Journal of BUON **14:** 7-18, 2009

REVIEW ARTICLE

Molecular pathogenesis of borderline and invasive ovarian tumors

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Summary

Ovarian cancer still ranks first as the leading cause of death among gynecological malignancies. Malignant transformation of normal ovarian epithelial cells is caused by genetic alterations that disrupt regulation of proliferation, programmed cell death, and senescence. The vast majority of ovarian tumors arise due to accumulation of genetic damage, but the specific genetic pathways for the development of epithelial ovarian tumors, borderline and malignant, are largely unknown. Our results show that in progressive stages of carcinoma, the oxidative stress can contribute to the uncontrolled tumor expansion. Circulating levels of antioxidants may be important to consider when evaluating a woman's risk of cancer, even among women who are at higher predicted risk. The purpose of this article was to review the current approaches to molecular pathogenesis of borderline and invasive epithelial ovarian tumors.

Key words: borderline tumors, molecular pathogenesis, ovarian cancer

Introduction

Ovarian epithelial tumors are thought to arise from the simple cuboidal surface epithelium of the ovary and account for 75% of all ovarian tumors, and 90-95% of ovarian malignancies. They are classified into 3 main groups: benign, borderline (low malignant potential), and invasive, reflecting their clinical behavior [1]. Each group includes several histological subtypes: serous, mucinous, endometrioid, Brenner (transitional cell), small cell, undifferentiated, and mixed mesodermal, corresponding to the different types of epithelia in the organs of the female reproductive tract [1-3]. In developed countries, serous and mucinous subtypes account for about 60% and 30% of all ovarian epithelial tumors, respectively [4].

Little is known about the behavior of the ovarian surface epithelium (OSE), which participates actively in the mechanism of gonadotropin-induced ovulatory follicular rupture and plays a central role in ovarian cancer etiology [5,6]. Recent literature reveals that the proliferation and migration of the OSE are regulated by hormones, growth factors, and cytokines. Gonadotropins, including follicle-stimulating hormone (FSH) and luteinizing hormone (LH), have been implicated in OSE proliferation, migration, and protection from apoptosis in humans and animals *in vivo* and *in vitro* [7-9]. Steroid hormones such as estrogen, progesterone and androgen also modulate the OSE [5]. Beside these, other regulators of the OSE include epidermal growth factor (EGF) [10] and platelet-derived growth factor (PDGF) [11].

Malignant transformation of a normal ovarian epithelial cell is caused by genetic alterations that disrupt regulation of proliferation, programmed cell death, and senescence. About 10% of epithelial ovarian cancers arise in women who have inherited mutations in cancer susceptibility genes such as BRCA1 or BRCA2 [12-14]. The lifetime risk for ovarian cancer in women with BRCA1 mutations is estimated to 40-50%, and is slightly lower (10-20%), in women who carry BRCA2 gene mutations [15,16].

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Received 14-10-2008; Accepted 05-11-2008

The vast majority of ovarian cancers arise due to the accumulation of genetic damages over the course of a lifetime, and are referred to as sporadic cancers, but the specific genetic pathways for the development of epithelial ovarian tumors are largely unknown. It is unclear whether different histological subtypes of ovarian tumors have different pathogenetic pathways. Using a molecular genetic approach, Campbell et al. demonstrated that endometriosis may be the precursor of the majority of endometrioid and clear cell ovarian carcinomas [17]. In addition, Obata et al. showed frequent PTEN/MMAC mutations in endometrioid, but not serous or mucinous epithelial ovarian tumors [18]. These data suggest that tumors with different histological subtypes may arise through distinct developmental pathways.

The pathogenetic pathways of serous and mucinous tumors remain largely unknown and precursors of these tumors have not been identified. However, there are several uncommon histopathological features which may lend insight into the pathogenesis of epithelial ovarian tumor development. First, benign, borderline, and invasive-appearing areas are seen only in a small percentage of low-grade serous ovarian carcinomas. Second, incidental microscopic serous carcinomas, which are high grade, can be identified in grossly normal ovaries [19]. Third, high-grade serous carcinoma can be found on the surface of the ovary with little or no stromal invasion [20].

A high number of ovarian tumors may develop from ovarian inclusion cysts which arise from ovarian surface epithelium invaginated into the cortex of the ovary. The epithelial lining of the cyst may evolve into a mucinous or serous cystadenoma through distinct pathogenetic pathways. Mucinous cystadenoma may give rise to mucinous borderline ovarian tumors (BOTs). A subset of these tumors may progress to invasive low-grade mucinous carcinoma and subsequently into high-grade carcinoma [21].

Serous cystadenoma may give rise to serous BOTs. However, a majority of serous BOTs may develop directly from ovarian inclusion cysts, i.e. without the intervening stage of cystadenoma. In contrast to mucinous BOTs, only a small percentage of serous BOTs may progress to low-grade carcinoma and subsequently to high-grade carcinoma [21].

Ovarian borderline (low malignant potential) tumors are a puzzling group of neoplasms that do not fall neatly into benign or malignant categories. Their behavior is enigmatic, their pathogenesis unclear, and their clinical management controversial, especially for serous borderline tumors, the most common type of ovarian borderline tumor. Clarifying the nature of borderline tumors and their relationship to invasive carcinoma has puzzled investigators since this category was created over 30 years ago. Much of the confusion and controversy concerning these tumors is due to lack of understanding of their pathogenesis and an absence of a model for the development of ovarian carcinoma. This review summarizes recent molecular studies of ovarian borderline tumors and invasive carcinomas.

Etiology of genetic alterations

Most epithelial ovarian carcinomas are thought to develop due to accumulation of a series of genetic alterations over lifetime. The causes of the genetic damage that underline the development of these cancers are not completely understood, but epidemiologic and molecular studies have begun to shed some light on the etiology of ovarian cancer. There is evidence to suggest that sporadic ovarian cancer generally is a monoclonal disease that originates in the ovarian surface epithelium or underlying inclusion cysts [22]. It has been suggested that ovulation may be the main cause of mutation in the ovarian epithelium. Several lines of evidence link ovulation and epithelial ovarian cancer. First, most animals, such as rats and mice, ovulate reflexively when stimulated appropriately and have a low incidence of epithelial ovarian cancer. In contrast, chickens and women ovulate repetitively and have the highest incidence of epithelial ovarian cancer. The observation that pregnancy and oral contraceptive pill use, which decrease lifetime ovulatory cycles, are protective against ovarian cancer [23] is also consistent with the theory that ovulation is the main driving force underlying the accumulation of genetic damage in the ovarian epithelium.

It is not known why repetitive ovulation facilitates the development of ovarian cancer, and several factors, including stimulation by gonadotropins, may play a role [24,25]. One theory is that mutations in the epithelium may result from errors in DNA synthesis that occur during proliferation required to repair ovulatory defects. There is evidence to suggest that spontaneous mutations are more likely to occur in cells that are proliferating relative to those at rest [26]. Spontaneous errors in the process of DNA synthesis may occur about once every million bases. Several families of DNA repair genes exist, but some types of mutations more readily elude surveillance and repair and become fixed in the genome. Furthermore, the efficiency of these DNA repair systems may vary between individuals due to inherited differences in the activity of various alleles of DNA repair genes.

Five years of oral contraceptive use decreases the risk of ovarian cancer by approximately 50%, while decreasing lifetime ovulatory cycles by only 10-20% [23]. Recently, it has been shown that administration of the progestin levonorgestrel, either alone or in combination with estrogen, stimulates apoptosis of ovarian epithelial cells in macaques [27]. This suggests that the progestagenic milieu of pregnancy and the pill will protect against ovarian cancer by increasing apoptosis of ovarian epithelial cells, thereby cleansing the ovary of cells that have acquired genetic damage.

Mechanisms of malignant transformation

The mutations that lead to the development of ovarian and other cancers primarily target genes involved in regulating proliferation, programmed cell death (apoptosis) and senescence-processes that determine the number of cells in a population. The rate of proliferation is a major determinant of the number of cells in a population. Malignant tumors are characterized by alterations in genes that control proliferation. There is increased activity of genes that stimulate proliferation (oncogenes) and loss of growth inhibitory genes (tumor suppressors) (Table 1).

Oncogenes encode proteins normally involved in stimulating proliferation, but when these gene products are overactive they contribute to the process of malignant transformation. Oncogenes can be activated via several mechanisms. In some cancers, amplification of oncogenes with resultant overexpression of the corresponding protein has been noted. Some oncogenes may become overactive when affected by point mutations. Finally, oncogenes may be translocated from one chromosomal location to another and then come under the influence of promoter sequences that cause overexpression of the gene.

Peptide growth factors in the extracellular space can stimulate a cascade of molecular events that leads to proliferation by binding to cell membrane receptors. Peptide growth factors usually act in the local environment where they have been secreted. It has been shown that ovarian cancers produce or are capable of responding to various peptide growth factors. For example, epidermal growth factor (EGF) [28] and transforming growth factor-α (TGF-α) [29] are produced by some ovarian cancers that also express the receptor that binds these peptides (EGF receptor) [30]. Cell membrane receptors that bind peptide growth factors are composed of an extracellular ligand-binding domain, a membrane-spanning region, and a cytoplasmic ty-

Table 1. Classes of genes involved in growth regulatory pathways and malignant transformation

Factors	*Corresponding receptors*
Growth stimulatory genes (oncogenes)	
Peptide growth factors	Examples
Epidermal growth factor (EGF) and transforming growth factor-α (TGF-α)	EGF receptor
Heregulin	*erbB2* (HER-*2neu*), *erbB3, erbB4*
Insulin-like growth factors (IGF-I and IGF-II)	IGF-I and -II receptors
Platelet-derived growth factor (PDGF)	PDGF receptor
Fibroblast growth factors (FGFs)	FGF receptors
Macrophage-colony stimulating factor (M-CSF)	M-CSF receptor (*fms*)
Cytoplasmic factors	Examples
Non-receptor tyrosine kinases	*abl, src,* PIK3CA
G proteins	K-*ras,* H-*ras*
Serine/threonine kinases	AKT2
Nuclear factors	Examples
Transcription factors	*myc, jun, fos*
Cell cycle progression factors	Cyclins, E2F
Growth inhibitory genes (tumor suppressor genes)	
Extranuclear factors	Examples
Cell membrane factors	Transforming growth factor-β1-3 and its type I and II receptors
Cell adhesion factors	Cadherins, APC
Phosphatases	PTEN
Nuclear factors	Examples
Cell cycle inhibitors	*Rb, p53, p16, p27*
Unknown function	*BRCA1*, *BRCA2*

rosine kinase domain. Binding of a growth factor to the extracellular domain results in aggregation and conformational shifts in the receptor and activation of the inner tyrosine kinase [31]. This kinase phosphorylates tyrosine residues on both the growth factor receptor (autophosphorylation) and targets in the cell interior, leading to activation of secondary signals. For example, phosphorylation of phospholipase C leads to breakdown of cell membrane phospholipids and generation of diacylglycerol and inositol triphosphate, both of which play a role in the propagation of the mitogenic signal.

The role of the EGF receptor family of transmembrane receptors and their ligands in growth regulation and transformation has been a prominent focus in cancer research. This family of receptors is also often referred to as the *erb*B family because the first member identified was the *v-erb*B oncogene. The second member of the family (*erb*B2*)* was initially called *neu* because it was found to the transforming gene responsible for the generation of neuroblastomas in rats treated with a chemical carcinogen. This human EGF receptor-like molecule was named both HER-2/*neu* and *erb*B2. The level of HER-2/*neu* is increased in some human breast, ovarian, and other cancers due to amplification [32]. HER-2/*neu* may also be overexpressed due to alterations in regulation of transcription in the absence of gene amplification. In recent years an anti-HER-2/*neu* antibody that induces breast cancer regression has been approved for clinical use by the Food and Drug Administration (FDA) [33]. It is possible that this approach might also benefit some women whose ovarian cancers overexpress HER-2/*neu*.

Mounting evidence also suggests that insulin-like growth factor (IGF) plays important roles in carcinogenesis and tumor progression [34-37]. In addition, ovarian cancers produce basic fibroblast growth factor (FGF) and its receptor, and basic FGF acts as a mitogen in some ovarian cancers [38]. Moreover, ovarian cancers produce macrophage-colony stimulating factor (M-CSF) [39] and serum levels of M-CSF are elevated in some patients [40] since the M-CSF receptor (*fms*) is expressed by many ovarian cancers [41]. In addition, M-CSF could act in a paracrine fashion to stimulate recruitment and activation of macrophages. Since macrophages products such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) have been shown to stimulate proliferation of some ovarian cancer cell lines [42,43], the potential for paracrine stimulation of the cancer by macrophages also exists. In addition to the expression of peptide growth factors and their receptors, ascites of patients with ovarian cancer contains phospholipid factors that stimulate proliferation of ovarian cancer cells [44,45]. Despite circumstantial evidence demonstrating the potential for autocrine and paracrine growth regulation of ovarian cancer cells by peptide growth factors, it remains unclear whether alterations in the expression of growth factors are critical in the development of ovarian cancer. Peptide growth factors may function as necessary cofactors rather than as the driving force behind malignant transformation.

If proliferation is to occur in response to signals generated in the cytoplasm, these events must lead to activation of nuclear factors responsible for DNA replication and cell division. Expression of several genes that encode nuclear proteins increases dramatically within minutes of treatment of normal cells with peptide growth factors. After that, the products of these genes bind to specific DNA regulatory elements and induce transcription of genes involved in DNA synthesis and cell division. However, when inappropriately overexpressed, these transcription factors can act as oncogenes. Among the nuclear transcription factors involved in the stimulating proliferation, amplification and/or overexpression of members of the *myc* family has most often been implicated in the development of human cancers [46]. Amplification of the c-*myc* oncogene occurs in some epithelial ovarian cancers. In the study of Tashiro et al. [47] the incidence of c-*myc* overexpression was observed in 37% of the cases. c-*myc* overexpression was more frequently observed in advanced stage serous adenocarcinomas, suggesting a role for tumor progression.

Oxidative stress and cell signaling

Oxidative stress is defined as an imbalance between the level of prooxidants (reactive oxygen species, ROS) and an antioxidant defence system, in favor of the former and resulting in irreversible cell damage [48-50]. Cellular oxidative stress can modify intercellular communication, protein kinase activity, membrane structure and function, and gene expression, and result in modulation of cell growth.

Cell signaling is a process enabling information to be transmitted from outside of a cell to various functional elements inside the cell. Signals sent to the transcription machinery responsible for expression of certain genes are normally transmitted to the cell nucleus by a class of proteins called transcription factors. Transcription factors are low molecular weight proteins that can bind with the promoter region of a gene. Activation of transcription factors is an important signaling pathway for the regulation of gene transcription by ROS. Transcription factors regulate the transcription of genes

involved in the development, growth, and aging of cells. The regulation of subcellular localization from cytoplasm to nucleus is the first step of transcription factor activity. Oxidative stress is believed to be involved in this process. Nuclear factor kappa B (NFκB) and AP-1, by direct oxidation and phosphorylation, are two transcription factors that are modulated by oxidative stress. The AP-1 transcription factor is a dimer of a protein complex joined by c-*fos*, c-*jun, jun*-B and *jun*-D. AP-1 controls genes required for cell growth and its activity is increased by compounds that induce cellular proliferation. ROS can cause activation of AP-1 as well as new synthesis of AP-1. Oxidative stress can also induce the immediate early protooncogenes c-*fos*, *jun*-B, c-*jun,* and *jun*-D, and thus increase AP-1 transcription factor activity.

Therefore ROS may play a central role in signal transfer system. High levels of ROS may alter signal pathways by oxidative damage of the cell membrane, changes in enzyme activity, and/or the activation of transcription factors. ROS regulate genes via protein kinase C (PKC) activation, oxidative damage, and/or ROS direct activation of transcription factors. The mediation of ROS on gene transcription may also inhibit normal cell apoptosis by modulation of *myc, bcl-2* and *p53* expression and result in an increase in cell number.

In fact, it is well known that ROS elicit a wide spectrum of cellular responses, depending on intracellular ROS level. A low dose of ROS controls normal cellular signaling pathways while an intermediate dose results in either temporary or permanent growth arrest. Obviously, a high dose of ROS causes cell death via either apoptotic or necrotic mechanisms. Recent studies provide strong evidence that oxidative stress has a crucial role in normal aging, and may contribute to pathologic process associated with aging including neoplasia, cataracts and neurodegenerative diseases. As such, development of safe and effective antioxidants, or agents to increase expression of antioxidant enzymes, may be useful in preventing some of these age-related pathologies.

The role of oxidative stress in carcinogenesis

Carcinogenesis is thus a complex multisequential process leading a cell from a healthy to a precancerous state and finally to an early cancerous stage. Cancer development includes 3 major steps, initiation, promotion and progression in which oxidative stress is involved.

When produced in excess, ROS can seriously alter the structure of biomolecules, such as proteins, lipids, lipoproteins, and DNA. Oxidative DNA damage may participate in ROS-induced carcinogenesis [4]. A common form of damage is the formation of hydroxylated bases of DNA, which are considered an important event in chemical carcinogenesis [51]. This adduct formation interferes with normal cell growth by causing genetic mutations and altering normal gene transcription. Several different pathways by which oxidative DNA damage leads to mutations have been proposed, including chemical modification of nucleotide moieties in DNA causing alteration in their hydrogen bonding, exacerbation of polymerase-specific hot spots, conformational change in the DNA templates, and the induction of a DNA polymerase conformation that is error-prone [52]. Formation of 8-hydroxy-2'-deoxyguanosine (8-OhdG), an oxidative modification of DNA produced by hydroxylation in the C-8 position of deoxyguanosine residues by the hydroxyl radical [53], has been used as a measurement of oxidative DNA damage.

Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroperoxides [54]. Lipid peroxyl radicals can subsequently propagate into malondialdehyde (MDA). The formation of lipid damage may results in several possible sequelae including protein oxidation [54]. These lipid radicals can diffuse through membranes, thus modifying the structure and function of the membrane and resulting in a loss of cell homeostasis. In addition, lipid peroxides may result in the interaction with cellular DNA and cause the formation of DNA - MDA adducts.

Proteins are also easily attacked by ROS directly or indirectly through lipid peroxidation. Protein radicals can be rapidly transferred to other sites within the protein infrastructure. This can result in further modification of enzyme activity, stimulation or inhibition [55,56]. In addition to enzymes, damage to the membrane transport proteins may produce cellular ionic homeostasis and lead to alterations in intracellular calcium and potassium that will trigger a series of changes in the cell [57]. Changes to receptor proteins and gap junction proteins may also modify signal transfer in cells. In selective cases alterations of protein structure may allow the target protein to be further attacked by proteinases [58]. Thus protein oxidative damage can result in the modifications in structure, enzyme activity, and signaling pathways.

Signal transduction or cell signaling is a process enabling information to be transmitted from the outside of a cell to various functional elements inside the cell. Signals sent to the transcription machinery responsible for expression of certain genes are normally transmit-

ted to the cell nucleus by a class of proteins called transcription factors. Many recent studies have shown that numerous oxidation-reduction reactions in the cell are involved in regulating several cell functions. According to their nature, quantity, source, and production kinetics in the cell, ROS affect cell regulation differently. The boundary between positive and negative ROS effects is hard to define according to the cell type studied [59]. The same concentration may or may not trigger deregulation of signal transmission, with desirable or undesirable effects. For example, activation of $NF_{\kappa}B$ is positive when it triggers apoptosis but negative when it causes expression of genes coding for proinflammatory agents (cytokines).

This duality depends on the cell type and also on the cell's antioxidant status. In this perspective, glutathione plays a prime role in maintaining a redox status that is optimal for the cell and in regulating transcription genes. In terms of cancer prevention, antioxidant strategies enabling the cell to maintain this optimal state as long as possible can be envisaged.

Our studies [60,61] show that patients with polypus or myoma, or either form of hyperplasia or adenocarcinoma have enhanced lipid peroxidation and altered activities of antioxidant enzymes in the peripheral blood circulation. Although alterations vary with the enzyme type and diagnosis, both reduction in antioxidants and elevation of lipid peroxidation were observed in general. The lowered activity of antioxidant enzymes in gynecological cancer patients could be a result of disturbed redox status, while elevated lipid peroxidation seems to be a consequence of the disease rather than its cause.

It is known that in response to acute oxidative stress antioxidants may be consumed to prevent oxidative damage, and then may be supplied through the antioxidant network. However, in the cases of observed gynecological pathologies it seems that prolonged oxidative stress elevates free radical production and induces consumption of antioxidants, which in turn further aggravates the free radical damage and increases the chance of developing uterine cancer. Indeed, the results obtained in our studies show that the observed changes of antioxidant status in peripheral blood circulation of gynecological cancer patients are more pronounced in premalignant (hyperplastic) and malignant (adenocarcinoma) lesions, compared with benign uterine changes (polypus and myoma) [60,61]. Further investigation should determine whether lipid hydroperoxide levels and antioxidant enzymes activities in blood of such patients might be used as an additional parameter in clinical evaluation of gynecological disorders.

Also, our results [48-50] show that in progressive stages of breast carcinoma, the oxidative stress which significantly increases in later phases of aging can contribute to this uncontrolled tumor expansion. Circulating levels of antioxidants may be important to consider when evaluating a woman's risk of breast cancer, even among women who are at higher predicted risk, based on predictive models or family history [48,50].

Focality studies on borderline and invasive ovarian tumors

While the majority of borderline tumors are confined to a single ovary at the time of diagnosis, 30-40% will present as bilateral or late-stage disease. Whether bilateral or late-stage BOTs are derived from a single ovarian tumor that metastasizes or "seeds" the other ovary and peritoneum, or is the result of a "field defect" that causes multiple primary tumors to occur simultaneously, is unknown. Mok et al. used the pattern of X chromosome inactivation to address this question in bilateral and advanced-stage serous BOTs [21]. With that approach Lu et al. used *Hpa*II restriction endonuclease digestion, followed by PCR amplification of the androgen receptor (AR) locus located on chromosome Xq11-12, to differentiate the active from the inactive X chromosome [62]. In 2 of 8 patients in that study, the left and right ovarian tumor sites had different AR alleles inactivated, indicating that the bilateral tumors originated independently. In the third patient, the X inactivation pattern in the left ovarian tumor differed from the two peritoneal implants, suggesting that the implants were separate primary tumors, and not metastatic from the left ovarian tumor [62]. The remaining 5 patients had the same pattern of X inactivation. Those results suggest that bilateral and advanced-stage serous BOTs may be multifocal in origin, which is in contrast to invasive epithelial ovarian cancer, which has been shown to be unifocal in origin [63].

Mutation analysis on K-*ras* and p53 genes

Molecular genetic studies aimed at delineating the pathogenesis of BOTs have highlighted the importance of K-*ras* signaling pathway. Activating mutations in K-*ras* and one of its downstream mediators, BRAF, have been identified in a variety of human cancers, and mutations of either K-*ras* or BRAF result in constitutive activation of the RAS/RAF/mitogen-activated protein (MAPK)/MEK signaling pathway [64].

Single-strand conformation polymorphism (SSCP) analysis founded K-*ras* mutations in 63% of

mucinous borderline ovarian tumors and 75% of invasive mucinous ovarian cancers [65]. These data suggest that K-*ras* mutations are involved in the development of mucinous BOTs and support the notion that mucinous BOTs may represent a pathological continuum between benign and frankly malignant mucinous cancers. In contrast to mucinous tumors, both serous BOTs and serous invasive ovarian cancers demonstrated a lower K-*ras* mutation rate. Serous BOTs showed a significantly higher K-*ras* and BRAF mutation rates than serous invasive cancers [21,52,53]. Mutations in either codons 12 and 13 of K-*ras* or codon 599 of BRAF occur in two thirds of serous BOTs and low-grade serous carcinomas [66,67]. In contrast, none of 112 high-grade serous carcinomas contained K-*ras* or BRAF mutations [4]. These data suggest that serous BOTs and invasive serous carcinomas may have different pathogenetic pathways, and only a small percentage of BOTs may progress to invasive cancers. In view of the absence of K-*ras* and BRAF mutations in high-grade serous carcinoma, it would seem that the development of high-grade serous carcinoma involves a pathway not related to the mutations in the RAS/RAF/MEK/MAPK signaling pathway. This conclusion is supported by the finding of p53 mutations in >50% of high-grade ovarian serous carcinomas and the rare finding of mutant p53 in serous BOTs and low-grade serous carcinomas [68,69]. Among mucinous tumors, 2 of 15 (13%) BOTs had mutations in p53, compared to 40% of the invasive mucinous cancers [70]. This further suggests that mucinous BOTs and mucinous invasive carcinomas may represent a continuum.

The biological effects of mutations in K-*ras* and BRAF in the development of low-grade carcinoma are likely mediated by the constitutive activation of MARK, the downstream target of the KRAS/BRAF/MEK/MAPK (extracellular signal-regulated kinase) signaling pathway [71]. This is supported by the observation that activating mutations in these genes are oncogenic in experimental cell culture systems [72], probably through a constitutive activation of MAPK which in turn regulate many downstream targets that are important for tumor development [73]. Activated MAPK or extracellular signal-regulated kinase can also be observed in conventional high-grade serous carcinomas, probably through an epigenetic mechanism other than activating mutations of K-*ras* and BRAF [71], because mutations in both genes are rarely found in high-grade serous carcinomas.

What is the biological significance of activation in the MAPK signaling pathway in serous BOTs and low-grade serous carcinoma? Pohl et al. [74] applied long serial analysis of gene expression to identify genes that are regulated by activated MAPK in low-grade serous carcinoma cells that harbor BRAF mutation. The most striking changes after MEK (upstream regulator of MAPK) inhibition were downregulation of cyclin D1, COBRA1 and transglutaminase-2 and upregulation of tumor necrosis factor-related apoptosis-inducing ligand, thrombosporin-1, optineurin, and palladin. Among all the differentially expressed genes, cyclin D1 showed the greatest alteration of gene expression. Cyclin D1 plays an important role in the cell cycle transition from G1 to S phase through its association with cyclin-dependent kinases 4 and 6. In ovarian tumors, overexpression of cyclin D1 is associated with low-grade tumors [75] because cyclin D1 is a downstream target of active MAPK, which is constitutively expressed in most low-grade ovarian tumors because of frequent activating mutations of K-*ras* and BRAF. New experiments in future are necessary to determine whether mutations of K-*ras* and BRAF are sufficient to initiate the development of serous BOTs or whether additional genetic "hits" are required in tumorigenesis. Furthermore, because the mitogen-activated protein kinase inhibitor CI-1040 can inhibit the KRAS/BRAF/MEK/MAPK pathway, it is likely that this compound and other emerging MEK inhibitors may be an effective therapeutic agent for patients with serous BOTs and low-grade serous carcinomas. *In vitro* CI-1040-treated ovarian serous tumors harboring either K-*ras* or BRAF mutations showed marked growth suppression (G1 cell cycle arrest) compared with tumors containing wild type K-*ras* and BRAF. It will be important to determine if treatment with CI-1040 can prolong disease-free interval and overall survival in patients with advanced-stage serous BOTs.

Loss of heterozygosity on multiple chromosome arms

Loss of heterozygosity (LOH) has been used widely to define minimally deleted regions where tumor suppressor genes may reside. Using 105 microsatellite markers to perform detailed deletion mapping on chromosomes 1, 3, 6, 7, 9, 11, 17, and X in BOTs, invasive ovarian carcinomas and serous surface carcinoma of the ovary, the authors identified several common loss regions [76-80]. Besides the AR locus on the X chromosome [76], BOTs showed significantly lower LOH rates (0-18%) in all loci screened, suggesting that LOH at autosomes is less significant in the development of BOTs. The significance in LOH at the AR locus in BOTs and invasive cancers remains to be determined.

LOH at p73 locus on 1p36 was found in both

high- and low-grade ovarian carcinomas as well as in the surface serous carcinomas, but not in BOTs. LOH rates at 3p25, 6q25.1-26, and 7q31.3 were significantly higher in high-grade serous carcinomas, as compared to low-grade serous carcinomas, mucinous carcinomas, and BOTs [77-79]. LOH rates at a 9 cM region on 6q23-24 were significantly higher in surface serous carcinoma than in serous ovarian tumors [80]. LOH at a 4 cM region on chromosome 11p15.1 and an 11 cM region on chromosome 11p15.5 was found only in serous invasive tumors. Furthermore, LOH rates in these two regions were significantly higher in high-grade serous tumors than in low-grade serous tumors [81]. Multiple, minimally deleted regions have been identified on chromosome 17. Significantly higher LOH rates were identified at the p53 locus on 17p13.1 and the NF1 locus on 17q11.1 in high-grade serous carcinomas, as compared to low-grade and serous BOTs and all mucinous tumors [82]. LOH at the region between THRA1 and D17S1327, including BRCA1 locus on 17q21, was found exclusively in high-grade serous tumors [83].

Two independent studies using comparative genomic hybridization have shown that the level of chromosomal imbalance in serous BOTs and low-grade serous carcinomas is similar to each other and is significantly lower than that of high-grade serous carcinomas, reflecting a lesser degree of chromosomal instability in serous BOTs and low-grade serous carcinomas compared with high-grade serous carcinoma [84,85]. Digital PCR analysis showed an increased allelic imbalance index paralleling the progression from serous BOTs to invasive low-grade serous carcinoma [66]. Specifically, allelic imbalance of chromosome 1p, which harbors tumor suppressor genes, including MYCL1 and NOERY/ARH1, is frequently found in serous BOTs [66,86]. Allelic imbalance at certain chromosomal regions in the areas of serous BOTs can be found in the adjacent invasive low-grade serous carcinomas [66,86], further supporting the tumor progression model of serous BOTs.

The findings of significantly higher LOH rates at multiple chromosomal sites in serous compared to mucinous subtypes suggest that serous and mucinous tumors may have different pathogenetic pathways.

Differential expression patterns of novel and known genes

Using RNA fingerprinting, reverse transcriptase polymerase chain reaction (RT-PCR), Northern blot analysis, Western blot analysis, and immunohistochemistry, several novel and known genes have been identified that are expressed differentially among normal ovarian surface epithelial cells, the epithelial lining of benign ovarian cysts, serous and mucinous BOTs, and invasive ovarian cancers.

Recently, high-density oligonucleotide microarrays have been done to profile gene expression in serous BOTs and serous carcinomas of the ovary [85,86]. Meinhold-Heerlein et al. [85] showed that well-differentiated serous carcinomas, which are equivalent to low-grade serous carcinomas, showed a similar profile to serous BOTs compared with moderately and poorly differentiated carcinomas that correspond to high-grade serous carcinomas. Among the differentially expressed genes, a cell cycle regulator, *p21/WAF1*, is consistently expressed in the majority of serous BOTs and low-grade carcinomas but not in high-grade carcinomas. Gilks et al. [87] reported a complete separation between serous BOTs and conventional high-grade serous carcinomas based on unsupervised clustering analysis of gene expression. In their study, the genes that were most differentially expressed in serous BOTs included *mucin 10* (subtype B), *kallikrein 6, B7 protein, claudin 10,* and *keratin 17*. Moreover, the authors found that many genes previously identified as upregulated in ovarian carcinoma relative to normal ovarian surface epithelium were expressed at even higher levels in serous BOTs. Those genes included *mucin 1, mesothelin, HE4, PAX 8,* and *apolipoprotein J/clusterin.* These genes' expression profiles further showed that serous BOTs and low-grade serous carcinoma have a similar expression signature, which is distinct from conventional high-grade serous carcinoma.

Using RNA fingerprinting, several differentially expressed sequences which are downregulated in ovarian cancer cells, have been identified. One of them corresponds to an extracellular matrix protein, *osteonectin (SPARC). SPARC* has been demonstrated to suppress ovarian cell carcinoma cell growth *in vitro* and *in vivo* [88]. High levels of *SPARC* expression were observed in normal ovarian surface epithelium and in the epithelial lining of benign cysts and cystadenomas. In comparison, both serous and mucinous invasive carcinomas had an even lower expression.

Another differentially expressed gene is a novel gene named *DOC-2/hDAB2* [89]. This gene encodes a 105 kDa signal transduction protein which contains a phosphotyrosine interacting domain and multiple SG3 binding motifs. *In situ* immunohistochemistry showed that the surface epithelium of the ovaries and the epithelial lining of benign cysts and cystadenomas demonstrated high levels of *DOC-2/hDAB2* expression. A significantly lower level of expression was identified in both high-grade and low-grade serous invasive carci-

nomas. In contrast, both mucinous BOTs and invasive mucinous carcinomas showed high levels of *DOC-2/hDAB2* expression [90]. When *DOC-2/hDAB2* was transfected into the ovarian carcinoma cell line which has been shown to down-regulate *DOC-2/hDAB2,* the stable transfectants showed significantly reduced growth rate and diminished ability to form tumors in nude mice. These data suggest that downregulation of *DOC-2/hDAB2* may play an important role in the development of serous ovarian tumors. They also imply that serous and mucinous ovarian tumors may have different pathogenetic pathways.

Expression of *p53* and *HER-2/neu* was also examined by immunohistochemistry and Western blot analysis. Overexpression of *p53* was detected in 60% of high-grade invasive carcinomas and 40% of low-grade invasive carcinomas. In contrast, overexpression of *p53* was not observed in any of the BOTs, benign tumors, or normal ovaries. Overexpression of *HER-2/neu* in ovarian cancers has been shown to correlate with poor prognosis of the disease and may be an early event in the development of a subset of ovarian tumors [90].

p73 is a gene that exhibits high sequence homology and similar gene structure to the tumor suppressor gene *p53.* When overexpressed in transfection systems, *p73* can transactivate *p53*-responsive genes and induce apoptosis. RT-PCR and Western blot analysis showed that borderline and invasive ovarian tumors have significantly higher levels of *p73* expression than normal ovarian surface epithelial cells [91]. These findings suggest that upregulation of *p73* may be involved in the development of borderline and invasive ovarian tumors.

Expression of several hormone receptor genes, including estrogen receptor-alfa (ER-α), estrogen receptor-beta (ER-β), AR, and progesterone receptor (PR) has also been examined by RT-PCR and immunohistochemistry in normal ovaries, benign cysts, cystadenomas, and invasive carcinomas. Both ER-α and ER-β are expressed at high levels in all of these tissues. In contrast, AR was expressed at significantly lower levels in invasive carcinomas than in BOTs and benign tumors, and PR was expressed at significantly lower levels in both BOTs and invasive carcinomas. These results suggest that downregulation of AR may be important for the development of invasive carcinomas and downregulation of PR may be important for the development of both BOTs and invasive carcinomas.

Conclusion

Epithelial ovarian cancer continues to be the leading cause of death among gynecologic malignancies. The establishment of preventive strategies, early diagnostic methods, and effective therapies to treat recurrent ovarian tumors creates a pressing need to understand its pathogenesis and to identify molecular biomarkers for prediction, diagnosis, as well as therapy. Cancer is a disease involving multistep dynamic changes in the genome. However, the genetic alteration events as well as their cooperation that promotes malignant transformation and growth in ovarian carcinoma is very complex. New therapies aiming to inhibit the action of engaged transcription factors should be defined specifically for cancer treatment.

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