



Review

## Gene Mutations in Animal Models: Do Tumor Suppressor Genes, *brca1* and *brca2*, Play a Role in Ovarian Carcinogenesis?

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Ovarian cancer is the most lethal cause of death from gynecological malignancies in the Western world. Over 90% of human ovarian cancers arise in the ovarian surface epithelium (OSE). The OSE surrounding the ovary is simple mesothelium and squamous to flat-cuboidal mesothelial cells. This cell type of ovary has both epithelial and mesenchymal potential. Also OSE cells are regulated by many factors such as cytokines, growth factors, and multiple hormones. Nevertheless OSE function is poorly understood. In particular, ovarian cancers are closely related with hereditary predisposition. Hereditary ovarian tumors are commonly associated with mutations in tumor suppressor genes such as *brca1* and *brca2* genes. These genes play a role in maintenance of genome integrity, DNA repair, cell cycle control and apoptosis. Mutations in *brca1* and/or *brca2* may lead to carcinogenesis through distinct molecular pathways like estrogen-mediated proliferation, the presence of a p53 mutation, and the modulation of the activity of NF- $\kappa$ B. Especially the dysfunction of *brca1* triggers the inactivation of p53 and a higher proportion of a p53 mutation is commonly linked to *brca*-linked ovarian tumorigenesis. The dysfunction of *brca1* and/or *brca2* can arise from multiple mechanisms in the regulation of both JNK and ERK1/2 signaling. For more effective diagnosis and therapy of ovarian cancer, the role of *brca1* and/or *brca2* in ovarian cancer has to be distinctively elucidated by the animal models in which the gene functions are deleted in mouse OSE cells and by the mechanisms by which these genes affect ovarian carcinogenesis.

**Key words:** Ovarian surface epithelium, tumor suppressor gene, *brca1/2*, animal models

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### Ovarian surface epithelium

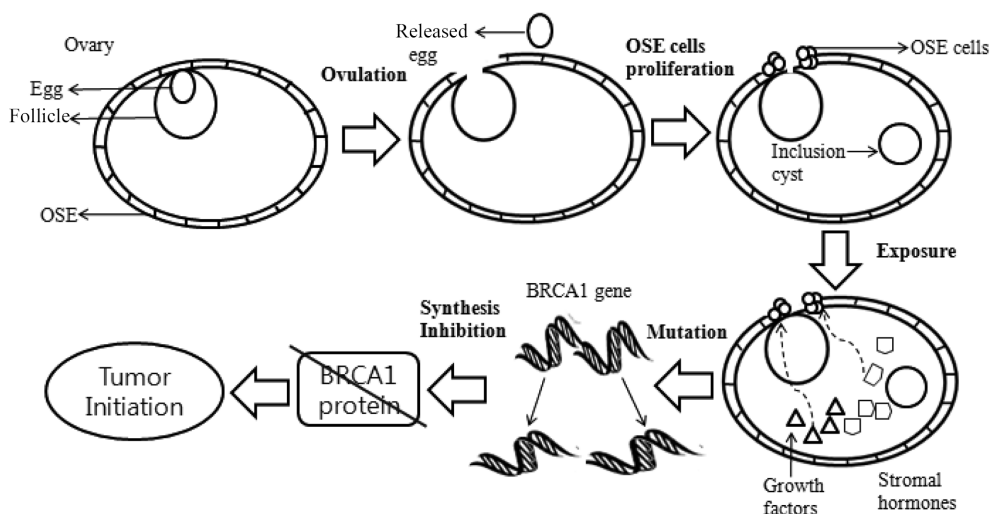
The mammalian ovary is covered with a singular layer of epithelial cells that forms the outer layer of the ovary. This ovarian surface epithelium (OSE) is a simple mesothelium and is monolayered squamous to cuboidal epithelium (Choi and Auersperg, 2003). Also, it is separated from the underlying ovarian stroma by a basement membrane upon the tunica albuginea and is held together by desmosomes and gap or tight junctional complexes (Murdoch and McDonnell, 2002). The adult OSE arise from a common embryonic origin, the celomic epithelium, during embryonic development.

In culture, human OSE cell lines form cobblestone epithelial

monolayer. This OSE cells represent not only epithelial but also mesenchymal intermediated filament proteins (keratin and vimentin) (Wright *et al.*, 2002). Besides it manufactures epithelial (laminin and collagen IV) and mesenchymal (collagen and III) components of extracellular matrix (ECM) (Wong and Leung, 2007).

Ovary has many hormones, growth factors, and cytokines, which are regulated by interactions between OSE and surrounding cells. The OSE function is not well understood but it is very important in normal ovarian physiology. In reproductive cycle, it is believed that OSE participates in the cyclical ovulation ruptures and repair (Auersperg *et al.*, 2001). Also, the OSE produces proteolytic enzymes, which are degraded between the tunica albuginea and underlying follicular theca (Wong and Leung, 2007). After ovulation, OSE cells reconstitute an intact simple mesothelium and repairs ruptured surface (Leung and Choi, 2007). At this time, the chance of, DNA damage in OSE cells increases resulting in risk of ovarian cancer increase (Figure 1).

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**Figure 1.** Scheme of ovarian cancer development derived from *brca1* mutation in OSE cells. The ovary is covered with simple OSE cells. These OSE cells are ruptured by ovulation and destroyed to release eggs. Ruptured OSE cells are recomposed by OSE cell proliferation. At this time, OSE cells can be created in an inclusion cyst. These inclusion cysts can more effectively be contacted with growth factors or stromal hormones by stromal layers. At results, *brca1* gene appears to lead to mutation and inhibition of *brca1* protein synthesis. When DNAs are damaged, deficient *brca1* protein brings about scarcity of DNA repair function. Ultimately, this reason may cause the initiation of various types of ovarian tumors, i.e., serous, endometriod, mucinous, or clear cell ovarian tumors.

About 90% of epithelial ovarian cancers are thought to arise from the OSE (Clark-Knowles *et al.*, 2007). Epithelial ovarian cancer is the fifth most common cause of cancer in women but primary cause of death from gynecological malignancies.

## Epithelial ovarian cancer

Ovarian cancer is the primary and most lethal cause of death from gynecological malignancies in the Western world (Auersperg *et al.*, 1998; Auersperg *et al.*, 2001). In women, ovarian cancer is the 8<sup>th</sup> most common cancer, but ranks 5<sup>th</sup> in cancer associated deaths (O'Donnell *et al.*, 2010). Annually, 2,500 new cases are diagnosed and 1,550 women die from the disease in Canada (Canadian Cancer Statistics 2002). A high fatality rate may be due to the lack of effective screening methods and the paucity of symptoms and signs in early stage of the disease (Holschneider and Berek, 2000; Bast *et al.*, 2002; Luo *et al.*, 2003). Despite intense research efforts, the mechanism of transformation and development of ovarian cancer is not well elucidated. Epithelial ovarian carcinomas, which comprise over 90% of human ovarian cancer, arise in the OSE. The etiology and early events in the progression of these carcinomas are poorly understood because there are no appropriate animal models, and because methods to culture OSE have become available only recently (Auersperg *et al.*, 2001; Auersperg, 2003; Urban, 2003).

## Gene mutations in epithelial ovarian tumors

Hereditary ovarian tumors are commonly associated with mutations in *brca1* or *brca2* genes, which are play role maintenance of genome integrity, DNA repair, cell cycle control and apoptosis (Wong and Auersperg, 2003). The lifetime risk of ovarian cancer by age 70 increases from 1.4% in the general population to 28-60% with a *brca1* mutation and to 11-27% with a *brca2* mutation (Frank and Critchfield, 2001). Despite the presence of *brca1* germline mutations in all tissues, cancer susceptibility phenotype is highly tissue-specific due to hormonal influences. Histo-pathology of normal ovaries from healthy women with hereditary predisposition showed increased numbers of inclusion cysts and clefts, and subtle changes in nuclear chromatin in the OSE with *brca1* and *brca2* mutations (Salazar *et al.*, 1996; Sherman *et al.*, 1999; Werness *et al.*, 1999; Barakat *et al.*, 2000).

In the OSE cells from women with family histories of ovarian cancer (FH-OSE), epithelial-mesenchymal conversion in response to the culture environment is reduced and the epithelial phenotype is more persistent, suggesting a state of autonomy intermediate between OSE cells with no family histories (NFH) and ovarian carcinoma cell lines (Auersperg *et al.*, 1995; Dyck *et al.*, 1996; Wong *et al.*, 1998; Wong *et al.*, 2001). FH-OSE cells immortalized by SV-40 large T-antigen show an increased telomeric shortening and reduced

growth potential, which causes premature cellular senescence (Kruk *et al.*, 1999). The increased genetic instability may explain the earlier onset of ovarian cancer in women with family histories, indicating that phenotypic changes distinct from DNA repair occur in OSE from women with hereditary predisposition to ovarian cancer. In addition, these alterations might represent some of the earliest carcinogenic changes and lead to the discovery of markers for early detection of ovarian carcinoma (Maines-Bandiera and Auersperg, 1997; Sundfeldt *et al.*, 1997).

### ***In vivo* models of epithelial ovarian cancer**

The establishment of animal models for epithelial ovarian cancer has proved to be difficult. In contrast to diverse *in vitro* models, which are available as OSE cultures from many species (i.e., human, mouse, rat, rabbit, cow, pig, etc.), *in vivo* animal models for epithelial ovarian cancer have been very limited because most human ovarian tumors appear to be derived from OSE, whereas ovarian cancers in other species are derived from granulosa, stromal, and germ cells. Thus, the commonly-used laboratory animals do not develop epithelial ovarian tumors. As an animal model, it was first reported that aging hens, which spend most of their life ovulating, have a high incidence of ovarian adenocarcinomas that closely resemble those of humans (Fredrickson, 1987).

Early lethality in embryos, growth defects, and chromosome abnormalities were observed in transgenic mice with homozygous deletion of *brca1* or *brca2* involved in p53-associated DNA repair (Brugarolas and Jacks, 1997; Ludwig *et al.*, 1997; Deng and Brodie, 2001). Mammary tissue-specific knockout mice were generated to elucidate a role of *brca1* and *brca2* genes; inhibited branching morphogenesis and increased incidence of breast tumors at age one year were induced in these mice (Bennett *et al.*, 2000; Ludwig *et al.*, 2001). Attempts to develop transgenic mouse models with an OSE-specific promoter (OSP) have been hampered with the fact that OSE is a simple, rather primitive epithelium with no known tissue-specific markers that would provide the required promoters. However, the discovery of OSP-1, a retrovirus-like element which, in the rat, is expressed only in the ovary, has given promising results (Selvakumaran *et al.*, 2001). In one study, the simultaneous overexpression of any two of three oncogenes, *c-myc*, *K-ras*, and *Akt*, resulted in the formation of epithelial ovarian tumors in p53-deficient homozygote mice, indicating that the mouse OSE is the precursor tissue for these ovarian carcinomas and that introduction of two oncogenes is sufficient for the formation

of ovarian tumors which resemble human ovarian tumors (Orsulic *et al.*, 2002). Recently, using a single intrabursal administration of recombinant Cre-expressing adenovirus, we demonstrated that concurrent inactivation of p53 and Rb1 is sufficient for reproducible induction of ovarian epithelial carcinogenesis in mice homozygous for conditional gene alleles (Flesken-Nikitin *et al.*, 2003).

### ***Brca1* and *brca2* as tumor suppressor genes**

Among genes potentially involved in ovarian carcinogenesis, germline mutations of the *brca1* and *brca2* genes, located on chromosome 17q12-21 and 13q12-13 respectively, are estimated to be responsible for the great majority of familial ovarian and breast cancers (Abel *et al.*, 1993; Lalle *et al.*, 1994; Miki *et al.*, 1994; Wooster *et al.*, 1994; Wooster *et al.*, 1995; Xu and Solomon, 1996; Healy, 1997; Scully, 2000; Scully and Livingston, 2000; Boyd, 2003; Scully and Puget, 2003), and have been detected in 90% of familial breast/ovarian cancers (Alberg and Helzlsouer, 1997). The human *brca1* gene encodes a nuclear protein of 1863 amino acids (220-kDa), whereas the *brca2* protein is composed of 3418 amino acids; both proteins are important in cellular responses to DNA damage and in maintaining genomic integrity (Chen *et al.*, 1998; Patel *et al.*, 1998; Scully and Livingston, 2000; Venkitaraman, 2000; Yu *et al.*, 2000; Moynahan *et al.*, 2001; Shamoo, 2003; Somasundaram, 2003). In the mouse, *brca1* encodes a protein of 1812 amino acids with approximately 58% identity to the human protein (Lane *et al.*, 1995; Marquis *et al.*, 1995). The *brca1*-regulated gene products have been implicated directly or indirectly in cell cycle regulation and DNA repair (Xu *et al.*, 1999; Somasundaram, 2003). In particular, a series of allelic mutations in the tumor suppressor *brca1* have been created to study mechanisms underlying *brca1*-associated tumorigenesis. These studies indicate that *brca1* is essential in maintaining genome integrity through its involvement in DNA damage repair, G(2)-M cell-cycle checkpoint and centrosome duplication (Xu *et al.*, 1999; Haber, 2000; Hartman and Ford, 2002; Jasin, 2002). The loss of *brca1* triggers multiple genetic alterations, including the inactivation of p53 and activation of a number of oncogenes, that ultimately result in mammary tumorigenesis (Deng and Scott, 2000; Brodie and Deng, 2001; Brodie *et al.*, 2001; Venkitaraman, 2002; Deng and Wang, 2003). Although much progress has been made toward the understanding of the function of these genes through genetic, biochemical, and structural studies, a definitive elucidation as to the roles of these genes in ovarian carcinogenesis has

not been achieved.

In the majority of mutations predicted to result in protein truncations that delete the C-terminal of *brca1*, deficiency of *brca1* results in early embryonic lethality in mice due to developmental delay and cellular proliferation defects at 7.5 to 13.5 day of embryonic development (Gowen *et al.*, 1996; Hakem *et al.*, 1996; Liu *et al.*, 1996; Ludwig *et al.*, 1997; Shen *et al.*, 1998; Deng and Brodie, 2001). To overcome the early lethality, transgenic mice have been generated by flanking *brca1* exon 11 with loxP sites (Xu *et al.*, 1999; Deng, 2002; Weaver *et al.*, 2002). In these studies, the deletion of the exon by EIIA-Cre, which expresses Cre in the germline, resulted in p53-dependent lethality at late gestation. It is of interest that the tumors are highly diverse in histopathology and displayed extensive genetic/molecular alterations, including overexpression of ErbB2, c-Myc, p27, and Cyclin D1, and downregulation of p16 (Deng, 2002). The *brca1* and *brca2* deletion restricted to mammary epithelium was achieved using the highly restricted whey acidic protein (WAP) promoter and the somewhat less specific mouse mammary tumor virus-long terminal repeat (MMTV-LTR) to direct expression in the *brca1*Ko/Co/Wap-cre and *brca1*Ko/Co/MMTV-cre mice (Xu *et al.*, 1999). Notably, the embryonic lethality of *brca1* knockout mice is rescued by the human transgene, and the expression of human *brca1* transgene mirrors the endogenous murine gene (Chandler *et al.*, 2001).

*brca2* deficiency also resulted in early embryonic lethality at 7.5 to 10.5 day of embryonic development in the mouse when disrupting mutations produce truncations that occur 5' to exon 10 and 11 (Ludwig *et al.*, 1997; Sharan *et al.*, 1997; Suzuki *et al.*, 1997; Bennett *et al.*, 2000). The conditional knockout of *brca2* in mammary epithelium, *brca2* ox/7/WAP cre mice, created a mutation in exons 3 and 4; in these mice, 20 out of 26 (77%) *brca2* ox/7/WAP-cre mice developed mammary tumors (Ludwig *et al.*, 2001). The targeted *brca2* exons 11 or 27 may play a critical role involved in Rad51 (a protein essential to homologous recombination and DNA double strand-break repair) (Jonkers *et al.*, 2001; Yang *et al.*, 2002; Donoho *et al.*, 2003). Although the phenotypic consequences of their disruptions are similar, *brca1* and *brca2* play distinct roles in the mechanisms that lead to the repair of DNA double-strand breaks (Venkitaraman, 2001).

Mammary (and other) tumors occur at long latency as compared to oncogene-induced mouse tumors. p53 deficiency is highly cooperative with both *brca1* and *brca2* in promoting tumorigenesis (Xu *et al.*, 1999; Jonkers *et al.*, 2001). Analysis of *brca1*-associated mammary tumors reveals significant similarities to *brca1*-associated breast cancer in regard to high tumor grade, hormone receptor negativity,

a high incidence of p53 mutations, and genetic instability (Xu *et al.*, 1999; Moynahan, 2002). With the exception of mammary tumors observed in few *brca1* tissue-specific knockout mice, none of the heterozygous mutant mice have shown susceptibility to mammary tumors (Xu *et al.*, 1999). In addition, heterozygous females of *brca2* develop normally and no tumors are prone to this model (Ludwig *et al.*, 1997; Sharan *et al.*, 1997; Suzuki *et al.*, 1997). The magnitude of the developmental defect was correlated to the amount of cre-mediated deletion; however, no developmental defects were noted in the K14cre/*brca2*F11/F11 or the *brca2* ox/7/WAP-cre mice (Jonkers *et al.*, 2001; Ludwig *et al.*, 2001).

## Brca1 and brca2 in ovarian cancer

Ovarian carcinomas associated with germline *brca* mutations have a significantly higher growth fraction than do sporadic cancers (Koul *et al.*, 2000; Levine *et al.*, 2002). In addition to the germline mutations of these genes, somatic mutations also appear to involve ovarian cancer development (Hosking *et al.*, 1995; Chan *et al.*, 2002), but gene expression profiling of hereditary and sporadic ovarian cancers reveals unique *brca1* and *brca2* signatures (Hedenfalk, 2002). The survival of patients with ovarian cancer is affected by *brca* germline mutation, at least in the early years after diagnosis (Ben David *et al.*, 2002; Cass *et al.*, 2003). In consideration of clinicopathological features, there was a significantly higher proportion of tumors with serous adenocarcinoma and of cases of advanced stages in the *brca1* or *brca2* cases compared to controls (Sekine *et al.*, 2001).

Multiple mechanisms cause nearly universal dysfunction of *brca1* and/or *brca2* in hereditary and sporadic ovarian carcinoma. Ovarian cancers with *brca2* dysfunction often have simultaneous *brca1* dysfunction (Hilton *et al.*, 2002). This study demonstrated that an inhibition of ERK1/2 activation by dominant negative and PD98059 resulted in increased apoptosis after *brca1* expression in MCF-7 cells. Furthermore, *brca1*-induced apoptosis involved activation of JNK, induction of Fas-L/Fas interaction, and activation of caspases 8 and 9 (Hilton *et al.*, 2002). The response to *brca1* expression is determined by the regulation of both the JNK and ERK1/2 signaling pathways in cells (Yan *et al.*, 2002). Mutations in *brca1* and *brca2* may lead to carcinogenesis through distinct molecular pathways that also appear to be involved in sporadic cancers. Sporadic carcinogenic pathways may result from epigenetic aberrations of *brca1* and *brca2* or their downstream effectors (Reedy *et al.*, 2001; Jazaeri *et al.*, 2002). The *brca*-mediated tumorigenesis appears to be related to estrogen-

mediated proliferation of breast and ovarian epithelium and to the distinctive genomic context of the *brca* genes (Welch and King, 2001). Clonal loss of the wild-type *brca2* allele, as well as the same somatic mutation of the TP53 gene, was evident in both histologic components. These results indicate that hereditary ovarian carcinoma may result from a mutation in *brca2* and that both histologic elements of this tumor arise from the same progenitor cell (Sonoda *et al.*, 2000).

The presence of a p53 mutation in 80% of these cancers indicates that p53 mutation is common but not required for *brca*-linked ovarian tumorigenesis; notably, a significantly higher proportion of the p53 mutations in *brca2*-linked cancers was deletions or insertions, compared with the more typical spectrum of missense mutations seen in *brca1*-linked cancers. In addition, *brca*-linked ovarian carcinomas appear to develop through a unique pathway of tumorigenesis that does not involve mutation of K-ras or amplification of *ErBb-2*, C-myc, or *Akt2* (Rhei *et al.*, 1998). The cells infected with an adenovirus expressing *brca1* up-regulate the endogenous expression of NF- $\kappa$ B target genes *Fas* and *INFbeta*, suggesting that *brca1* may play a role in cell life-death decisions following cell stress by modulation of the activity of NF- $\kappa$ B (Benezra *et al.*, 2003). A subset of the identified factors activated the *brca1* promoter, suggesting that identification of these factors is an important step in the clarification of the mechanism of breast carcinogenesis (Thakur *et al.*, 2003).

The role of *brca1* and/or *brca2* in ovarian carcinogenesis has not been elucidated in an animal model or, specifically, in mouse OSE cells. Direct, targeted deletion of these genes by loxP-Cre system in mouse OSE cells will provide a much-needed model to elucidate the roles of *brca1* and *brca2* of ovarian cancer development. Thus, the first phase of this research is to use a novel strategy to delete gene functions of *brca1* and/or *brca2* in mouse OSE cells; the second phase is to investigate the mechanisms by which these genes affect ovarian carcinogenesis.

## Summary

It is anticipated that establishment of new and accurate animal models with transgenes and knockout of tumor suppressor genes results in a better understanding role of *brca1* and *brca2* genes in ovarian carcinogenesis. In addition, these novel animal models provide necessary tools for testing hypotheses related to gene therapy by reconstitution of tumor suppressor genes in ovarian neoplasia associated with their deficiency. Thus, new preclinical mouse models will advance development of novel diagnostic, therapeutic, and preventive

approaches targeting ovarian cancer associated with tumor suppressor gene deficiency. With development of appropriate mouse models, we are able to apply these models for studying the role of endocrine factors in ovarian cancers. Furthermore, the development of new genetic mouse models will allow to evaluate similarities and differences between ovarian cancers and other tumors, such as breast and endometrial tumors, derived from the *brca1* and *brca2* mutations.

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