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Animal models of ovarian cancer

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Abstract

Ovarian cancer is the most lethal of all of the gynecological cancers and can arise from any cell type of the ovary, including germ cells, granulosa or stromal cells. However, the majority of ovarian cancers arise from the surface epithelium, a single layer of cells that covers the surface of the ovary. The lack of a reliable and specific method for the early detection of epithelial ovarian cancer results in diagnosis occurring most commonly at late clinical stages, when treatment is less effective. In part, the deficiency in diagnostic tools is due to the lack of markers for the detection of preneoplastic or early neoplastic changes in the epithelial cells, which reflects our rather poor understanding of this process. Animal models which accurately represent the cellular and molecular changes associated with the initiation and progression of human ovarian cancer have significant potential to facilitate the development of better methods for the early detection and treatment of ovarian cancer. This review describes some of the experimental animal models of ovarian tumorigenesis that have been reported, including those involving specific reproductive factors and environmental toxins. Consideration has also been given to the recent progress in modeling ovarian cancer using genetically engineered mice.

Introduction

Despite improved knowledge of the etiology of ovarian cancer, aggressive cytoreductive surgery, and modern combination chemotherapy, there has been little change in the mortality statistics over the last 30 years, and approximately 60% of the women who develop ovarian cancer will die from their disease. Lack of an adequate screening test for early disease detection and the rapid progression to chemoresistance have prevented appreciable improvement in the five year survival rate of patients with ovarian cancer.

Experimental models for human diseases are of crucial importance not only to understand the biological and genetic factors that influence the phenotypic characteristics of the disease but to utilize as a basis for developing rational intervention strategies. Ovarian cancer cell lines derived from ascites or primary ovarian tumors have been used extensively and can be very effective for studying the processes controlling growth regulation and chemosensitivity. Our limited knowledge of the initiating events of ovarian cancer has restricted the development of models in which the early pathogenic events for ovarian cancer can be studied. However, there are a few animal models that develop ovarian tumors spontaneously, and others where the manipulation of various reproductive factors or exposure to environmental toxins have been shown to promote ovarian tumorigenesis. Finally, the recent identification of promoters that can drive gene expression in the ovarian surface epithelium is providing new opportunities for the generation of genetically engineered mouse models of ovarian cancer. Here we describe some of the models that have been developed to investigate ovarian cell transformation.

Spontaneous and Non-epithelial Ovarian Tumorigenesis

There are few animal models that develop ovarian tumors spontaneously. Hens maintained under intensive egg-laying conditions develop ovarian adenocarcinomas; however such tumors are uncommon in hens less than 2 years of age [1]. Ovarian tumors will also arise spontaneously with age in some strains of mice [2], and in Wistar and Sprague-Dawley rats [3,4]. These tumors show a wide variety of histologic sub-types, including tubular adenoma, adenocarcinoma, papillary cystadenoma, mesothelioma, granulosa cell tumor, and polycystic sex cord/stromal tumor. However, the low incidence and/or the length of time required for the appearance of tumors in all of these models render them poorly feasible for experimental studies of ovarian carcinogenesis.

Some strains of mice, including C3HeB/Fe and C3HeB/De, show a high incidence of spontaneously occurring granulosa cell tumors and tubular adenomas [5]. Strain HAN:NMRI develop spontaneous Sertoli cell-like tumors and (DBA × Ce)F1 hybrids have a high incidence granulosa cell tumors [5]. Granulosa cell tumors also appear spontaneously at 4–6 weeks of age in SWR/J and in SWR/Bm inbred strain mice, with a maximum incidence reached by 10 weeks [6]. In some SWXJ strains, granulosa cell tumors occur spontaneously, and in others granulosa tumors can only be induced by treatment with dehydroepiandrosterone [7].

Spontaneous germ cell tumors are less common, but have been reported in LT/Sv and related strains of mice. These mice have a high frequency of spontaneous ovarian teratomas arising from follicular oocytes that undergo parthenogenetic activation. In some strains, this defect appears to be associated with an arrest of the oocytes at metaphase of meiosis I [8]. Teratomas arising from parthenogenetic activation of oocytes also occur in c-*mos*-deficient oocytes, which fail to maintain meiotic arrest after oocyte maturation [9,10].

Mice generated to be deficient in the tumor suppressor gene Lats1 exhibit a lack of mammary gland development, infertility and growth retardation. Accompanying these defects are hyperplastic changes in the pituitary and decreased serum hormone levels. The reproductive hormone defects of Lats1-/- mice are reminiscent of isolated LH-hypogonadotropic hypogonadism and corpus luteum insufficiency in humans. Lats1-/- mice develop soft-tissue sarcomas and ovarian stromal cell tumors [11].

The Ovarian Surface Epithelium

Although ovarian cancer in humans can arise from any of the cell types found in the ovary, almost 90% are derived from the ovarian surface epithelium (OSE) [12]. The OSE covers the entire ovarian surface, and varies morphologically from simple squamous to cuboidal to low pseudostratified columnar [13,14]. Embryologically derived from the mesodermal epithelium of the gonadal ridges, OSE cells are continuous with the flattened mesothelium of the peritoneum [15] and are separated from the underlying stromal compartment of the ovary by a basement membrane. Immunohistochemical staining has shown that OSE cells express cytokeratin, desmoplakin, transforming growth factor-α (TGF-α) and receptors for estrogen, progesterone and epidermal growth factor (EGF) [16–20]. Despite their rather unremarkable appearance *in vivo*, it is believed that OSE cells actively participate in the ovulatory process. Studies in rabbits and sheep have shown that OSE release proteolytic enzymes that degrade the basement membrane and the underlying apical follicular wall, weakening the ovarian surface to the point of rupture [21]. The OSE cells directly over the point of rupture undergo apoptotic cell death before ovulation [22] and the wound created at the ovulatory site surface is repaired by rapid proliferation of OSE cells from the perimeter of the ruptured follicle [23]. The biology, endocrinology and pathology of the ovarian surface epithelium have recently been reviewed in detail [24].

Although the ovarian surface is generally smooth in early reproductive life, with aging the ovary becomes more convoluted. Invaginations of the epithelium result in crypts or gland-like structures that can become pinched off to form epithelial inclusion cysts within the underlying stromal compartment [25]. This may occur following the postovulatory proliferation of OSE, follicular attrition, and/or from inflammation caused by carcinogens or chemical irritants like talcum powder [26]. The incidence of inclusion cysts increases with advancing age and are common in postmenopausal women. Although generally benign in nature, these epithelial rearrangements are widely thought to be the potential origin of many epithelial cancers. The more frequent appearance of epithelial invaginations and inclusion cysts in women with hereditary risk of ovarian cancer has strengthened this hypothesis [27]. In addition, some microscopic borderline and malignant tumors have been observed to arise directly within these sites, and they are often associated with dysplasia in similar sites elsewhere in the same or contralateral ovary [28,29].

Xenografts of OSE Cells Transformed *in vitro*

OSE cells have been implicated as the cell of origin for the majority of ovarian cancers based primarily on histological and immunohistochemical analyses of patient samples, but several recent experimental models manipulating these cells *in vitro* have provided additional support for this concept. Primary culture of human OSE was first reported by Auersperg et al. in 1984 [30], and her group has since developed several *in vitro* models of ovarian epithelial carcinogenesis. Introduction of Kirsten murine sarcoma virus into rat OSE cells results in endometrioid tumors following subcutaneous or intraperitoneal injection into immunosuppressed rats [31]. Transfection of SV40 T antigen early genes induces immortalization of human OSE cells that delays, but does not prevent, the senescence that normally occurs after a few passages [32]. Introduction of E-cadherin into these T antigen-immortalized cells induces epithelial differentiation [33] and the cells formed transplantable, invasive adenocarcinomas when injected into SCID mice [34]. In contrast to T antigen-immortalized cells, introduction of the human papilloma virus E6 and E7 genes into human OSE cells results in the spontaneous progression from a benign to invasive phenotype [35].

Unlike human OSE, rat and mouse OSE do not senesce. Rat OSE cells that have spontaneously immortalized but are not tumorigenic (eg. ROSE 199 cells; [36]) have been used in a variety of experiments, including some to characterize the cellular features when SV40 T antigen or H-*ras* is introduced into immortalized cells and following the formation of tumors when these cells are xenografted into nude mice [37]. Repeated subculture of rat and mouse OSE cells to maintain continued proliferation results in spontaneous malignant transformation, as characterized by loss of contact inhibition, substrate-independent growth and the ability to form tumors in nude mice [38,39]. In a variation of the above *in vitro* transformation approaches, Orsulic and colleagues used the RCAS retroviral vector to introduce oncogenes into OSE cells from transgenic mice bearing the RCAS receptor TVA and the cells were evaluated for tumorigenicity by injection into immune-deficient or syngeneic animals [40]. The investigators found that p53 deficiency in combination with two oncogenes from among C-*MYC*, *K-RAS*, or *AKT* were required to achieve transformation.

While these models allow an evaluation of oncogenes whose activation may contribute to the development of epithelial ovarian cancer, this approach does not allow the investigation of the early events in ovarian tumorigenesis inherent in mice when the tumors arise *in situ*. However, the establishment of *in vitro* models of normal and transformed OSE cells has provided the opportunity to use molecular approaches such as microarray or suppression subtractive hybridization to identify differential gene expression patterns that can distinguish normal OSE and ovarian cancer cells [41,42]. These data will be useful for the elucidation of molecular events associated with OSE cell transformation.

Xenografts of Cancer Cells

Xenograft models, where ovarian cancer cells have been injected either subcutaneously or into the peritoneal cavity have been used extensively for the testing of novel therapeutics or modified regimens for administration of standard chemotherapeutic drugs [43–45]. Some mouse models take advantage of the presence of a bursa, a sac-like structure that envelops rodent ovaries. For decades, researchers have used the intra-bursal space for transplants of xenografted ovaries, or to facilitate direct exposure of the ovary to various factors. For the generation of mouse models of ovarian cancer, the injection of ovarian cancer cells into the intra-bursal space results in tumor formation that can perhaps be viewed as more physiological (Figure 1), as the cancer cells are placed directly in the environment where ovarian tumors normally arise [46].

Reproductive Factors and Ovarian Tumorigenesis

Unlike most other cancers, the series of events involved in the initiation, progression and metastasis of ovarian cancer is not yet established. It is not clear if malignancies arise from benign or borderline tumors or if they develop *de novo* from the surface epithelium or inclusion cysts, as there is evidence for both [47]. The incidence of ovarian cancer climbs dramatically in women around the age at which they reach menopause. The reason for this is not clear, but two of the major changes associated with menopause form the foundation for hypotheses regarding the origin of ovarian tumors: 1) the depletion of oocytes or germ cells, which is the underlying cause of menopause, and 2) a significant increase in the pituitary's production of the gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), that arises as a consequence of the reduced follicular estrogen levels. In addition to the loss of germ cells and the associated alterations in hormone levels which normally occur at menopause, there are a number of non-menopausal factors that have been shown to have physiological relevance in epithelial ovarian tumorigenesis, including ovulation. Each of these will be discussed in the context of the animal models that have resulted from the experimental manipulations of these factors.

Ovulation

The "incessant ovulation hypothesis" proposes that continuous ovulation, with its successive rounds of surface rupture and OSE cell mitosis to repair the wound, renders the cells susceptible to malignant transformation [48].

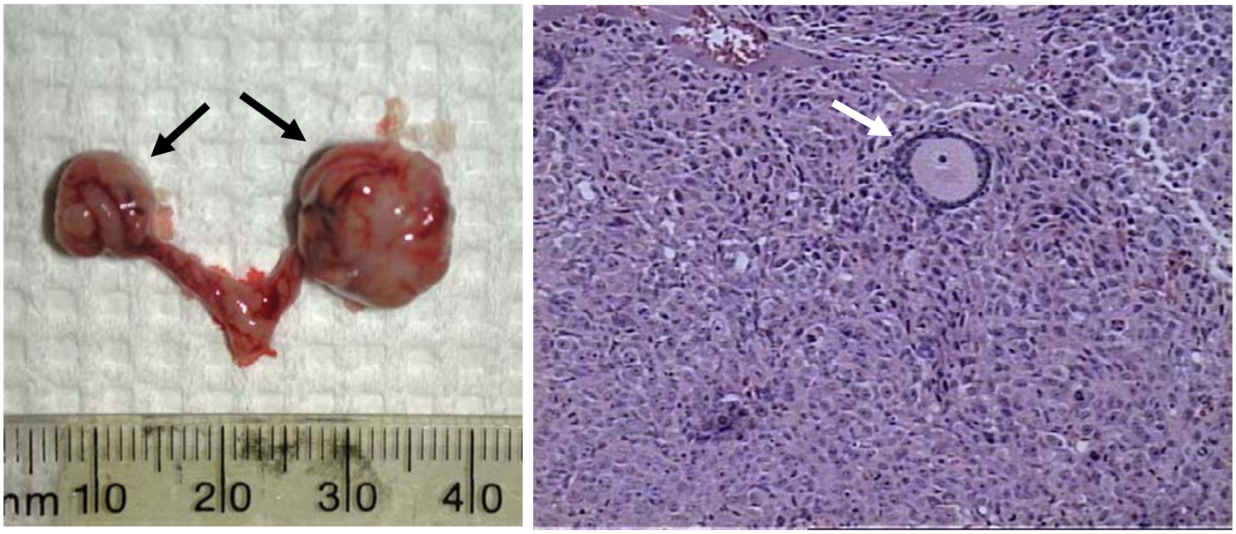

Figure 1
Development of ovarian tumors following injection of ES-2 ovarian cancer cells under the bursal membrane of nude mouse ovaries. Left figure- Proliferating cancer cells invade the normal tissue and increase the ovarian mass to diameters > 10-fold in size (indicated by arrows). Right figure- A single follicle containing a growing oocyte, indicated by an arrow, is clearly visible in the mass of tumor tissue.

Anecdotal support for this hypothesis comes from the observation that intensive egg-laying domestic hens frequently develop peritoneal carcinomata that is presumably of ovarian origin [1]. Epidemiological studies indicate that circumstances that decrease the number of ovulations, i.e., pregnancy, oral contraceptive usage, duration of lactation and early menopause, all substantially reduce the risk of ovarian cancer [49,50].

Inherent in the incessant ovulation hypothesis for ovarian cancer risk is the premise that repetitive damage of the OSE at ovulation and/or the subsequent mitotic repair following ovulation increases the risk of developing ovarian cancer. Experimental evidence to support the susceptibility of OSE cells to mutagenic events during mitosis is provided by studies showing that primary cultures of normal rat and mouse OSE cells which have been repeatedly subcultured to maintain continued proliferation acquire features associated with malignant transformation, including loss of contact inhibition, substrate-independent growth and the ability to form tumors in nude mice [38,39].

The risk generated by incessant ovulation may also be associated with the formation of epithelial cell-lined inclusion cysts that are frequently found in the ovarian stroma of perimenopausal women. As noted above, these inclusion cysts may form as a result of the process of ovulation and the pinching off of deep clefts [47]. In mice, the lifetime total number of ovulations is associated with a marked increase in OSE invagination and stratification [51], although the incidence of inclusion cysts was more related to age than to number of ovulations. Therefore, unlike in humans, an association between number of ovulations and ovarian cancer risk has not been demonstrated in rodents.

Gonadotropins

An alternative, but not mutually exclusive, hypothesis for the mechanism of ovarian carcinogenesis proposes that the development of ovarian tumors is related to excessive gonadotropin production associated with the onset of menopause or premature ovarian failure [52]. The median age for epithelial ovarian cancer is 60–65 years, with only 10–15% of the tumors appearing in premenopausal women [53]. Serum FSH and LH levels reach their peak during perimenopausal and postmenopausal years and remain elevated thereafter [54]. High circulating levels of pituitary gonadotropins may increase the risk of ovarian cancer by stimulating the growth of ovarian epithelial cells, since normal human OSE cells and epithelial inclusions have been found to express receptors for FSH [55] and LH/hCG [56]. Enhanced cell proliferation in response to FSH and/or LH/hCG has been reported for primary cultures of rabbit [57], mouse [58] and human [56] OSE cells. Schiffenbauer and colleagues [59] found that human epithelial ovarian cancers progressed faster in ovariectomized mice due to elevated FSH and LH levels, which promoted increased vascular endothelial growth factor expression and tumor neovascularization.

The gonadotropin theory of ovarian tumorigenesis suggests that elevated gonadotropin concentrations contribute to the development of ovarian tumors. This theory is based on the initial observation of Biskind and Biskind in 1944 [60] who reported that transplantation of ovaries into the splenic pulp of adult rats led to the development of ovarian tumors. The tumorigenesis was attributed to inactivation of estrogen in the liver, and the consequent elevation of gonadotropin levels due to the lack of steroid feedback on the pituitary. Several transgenic or knockout animal models in which gonadotropin levels are elevated also result in ovarian tumorigenesis. For example, when inhibin, the ovarian protein that inhibits the production of FSH, is made deficient in mice, gonadal stromal tumors arise [61]. Transgenic mice generated to have chronic LH hypersecretion develop granulosa cell tumors or luteomas, depending on the background strain [62,63]. Mice with disruption of the FSH receptor are acyclic and sterile, with very small, underdeveloped ovaries; they exhibit hypergonadotropic-hypogonadism with high levels of circulating FSH and LH similar to the postmenopausal state in women. By 12 months, more than 92% of these animals developed various kinds of ovarian pathology, including neoplasms of sex cord-stromal type as well as cysts, suggesting that FSH receptor insensitivity in the face of prolonged elevated levels of gonadotropins may be contributing to the development of ovarian granulosa or stromal tumors [64]. None of the animal models with targeted manipulation of gonadotropin secretion or action appear to promote ovarian epithelial tumorigenesis.

Steroid hormones

In the developing fetal ovary, marked OSE cell proliferation occurs at 16 to 20 weeks of gestation, coincident with the appearance of steroid-producing cells in the ovarian cortex [65]. Adult human OSE cells express receptors for estrogen, progesterone and androgens [66,67], and human OSE cell proliferation can be stimulated by androgens [68]. In contrast, human OSE cells in culture are reportedly unaffected by estradiol or progesterone [66], which would suggest that these steroid hormones do not have a significant role in ovarian tumorigenesis. However, a recent study has found that menopausal women who have taken hormone replacement therapy using estrogen only are at an increased risk of ovarian cancer [69]. In animals, continuous exposure to estradiol stimulates sheep OSE cell proliferation [70], while in guinea pigs and rabbits, it results in the formation of a papillary ovarian surface resembling human serous neoplasms of low malignant potential [71,72]. The mechanisms by which estrogen may contribute to ovarian cancer risk is unknown, but could be direct action on the OSE cells, or may be indirect, as estrogen reduces GnRH receptor expression in both OSE and ovarian cancer cells, thereby suppressing the growth inhibitory effects of GnRH [73]. Estrogen also modulates levels of hepatocyte growth factor which stimulates OSE cell growth [74].

A number of studies, largely epidemiological, provide support for the hypothesis that androgens are involved in ovarian carcinogenesis. Over 80% of tumors express AR [75] and an increased risk of ovarian cancer was found in women with elevated circulating levels of androgens [76]. Testosterone-stimulated growth of OSE cells in guinea pigs caused the formation of benign cysts, small adenomas in the ovarian parenchyma, and papillomas on the ovarian surface [77]. Androgens may promote ovarian tumorigenesis in part by decreasing TGF-β receptor levels, thereby allowing ovarian cancer cells to escape TGF-β growth inhibition [78].

Germ cell deficiency/depletion

Aging and hereditary risk are associated with a more frequent incidence of epithelial invaginations and inclusion cysts, putative preneoplastic precursor lesions, but the underlying mechanisms for these epithelial-stromal rearrangements are unknown. OSE cell hyperplasia with stromal invasion has been reported in a diverse array of experimental situations, all of them involving loss of germ cells and consequent failure of follicle development. For example, mutations at the *W* (*Kit*) or *Sl* (*Kitl*) loci result in sterility by preventing the normal proliferation and migration of germ cells during fetal development [79]. Germ cell deficiency *in vivo*, as is found in W^x/W^v mice, results in bilateral ovarian tubular adenomas in more than 95% of the animals by 5 months of age [80,81]. The tumors arise from interstitial cell hyperplasia, with proliferation and invasion of the ovarian surface epithelium into the stromal compartment of the ovary. Invasive epithelial tubules are also found in Sl/Sl^t germ cell deficient mice by 7 months of age [82], and mice heterozygous for the Sl^d mutation, which carries a splicing defect, develop papillary structures and epithelial invaginations (Figure 2), similar to that seen in women [26]. Likewise, female mice homozygous for the germ cell deficient (*gcd*) mutation enter reproductive senescence prematurely due to a dearth of germ cells. By one year of age, 56% of homozygotes have developed ovarian tubulostromal adenomas while wild-type littermates are phenotypically normal [83].

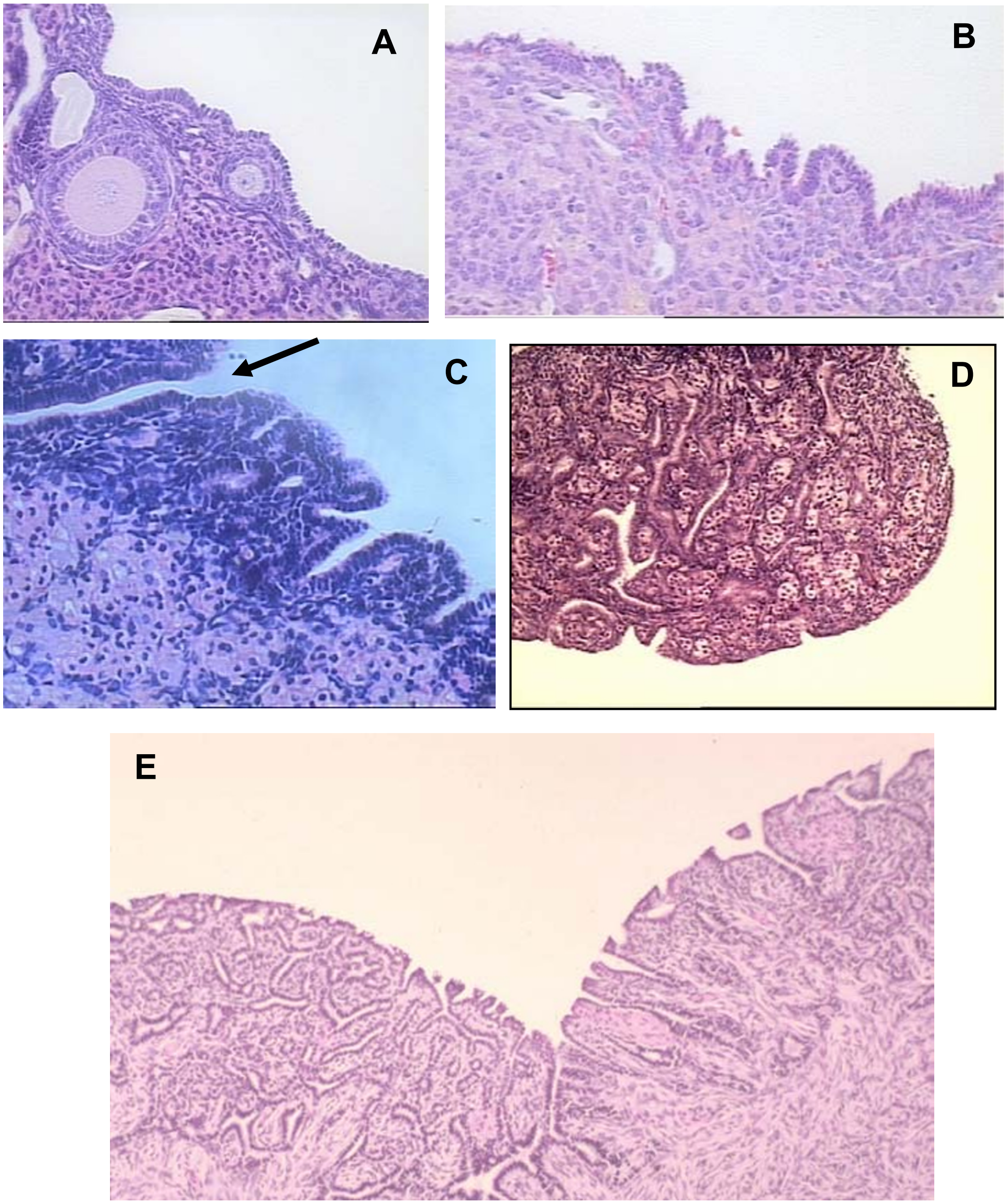

Figure 2
Morphology of the ovarian surface epithelium in wild-type (A; 12 months), *Sl^d* heterozygous (B, C; 12 months) and homozygous (D; 6 months) mice. Ovaries from wild-type mice contain developing follicles and a covering layer of columnar OSE. In 12-month-old *Sl^d* heterozygous mice, there is a depletion of follicles, and the ovarian surface has become very convoluted (B), with this papillary surface sometimes leading to deep invaginations, as indicated by the arrow (C). By 6 months of age, the ovaries of homozygous *Sl^d* mice are completely abnormal, with no recognizable ovarian structures, and are composed primarily of invasive epithelial tubules. (E) Human ovarian papillomatosis, for comparison.

Therefore, it appears that oocyte depletion is associated with formation of epithelial structures that resemble the preneoplastic lesions in human ovaries.

Experimental ovarian tumorigenesis has been investigated in inbred and hybrid strains of mice and induced by a diversity of mechanisms including X-irradiation, oocytotoxic xenobiotic chemicals, ovarian grafting to ectopic or orthotopic sites, neonatal thymectomy, genetic defects reducing germ cell populations, and aging [reviewed in [84]]. While germ cell deficiency seems to be a required element for the development of epithelium-derived adenomas, the mechanisms by which germ cell loss contributes to tumorigenesis in these models remain unclear. Ovarian follicles do not develop in the absence of oocytes, indicating that the oocyte directs the development of follicles. Pathogenetic factors that prematurely destroy or diminish the numbers of germ cells lead to failure in follicle development and a resulting decrease in sex steroid hormone secretion (notably estradiol) leading to a compensatory over-production of pituitary gonadotropins, which places the ovary at an increased risk to develop tumors. Therefore oocyte depletion, similar to that which occurs naturally by the time of menopause, may be a contributing factor to the oncogenic behavior of the surface epithelial cells.

The intense proliferation of OSE and stromal (interstitial) cells with the development of unique tubular adenomas in response to sterility seems to require both the lack of germ cells/follicles and the increased production of gonadotropins. Elevated gonadotropins alone resulted in granulosa cell tumors or luteomas [62,63]. Oocyte destruction by gamma irradiation in hypogonadal mice deficient in gonadotropins did not result in the development of tubular adenomas [85]. Similarly, the experimental suppression of gonadotropin levels in W^x/W^v mice was sufficient to prevent the development of ovarian tubular adenomas from the surface epithelium [86], suggesting that both oocyte loss/destruction and elevated gonadotropins are necessary for epithelial tumorigenesis.

Environmental Carcinogens

Although the more established hypotheses that have been proposed to explain increased risk of developing ovarian cancer are related to the number of ovulations or to increased hormone levels, there are additional risk factors that have been identified, including a number of environmental carcinogens. While these factors have been reported to have effects on the ovarian surface epithelium, they are usually also associated with follicular destruction and/or ovotoxicity, so indirect actions due to altered gonadotropin levels cannot be eliminated. Use of perineal talc has been identified as a risk factor, possibly due to its ability to ascend the genital tract and affect the ovarian surface [87]. Indeed, direct exposure of rat ovaries to talc results in focal areas of papillary change in the ovarian surface epithelium, as well as ovarian cysts [88]. Exposure of rhesus and cynomolgus monkeys to the environmental pollutant, hexachlorobenzene results in both reproductive failure and notable alterations in the size, shape and degree of stratification of the OSE cell layer [89]. More recent studies have shown that the insecticide methoxychlor increases both the height of the OSE cell layer and the percentage of atretic follicles in exposed mice [90]. In rodent studies, ovarian toxicity and/or carcinogenicity has been documented for at least eight chemicals that result in follicular necrosis, tubular hyperplasia, granulosa cell tumors and benign mixed tumors [91,92]. N-ethyl-N-nitrosourea administered to rats intraperitoneally or transplacentally increases the incidence of ovarian tubular adenomas [93]. The mechanisms by which these environmental carcinogens enhance the risk of ovarian tumors remain unexplored.

Transgenics and Targeted Approaches to Transform the Ovarian Epithelium

The ideal model to investigate the pathogenic events associated with early ovarian tumorigenesis would be a mouse model in which the tumor arises directly from the OSE cells. This model would differ from current xenograft models in that transgenic mice with defined genetic lesions could be studied at various stages as they inevitably develop ovarian cancer *in situ*. In addition, the development of a genetic model would permit the direct testing of oncogenes and tumor suppressors for their contribution to the initiation and progression of overt malignancies in the mouse ovary. Finally, a number of different factors could be altered such as the genetic background of the mouse strain, the frequency of ovulation and the levels of various hormones to determine their impact on the development of tumors in the susceptible transgenic mouse line.

One approach to alter gene expression directly in the OSE cells would be to take advantage of the fact that these cells readily take up and express genes delivered by intra-bursal injection of adenoviruses [94,95]. This method has the potential advantage of mimicking somatic mutations that contribute to early ovarian tumorigenesis. One recent report used intra-bursal adenovirus delivery and Cre-loxP mediated gene inactivation to render OSE cells deficient in two key tumor suppressor genes: p53 and Rb [95]. The p53 tumor suppressor gene is the most frequently mutated gene in human neoplasms. Mutations and/or over-expression of p53 have been described in 26–62% of ovarian cancers, particularly serous ovarian carcinomas [reviewed in [96]]. Aberrations in the Rb pathway have been reported [97]; however, direct evidence for their contribution to ovarian epithelial tumorigenesis is lacking. In this model, recombinant adenovirus expressing Cre was injected under the ovarian bursal membrane of double transgenic mice bearing floxed copies of *p53* and *Rb*. Concurrent inactivation of p53 and Rb was sufficient for reproducible induction of ovarian epithelial carcinogenesis in mice homozygous for the conditional alleles. While less than 15% of mice with inactivation of either *Rb* or *p53* developed tumors, 33 of 34 mice with deficiencies in both genes succumbed to their ovarian cancers at a median of 227 days, with 24% having abdominal ascites.

The major impediment to the development of transgenic models of ovarian cancer is the lack of specific promoters able to direct gene expression to OSE cells. Previous models of ovarian cancer have resulted in granulosa cell tumors using promoters, such as inhibin-alpha subunit promoter, that are active in this cell type to drive the expression of the large T antigen of SV40 [98,99]. Recent studies have identified two other promoters that may prove to be useful for the generation of transgenic models of ovarian cancer. The Ovarian Specific Promoter (OSP-1) was developed from a retrovirus-like element specifically expressed in the rat ovary. The promoter drives gene expression specifically in normal and neoplastic ovarian epithelial cells [100] and expression of *lacZ* driven by OSP-1 in transgenic mice was restricted to the ovary as determined by X-gal staining of multiple organs [101]. Immunohistochemical detection of β-galactosidase showed *lacZ* expression mainly in the granulosa cells and ovarian surface epithelial cells. However, transgenic mice in which OSP-1 drives the expression of the early region of SV40 virus developed tumors in a variety of tissues, including unilateral granulosa cell tumors in two of three female founder mice. Thus, although transcription from the OSP-1 promoter occurs predominantly in the ovary, this promoter is sufficiently "leaky" in cells in other tissues to permit their tumorigenic conversion by SV40 TAg.

The first transgenic model of epithelial ovarian cancer was recently reported and used the upstream region of the Mullerian inhibitory substance type II receptor (*MISIIR*) gene to drive tissue-specific expression [102]. MISIIR is a single transmembrane serine/threonine kinase that shares homology with the TGFβ-receptor [103,104]. Expression of *MISIIR* has been reported to be restricted to mesenchymal cells surrounding the Mullerian duct during embryogenesis, tubular and follicular structures of fetal gonads, Sertoli and Leydig cells of adult testis, and granulosa cells of adult ovary [103,105,106]. More recently, expression of *MISIIR* in established human ovarian cancer cell lines as well as cell lines derived from the ascites of patients with ovarian carcinomas has been demonstrated [107]. Transgenic mice in which the 5' upstream regulatory sequences of the mouse *MISIIR* gene were used to target expression of the SV40 *TAg* specifically to the epithelium of the female mouse reproductive tract, including the OSE, developed ovarian carcinomas with metastatic spread to peritoneal organs by 3 months of age. Female transgenic mice developed bilateral ovarian tumors in ~50% percent of cases. Histologically, these tumors were poorly differentiated carcinomas with occasional cysts and papillary structures present at the surface of the ovary. These tumors disseminated intraperitoneally, invaded the omentum and formed ascites in a manner that resembles human ovarian carcinomas. The demonstration that the *MISIIR* promoter can be used successfully to drive gynecological tissue-specific transgene expression in mice and that this often results in the formation of ovarian carcinoma offers very promising opportunities for testing the efficacy of chemotherapeutic and chemopreventive agents in a heritable model of epithelial ovarian cancer.

Conclusions

The two most pressing problems in the management of ovarian cancer are the lack of adequate diagnostic or screening strategies, and the recurrence of disease that is often chemoresistant. In part, the deficiency in diagnostic tools is due to the lack of markers for the detection of preneoplastic or early neoplastic changes in the OSE cells. The generation of animal models in which OSE cells undergo neoplastic transformation *in vivo* will provide much-needed opportunities to investigate the cellular and molecular changes associated with the initiation of OSE cell transformation, as well as to provide models in which prevention, diagnostic, screening and therapeutic strategies can be developed.

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