

Review

Early events in ovarian oncogenesis

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Abstract

Ovarian cancer represents the most lethal of the gynecological neoplasms. The molecular and genetic events associated with early ovarian oncogenesis are still largely unknown, thus contributing to the lack of reliable biomarkers for disease detection. Since the majority of ovarian tumors are diagnosed at an advanced stage, the availability of early ovarian cancer tissue samples for molecular analyses is very limited. In this review, problems encountered in the study of early ovarian cancer are presented, along with the controversies concerning precursor lesions and stepwise progression towards ovarian malignancy. Experimental modeling in the development of ovarian cancer is also described, as well as genetic and epigenetic alterations associated with early ovarian cancer. Lastly, examples of technological advances in the study of early ovarian cancer are discussed. Hopefully, the increasing knowledge about molecular and genetic events involved in the early stages of ovarian tumorigenesis will provide the basis for management of ovarian cancer in the future.

Introduction

The term ovarian cancer applies in general to malignant tumors arising from the modified peritoneal mesothelium that covers the surface of the ovary [1]. These epithelial ovarian cancers (EOC) make up 90% of all human ovarian malignant tumors and display a wide range of histological features, usually recapitulating the morphology of the endocervix, endometrium, or Fallopian tubes that are embryologically related to the surface epithelium [2,3]. Ovarian cancer is the most prevalent cause of death from a gynecological malignancy among women in the Western world, primarily reflecting the fact that it produces vague symptoms, resulting in diagnosis at an advanced stage [4]. In contrast to breast cancer, where most cases are detected at an early stage, only 25% of ovarian cancers are diagnosed at stage I, when the cure rate is almost 90%. The cure rate for ovarian cancer diagnosed at an advanced stage is less than 20% [5]. Ovarian cancer mortality has not significantly decreased due to our poor understanding

of the underlying biology, which in turn has contributed to a lack of reliable biomarkers for disease detection. Therefore, endeavors to improve survival continue to focus on the development of novel chemotherapeutic agents and strategies for earlier detection and prevention of this devastating disease [6].

Problems encountered in the study of early ovarian cancer

There are many difficulties associated with studying early events in ovarian oncogenesis. Only few suitable *in vitro* and *in vivo* models of ovarian neoplasms have been described thus far. The most commonly used experimental animals do not develop EOC. They instead get non-epithelial (sex cord-stromal or germ cell) ovarian tumors, whose underlying biology is ultimately different from that of EOC. The methodology to culture human ovarian surface epithelium (OSE), which presumably gives rise to EOC, has become accessible only recently [7,8]. Human

OSE (HOSE) cells are especially fragile and, being easily removed by handling the ovary or allowing it to dry, are often absent in surgical samples, and thus unavailable for consecutive molecular analyses. Hence, it is difficult to attain HOSE cells, maintain them in tissue culture, and analyze under standardized conditions. Despite these experimental difficulties, the first transgenic mouse model of EOC has been successfully described just recently [9]. The specific location of the ovary, deep in the pelvis, excludes the use of invasive screening procedures. Consequently, the vast majority of ovarian tumors available to investigators through tumor banks come from patients with advanced disease. The availability of early ovarian cancer tissue samples, especially stage I, is unfortunately very limited. Since there is little information on the sequence of molecular and genetic alterations associated with initiation and progression from early- to advanced-stage ovarian cancer, an attempt will be made in this review to summarize findings with regard to early events in ovarian oncogenesis.

Genetics of ovarian cancer

It is well known that ovarian cancer has a large number of genetic changes involving both activation of oncogenes and loss of tumor-suppressor genes [10], thus complicating determination of the importance of an individual gene's alteration. Even though numerous genes with altered expression in EOC are under investigation worldwide, only few are likely to be causal and will provide new targets for diagnosis and therapy of this malignancy [11,12]. The question of how to prove the causality of a candidate gene to some aspect of the disease phenotype will most probably be answered through the use of genetically engineered mice. Ovarian cancer has been referred to as very complex and heterogeneous. Because of this, tumors with the same histological features that arise in different patients may display diverse alterations with different patterns of oncogene activation or tumor-suppressor gene loss [5].

Precursors of ovarian cancer

Contrary to the multistep progression model of colorectal tumorigenesis, in which a distinct series of morphologically well-defined lesions have been described [13], the ovarian cancer research community disagrees as to whether inclusion cysts [14], benign ovarian tumors [15] or borderline (low malignant potential) tumors [16] are precursors of the EOC. Consequently, this controversy about the source of EOC has hampered our efforts to dissect and elucidate steps in ovarian oncogenesis. Although the mainstream idea is that EOC's arise from the surface epithelium, some investigators have questioned this dogma by putting forward an alternative hypothesis that EOC develops from the secondary Müllerian system [3,17]. With regard to inclusion cysts, Salazar and col-

leagues had a unique opportunity to be among the first to analyze in detail histological features of the ovaries prophylactically removed from healthy women at an inherited risk of ovarian malignancy [18]. In addition to increased formation of inclusion cysts, many other abnormalities were observed in ovaries obtained in prophylactic oophorectomies. Those include surface epithelial pseudostratification, papillomatosis, deep cortical invaginations of the surface epithelium, stromal abnormalities, and ultrastructural changes in OSE cells. These histological lesions were thought to be early substrates from which ovarian cancer could develop.

It is also controversial whether benign, borderline and malignant tumors are a part of a disease continuum of stepwise progression toward ovarian cancer or if they represent separate disease entities, each arising *de novo* [17]. Certain molecular changes, such as telomerase expression, alterations in DNA methylation levels and LOH are associated with both borderline and malignant, but not benign ovarian tumors, and will be discussed later on in this review [19].

Epigenetic alterations in early ovarian cancer

Most recently, epigenetic changes have been recognized as an important alternative mechanism of gene inactivation in ovarian cancer. The evidence of hypermethylation of the promoter region of *BRCA1*, which is known as breast and ovarian cancer susceptibility gene and tumor-suppressor gene involved in the maintenance of genomic integrity, corroborates the view that this epigenetic alteration may play a determinant role in inactivation of tumor-suppressors and ultimately contributing to the initiation or malignant progression of tumors. Consequently, hypermethylated *BRCA1* represents potential biological marker for early ovarian cancer diagnosis and development of methylation-targeted drugs for its treatment [20]. HPLC analysis revealed the absence of alterations in DNA methylation of a specific locus *MyoD1* in ovarian cystadenomas, but their presence in both borderline tumors and carcinomas [21]. These results lend support to the idea that such changes are early events in ovarian oncogenesis and emphasize the merit of distinguishing borderline ovarian neoplasms from cystadenomas, despite their similar non-aggressive clinical behavior. Therefore, borderline tumors of the ovary may be considered a distinct disease entity.

Telomerase activity in ovarian cancer

Another example of a molecular change shared by both borderline and malignant but not benign ovarian tumors is expression of the enzyme telomerase [22]. Activation of this reverse-transcriptase that synthesizes telomeric sequences, and is important for DNA replication and chromosome protection, is strongly associated with can-

cer. It is absent from most normal somatic cells and is present in about 90% of all human tumors. In particular in the ovary, the first description of differences in telomeric instability between normal cells obtained from a tissue with high, site-specific cancer risk due to a genetic predisposition and similar cells from a control group has been provided recently [23]. These HOSE cell lines derived from the ovaries of women with family histories of breast/ovarian cancer have proven to be a useful culture model system for studying ovarian oncogenesis. HOSE cells from cancer-prone individuals, in comparison to those of individuals at normal risk had reduced growth potential, increased telomeric instability and shorter telomeres, suggesting that a family history of ovarian malignancy confers increased telomeric instability to the normal surface epithelium. Moreover, telomeric lengths in HOSE cells from cancer-prone individuals were shorter than those found in ovarian cancer, confirming the association of decreased mean telomeric length with malignant transformation [24].

Modeling ovarian cancer

The incessant ovulation hypothesis of ovarian cancer etiology proposed in 1971 suggests that ovulatory rupture and repair predispose OSE to mutation [25]. It is supported by epidemiological data that the accumulated number of menstrual cycles correlates with the risk of ovarian cancer [26]. The oral contraceptives and pregnancy protect against ovarian cancer, probably by a decrease in lifetime ovulations and/or increasing apoptosis of OSE cells, thereby cleansing the ovary of cells with acquired genetic damage [27,28].

More than twenty years ago, rat OSE was the first OSE of any species to be established and maintained in tissue culture. Concurrently, the first demonstration of the susceptibility of OSE to an oncogenic virus was provided [29]. These cells were shown to be transformable by Kirsten murine sarcoma virus (Ki-MSV) and to produce highly malignant ovarian tumors in immunosuppressed rats.

Furthermore, for an accurate historical perspective on animal models of ovarian cancer, the work of Nicosia et al. on cultured rabbit OSE needs to be highlighted. Their morphological studies suggested variability in the proliferative activity of OSE during the reproductive life cycle and role of local factors and/or reproductive patterns in regulating the growth and development of this crucial component of the ovary [30,31].

In the 1990s, experimental support for the incessant ovulation hypothesis of ovarian cancer etiology was obtained by isolating rat OSE (ROSE) cells and subjecting them to repeated subculture *in vitro* to mimic the growth of these cells following ovulation [32,33]. This strategy resulted in

malignant transformation of ROSE as assessed by tumorigenicity in ten out of thirty independent attempts. A situation was provided where independent events leading to malignant transformation occurred in cells with originally the same genetic constitution. This not only made available related normal/tumor cell pairs, which is an important asset when techniques of molecular biology are applied to uncover genetic differences associated with malignant versus normal cells, but also gave the opportunity to determine the frequency of the same genetic event in multiple independent transformants. Using ROSE model, the Lost On Transformation 1 (*LOT1*), a growth-suppressor gene involved in EGFR signaling pathway that encodes a nuclear zinc-finger protein was discovered. Moreover, by the technique of genome scanning, the cathepsin B gene was found to be amplified and/or overexpressed in malignant transformed ROSE cells. There is evidence suggesting involvement of these genes in human ovarian oncogenesis [34,35].

Genes associated with early ovarian cancer

In conjunction with the rat *in vitro* model of ovarian cancer, consolidative suppression subtractive hybridization (CSSH) has been developed and applied to look for gene expression differences between normal and transformed cells [36]. Cellular retinol-binding protein 1 (*CRBP1*), gene that was found to be down-regulated in this model, is considered critical to vitamin A (retinol) metabolism. A recent report by Kuppumbatti et al. indicated that alterations in retinol metabolism could be an early event in breast oncogenesis [37]. This information and the finding that treatment with fenretinide, a vitamin A derivative, prevented the occurrence of ovarian cancers in women [38] provided the basis to examine expression of *CRBP1* in the context of human ovarian cancer [39]. Vitamin A and derivatives have well-established roles in cancer prevention and treatment [6] and many studies have revealed the inverse association between ovarian cancer risk and vitamin A intake [40,41], however the mechanism of this association is still unclear. Analysis of microdissected human serous ovarian cancers has shown the loss of *CRBP1* expression in one-third of cases, which is comparable with the *in situ* hybridization breast cancer study [37], and with the loss of other genes in ovarian cancer [12]. This appears to be an early event in human ovarian oncogenesis, since there was no statistically significant difference in the loss of *CRBP1* expression between histological grades or clinical stages of disease in analyzed samples. Described change in the ability of cells to metabolize vitamin A could have implications with regard to ovarian cancer initiation and/or progression [36,39]. Nevertheless, the study of *CRBP1* in ovarian cancer has demonstrated how the novel technology of laser capture microdissection can improve the quality of the molecular analysis by eliminating contamination with stromal, vas-

cular and inflammatory cells that are often present in EOC specimens, and by isolating pure populations of ovarian malignant cells. In the future, results obtained using laser capture microdissection may allow for better identification of early events in ovarian oncogenesis and the advancement of therapeutic tools.

DOC2/Dab2 is a candidate tumor-suppressor gene that negatively regulates Ras-mediated cell growth and is thought to function in epithelial cell positioning control. Immunohistochemical analysis has shown that the expression of Dab2 is lost in 80% of ovarian carcinomas but this loss did not correlate with tumor grade [42]. *Dab2* inactivation has been proposed to be an early event in ovarian oncogenesis enabling the basement membrane-independent and disorganized proliferation of tumor cells [43]. Comprehensive histological analysis has also suggested loss of the basement membrane as an early event in the preneoplastic transformation of HOSE and in the early stages of tumorigenesis before tumor invasion and metastasis [44].

It has been previously reported that activation and overexpression of *BTAK/Aurora-A* gene is more frequent in early-stage/low-grade ovarian tumors [45]. *BTAK/Aurora-A* is a serine/threonine protein kinase, which is essential for chromosome segregation and centrosome functions. Immunohistochemical analysis has showed its preferential expression in less invasive ovarian tumors suggesting that alterations of *BTAK/Aurora-A* could be an early event in human ovarian oncogenesis and play an important role in development of a subset of ovarian cancers.

Mutations of *K-ras* oncogene are detected most frequently in mucinous adenocarcinomas but rarely in other common epithelial tumors of the ovary and therefore may be considered a biomarker of this histological subtype [46]. Studies have suggested not only a correlation to histological subtype, but also association of *K-ras* mutation with early steps in the progression of mucinous ovarian tumors [47,48]. Mutations of *K-ras* occur more frequently in borderline than in malignant ovarian serous carcinomas and they differ between these two categories of ovarian neoplasia [49]. According to Ortiz et al., serous borderline tumors arise through a different molecular pathway than invasive serous carcinomas and these lesions are perhaps unrelated [50].

As indicated earlier, problems encountered in the study of surface epithelium as the source of ovarian cancer are manifold. Normal OSE does not have any tissue-specific differentiation markers that would aid in the identification of alterations that precede the development of overt ovarian cancer [51]. During human ovarian oncogenesis, morphologically simple OSE transforms into highly com-

plex histological structures similar to Müllerian duct-derived epithelia. This is opposite to majority of other epithelial malignant tumors that during neoplastic transformation become less differentiated than the epithelium of their origin. This abnormal differentiation of OSE is associated with expression of *E-cadherin*, a cell-adhesion molecule acting as an epithelial differentiation marker and inducer of epithelial differentiation, which is not present in normal OSE [52]. In carcinomas other than ovarian, *E-cadherin* is frequently lost, suggesting possible tumor-suppressor role. In ovarian carcinoma, following the expression in primary tumors, the subsequent loss of *E-cadherin* plays a role in late-stage ovarian metastasis. Experimental data indicate that expression of *E-cadherin* is an early event in ovarian oncogenesis. It initiates early preneoplastic changes by giving OSE cells a more complex epithelial phenotype [53]. Similarly, hepatocyte growth factor and its receptor *Met (HGF-Met)* may also play a role in early stages of ovarian oncogenesis according to a study in HOSE cells obtained from women with family histories of breast/ovarian cancer [54].

Technological advances in the study of early ovarian cancer

A variety of molecular biology and high-throughput techniques are currently being used in order to analyze ovarian tumor tissues and cell lines. Spectral karyotyping, heterozygosity (LOH) and comparative genomic hybridization (CGH) techniques were used to look for chromosomal gains and/or losses and translocations in genomic structure of ovarian tumors. Those studies have shown that early-stage ovarian cancers have a smaller number of genetic changes than late-stage cases [55,56]. The discovery of frequent allelic losses for a specific chromosome location suggests that a tumor-suppressor gene for the malignancy in question maps closely to the locus being studied. It has been reported that LOH on chromosomes 7p, 7q, 9p and 11q [57], 13q and 17p [58] is an early event in ovarian tumorigenesis. Borderline tumors have similar patterns of LOH to that of early-stage malignant ovarian tumors, indicating inactivation of the same set of tumor-suppressor genes in the development of malignant and borderline forms [57]. Karyotypic abnormalities in ovarian cancer are very complex and difficult to interpret because of the advanced-stage of disease in most surgical specimens under analysis.

Techniques such as differential display, suppression subtractive hybridization, cDNA microarray and serial analysis of gene expression (SAGE) are used to examine gene expression profiles by comparing ovarian tumor and normal tissues or cell lines. Clinical stage or extent of disease at diagnosis is one of the most important prognostic factors in ovarian cancer [59]. It has been reasoned that if stage I disease is a precursor of advanced ovarian cancer,

comparing early- (stage I/II) and late-stage (stage III/IV) tumor specimens for changes in gene expression and identifying molecular differences between them should reveal biomarkers that are hallmarks of early disease and targets for early diagnosis and/or chemotherapeutic treatments [39,56,60,61].

Molecular profiling of ovarian cancer is dependent on several factors including age, sex, ethnicity and genetic background of a given patient, as well as on histological features, grade and stage of disease in the tissue samples analyzed. The investigations that have compared EOC's with sex cord-stromal or germ cell tumor samples have led to discrepancy in explanation of the significance of an individual gene and its potential role in early ovarian oncogenesis. Therefore, it is necessary to consider all these variables in order to identify molecular pathways and genes leading to ovarian cancer and to determine if each histological subtype evolves as a result of a separate set of molecular alterations. Additionally, heterogeneity of ovarian cancer can complicate the interpretation of gene expression studies. Thus, sample selection is another important issue and recently developed technique of laser capture microdissection can be used to isolate a defined cell population from specific areas of ovarian tissue [39].

While large data sets are obtained and analyzed, gene expression patterns become apparent, such as groups of genes coordinately up-regulated in ovarian cancer [62]. Additionally, studies of great numbers of EOC specimens have allowed researchers to decrease the complexity of cDNA microarrays by eliminating large subsets of genes that are not expressed or whose expression does not vary in EOC's as compared to normal ovarian tissue [63]. Molecular profiling of EOC's will significantly improve our current understanding of early ovarian oncogenesis and of the individual histological subtypes of EOC's.

Large-scale analysis of cellular protein profiles of normal and tumor tissues are also being utilized to reveal quantitative changes in expression and post-translational modifications of proteins that can be important hallmarks of early ovarian cancer. Surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) analysis and matrix-assisted laser desorption ionization time-of flight (MALDI-TOF) technology have the potential to distinguish patterns of alterations in thousands of small proteins (<20 kd). When linked to computer algorithms, SELDI-TOF can identify a pattern of protein changes in serum with high specificity and sensitivity for early ovarian cancer [64].

Conclusion

This review summarized molecular and genetic studies of early ovarian oncogenesis. It presented problems associ-

ated with studying this process, and emphasized the unavailability of early ovarian tumors and the significance of the OSE as the source of ovarian cancer. The review addressed the question as to whether ovarian cancer trails the multistep progression model of oncogenesis as originally described in colorectal cancer. It appears valid, based on experimental data, that preneoplasia precedes overt disease as in other solid tumor types. There has been also some controversy as to whether ovarian cancer originates from the surface epithelium or secondary Müllerian system. Based on the experimental induction of malignant transformation in rat, mouse, and human OSE, it is assumed that ovarian cancer develops from the surface epithelium. With the advancement of molecular biology methods, large amount of data has been produced related to gene expression and genetic abnormalities in ovarian cancer. However, in order to prove causality of a candidate gene to some aspect of the diseases phenotype, generation of mouse models of ovarian cancer by transgenesis will be required. In the meantime, HOSE cell lines, especially ones derived from the ovaries of women with family histories of breast/ovarian cancer, continue to serve as a reliable, convenient, and clinically relevant model system for further delineation of molecular pathways involved in the early stages of ovarian oncogenesis. In summary, any advance in our understanding of early events in ovarian neoplastic progression will only come from the application of modern techniques and analyses, such as differential display, comparative genomic hybridization, laser capture microdissection, suppression subtractive hybridization, serial analysis of gene expression and cDNA microarray, which provide a more sophisticated approach than that allowed by purely morphological methods. Consequently improved knowledge about molecular events implicated in early stages of ovarian oncogenesis will provide a rational basis for management of ovarian cancer in the near future.

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