell like characteristics .Although there is evidence in some tumor types (such as melanoma) normal adult stem cells are the initial precursor cells to neoplastic transformation, definite evidence of these adult stem cells as the originator of ovarian cancer has been lacking. The term ''cancer stem cells'' usually refers to a defined population of tumor cells that express a distinct set of cell surface or intracellular markers which are universally expressed in many tissues. The term ''cancer initiating cells'' or ''tumor initiating cells'' have been used with CSCs but neither of these terms define the cells that initiate the tumor [61]. CSCs are usually characterized by their ability to renew and give rise to a progeny of cells that have high proliferative and invasive capacity. This phenomenon often described in the literature as ''asymmetric division'' defines a process whereby one daughter cell on division retains the characteristics of the parent cell while the other may not necessarily have the parental traits [61]. Hence, tumors that arise from CSCs consist of CSCs and a mixed population of cells which creates the full heterogeneous phenotype of the tumor. Within the tumor CSCs possess several key properties which includes, (i) unlimited proliferative potential; (ii) ability to renew indefinitely in an undifferentiated state; (iii) resistance to therapies; (iv) high DNA repair capacity; and (v) the ability to drive the expansion of tumor by cells that are deregulated at various stages of

differentiation. These properties of CSCs represent a critical target for new cancer therapy. Nonetheless creating and designing therapies against ovarian CSCs has proven complex because, (i) there are no CSC marker for ovarian cancer that can be specifically targeted and (ii) ovarian CSCs are protected by resistance mechanisms that make them less susceptible to conventional therapies. The first description of stem cells in ovarian cancer was reported in the ascites of an ovarian cancer patient, derived from a single cell which could sequentially propagate tumors over several generations [62]. CSCs have been isolated from ovarian tumors and cell lines based on their abilities to differentially efflux the DNA binding dyes [62].

The cyclic and repeated disruption and repair of the OSE with complex remodeling has led to a belief that there exists a population of somatic stem/progenitor cells within the OSE layer responsible for sustained wound healing [63]. Somatic stem cells are just the normal tissue cells with an ability to renew themselves by asymmetric division, and thereby they produce a set of daughter cells committed to rectify the damage by regeneration and repair. Both human and bovine OSE cells exhibited positive staining for Kit ligand (KL) and its tyrosine kinase receptor *c-kit* which are typical stem cell factors [64].

Increasing evidence supports the hypothesis that OSE tumour growth capacity depends on Cancer Stem Cells (CSC's) that arise from a small proportion of OSE stem cells [64]. It has been reported that CSC's are responsible for aggressiveness of the disease, metastasis and resistance to chemotherapy. In EOC cell, a small subset of tumor stem cells, called Side Population (SP), actually proliferates as CSC's and the rest of the population behaves like stem cells destined for damaged tissue repair. SP can be detected by their ability to efflux the DNA-binding dye Hoechst 33342 through an ABC membrane transporter [64]. In a mouse model, several proliferative markers were used to distinguish the quiescent population of OSE cells from highly proliferative cells surrounding the post-follicular wounded region in OSE layer [65]. A significant result was that two nuclear proliferation markers, bromo-dUracil (BrdU) and Histone 2B green fluorescent protein (2HB-GFP) were retained by a quiescent cell population for up to four months, while another population within the same tissue rapidly lost the markers in a short period [65]. With high resolution confocal microscopy these authors demonstrated the possibility of asymmetric division of coelomic epithelial cells, which is highly characteristic of stem cells. The conclusion was that those cells which lost the markers were highly proliferative somatic stem cells showing asymmetric division, and were distinct from the OSE tissue specific cells that were dividing less and as a result maintained the nuclear markers for longer periods [65].

The Morphologic and Molecular Heterogeneity of Epithelial Ovarian Cancer

One of the major problems in elucidating the pathogenesis of ovarian cancer is that it is a heterogeneous disease composed of different types of tumors with widely differing clinicopathologic features and behavior. Based on a series of morphologic and molecular genetic studies, we have proposed a dualistic model that categorizes various types of ovarian cancer into two groups designated type I and type II. Type I tumors are clinically indolent and usually present at a low stage. They exhibit a shared lineage between benign cystic neoplasms and the corresponding carcinomas often through an intermediate (borderline tumor) step, supporting the morphological continuum of tumor progression in these neoplasms [66]. This stepwise sequence of events parallels the adenoma-carcinoma sequence that occurs in colorectal carcinoma. Type I tumors include low- grade serous, low-grade endometrioid, clear cell and mucinous carcinomas. In contrast to the clear-cut and distinctive morphologic differences among type I tumors, the morphologic differences among the type II tumors are more subtle and as a result there is considerable overlap in the diagnosis of these tumors by different pathologists. Type II tumors exhibit papillary, glandular, and solid patterns and are diagnosed as high-grade serous, high-grade endometrioid and undifferentiated carcinomas depending on the dominant pattern. Generally, most pathologists classify them as high-grade serous carcinomas even though they bear little resemblance to tubal-type epithelium (the basis for typing a tumor as serous); arguably many of those lacking distinctive serous or endometrioid features could be classified as "high-grade adenocarcinoma" [66].

In addition to these neoplasms, malignant mixed mesodermal tumors (carcinosarcomas) are included in the type II category because they have epithelial components identical to the pure type II carcinomas. Type II tumors are highly aggressive and almost always present in advanced stage. Since they account for approximately 75% of all epithelial ovarian carcinomas and have relatively similar morphologic features and a uniformly poor outcome, ovarian cancer has been erroneously regarded as a single disease. The morphologic differences between type I and type II tumors are mirrored by marked differences in their molecular genetic features [66]. As a group, type I tumors are genetically more stable than type II tumors and display specific mutations in the different histologic cell types21. Thus, *KRAS, BRAF, and ERBB2* mutations occur in approximately two thirds of low-grade serous carcinomas whereas *TP53* mutations are rare in these tumors. Low-grade endometrioid

carcinomas have aberrations in the Wnt signaling pathway involving somatic mutations of *CTNNB1* (encoding β-catenin), *PTEN* and *PIK3CA*7. Mucinouscarcinomas have *KRAS* mutations in more than 50% of specimens [66].

Clear cell carcinoma is unique in that it has a high percentage of *PIK3CA* activating mutations when purified tumor samples and cell lines are analyzed22. There is little available molecular genetic data on transitional cell (Brenner) tumors. High-grade serous carcinoma, the prototypic type II tumor, is characterized by very frequent *TP53* mutations (>80% of cases) and *CCNE1* (endcoding cyclin E1) amplification but rarely mutations that characterize most type I tumors such as *KRAS, BRAF, ERBB2, PTEN, CTNNB1 and PIK3CA*7 [66]. Although only a small number of malignant mixed mesodermal tumors have been analyzed molecularly, the few that have been display a similar molecular genetic profile. In summary, type I tumors, as a group, are genetically more stable than type II tumors and display a distinctive pattern of mutations that occur in specific cell types (low-grade serous, low-grade endometrioid, clear cell and mucinous). In contrast, the type II tumors (high-grade serous, high-grade endometrioid, malignant mixed mesodermal tumors and undifferentiated carcinomas) show greater morphologic and molecular homogeneity, are genetically unstable and have a very high frequency of *TP53* mutations. These findings suggest that different types of ovarian carcinomas develop along different molecular pathways [66].

Biomarkers as prognostic and therapeutic tool in ovarian Cancer

CA-125

Bi-manual examination, CA-125 and transvaginal ultrasonography together are sensible tools to detect only 30–45% of women with early-stage disease. Recent developments in proteomic and genomic research have identified a number of potential biomarkers. Although panels of tumor markers and proteomic-based technologies may improve the positive predictive value, all markers require validation and interfacing with newly developed diagnostic imaging technologies. While a large amount of information on miRNAs has been promising, much remains to be elucidated [67]. 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 [68]. 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. 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 [69].

For detection of ovarian cancer in postmenopausal women, the CA-125 cut-point of 35 units/ml has been used. The 98th percentile in this population yielded a 2% false-positive rate, whereas the same cut-point in premenopausal women resulted in substantially higher false-positive rates. Baseline CA-125 values and clinical and demographic data from 3692 women participating in a screening study, which was conducted by the National Cancer Institute, recommended to achieve a 2% false-positive rate in ovarian cancer screening trials and in high-risk women, the cutoff point for initial CA-125 testing should be personalized primarily for menopausal status: 50 units/ml for premenopausal women, 40 units/ml for premenopausal on oral contraceptive, and 35 units/ml for postmenopausal women [70].

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 [71]. 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–specificity.

HE4

This protein has a WAP-type four disulfide-core and is encoded by the *WFDC2* gene found on chromosome 20q1213.1 [72]. It is elevated in ovarian cancer and there is increased HE4 mRNA expression in different types of EOC's [73;74]. HE4 is overexpressed in specific subtypes of ovarian cancer, 100% in endometrioid and 93% in serous ovarian cancer, possibly enabling one to distinguish among several tumor types. Alone, the sensitivity of this marker has been reported to be 95% with a specificity of 72.9% [75]. Moore *et al*. reported a sensitivity of 76.5% and specificity of 95% when CA-125 and HE4 were combined to differentiate benign from malignant lesions in a retrospective study. Recently, in a prospective trial, this panel yielded a sensitivity of 93.8%, a specificity of 74.9% and a negative predictive value of 99% [76]. Equally important, HE4 is less likely to be elevated falsely in the setting of benign neoplasms as compared with serum CA-125, and it can be used to differentiate endometriomas from malignant ovarian tumors, with sensitivity of 71% as compared with sensitivity of 64% of CA-125. HE4 is also noted as a potential marker for adenocarcinoma of the endometrium [77]. Recently, two meta-analysis studies

independently published a similar conclusion that HE4 is a valuable marker in the diagnosis of ovarian cancer [78;79]. HE4 has recently obtained US FDA approval for monitoring of disease recurrence or progression but not for screening. In summary, HE4 was superior to CA-125 in separating benign, borderline ovarian tumors, cancers of the fallopian tubes, as well as early-stage EOC.

Mesothelin

Mesothelin is a glycosylphosphatidylinositol-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% [80]. In a study consisting of 44 ovarian tumor specimens, Obulhasim *et al*. found that mesothelin was expressed in 100% of serous cystadenocarcinoma and 100% of serous borderline tumors of the ovary. The average methylation of CpG sites in ovarian tumors ranged from 6 to 56% in mesothelin-positive, and from 13 to 79% in mesothelin-negative samples. The authors identified diverse levels of methylation/ hypomethylation at CpG sites in the mesothelin promoter region in ovarian cancer [81]. 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 detected more 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 [82].

Transferrin

Transferrin (79 kDa) is an iron-binding transport protein responsible for transporting iron from sites of iron absorption and heme degradation to areas of storage and utilization. Transferrin has been previously reported to be decreased in the serum of patients with ovarian cancer [83]. All of these molecules have been shown to play an important role in oxidative stress, for which there are extensive data linking it to carcinogenesis [84]. It functions as promoter of tumor development and survival via antiapoptotic effects [85]. Combination of CA-125, transferrin, TTR and ApoA1 using proteomic analysis yielded a sensitivity of 89% at specificity of 92% for early detection screening [86].

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. OPN has the ability to inhibit apoptosis. Kim *et al*., in a study consisting of 107 plasma samples using cDNA array, found significantly higher levels of OPN expressed in invasive ovarian cancer and borderline ovarian tumors [87]. OPN is involved in metastasis and tumor progression, useful to monitor recurrence. OPN as a sole biomarker has a sensitivity of 81.3%, when combined with CA-125 the panel yielded a sensitivity of 93.8%, but a low specificity of 33.7% [88].

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 [89]. 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 [90].

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 [91].

B7-H4

B7-H4 is a 282 amino acid protein, which is expressed on the surface of a variety of immune cells and functions as a negative regulator of T-cell responses. B7-H4 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 [92]. Using ELISA to analyze the level of B7-H4 protein in more than 2500 serum samples, ascites fluids and tissue lysates, Simon *et al*. found high levels of B7-H4 protein in ovarian cancer tissue lysates when compared with normal tissues. B7-H4 was present at low levels in all sera, but showed an elevated level in serum samples from ovarian cancer patients when compared with healthy controls or women with benign gynecologic diseases. 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 [93].

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 [94]. Confirmation of proteomics data with immunoassays in some early-stage cases revealed a substantial increase of SAA levels in plasma in comparison with the values of healthy controls. When combined with CA-125 the panel yielded an accuracy rate of 95.2% for ovarian cancer screening [95].

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. Of the 15 family members that are encoded by a group of genes tandemly localized on chromosome 19q13.3–4, 12 KLK are over expressed in ovarian cancer at the mRNA and/or protein level [96]. KLK6 and KLK10 were elevated in ovarian cancer tissues that had low levels of CA-125 [97], elevated KLK11 was found in 70% of ovarian cancer sera at a specificity of 95% [98].

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. Although these results indicate improvement in sensitivity, preliminary data from different laboratories suggest that OVX1 may be unstable unless serum is rapidly separated, which could complicate its use in population screening if samples are sent by post [99]. In a study of 201 serum samples, Donach *et al*. found a sensitivity of 88% and specificity of 92.5% using the artificial neural network with a panel of OVX1, M-CSF, CA19–19 and CA 72–74 [100].

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 [101]. VEGF levels were significantly elevated in the sera and cyst fluids of carcinoma patients compared with patients who had benign neoplasms. High VEGF levels in ascitic fluids appeared to be significantly associated with shorter disease-free survival and overall survival. The elevated VEGF levels in sera and tumor effusions of patients with Fédération Internationale de Gynécologie Obstétrique (FIGO) stages I/II indicated that angiogenesis promoted by VEGF is a continuous process, independent of clinical advancement of the disease [102]. When combined with CA-125 the sensitivity is 77% and specificity is 87% [103]. VEGF inhibition has been shown to inhibit tumor growth and ascites production, and to suppress tumor invasion and metastasis [104].

miRNAs

In parallel with the efforts to identify potential protein biomarkers, 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 [105]. miRNAs globally influence gene expression, which ultimately determines cellular behavior by targeting complementary gene transcripts for translational repression or degradation of the mRNA transcript [106]. Similar to other cancers, the initiation and development of ovarian cancer is characterized by disruption of oncogenes and tumor suppressor genes by both genetic and epigenetic mechanisms [107]. Previous miRNA-expression profiling studies of ovarian cancer have defined differentially expressed miRNAs in ovarian cancer relative to the corresponding normal control, and various miRNAs may represent potential targets for detection, diagnosis, prognosis and therapy [108]. There is a variety of tumor miRNA-expression patterns including genetic alterations, epigenetic regulation or altered expression of transcription factors, which finally target the miRNA genes. In cancer cells, transcriptional gene silencing has frequently been associated with epigenetic defects [109]. 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 [110]. Recent efforts have been focused on establishing miRNA as novel molecular biomarkers for ovarian cancer and defining peripheral blood-derived miRNA as novel circulating biomarkers [111].

Current & future technologies of interest to identify unique ovarian cancer biomarkers

Microarrays

Microarray technology is one of the powerful tools to study genome-wide expression of genes. DNA microarray consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing a specific DNA sequence. This can be a short section of a gene that is used to hybridize a cDNA sample [112]. Genome-wide expression profiling using DNA microarray technology has enhanced the understanding of the genes that influence ovarian cancer development, histopathologic subtype,

progression, response to therapy and overall survival. Both diagnostic and prognostic information can be obtained by this method [112]. For example, osteopontin and kallikrein 10 [113] are markers that have been identified by microarray analysis.

Microarray-based expression analysis is considered an ideal strategy for identifying candidate miRNAs. The small size of the mature miRNA is less susceptible to nuclease degradation. In addition, the small size makes it possible to extract miRNAs from paraffin-embedded formalin-fixed tissue blocks, which makes large archives of fixed tissue available for molecular analysis. The use of microarray can therefore generate molecular signatures of the disease stages. The expression data are archived in a standardized base, both National Center for Biotechnology Information – Gene Expression Omnibus and Array Express databases have been used for storage of miRNA microarray data [114].

Microvesicles & exosomes analysis

Microvesicles are generated by the outward budding and fission of membrane vesicles from the cell surface occurring in normal and pathologic cells, and more frequently, in tumor cells. Microvesicles can be widely detected from bodily fluids such as blood, urine, cerebrospinal fluid and ascites from which high-quality RNA, DNA and protein can be extracted and purified for analysis. In addition, by transferring these bioactive molecules, they are now thought to have vital roles in tumor invasion and metastases in cancer progression [115]. Exosomes are one of many different sub-populations of microvesicles. Exosomes originate predominantly from preformed multivesicular bodies that are released upon fusion with the plasma membrane. Exosomes can also contain proteins, enzymes, miRNAs and mRNAs; and thus, exosomes served as bioactive shuttle vesicles that constitute a mode of selective transmitting of information between cells [116]. An inappropriate release of miRNAs via exosomes may cause significant alterations in biologic pathways that affect disease development. Exosomes play an important role in cell to cell communication. They transfer proteins, mRNA and miRNA into recipient cells. The interplay via the exchange of exosomes between cancer cells, and between cancer cells and the tumor stroma may promote the transfer of oncogenes (e.g., b-catenin, CEA, HER2, Melan-A/Mart-1 and LMP-1) and onco-mi-RNAs (e.g., let7, miR-1, miR-15, miR-16 and miR-375) from one cell to another, leading to the modulation of the activity of cellular signaling pathways in the recipient cells [117].

Genomic technologies have identified biomarkers and potential therapeutic targets for ovarian cancer. Comprehensive functional validation studies of the biological and clinical implications of these biomarkers are needed to advance them toward clinical use [118]. Amplification of chromosomal region 5q31–5q35.3 has been used to predict poor prognosis in patients with advanced stage, high-grade serous ovarian cancer. In this study, we further dissected this large amplicon and identified the overexpression of FGF18 as an independent predictive marker for poor clinical outcome in this patient population. Using cell culture and xenograft models, we show that FGF18 signaling promoted tumor progression by modulating the ovarian tumor aggressiveness and microenvironment [118]. FGF18 controlled migration, invasion, and tumorigenicity of ovarian cancer cells through NF-κB activation, which increased the production of oncogenic cytokines and chemokines. This resulted in a tumor microenvironment characterized by enhanced angiogenesis and augmented tumor-associated macrophage infiltration and M2 polarization. Tumors from ovarian cancer patients had increased FGF18 expression levels with microvessel density and M2 macrophage infiltration, confirming our in vitro results. These findings demonstrate that FGF18 is important for a subset of ovarian cancers and may serve as a therapeutic target [118].

Ongoing research on ovarian cancer

Ovarian cancer remains 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 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. Mass spectrometry & quantitative proteome analysis Mass, Microarray techniques, and Microvesicles & exosomes are the most recent techniques employed for the sensitivity and identification of biomarkers to predict early detection of ovarian cancer. Moreover, mutation of both BRCA1, and BRCA2 genes and their prevalence to ovarian carcinoma is also ongoing area of research.

Abbreviations

Ovarian Surface Epithelium (OSE)**,** ovarian cancer (OC)**,** cancer antigen 125 (CA125), Estrogen Receptor α (ERα)**,** Progesterone Receptor (PR)**,** Epithelial Ovarian Cancer (EOC), Serous Borderline Tumor (SBT), Atypical Proliferative Serous Tumor (APST), Micropapillary Serous Carcinoma (MPSC), Low-Grade Serous Carcinoma (LGSC), High-Grade Serous Carcinoma (HGSC), *ZNF217* (zinc finger protein 217), serous tubal intraepithelial carcinomas (STICs), *BRCA1; BRCA2* (Breast Cancer Antigen 1;2), Cancer Stem Cells (CSC's), Side Population (SP), bromo-dUracil (BrdU), Histone 2B green fluorescent protein (2HB-GFP).

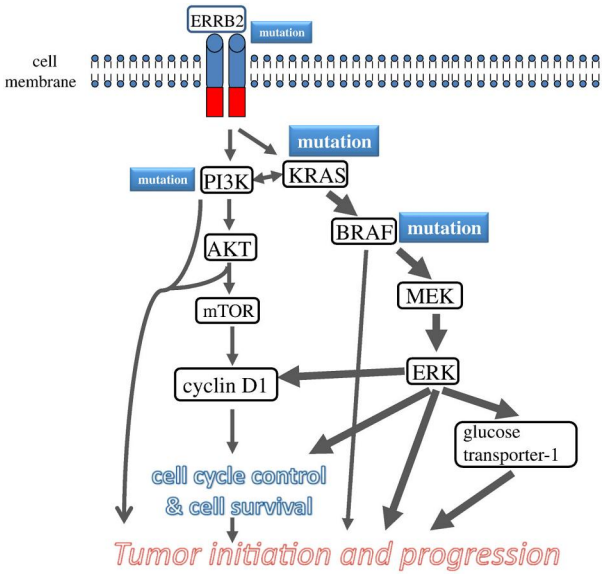

Fig. 1 Schematic illustration of pathway alterations involved in the development of low-gradeserous carcinoma. The cardinal molecular genetic changes include somatic mutations in KRAS, BRFA and occasionally ERRB2 (encoding Her2/Neu) and PIK3CA. The mutated gene products constitutively activate the signaling pathways that regulate cellular proliferation and survival and promote tumor initiation and progression through several mechanisms including up regulation of glucose transporter-1. The size of the boxes containing specific genes reflects the relative frequency of the mutation and the thickness of the arrows indicates the relative contribution of the pathway alterations to tumor development. Kurman and Shih (2011)

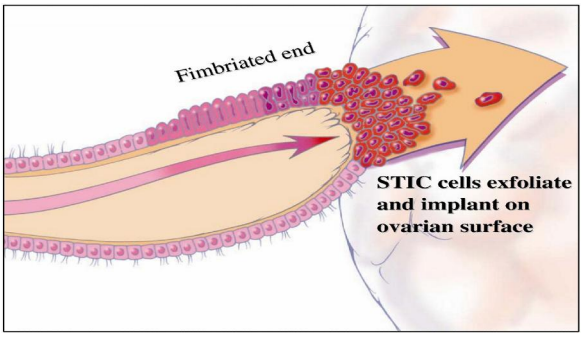

Fig. 2. Spread of serous tubal intraepitelial carcinoma (STIC) from the fimbria to the ovarian surface. Adapted and reprinted with permission from American J Surg Pathol 2010;34:433-443. Kurman RJ, Shih IM. Kurman RJ, Shih IM. (2010).

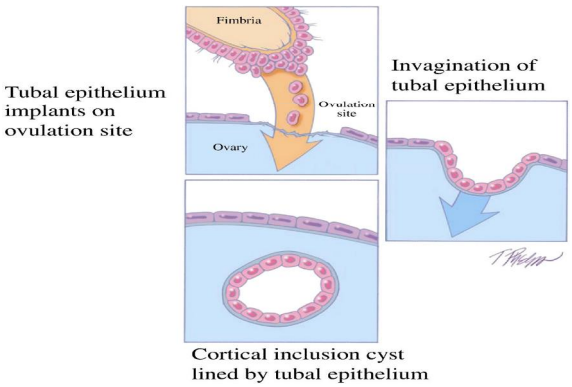

Fig.3. Development of a cortical inclusion cyst from tubal epithelium. Adapted and reprinted with permission from American J Surg Pathol 2010;34:433-443. Kurman (2010).

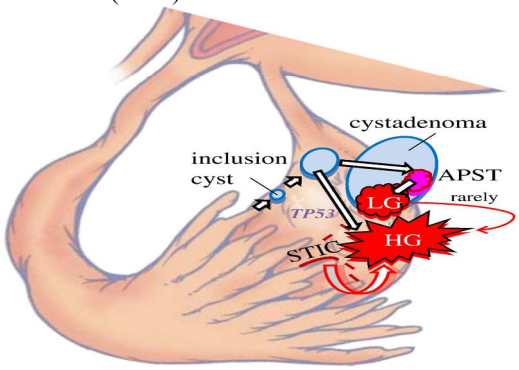

Fig. 4 Development of low-grade [type I pathway with *KRAS* or *BRAF* mutation) and high-grade serous carcinoma [type II pathway with *TP53* mutation] from tubal epithelium by way of a cortical inclusion cyst and cystadenoma or an intraepithelial carcinoma (STIC) implanting directly on the ovary developing into a high-grade serous carcinoma. Reprinted with permission from American J Surg Pathol 2010;34:433-44. Kurman and Shih, et al (2010).

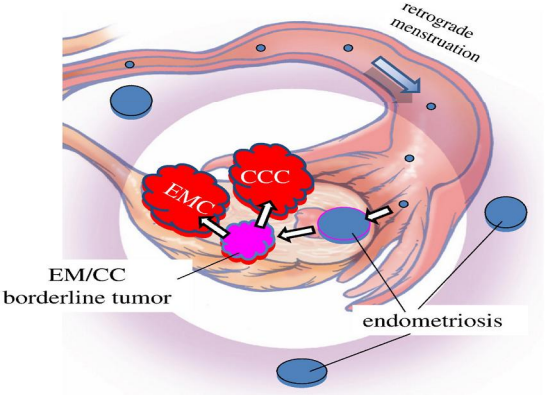

Fig.5: Development of low-grade endometrioid and clear cell carcinoma from endometriosis by retrograde menstruation. Reprinted with permission from American J Surg Pathol Kurman and Shih (2010).

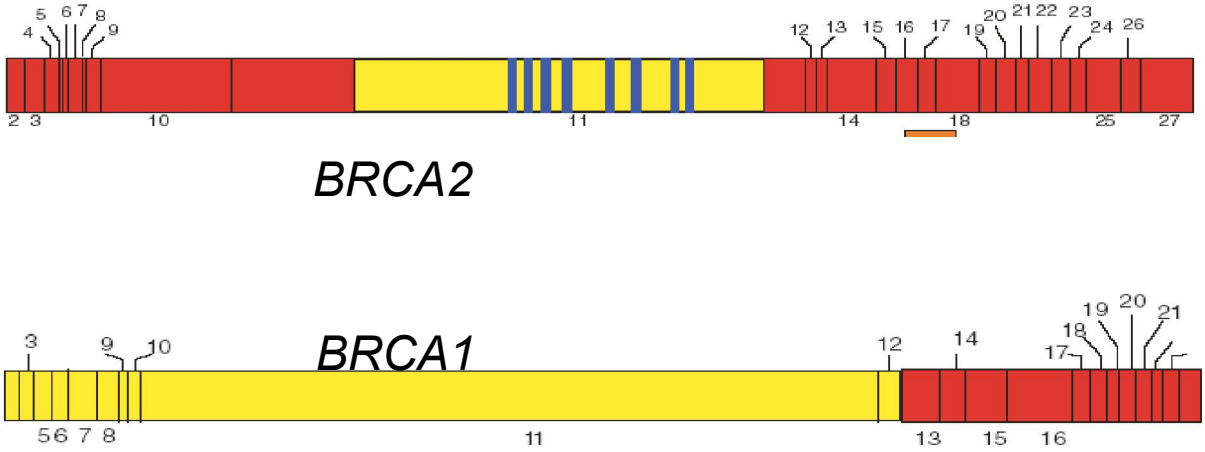

Fig.6.**Mutations in *BRCA* genes** give individuals a predisposition to more ovarian cancer (in yellow), or more breast cancer (in red) risks. Taken from Sowter and Ashworth (2005).

References

1. Gaytan M, Sanchez MA, Morales C, Bellido C, Millan Y, Martin de Las MJ, Sanchez-Criado JE & Gaytan F. 2005. Cyclic changes of the ovarian surface epithelium in the rat. Reproduction **129** 311-321.
2. Beral V, Bull D, Green J & Reeves. 2007. Ovarian cancer and hormone replacement therapy in the Million Women Study. Lancet **369** 1703-1710.
3. Jacobs and Bast, Jr. 1989 Jacobs I & Bast RC, Jr. 1989. The CA 125 tumour-associated antigen: a review of the literature. Hum.Reprod. **4** 1-12.
4. 4.Maines-Bandiera and Auersperg 1997 Maines-Bandiera SL & Auersperg N. 1997. Increased E-cadherin expression in ovarian surface epithelium: an early step in metaplasia and dysplasia? *Int. J.* Gynecol. Pathol. **16** 250-255.
5. Kabawat SE, Bast RC, Jr., Bhan AK, Welch WR, Knapp RC & Colvin RB. 1983. Tissue distribution of a coelomic-epithelium-related antigen recognized by the monoclonal antibody OC125. Int.J.Gynecol.Pathol. **2** 275-285.
6. 6 Jacobs and Bast, Jr 1989 Jacobs I and Bast RC, Jr. 1989. The CA 125 tumour-associated antigen: a review of the literature. *Hum.Reprod*. **4** 1-12.
7. Byskov AG, Skakkebaek NE, Stafanger G & Peters H. 1977 Influence of ovarian surface epithelium and rete ovarii on follicle formation. J.Anat. **123** 77-86.
8. Hirshfield AN. 1991 Development of follicles in the mammalian ovary. Int.Rev.Cytol. **124** 43-101.
9. Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC 2001 Ovarian surface epithelium: biology, endocrinology, and pathology. Endocr Rev 22:255–288.
10. MacCalman CD, Farookhi R & Blaschuk OW. 1994 Estradiol regulates E-cadherin mRNA levels in the surface epithelium of the mouse ovary. Clin.Exp.Metastasis **12** 276-282.
11. Ryan PL, Valentine AF & Bagnell CA. 1996 Expression of epithelial cadherin in the developing and adult pig ovary. Biol. Reprod. **55** 1091-1097.
12. Makrigiannakis A, Coukos G, Christofidou-Solomidou M, Gour BJ, Radice GL, Blaschuk O & Coutifaris C. 1999 N-cadherin-mediated human granulosa cell adhesion prevents apoptosis: a role in follicular atresia and luteolysis? Am.J.Pathol. **154** 1391-1406.
13. Peralta SA, Knudsen KA, Jaurand MC, Johnson KR, Wheelock MJ, Klein-Szanto AJ & Salazar H. 1995 The differential expression of N-cadherin and E-cadherin distinguishes pleural mesotheliomas from lung adenocarcinomas. Hum.Pathol. **26** 1363-1369.
14. Davies BR, Worsley SD & Ponder BA. 1998 Expression of E-cadherin, alpha-catenin and beta-catenin in normal ovarian surface epithelium and epithelial ovarian cancers. Histopathology **32** 69-80.
15. Maines-Bandiera SL & Auersperg N. 1997 Increased E-cadherin expression in ovarian surface epithelium: an early step in metaplasia and dysplasia? Int.J. Gynecol. Pathol. **16** 250-255.
16. Van der Linden PJ, de Goeij AF, Dunselman GA, Arends JW & Evers JL. 1994 P-cadherin expression in human endometrium and endometriosis. Gynecol. Obstet. Invest **38** 183-185.
17. Wong AS, Maines-Bandiera SL, Rosen B, Wheelock MJ, Johnson KR, Leung PC, Roskelley CD & Auersperg N. 1999 Constitutive and conditional cadherin expression in cultured human ovarian surface epithelium: influence of family history of ovarian cancer. Int.J.Cancer **81** 180-188.
18. Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC 2001 Ovarian surface epithelium: biology, endocrinology, and pathology. Endocr Rev **22** 255–288.
19. Osterholzer HO, Johnson JH, Nicosia SV 1985 An autoradiographic study of rabbit ovarian surface epithelium before and after ovulation. Biol Reprod **33** 729–738.
20. Auersperg N, Edelson MI, Mok SC, Johnson SW, Hamilton TC 1998 The biology of ovarian cancer. Semin Oncol 25 281–304.
21. Murdoch WJ and van Kirk EA 2002 Steroid hormonal regulation of proliferative, p53 tumor suppressor, and apoptotic responses of sheep ovarian surface epitheial cells. Molecular and and cellular endocrinology. **186** 61-67.
22. Auersperg N. 2013. Ovarian surface epithelium as a source of ovarian cancers: unwarranted speculation or evidence-based hypothesis? Gynecol.Oncol. **130**: 246-251.
23. Wood P.A. and W.J.Hrushesky 2005. Sex cycle modulates cancer growth. Breast Cancer Res.Treat. **91**: 95-102.
24. Fathalla MF. 1971 Incessant ovulation—a factor in ovarian

neoplasia? Lancet **2** 163.
25. Salina Yahya Saddick (In Press 2014) Ovarian Surface Epithelium receptors during pregnancy and estrus cycle of rats with emphasis on steroids and gonadotropins fluctuation. Saudi Journal of Biological Sciences.
26. Hilliard T.S., D.A.Modi, and J.E.Burdette 2013 Gonadotropins activate oncogenic pathways to enhance proliferation in normal mouse ovarian surface epithelium. Int.J. Mol.Sci. **14** 4762- 4782.
27. Stewart S.L., T.D.Querec, B.N.Gruver, B.O'Hare, J.S.Babb, and C.Patriotis 2004 Gonadotropin and steroid hormones stimulate proliferation of the rat ovarian surface epithelium. J.Cell Physiol **198** 119-124.
28. Salina Yahya Saddick 2012 *In vitro* regulation of sheep ovarian surface epithelium (OSE) proliferation by local ovarian factors. Saudi Journal of Biological Sciences **19** 285–290.
29. Salina Y. Saddick 2013 *In vivo* and *in vitro* studies on apoptosis in OSE cells and inclusion cysts of pregnant heifers. Saudi Journal of Biological Sciences **20** 281–289.
30. Robert J. Kurman, and Ie-Ming Shih 2011 Molecular Pathogenesis and Extraovarian Origin of Epithelial Ovarian Cancer. Shifting the Paradigm. Hum Pathol. **42**(7) 918–931.
31. Burks RT, Sherman ME, Kurman RJ. 1996 Micropapillary serous carcinoma of the ovary. A distinctive low-grade carcinoma related to serous borderline tumors. Am J Surg Pathol. **20** 1319–1330.
32. Shih I-M, Kurman RJ. 2004 Ovarian tumorigenesis- a proposed model based on morphological and molecular genetic analysis. Am J Pathol. **164** 1511–1518.
33. Senturk E, Cohen S, Dottino PR, Martignetti JA. 2010 A critical re-appraisal of BRCA1 methylation studies in ovarian cancer. Gynecol Oncol. **119** 376–383.
34. May T, Virtanen C, Sharma M, Milea A, Begley H, Rosen B, Murphy KJ, Brown TJ, Shaw PA. 2010 Low malignant potential tumors with micropapillary features are molecularly similar to low grade serous carcinoma of the ovary. Gynecol Oncol. **117** 9–17.
35. Senturk E, Cohen S, Dottino PR, Martignetti JA. 2010 A critical re-appraisal of BRCA1 methylation studies in ovarian cancer. Gynecol Oncol. **119** 376–383.
36. May T, Virtanen C, Sharma M, Milea A, Begley H, Rosen B, Murphy KJ, Brown TJ, Shaw PA. 2010 Low malignant potential tumors with micropapillary features are molecularly similar to low gradeserous carcinoma of the ovary. Gynecol Oncol. 117-119
37. Meinhold-Heerlein I, Bauerschlag D, Hilpert F, Dimitrov P, Sapinoso LM, Orlowska-Volk M,Bauknecht T, Park TW, Jonat W, Jacobsen A, Sehouli J, Luttges J, Krajewski M, Krajewski S,Reed JC, Arnold N, Hampton GM 2005 Molecular and prognostic distinction between serous ovariancarcinomas of varying grade and malignant potential. Oncogene **24** 1053–65.
38. Singer G, Oldt R 3rd, Cohen Y, Wang BG, Sidransky D, Kurman RJ, Shih IeM 2003 Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst. **95** 484–6.
39. Mok SC, Bell DA, Knapp RC, Fishbaugh PM, Welch WR, Muto MG, Berkowitz RS, Tsao SW 1993 Mutation of K-ras protooncogene in human ovarian epithelial tumors of borderline malignancy. Cancer Res. **53** 1489–92.
40. Sieben NL, Macropoulos P, Roemen GM, Kolkman-Uljee SM, Fleuren G Jan, Houmadi R, Diss T,Warren B, Al Adnani M, De Goeij AP, Krausz T, Flanagan AM 2004 In ovarian neoplasms, BRAF, but not KRAS, mutations are restricted to low-grade serous tumours. J Pathol. **202** 336–40.
41. Mayr D, Hirschmann A, Lohrs U, Diebold J. 2006 KRAS and BRAF mutations in ovarian tumors: Acomprehensive study of invasive carcinomas, borderline tumors and extraovarian implants. Gynecol Oncol. **103** 883–7.
42. Jones S, Wang TL, Shih IM, Mao TL, Nakayama K, Roden R, Glas R, Slamon D, Diaz LA Jr.Vogelstein B, Kinzler KW, Velculescu VE, Papadopoulos N 2010 Frequent mutations of chromatinremodeling gene ARID1A in ovarian clear cell carcinoma. Science **330** 228-231.
43. Ahmed AA, Etemadmoghadam D, Temple J, Lynch AG, Riad M, Sharma R, Stewart C, Fereday S, Caldas C, Defazio A, Bowtell D, Brenton JD 2010 Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. J Pathol. **221** 49–56.
44. Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, Cristiano BE,Pearson RB, Phillips WA 2004 Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res. **64** 7678–81.
45. Sato N, Tsunoda H, Nishida M, Morishita Y, Takimoto Y, Kubo T, Noguchi M 2000 Loss of heterozygosity on 10 q23.3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. Cancer Res. **60** 7052–6.
46. Jiang X, Morland SJ, Hitchcock A, Thomas EJ, Campbell IG. 1998 Allelo typing of endometriosis with adjacent ocarian carcinomna reveals evidence of a common lineage. Cancer Research **58** 1707–1712.
47. Piek JM, van Diest PJ, Zweemer RP, Jansen JW, Poort-Keesom RJ, Menko FH, Gille JJ, Jongsma AP, Pals G, Kenemans P, Verheijen RH. 2001 Dysplastic changes in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer. J Pathol. **195** 451–456.
48. Piek JM, van Diest PJ, Zweemer RP, Kenemans P, Verheijen RH. 2001 Tubal ligation and risk of ovarian cancer. Lancet **358** 844.
49. Piek JM, Verheijen RH, Kenemans P, Massuger LF, Bulten H, van Diest PJ. 2003 BRCA1/2-relatedovarian cancers are of tubal origin: a hypothesis. Gynecol Oncol. **90** 491
50. Kurman RJ, Shih IM. 2010 The origin and pathogenesis of epithelial ovarian cancer: a proposedunifying theory. Am J Surg Pathol. **34** 433–443.
51. Beral V, Bull D, Green J, Reeves G. 2007 Ovarian cancer and hormone replacement therapy in the Million Women Study. Lancet **369** 1703–1710.
52. Risch HA, Weiss NS, Lyon JL, Daling JR, Liff JM 1983 Events of reproductive life and the incidence of epithelial ovarian cancer. Am J Epidemiol. **117** 128–139.
53. Pothuri B, Leitao MM, Levine DA, Viale A, Olshen AB, Arroyo C, Bogomolniy F, Olvera N, LinO, Soslow RA, Robson ME, Offit K, Barakat RR, Boyd J. 2010 Genetic analysis of the early natural history of epithelial ovarian carcinoma. PLoS One. **5** e10358.
54. Veras E, Mao TL, Ayhan A, Ueda S, Lai H, Hayran M, Shih IeM, Kurman RJ. 2009 Cystic and adenofibromatous clear cell carcinomas of the ovary:distinctive tumors that differ in their pathogenesis and behavior: a clinicopathologic analysis of 122 cases. Am J Surg Pathol. **33** 844–853.
55. Bulun SE. 2009 Endometriosis. N Engl J Med. **360** 268–279.

Martin DC. 1997 Cancer and endometriosis: do we need to be concerned? Semin Reprod Endocrinol. **15** 319–324.
56. Riopel MA, Ronnett BM, Kurman RJ. 1999 Evaluation of diagnostic criteria and behavior of ovarian intestinal-type mucinous tumors: atypical proliferative (borderline) tumors and intraepithelial, microinvasive, and metastatic carcinomas. Am J Surg Pathol. **23** 617–635.
57. Seidman, JD.; Cho, KR.; Ronnett, BM.; Kurman, RJ. 2011 Surface epithelial tumors of the ovary. In: Kurman, RJ.; Ellenson, LH.; Ronnett, BM., editors. Blaustein's Pathology of the Female Genital Tract. 6th. Springer-Verlag; New York 679-784.
58. Seidman JD, Khedmati F. 2008 Exploring the histogenesis of ovarian mucinous and transitional cell (Brenner) neoplasms and their relationship with Walthard cell nests: a study of 120 tumors. Arch Pathol Lab Med. **132** 1753–1760.
59. Murdoch WJ & McDonnel AC. 2002 Roles of the ovarian surface epithelium in ovulation and carcinogenesis. Reproduction **123** 743-750.
60. Tutt A & Ashworth A. 2002 The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. Trends Mol.Med. **8** 571-576.
61. Bapat SA, Mali AM, Koppikar CB, Kurrey NK. 2005 Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res. **65** (8) 3025-9.
62. Nuzhat Ahmed, Khalid Abubaker, Jock Findlay, and Michael Quinn 2013 Cancerous Ovarian Stem Cells: Obscure Targets for Therapy but Relevant to Chemoresistance. Journal of Cellular Biochemistry **114** 21–34.
63. Szotek, P.P., Chang, H.L., Brennand, K., Fujino, A., Pieretti-Vanmarcke, R., Celso, C.L., Dombkowski, D., Preffer, F., Cohen, K.S., Teixeira, J. & Donahoe, P.K. 2008 Normal ovarian surface epithelial label-retaining cells exhibit stem/progenitor cell characteristics. Proceedings in National Academy of Science U.S.A., **105** (34) 12469-12473.
64. Parrott JA, Kim G & Skinner MK. 2000 Expression and Action of Kit Ligand/Stem Cell Factor in Normal Human and Bovine Ovarian Surface Epithelium and Ovarian Cancer. Biology of Reproduction **62** 1600–1609.
65. Pan Y & Huang X. 2008 Epithelial Ovarian Cancer Stem Cells---A Review. International Journal of Clinical and Experimental Medicine **1** 260-266.
66. Robert J. Kurman, and Ie-Ming Shih 2010 The Origin and Pathogenesis of Epithelial Ovarian Cancer- a Proposed Unifying Theory. Am J Surg Pathol. **34** (3) 433–443.
67. Long Nguyen, Segundo Joel Cardenas-Goicoechea, Pierre Gordon, Christina Curtin, Mazdak Momeni, Linus Chuang1 & David Fishman 2013 Biomarkers for early detection of ovarian cancer. Women Health **9** (2) 171–187.
68. Bast RC Jr, Klug TL, St John E. 1983 A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N. Engl. J. Med. **309** (15) 883–887.
69. Rossing MA, Wicklund KG, Cushing- Haugen KL, Weiss NS. 2010 Predictive value of symptoms for early detection of ovarian cancer. J. Natl Cancer Inst. **102** (4) 222–229.
70. Skates SJ, Mai P, Horick NK *et al.* 2011 Large prospective study of ovarian cancer screening in high-risk women: CA125 cut-point defined by menopausal status. Cancer Prev. Res. (Phila.) **4** (9) 1401–1408.
71. Drapkin R, Von Horsten HH, Lin Y *et al.* 2005 Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res. **65** (6) 2162–2169.
72. Galgano MT, Hampton GM, Frierson HF Jr. 2006 Comprehensive analysis of HE4 expression in normal and malignant human tissues. Mod. Pathol. **19** (6) 847–853.
73. Lu KH, Patterson AP, Wang L. 2004 Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin. Cancer Res. **10** (10) 3291–3300.
74. Hough CD, Sherman-Baust CA, Pizer 2000 Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. *Cancer Res.* **60** (22) 6281–6287.
75. Moore RG, Brown AK, Miller MC 2008 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.
76. Moore RG, Miller MC, Disilvestro P. 2011 Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. Obstet. Gynecol. **118** (2 Pt 1) 280–288.
77. Huhtinen K, Suvitie P, Hiissa 2009 Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. Br. J. Cancer **100** (8) 1315–1319.
78. Wu L, Dai ZY, Qian YH, Shi Y, Liu FJ, Yang C. 2012 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.
79. Yu S, Yang HJ, Xie SQ, Bao YX. 2012 Diagnostic value of HE4 for ovarian cancer: a meta-analysis. Clin. Chem. Lab. Med. **50** (8) 1439–1446.
80. Mcintosh MW, Drescher C, Karlan B 2004 Combining CA 125 and SMR serum markers for diagnosis and early detection of ovarian carcinoma. Gynecol. Oncol. **95** (1) 9–15.
81. Obulhasim G, Fujii H, Matsumoto T 2010 Mesothelin gene expression and promoter methylation/hypomethylation in gynecological tumors. Eur. J. Gynaecol. Oncol. **31** (1) 63–71.
82. Badgwell D, Lu Z, Cole L Fritsche H, Atkinson EN, Somers E, Allard J, Moore RG, Lu KH, Bast RC Jr. 2007 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.
Ahmed N, Oliva KT, Barker G. 2005 Proteomic tracking of serum protein isoforms as screening biomarkers of ovarian cancer. Proteomics **5**(17) 4625–4636
83. Toyokuni S. 2006 Novel aspects of oxidative stress-associated carcinogenesis. Antioxid. Redox Signal. **8**(7–8) 1373–1377.
84. Koshkaryev A, Piroyan A, Torchilin VP. 2012 Increased apoptosis in cancer cells *in vitro* and *in vivo* by ceramides in transferrin-modified liposomes. Cancer Biol. Ther. **13**(1) 50–60.
85. Su F, Lang J, Kumar A 2007 Validation of candidate serum ovarian cancer biomarkers for early detection. Biomark. Insights **2** 369–375.
86. Kim JH, Skates SJ, Uede T 2002 Osteopontin as a potential diagnostic biomarker for ovarian cancer. JAMA **287** (13) 1671–1679.
87. Nakae M, Iwamoto I, Fujino T Maehata Y, Togami S, Yoshinaga M, Douchi T. 2006 Preoperative plasma

osteopontin level as a biomarker complementary to carbohydrate antigen 125 in predicting ovarian cancer. J. Obstet. Gynaecol. Res. **32** (3) 309–314.
88. Slack-Davis JK, Atkins KA, Harrer C, Hershey ED, Conaway M. 2009 Vascular cell adhesion molecule-1 is a regulator of ovarian cancer peritoneal metastasis. Cancer Res. **69** (4) 1469–1476 .
89. Yurkovetsky Z, Skates S, Lomakin A. 2010 Development of a multimarker assay for early detection of ovarian cancer. J. Clin. Oncol. **28** (13) 2159–2166.
90. Gadomska H, Grzechocinska B, Janecki J, Nowicka G, Powolny M, Marianowski L. 2005 Serum lipids concentration in women with benign and malignant ovarian tumours. Eur. J. Obstet. Gynecol. Reprod. Biol. **120** (1) 87–90.
91. Tringler B, Liu W, Corral L. 2006 B7-H4 overexpression in ovarian tumors. Gynecol. Oncol. 100 (1) 44–52.
92. Simon I, Zhuo S, Corral L. 2006 B7-h4 is a novel membrane-bound protein and a candidate serum and tissue biomarker for ovarian cancer. Cancer Res. **66**(3) 1570–1575.
93. Urieli-Shoval S, Finci-Yeheskel Z, Dishon S. 2010 Expression of serum amyloid a in human ovarian epithelial tumors: implication for a role in ovarian tumorigenesis. J. Histochem. Cytochem. **58** (11) 1015–1023.
94. Moshkovskii SA, Vlasova MA, Pyatnitskiy MA. 2007 Acute phase serum amyloid A in ovarian cancer as an important component of proteome diagnostic profiling. Proteomics Clin. Appl. **1**(1) 107–117.
95. Borgono CA, Diamandis EP. 2004 The emerging roles of human tissue kallikreins in cancer. Nat. Rev. Cancer **4** (11) 876–890.
96. Rosen DG, Wang L, Atkinson JN. 2005 Potential markers that complement expression of CA125 in epithelial ovarian cancer. Gynecol. Oncol. **99** (2) 267–277.
97. Diamandis EP, Okui A, Mitsui S. 2002 Human kallikrein 11: a new biomarker of prostate and ovarian carcinoma. Cancer Res. **62** (1) 295–300.
98. Tainsky MA. 2009 Genomic and proteomic biomarkers for cancer: a multitude of opportunities. Biochim. Biophys. Acta **1796** (2) 176–193.
99. Donach M, Yu Y, Artioli G. 2010 Combined use of biomarkers for detection of ovarian role for microRNA-31 in inhibiting the proliferation of serous ovarian carcinomas and other cancers. Cancer Res. **70** (5) 1906–1915.
100. Kraft A, Weindel K, Ochs A 2009 Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. Cancer **85** (1) 178–187.
101. Harlozinska A, Sedlaczek P, Kulpa J. 2004 Vascular endothelial growth factor (VEGF) concentration in sera and tumor effusions from patients with ovarian carcinoma. Anticancer Res. **24** (2C) 1149–1157.
102. Li L, Wang L, Zhang W. 2004 Correlation of serum VEGF levels with clinical stage, therapy efficacy, tumor metastasis and patient survival in ovarian cancer. Anticancer Res. **24** (3b) 1973–1979.
103. Byrne AT, Ross L, Holash J. 2003 Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. Clin. Cancer Res. **9** (15) 5721–5728.
104. Lopez J, Percharde M, Coley HM, Webb A, Crook T. 2009 The context and potential of epigenetics in oncology. Br. J. Cancer **100** (4) 571–577.
105. Creighton CJ, Fountain MD, Yu Z. 2010 Molecular profiling uncovers a p53-associated role for microRNA-31 in inhibiting the proliferation of serous ovarian carcinomas and other cancers. Cancer Res. **70** (5) 1906–1915.
106. Iorio MV, Visone R, Di Leva G. 2007 MicroRNA signatures in human ovarian cancer. Cancer Res. **67** (18) 8699–8707.
107. Zhang L, Volinia S, Bonome T. 2008 Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. Proc. Natl Acad. Sci. USA **105** (19) 7004–7009.
108. Bartels CL, Tsongalis GJ. 2009 MicroRNAs: novel biomarkers for human cacer. Clin. Chem. **55** (4) 623–631.
109. Chen X, Ba Y, Ma L. 2008 Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. **18** (10), 997–1006.
110. Kuhlmann JD, Rasch J, Wimberger P, Kasimir-Bauer S. 2012 MicroRNA and the pathogenesis of ovarian cancer – a new horizon for molecular diagnostics and treatment? Clin. Chem. Lab. Med. **50** (4) 601–615.
111. Chon HS and Lancaster JM. 2011 Microarray-based gene expression studies in ovarian cancer. Cancer Control **18** (1) 8–15.
112. Shvartsman HS, Lu KH, Lee J. 2003 Overexpression of kallikrein 10 in epithelial ovarian carcinomas. Gynecol. Oncol. **90** (1) 44–50 .
113. Thomson JM, Parker JS, Hammond SM. 2007 Microarray analysis of miRNA gene expression. Methods Enzymol. **427** 107–122.
114. Muralidharan-Chari V, Clancy JW, Sedgwick A, D'souza-Schorey C. 2010 Microvesicles: mediators of extracellular communication during cancer progression. J. Cell Sci. **123** (Pt 10) 1603–1611.
115. Hu G, Drescher KM, Chen XM. 2012 Exosomal miRNAs: biological properties and therapeutic potential. Front. Genet. **3** 56.
116. Kharaziha P, Ceder S, Li Q, Panaretakis T. 2012 Tumor cell-derived exosomes: a message in a bottle. Biochim. Biophys. Acta **1826**(1) 103–111.
117. Wei Wei, Samuel C. Mok, Esther Oliva Sung-hoon Kim, Gayatry Mohapatra, and Michael J. Birrer 2013 FGF18 as a prognostic and therapeutic biomarker in ovarian cancer. The Journal of Clinical Investigation **123** 4463-4448.

2/19/2014