0163-769X/01/$03.00/0
Endocrine Reviews 22(2): 255–288

Ovarian Surface Epithelium: Biology, Endocrinology, and Pathology*

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ABSTRACT

The epithelial ovarian carcinomas, which make up more than 85% of human ovarian cancer, arise in the ovarian surface epithelium (OSE). The etiology and early events in the progression of these carcinomas are among the least understood of all major human malignancies because there are no appropriate animal models, and because methods to culture OSE have become available only recently. The objective of this article is to review the cellular and molecular mechanisms that underlie the control of normal and neoplastic OSE cell growth, differentiation, and expression of indicators of neoplastic progression. We begin with a brief discussion of the development of OSE, from embryonic to the adult. The pathological and genetic changes of OSE during neoplastic progression are next summarized. The histological characteristics of OSE cells in culture are also described. Finally, the potential involvement of hormones, growth factors, and cytokines is discussed in terms of their contribution to our understanding of the physiology of normal OSE and ovarian cancer development. (*Endocrine Reviews* **22:** 255–288, 2001)

I. Introduction

THE OVARIAN surface epithelium (OSE), also referred to in the literature as ovarian mesothelium (OM) (1, 2) or normal ovarian epithelium (NOE) (3), is the modified pelvic mesothelium that covers the ovary. It is composed of a single layer of flat-to-cuboidal epithelial cells with few distinguishing features (1, 4, 5). The OSE was previously referred to as the "germinal epithelium" as it was once mistakenly believed that it could give rise to new germ cells. Since this hypothesis was disclaimed, ovarian research has centered on those components of the ovary that carry out its important and highly complex endocrine and reproductive functions, in comparison to which the OSE appeared singularly uninteresting. As a result, the OSE remained among the least studied and, scientifically, most neglected parts of the ovary until the latter part of the 20th century. Its inconspicuous histological appearance and apparent lack of significant functions contributed to this situation. Interest in the OSE revived when it became apparent that approximately 90% of human ovarian cancers, viz the epithelial ovarian carcinomas, might arise in the OSE (1, 6–8). This group of cancers is the most lethal among ovarian neoplasms and is the prime cause of death from gynecological malignancies in the Western world. Until recently, the implication of OSE as the source of epithelial ovarian cancers was questioned (9) because it was based mainly on histopathological and immunocytochemical observations in clinical specimens. There were no experimental systems for the study of these neoplasms. Animal models were not available because, except in aging hens (10), ovarian tumors in species other than human do not arise in OSE but in follicular, stromal, or germ cells, and the biology of these tumors is fundamentally different from that of epithelial ovarian cancer. The establishment of culture systems posed problems because OSE is a minute part of the intact ovary, is difficult to separate from other cell types by physical or enzymatic means, has a very limited growth potential in culture, and has no tissue-specific markers for positive identification. Because of the resulting lack of experimental models, the etiology and early events in ovarian carcinogenesis are still among the least understood of all major human

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* This manuscript was supported by grants from the Canadian Institutes of Health Research and the National Cancer Institute of Canada with funds from the Terry Fox Run.

malignancies. In the 1980s, the first tissue culture systems for OSE from different species (6, 11–14), including human (15, 16), were developed. Subsequently, information about the normal functions of OSE and its relationship to ovarian cancer expanded rapidly and, recently, the capacity of cultured OSE to give rise to ovarian adenocarcinomas was demonstrated experimentally (17, 18). The results of these studies, which are summarized in the first part of this review, indicate that OSE is physiologically much more complex than would be predicted from its inconspicuous appearance, and they support the hypothesis that the ovarian epithelial cancers arise in this simple epithelium. In the second part of this review, we summarize some of the salient features of the ovarian epithelial carcinomas, *i.e.*, the group of tumors thought to be of OSE origin, with emphasis on their regulation and function by endocrine factors.

II. Embryonic Development

Early in development, the future OSE forms part of the celomic epithelium, which is the mesodermally derived epithelial lining of the intraembryonic celom. It overlies the presumptive gonadal area and, by proliferation and differentiation, gives rise to part of gonadal blastema (Fig. 1). Starting at about 10 weeks of development and continuing to the fifth month of human gestation, the fetal OSE changes from a flat-to-cuboidal simple epithelium with a fragmentary basement membrane to a multistratified, papillary epithelium on a well defined basement membrane, but it reverts to a monolayer by term. It has been postulated that the growth signals for fetal OSE include intragonadal steroid hormones because morphological evidence of steroid differentiation of ovarian stromal cells temporally parallels enhanced OSE growth and morphogenesis (1). There are differences between the OSE and extraovarian mesothelium during fetal development. These differences must be due to local factors acting in the region of the gonadal ridge, since OSE and extraovarian mesothelium are otherwise identical to their origin in celomic epithelium and face a similar environment as both line the pelvic cavity. One of the most interesting differences between these two parts of the pelvic mesothelium is the expression of CA125, a cell surface glycoprotein of unknown function, which, in the adult, is both an epithelial differentiation marker and a tumor marker for ovarian and Mullerian duct-derived neoplasms (19). CA125 is expressed by the oviductal, endometrial, and endocervical epithelia, as well as by the pleura, pericardium, and peritoneum of first and second trimester human fetuses and of adult women, but not by OSE. OSE is therefore the only celomic epithelial derivative that either never acquired this differentiation marker or lost it early in development (20). The former interpretation would support the idea that OSE is less differentiated and less committed to a mature mesothelial phenotype than the remainder of the pelvic peritoneum. The expression of CA125 in OSE-derived epithelial carcinomas indicates that the adult OSE has retained the competence of celomic epithelium to differentiate, at least under pathological conditions.

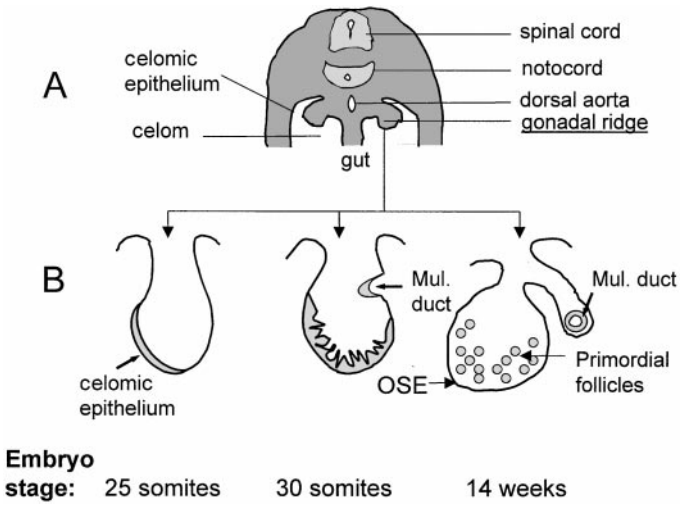

FIG. 1. Schematic representation of ovarian embryonic development. A, Cross-section through the dorsal part of a 13-mm human embryo; B, sequential changes in the gonadal ridge, which is covered by modified celomic epithelium (*shaded*). This epithelium proliferates and forms cords that penetrate into the ovarian cortex and give rise to the granulosa cells in the primordial follicles. The follicles become separated from the overlying ovarian surface epithelium (OSE) by stroma. The Mullerian ducts (Mul. duct) develop as invaginations of the celomic epithelium dorsolaterally from the gonadal ridges.

The fetal OSE is also a likely developmental source of the ovarian granulosa cells. There is still controversy whether granulosa cells are embryologically derived from OSE, from mesonephric tubules via the intraovarian rete, or from both, and to what degree these origins vary among species. There is good evidence though that in the human, OSE is the source of at least part of the granulosa cells. Furthermore, this distinction only becomes important in late stages of development because OSE and the intraovarian rete have a common origin in the celomic epithelium that overlies the urogenital ridges (21–25). In addition to its likely role as a progenitor of granulosa cells via the fetal OSE, the celomic epithelium in the vicinity of the presumptive gonads invaginates to give rise to the Mullerian (paramesonephric) ducts, *i.e.*, the primordia for the epithelia of the oviduct, endometrium, and endocervix. Thus, the celomic epithelium in and near the gonadal area represents an embryonic field with the capacity to differentiate along many different pathways. The relevance of this close developmental relationship between the Mullerian epithelia and the OSE to ovarian epithelial carcinogenesis will become apparent later in this review.

III. OSE in the Adult

A. Structure

In the mature woman, OSE is an inconspicuous monolayered squamous-to-cuboidal epithelium (Fig. 2). It is characterized by keratin types 7, 8, 18, and 19, which represent the keratin complement typical for simple epithelia. It expresses mucin antigen MUC1, 17β-hydroxysteroid dehydrogenase, and cilia, which distinguish it from extraovarian mesothelium, apical microvilli, and a basal lamina (6, 16, 26–28). Intercellular contact and epithelial integrity of OSE are maintained by simple desmosomes, incomplete tight junctions (6, 16), several integrins (29, 30), and cadherins (31, 32).

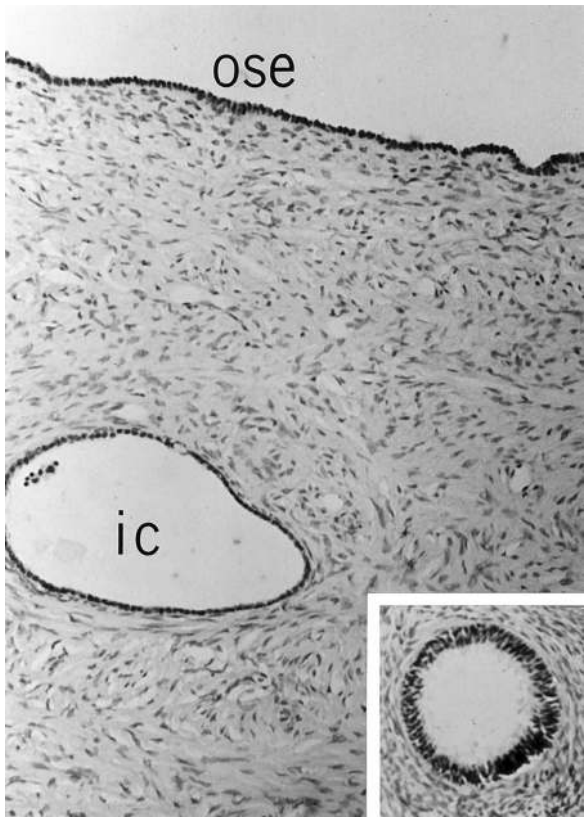

FIG. 2. Section through a normal adult ovarian cortex, showing OSE on top as a cuboidal monolayer and an epithelial inclusion cyst lined with OSE (IC). The *inset* illustrates an inclusion cyst that has undergone tubal metaplastic changes as indicated by the densely arranged, columnar epithelial cells. Hematoxylin and eosin, ×80.

The cadherins are a family of calcium-dependent adhesion molecules that mediate selective cell-cell adhesion and also indirectly influence gene expression through their close association with the catenins (32, 33). In the human, OSE, granulosa cells, and extraovarian mesothelium are connected by N-cadherin, which characterizes adhesive mechanisms of mesodermally derived tissues (32, 34–36). E-cadherin, which is the principal intercellular adhesion molecule in most epithelia, is constitutively present in human oviductal, endometrial, and endocervical epithelia (37) and also in mouse and porcine OSE (38, 39). In contrast, E-cadherin expression in the human OSE is limited to the rare regions where the cells assume columnar shapes, *i.e.*, where they approach the phenotype of metaplastic epithelium (31, 32, 36, 40). Thus, coexpression of E-cadherin with N-cadherin in human OSE is conditional and signifies a propensity toward the aberrant epithelial differentiation of metaplastic and neoplastic OSE (36). Factors regulating E-cadherin expression in female reproductive tissues appear to involve hormonal controls, since estrogen and progesterone were reported to increase E-cadherin mRNA levels in the immature mouse ovary and uterus *in vivo* (38, 41). E-cadherin is not only a differentiation marker for normal Mullerian epithelia, but also an inducer of epithelial differentiation (42). We recently created an epithelial tumorigenic OSE-derived cell line closely resembling ovarian serous adenocarcinoma cells by transfecting the gene for mouse E-cadherin into a nontumorigenic, SV40 large T antigen-immortalized OSE line (18). These results support the hypothesis that E-cadherin has an inductive influence in the aberrant epithelial differentiation of OSE in ovarian carcinogenesis. Like E-cadherin, P-cadherin is absent in the OSE of adult women but is present in the epithelia of Mullerian duct derivatives and in ovarian adenocarcinoma cell lines (36, 37, 43). Thus, the distribution of P-cadherin changes in association with tissue-specific morphogenetic events and pathological processes. Both receptor tyrosine kinases and receptor tyrosine phosphatases have been found to coimmunoprecipitate with cadherin-catenin complexes. These interactions may be important in the orchestration of different functions of OSE in various physiological and pathological circumstances (44, 45).

The OSE is separated from the ovarian stroma by a basement membrane and, underneath, by a dense collagenous connective tissue layer, the tunica albuginea, which is responsible for the whitish color of the ovary. It is thinner and less resilient than the tunica albuginea in the testis, but likely provides a partial barrier to the diffusion of bioactive agents between the ovarian stroma and OSE. The OSE differs from all other epithelia by its tenuous attachment to its basement membrane, from which it is easily detached by mechanical means. Until recently, the resulting loss of OSE in surgical specimens was responsible for the widely held opinion that OSE is frequently absent in ovaries of older women. Whether this loose attachment has any physiological consequences is not known. With age, the human ovary assumes increasingly irregular contours and forms OSE-lined surface invaginations (clefts) and epithelial inclusion cysts in the ovarian cortex. It has been suggested that the squamous and cuboidal forms of OSE cells on the ovarian surface represent cell groups that, respectively, have or have not undergone postovulatory proliferation (46). In addition, OSE cells tend to assume columnar shapes, especially within clefts and inclusion cysts. Whether these shape changes are the result of crowding or whether they reflect genetically determined metaplastic changes is not always clear, but they may be derived by either process. The importance of surface invaginations and inclusion cysts lies in the propensity of the OSE in these regions to undergo metaplastic changes, *i.e.*, to take on phenotypic characteristics of Mullerian (usually tubal) epithelium, which include columnar cell shapes and several markers found in ovarian neoplasms, including CA125 and E-cadherin (6, 31, 40, 47–49). Furthermore, OSE-lined clefts and inclusion cysts, rather than surface OSE, are not only common sites of benign metaplasia but also of early neoplastic progression (50–52). It has been suggested that the inclusion cysts form from OSE fragments that are trapped in or near ruptured follicles at the time of ovulation (53, 54). However, Scully (52) reported that inclusion cysts are more numerous in ovaries of multiparous women than in nulliparous women who ovulate more frequently, and the cysts are particularly numerous in women with polycystic ovarian disease, a condition that is characterized by anovulation or infrequent ovulation. He proposed as an alternative that inclusion cysts arise through inflammatory adhesions of surface OSE which becomes apposed at sites of surface invaginations, combined with localized stromal proliferation.

There is currently no definitive explanation for the predilection of inclusion cysts as preferred sites of neoplastic progression of OSE, but these preferential locations strongly suggest the presence of specific tumor-promoting microenvironmental factors in these sites. Two different scenarios, which are not mutually exclusive, can be envisaged: 1) OSE within inclusion cysts is not separated from underlying stroma by the tunica albuginea. Therefore this OSE likely has more access to stromally derived growth factors and cytokines as well as to blood-borne bioactive agents that may promote neoplastic progression. This hypothesis is supported by the observation that, in inclusion cysts located near the ovarian surface, metaplastic and dysplastic changes tend to be more pronounced on the side near the stroma than on the side adjacent to the tunica albuginea (51, 52). 2) Neoplastic progression in OSE-lined cysts and clefts may be promoted by autocrine mechanisms through OSE-derived cytokines and hormones, since these agents may accumulate to bioactive levels in such confined sites but not on the ovarian surface where they diffuse into the pelvic cavity. The hypothesis that these factors participate in autocrine loops is supported by the capacity of normal OSE to secrete bioactive cytokines including interleukin (IL)-1 and IL-6 (55) and by reports that IL-1 and IL-6 enhance the proliferation of ovarian carcinomas (56), that IL-1 causes changes in gene expression including the induction of tumor necrosis factor (TNF)-α, which is a mitogen for OSE (57, 58), and that human CG (hCG) is produced by normal and neoplastic OSE (47) and is also mitogenic for rabbit OSE cells (59). Finally, the proliferative response to cytokines of cervical cells (which are developmentally related to OSE) changes with immortalization so that the immortalized cells acquire a selective advantage over normal cells (60). Within inclusion cysts, such cytokines and hormones might act as immediate autocrine growth regulators, or they might cause secondary changes in gene expression that promote neoplasia.

B. Functions

The OSE transports materials to and from the peritoneal cavity and takes part in the cyclical ovulatory ruptures and repair. Most of these functions vary with the reproductive cycle and thus are likely to be hormone dependent (1, 6, 59). It is well established that OSE must proliferate to repair ovulatory defects in the ovarian surface, and Osterholzer *et al*. (59) demonstrated directly that in rabbit ovaries, this proliferative activity is both localized to the vicinity of the ovulatory site and peaks at, and immediately after, the time of ovulation. Several reports, based on electron microscopy and histochemistry, have suggested that the OSE contains lysosome-like inclusions and produces proteolytic enzymes, which may contribute to follicular rupture (61). These reports were supported by direct observations of protease secretion by cultured OSE (29). However, this concept has been questioned because of inconsistencies in the timing of the appearance of the dense lysosome-like granules in the OSE, their biochemical nature, and the observation that follicles denuded of overlying OSE can also rupture (reviewed in Ref. 62). Furthermore, electron microscopy in various species has revealed that OSE cells degenerate and slough off the follicular surface shortly before ovulatory rupture. There is evidence that this cyclic, localized loss of OSE near the time of ovulation is due to apoptosis that is induced by prostaglandins (63, 64) and perhaps mediated by the Fas antigen (65, 66). It is possible that, as the tunica albuginea in the area of the stigma thins and ultimately disappears before ovulation, the OSE in this region is exposed to stromal influences that induce apoptosis. However, the possibility cannot be ruled out that the OSE alters the tunica albuginea and underlying stroma in the area of incipient ovulation just before its disappearance. The proteolytic capacity of OSE might contribute to the remodeling, as well as the breakdown, of the ovarian cortex. OSE likely also takes part in the restoration of the ovarian cortex by the synthesis of both epithelial and connective tissue-type components of the extracellular matrix (ECM) (27, 29, 67) and by its contractile activity, which resembles the contractile capacity exhibited by connective tissue fibroblasts during wound healing (68). Like fibroblasts, which convert to myofibroblasts when engaged in tissue repair, OSE cells in culture contain smooth muscle actin (our unpublished observations). This is in keeping with their dual epithelio-mesenchymal phenotype and with the proposition that OSE cells, like many other cell types, acquire a regenerative rather than stationary phenotype when they are explanted into culture. Contraction by OSE cells may also play a role in the shrinkage of the ovaries that occurs with age and results in their typical convoluted shape and the formation of the OSE-lined clefts and inclusion cysts.

C. Differentiation

Normal OSE covering a nonovulating ovary is a stationary mesothelium with both epithelial and mesenchymal characteristics. In contrast to mesothelia elsewhere, OSE retains the capacity to alter its state of differentiation along pathways leading either to stromal or to ectopic (aberrant) epithelial phenotypes. In response to stimuli that initiate a regenerative (repair) response, such as ovulatory rupture *in vivo* or explantation into culture, OSE cells assume phenotypic characteristics of stromal cells. Alternatively, OSE acquires complex epithelial characteristics of the Mullerian duct-derived epithelia, *i.e.*, of the oviduct, endometrium, and endocervix, when it undergoes metaplasia, benign tumor formation, and neoplastic progression. Together, these characteristics show that the differentiation of OSE is not as firmly determined as in other adult epithelia and that OSE is closer to its pleuripotential mesodermal embryonic precursor form than other celomic epithelial derivatives.

Normal stationary OSE has no known tissue-specific differentiation markers. *In situ*, it can be distinguished from extraovarian mesothelium by the lack of CA125 (20) and by the differential expression of mucin, cilia, 17β-hydroxysteroid dehydrogenase, and several antigenic markers (5, 6, 47, 49, 69, 70). It has classical epithelial features, which include desmosomes, tight junctions, basement membrane, keratin, and apical microvilli, but other aspects of epithelial differentiation are less defined. For example, E-cadherin and CA125 in human OSE are rare while both markers occur in oviductal and endometrial epithelium, and CA125 is also secreted by extraovarian pelvic mesothelium and by abdom-

inal and pleural peritoneum (1, 6, 20, 69, 70). OSE cells also constitutively coexpress keratin with vimentin, which is a mesenchymal intermediate filament, expressed by most epithelial cells only in response to wounding, explantation into culture, or pathological conditions (71–73). Expression of the connective tissue collagen types I and III has been shown in cultured OSE but not *in situ* (27).

During postovulatory repair and in culture (see *Section IV*) OSE cells have the ability to modulate to a fibroblast-like form that reflects their close developmental relationship to ovarian stromal cells. The exact mechanisms regulating this conversion have not been defined. However, as shown later in this review, epidermal growth factor (EGF), collagen substrata, and ascorbate are all conducive to epithelio-mesenchymal conversion of OSE in culture. In addition, transforming growth factor (TGF)-β, which is an autocrine regulator of OSE growth (74), causes epithelio-mesenchymal conversion in a number of epithelial cell types (75). Similar epithelio-mesenchymal conversions occur *in vivo* in mesodermally derived cell types closely related to OSE, such as pleural mesothelial cells responding to injury (76) and the cells of the developing Mullerian duct during regression in response to Mullerian inhibiting substance (77). This capacity of OSE to undergo epithelio-mesenchymal conversion likely confers advantages during the postovulatory repair of the ovarian surface: it increases motility, alters proliferative responses and capacities to modify ECM, and renders the cells contractile (see below). Epithelio-mesenchymal conversion might also function as a homeostatic mechanism to accommodate OSE cells that become trapped within the ovary at ovulation, to allow them to become incorporated into the ovarian stroma as stromal fibroblasts. As a related hypothesis, an inability to undergo epithelio-mesenchymal conversion would preserve the epithelial forms within the ovarian stroma, which could lead to OSE cell aggregation and subsequent inclusion cyst formation (Fig. 3). Factors that have been shown in culture to enhance epithelio-mesenchymal conversion of OSE include EGF (16), ascorbate (our unpublished data), and growth in collagen gels and other three-dimensional matrices (68, 78) (see *Section V*). It is important to note that OSE at the site of ovulatory rupture is exposed to all these influences. In contrast to epithelio-mesenchymal conversion, which is part of normal OSE physiology, the differentiation of metaplastic and neoplastic OSE along the lines of Mullerian duct-derived epithelia is clearly a pathological process, based on complex epigenetic and genetic changes that will be discussed briefly in *Section IV*.

IV. Neoplastic Progression of OSE

A. Epidemiology and etiology of the epithelial ovarian carcinomas

Ovarian cancer is the fourth or fifth most common cause of death from all cancers among women in the Western world and the leading cause of death from gynecological malignancies. The epithelial ovarian carcinomas, *i.e.*, the group derived from the OSE, represent approximately 90% of all human ovarian malignant neoplasms, with the rest originating in granulosa cells or, rarely, in the stroma or germ cells. The poor 5-yr survival (30–40%) is largely due to the fact that most ovarian carcinomas are inoperable when first discovered and respond poorly to therapy (7). Although screening tests are available for patient follow-up and for the detection of advanced cases (79), there are no reliable means for early detection except for genetic screening in a small proportion of individuals (80), and to date no test has been shown to reduce mortality.

The etiology of the epithelial ovarian carcinomas is poorly understood. Over the years, environmental agents that have been implicated but not proven to play a role include diet, talc, industrial pollutants, smoking, asbestos, and infectious agents (7). Epidemiological studies point to possible racial and geographic, social, and hormonal causative factors (7, 81–83). There is convincing evidence that nulliparity and, probably, hyperovulation treatment for infertility increase the risk of ovarian cancer, while oral contraceptives and pregnancies are protective. These observations support the hypothesis, first proposed by Fathalla in 1971 (149) and subsequently supported by epidemiological and experimental data (84, 85; reviewed in Ref. 8), that frequent ovulation contributes to increased risk because the repeated rupture and repair of the OSE at the sites of ovulation provide an opportunity for genetic aberrations. Recently, it has been suggested that inflammation may be a contributing factor in ovarian cancer development, because tubal ligation and hysterectomies act as protective factors, perhaps by preventing passage of environmental initiators of inflammation (86). Another major known risk factor is a strong family history of ovarian cancer, which accounts for 5–10% of cases.

B. OSE in women with histories of familial ovarian cancer

At present, a strong family history of ovarian cancer is the most important and best-defined risk factor for development of this disease, and it is associated with 5–10% of ovarian epithelial carcinomas. The risk increases from 1.4% in the general population to 5% for women with one first-degree relative and to 8% for women with two first-degree relatives affected (first-degree relatives include parents, siblings, and children, while second-degree relatives include grandparents, uncles, aunts, cousins, and grandchildren). There is also a strong association with familial breast cancer, and a lesser association with familial cancers of the colon and endometrium. Three hereditary ovarian cancer syndromes with autosomal dominance (reviewed in Ref. 87) are listed below.

1. Hereditary site-specific ovarian cancer, where a family history of ovarian cancer only is associated with an overall 3.6-fold increase in risk. No specific gene responsible for this syndrome has been identified.

2. Hereditary nonpolyposis colon cancer/ovarian cancer (Lynch Syndrome II or HNPCC), where ovarian cancer occurs in families that also have a high incidence of carcinomas of the colon and endometrium. It is associated with mutations in the DNA mismatch repair genes hMSH1, hMSH2, hPMS1, and hPMS2 (88). In this syndrome, the increase in risk has not been defined.

3. Hereditary breast/ovarian cancer. There is a 50% increase in ovarian cancer risk among women with family histories of breast cancer and a similar increase in breast

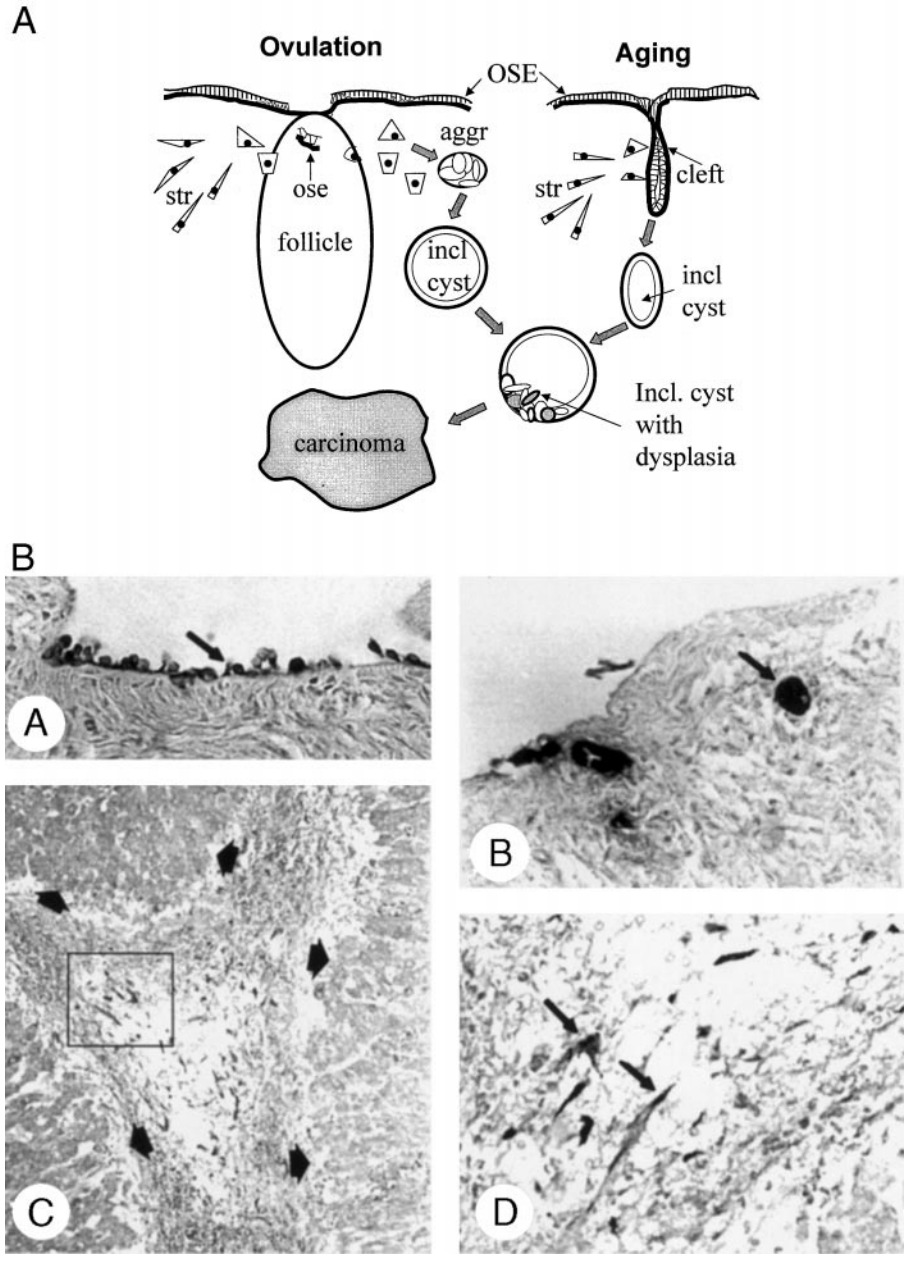

FIG. 3. Hypothesis: Epithelio-mesenchymal conversion of OSE cells may represent a homeostatic mechanism to incorporate cells that have been displaced from the ovarian surface into the stroma. If such conversion does not take place, the cells are more likely to form epithelial inclusion cysts, which are preferred sites of neoplastic progression. A, diagram outlining two paths by which OSE is displaced into the ovarian cortex. OSE fragments are displaced into or near the ruptured follicle at ovulation. OSE also lines surface invaginations, or clefts, which form as the ovary ages. If OSE cells undergo epithelio-mesenchymal conversion, they may migrate into, and become part of, the stroma *(str).* Alternatively, the cells remain epithelial, aggregate *(aggr),* and form inclusion cysts *(incl cyst).* Cysts may also form through the pinching off of surface clefts. Inclusion cysts are preferred sites of metaplastic and dysplastic changes that may lead to tumorigenesis. Importantly, the capacity of OSE to undergo epithelio-mesenchymal conversion is greatly reduced with malignant progression and, to a lesser degree, in women with a genetic predisposition to develop ovarian cancer (78). B, Illustration of some of the changes proposed in panel A. Paraffin sections of normal ovaries, stained immmunocytochemically for keratin as an OSE marker. OSE cells are shown on the ovarian surface (A), forming aggregates in the ovarian cortex (B), and as fibroblast-like cells in the center of a recently ovulated corpus luteum (C). Hematoxylin-eosin staining showed the central clot being invaded with fibroblasts (not shown). In parallel sections stained immunocytochemically (C), the fibroblast-shaped cells stain for keratin. D, Higher magnification of the area outlined by the *square* in panel C. The *arrows* in A, B, and D indicate darkly staining, keratin-positive cells. The *short arrows* in panel c indicate the boundaries between the luteal cells and the scar forming in the central region of the corpus luteum. Magnification: A, B, D, ×200; C, ×80.

cancer risk among women with family histories of ovarian cancer. Germline mutations in two genes involved in this syndrome, BRCA1 and BRCA2, appear to be responsible for a high proportion of cancers in women with familial cancer histories. The BRCA1 and BRCA2 proteins regulate DNA damage responses (89) and have been defined as tumor sup-

pressor genes. BRCA1, in particular, plays a major role in ovarian cancer susceptibility (90). Intensive screening for BRCA1 mutations is ongoing but the large size of the gene and the great variety of different mutations that have been found complicate screening and risk predictions (91). The observation that BRCA1 and BRCA2 germline mutations cause increases in cancer incidence predominantly in the breast, ovary, and prostate, although they are present in all tissues, points to interrelationships with hormonal influences. Interactions between BRCA1 and estrogen as well as PRL have indeed been reported in cancer cells (92–94), but there seems to be no information available on similar interactions in normal OSE. Importantly, not all of the carriers of these predisposing mutations develop ovarian cancer, which suggests a role for interactions with other, as yet unidentified, genetic and epigenetic influences.

There have been several contradictory reports on the occurrence of histological changes in the OSE of overtly normal ovaries that were removed by prophylactic oophorectomy from healthy women with histories of familial ovarian cancer. A nonblind study (95) demonstrated increased papillomatosis and pseudostratification of the OSE, as well as an increase in inclusion cysts and invaginations in ovaries from women with familial ovarian cancer. In another blind study, only nuclear changes were observed in the OSE of such women (96), while in two other reports no significant differences were observed (97, 98). Thus, it is still not clear whether, *in situ*, overtly normal OSE from women with family histories of ovarian cancer is distinct at the phenotypic level.

C. Epithelial ovarian carcinomas

1. Pathology. Histopathologically and immunocytochemically, ovarian carcinomas are among the most complex of all human malignancies (99, 100). One of the most unusual aspects of ovarian carcinogenesis is the change in differentiation that accompanies neoplastic progression. As discussed above, OSE is a simple, rather primitive epithelium with some stromal features, but as it progresses to malignancy it loses its stromal characteristics and acquires the characteristics of the Mullerian duct-derived epithelia, *i.e.*, the oviduct, endometrium, and uterine cervix. This aberrant differentiation occurs in such a high proportion of ovarian carcinomas that it serves as the basis for the classification of a high proportion of these cancers as serous (fallopian tube-like), endometrioid (endometrium-like), and mucinous (endocervical-like) adenocarcinomas (Fig. 4). Serous adenocarcinomas comprise approximately 80% of all epithelial ovarian cancers. Among the less common forms are clear cell carcinomas that express features resembling mesonephros. It has also been proposed that at least some endometrioid carcinomas may arise in endometriotic lesions derived from endometrial implants (101), and that some mucinous ovarian adenocarcinomas may actually be metastases of gastrointestinal malignancies because the mucus in these lesions is of the gastrointestinal rather than the endocervical variety (102).

At the cellular level, Mullerian differentiation is expressed by the appearance of altered cell shapes, E-cadherin, junctional complexes, epithelial membrane antigens, and secretory products including mucins (MUC1, MUC2, MUC3, and MUC4) and CA125 (6, 28, 31, 40, 99, 100). Histologically, the tumors form polarized epithelia, papillae, cysts, and glandular structures. Thus, unlike carcinomas in most other organs in which epithelial cells become less differentiated in the course of neoplastic progression than the epithelium from which they arise, the differentiation of ovarian carcinomas is more complex than that of OSE (Fig. 5). Only in the late stages do these specialized epithelial features diminish although they can persist even when the tumors are metastatic or in the ascites form (40). Tissue culture studies have shown that with neoplastic progression OSE cells not only develop complex epithelial phenotypes, but also become firmly committed to these phenotypes and unresponsive to signals causing mesenchymal conversion of normal OSE. Such unresponsiveness to environmental cues reflects the autonomy from normal control mechanisms that characterizes malignant tumors in general.

The high frequency of Mullerian differentiation-associated changes in early stages of ovarian cancer suggests that they might confer a selective advantage on the transforming OSE. The basis for such putative selective advantage(s) is currently being investigated. Possible hypotheses underlying this concept include the possibilities that 1) E-cadherin-mediated adhesion prevents anoikis in ovarian cancer cells when they seed the pelvic cavity (103); 2) with the Mullerian phenotype, OSE cells acquire changes in hormone/growth factor receptors and responsiveness that promote neoplastic progression (*e.g.*, estrogens are mitogenic for tubal and endometrial epithelium, but not for normal OSE) (104); 3) in contrast to the firmly attached, well vascularized epithelia of the oviduct and endometrium, normal OSE has only a tenuous attachment to underlying stromal components. Thus, Mullerian differentiation might enhance epithelio-mesenchymal exchanges of blood-borne and paracrine factors that support malignant transformation and growth.

Histopathologically detectable early malignant changes occur more frequently in OSE-lined clefts and inclusion cysts (Fig. 2) than on the ovarian surface that faces the pelvic cavity. The evidence for inclusion cysts as the preferred sites of ovarian carcinogenesis was reviewed by Scully (51, 52): 1) Most early carcinomas appear to be confined within the ovary without involvement of its surface; 2) tubal metaplasia is 10 times more common in epithelial inclusion cysts than on the ovarian surface; 3) inclusion cysts are significantly more numerous and the OSE lining them is 2–3 times more often metaplastic in women with contralateral epithelial ovarian tumors than in women without such cancers (105); 4) several ovarian carcinoma tumor markers (*e.g.*, CA125, CA19–9) are significantly more common in the epithelium of epithelial inclusion cysts than in the surface epithelium itself (20, 47, 106, 107). The localization of early malignant changes in crypts and cysts has given rise to speculations that neoplastic progression may be promoted by the particular microenvironment to which preneoplastic OSE is exposed within these confined spaces.

2. Genetic changes. The genetic basis of the epithelial ovarian carcinomas is too complex to be reviewed in detail here, but numerous excellent reviews exist on this subject. In brief,

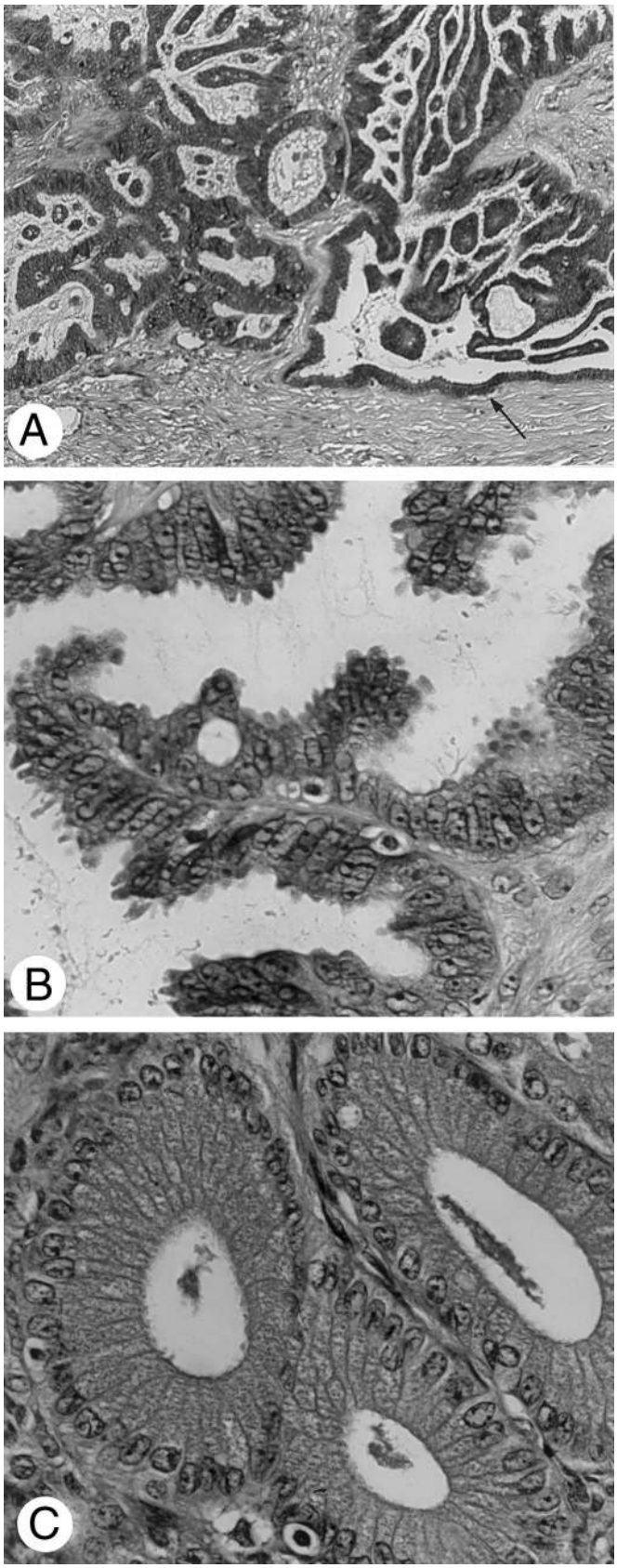

FIG. 4. Mullerian differentiation of ovarian tumors. A, Ovarian cortex with metaplastic OSE covering part of the ovarian surface (*arrow*). To the *left* and in the *upper part* of the figure, a tumor with numerous papillae and gland-like structures has formed. On the basis of its resemblance to the complex epithelium of the oviduct, this tumor is classified as a serous ovarian adenocarcinoma. B, Higher magnification of the tumor in panel A, illustrating the formation of papillae, cilia, and densely packed nuclei characteristic of serous type OSE-derived neoplasms. C, Mucinous differentiation of an ovarian tumor of borderline malignancy, resembling endocervix (and also celomic epithelium) in its differentiation. Hematoxylin and eosin. Magnification: A, ×80; B and C, ×300.

amplification, altered expression, and mutations in a number of oncogenes and tumor suppressor genes play a role in the development of ovarian epithelial neoplasms. Oncogenes that are frequently overexpressed or amplified in ovarian carcinomas include cMYC, particularly in serous adenocarcinomas (108); KRAS, especially in mucinous carcinomas that may exhibit enteric mucinous differentiation (109); and ERBB2, EGF-R, and cFMS (the receptor for colony-stimulating factor 1), all of which are associated with a poor prognosis (110–112). Recently, phosphatidyl inositol 3 kinase (PI3K) and its downstream effector AKT2 were also shown to be amplified in a significant proportion of ovarian carcinomas (113, 114). Among tumor suppressor genes, p53 is mutated in about 50% of late-stage tumors but rarely in low-stage tumors and borderline lesions (115), and the PI3K inhibitor PTEN is mutated in a significant proportion of endometrioid ovarian carcinomas (116). As mentioned in *Section IV.B.*, mutations in the tumor suppressor genes BRCA1 and BRCA2 appear to form the basis for most cases of familial ovarian cancer. The expression of a recently described tumor suppressor gene, NOEY2 (ARHI), is decreased specifically in carcinomas of the ovary and breast (117).

The epidemiology, histopathology, and clinical course of OSE-derived ovarian carcinomas differ profoundly from those of the mesotheliomas, which arise in extraovarian mesothelium, *e.g.*, in response to asbestos exposure, and lack Mullerian phenotypes. This difference reflects, among other factors, the different developmental histories of these two components of the pelvic peritoneum, which may include inductive signals emanating from the ovary and acting on the developing OSE (2, 6, 26).

V. OSE in Culture

A. Culture methods

The detailed procedures used for isolating and culturing normal human OSE were summarized previously (118) and have recently been described in detail (119). Briefly, in our laboratory, specimens for culture are obtained from overtly normal ovaries at surgery for nonmalignant gynecological diseases. Fragments of OSE are gently scraped from the ovarian surface with a rubber scraper or with the blunt side of a scalpel or other suitable instrument and immediately placed into sterile culture medium; it is imperative that the tissue remain sterile and does not dry, which happens very rapidly. OSE is also very loosely attached to the underlying ovarian cortex and is easily lost by excessive handling. If the surgery involves the removal of the ovaries, the OSE is obtained either by the surgeon while the ovaries are still *in situ*, or by a member of the research team after removal from the

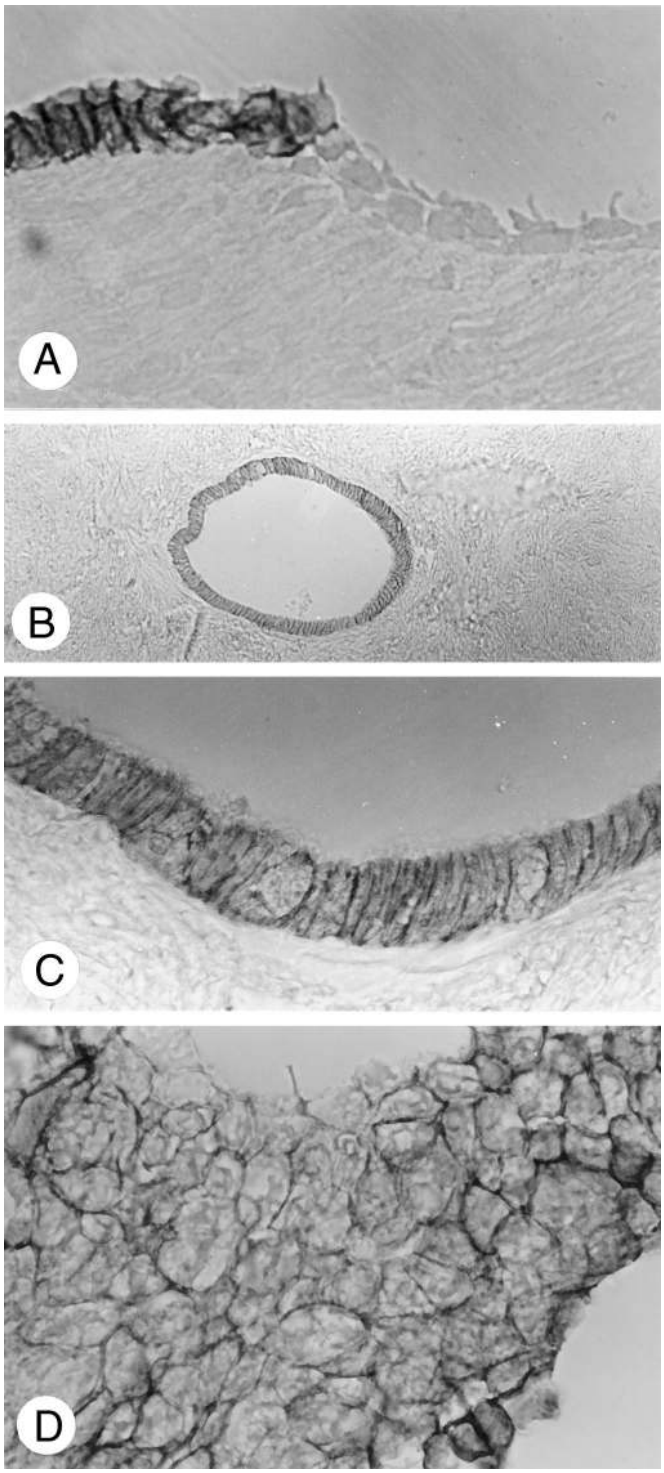

FIG. 5. E-cadherin expression by normal, metaplastic, and neoplastic OSE. Frozen sections, stained immunocytochemically for E-cadherin (40). A, Ovarian surface. Normal, flat-to-cuboidal OSE on the *right* is E-cadherin negative. On the *left*, the cells are columnar and E-cadherin positive. B, Epithelial inclusion cyst lined with metaplastic E-cadherin-positive OSE. C, Higher magnification of the epithelium lining the cyst in panel B. The cells are columnar, ciliated with interspersed secretory cells, resembling oviductal epithelium. D, Epithelial ovarian carcinoma with E-cadherin outlining intercellular junctions. Magnification: A, C, and D, ×300; B, ×80.

patient. OSE can also be obtained by the surgeon laparoscopically at the time of minor gynecological procedures that are carried out by this approach. The OSE fragments are cultured in medium 199-MCDB 105 (1:1) (Sigma, St. Louis, MO) with 15% FBS (HyClone Laboratories, Inc., Logan, UT). In addition, either 50 μg/ml gentamicin or 100 μg/ml of penicillin/streptomycin is added for the first few weeks. The cultures are left undisturbed for at least 4 days, grown to confluence, and then routinely passaged and split 1:3 when confluent, with 0.06% trypsin (1:250) and 0.01% EDTA. The cultures usually proliferate for three to four passages (1:3 splits) and then senesce. They are defined as senescent if they are composed of large flat cells that do not reach confluence over 1 month. OSE cells in low-passage culture can undergo epithelio-mesenchymal conversion, which tends to extend their life span by a few passages (Fig. 6) (27). This phenomenon varies in frequency and the underlying mechanisms have not been defined. Reduced-serum, and serum-free media were designed for human OSE and used to study mitogenic effects of growth factors and hormones (120, 121). Interestingly, rat OSE can be propagated in FBS-supplemented Waymouth medium 752/1 (11), while human OSE cells are stationary under these conditions but proliferate in FBS-supplemented media 199, MCDB 105, and MCDB 202 (15, 16, 118). For a long time there was no explanation for this phenomenon. However, it was reported recently that OSE proliferation is regulated by extracellular calcium by means of calcium-sensing receptors (122) and that human OSE proliferated only at calcium concentrations above 0.8 mM, whereas rat OSE grew at concentrations below this level. Waymouth medium has a calcium concentration of 0.8 mM, while the calcium concentrations of media 199, MCDB 105, and MCDB 202 range from 1.0 to 2.2 mM.

Markers to distinguish OSE from cell contaminants in culture include keratins 7, 8, 18, and 19, which distinguish OSE from other ovarian cell types (49, 71); 17β-OH steroid dehydrogenase and mucin, which distinguish it from extraovarian mesothelial cells; laminin, which together with keratin distinguishes OSE from stromal fibroblasts; and the absence of factor VIII and Ulex lectin receptors, which distinguish OSE from the morphologically similar endothelial cells (1, 16, 27).

B. Properties

1. Differentiation. Cultured OSE is highly responsive to environmental influences. Over several passages under standard culture conditions, freshly explanted OSE cells respond to the culture environment by modulating from an epithelial to a more mesenchymal phenotype (Table 1). Immediately upon explantation into primary culture they retain mesenchymal markers that are present *in vivo*, such as vimentin, and acquire additional mesenchymal characteristics, such as collagen type III secretion. They rapidly lose some epithelial differentiation markers, including villin and desmoplakin, but retain others, *e.g.*, keratin, for longer periods. With passages in culture, the cells may assume a more definitive fibroblast-like phenotype as indicated by a change to anterior-posterior polarity, reduced intercellular cohesion, gel contraction, increased secretion of collagen types I and III,

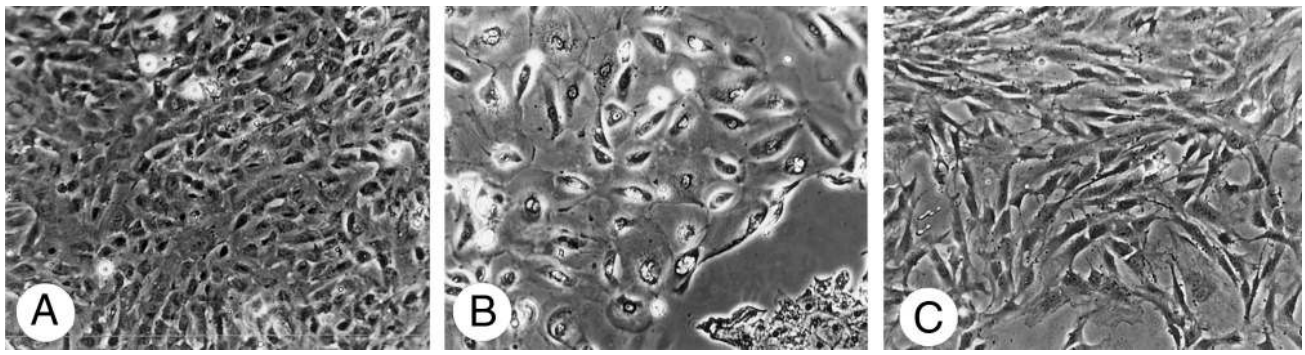

FIG. 6. Morphology of OSE in culture. A, Primary epithelial culture with a compact, cobblestone-like growth pattern. B, Passage 2 with flat epithelial OSE cells. Note a small group of granulosa cells in the *lower right corner*. C, Passage 5 with OSE cells that have undergone epithelio-mesenchymal conversion and have assumed fibroblast-like shapes. Such cells are initially keratin positive but tend to lose keratin with time and passages in culture (16, 78). Magnification: ×200.

TABLE 1. Comparison of epithelial and mesenchymal markers on OSE *in situ* and in culture

Markers	*In situ*	In culture	
		Primary culture	Passages 2–4
1. Epithelial markers			
Keratin	*Present*	Present	Diminished
Mucin	Present	ND	Present
Cytovillin	*Present*	Present	Absent
E-cadherin	Absent	Absent	Absent
Desmoplakin	*Present*	ND	Absent
Laminin	Present	Present	Present
Collagen IV	Present	Present	Present
2. Mesenchymal markers			
Vimentin	Present	Present	Present
Collagen type I	ND	ND	Present
Collagen type III	*Absent*	Present	Present
3. Morphology	*Epithelial*	Epithelial	Epithelial or mesenchymal

ND, Not determined. Features that change in culture are *italic*.

and loss of the epithelial marker keratin (27, 78, 123). Such epithelio-mesenchymal conversion is more consistent and prominent in three-dimensional than in two-dimensional culture (29, 78). It is enhanced by epithelial growth factor (16), collagen substrata (29), and ascorbate (our unpublished data). It varies widely in frequency between laboratories and within laboratories with time. The reasons for this variation and the precise mechanisms underlying the mesenchymal conversion of OSE have not been defined, but they most likely depend on as yet undefined serum factors. Similar epithelio-mesenchymal conversions occur in the culture of other mesodermally derived epithelia (reviewed in Refs. 87 and 124). Generally, cells respond to explantation into culture as they would to wounding and undergo changes in phenotype and in gene expression that are similar to those that occur in regenerative responses. In analogy, the response of OSE cells to explantation into culture likely mimics their normal response to ovulatory rupture. Thus, the phenotype observed in culture should perhaps be compared with that of regenerating OSE rather than to the phenotype of stationary OSE covering a nonovulating ovary.

2. ECM. Cultured OSE cells are profoundly influenced by the ECM and they, in turn, modulate ECM synthesis, lysis, and physical restructuring (29). OSE cells deposit epithelial as well as stromal ECM components which, in rat OSE, include banded collagen type I fibrils (67, 125, 126). Thus, OSE cells not only modulate to fibroblast-like forms morphologically, but have the capacity to autonomously produce complex connective tissue-type ECMs. Whether this autonomy contributes to the spread of OSE-derived tumors by providing tumor-derived stroma remains to be determined. Human OSE cells also secrete chymotrypsin-like and elastase-like peptidases, metalloproteases, and plasminogen activator inhibitor. Protease activity varies with the type of ECM on which the cells are maintained (27, 29). OSE cells from normal human ovaries do not appear to secrete plasminogen activator. Plasminogen activator detected in culture medium conditioned by OSE from an ovary with inflammatory disease may be derived from contaminating inflammatory cells (29, 127). OSE also expresses integrins that bind to laminin, collagens, fibronectin, and vitronectin and vary in type and amount with the substratum (29, 30). These properties are likely important in the roles of OSE in ovulation and postovulatory repair and may also influence the phenotypes of OSE-derived malignancies.

3. Intercellular adhesion. Similar to its *in vivo* phenotype (31, 32), intercellular contact of cultured OSE is maintained by N-cadherin, which is expressed constitutively while E-

cadherin is expressed only conditionally, when OSE cell shapes approach those of metaplastic epithelium (36). In contrast to the human, cultured rat OSE expresses E-cadherin consistently (128). N-cadherin-mediated adhesion appears to have an antiapoptotic effect in OSE of the rat (129), but whether it has a similar function in the human is not known. In general, expression of N-cadherin alone or of N- and E-cadherin together characterize adhesive mechanisms of mesodermally derived tissues (reviewed in Ref. 36).

C. Three-dimensional culture systems

Pathological changes in OSE, including neoplastic conversion and endometriosis, often involve three-dimensional formations such as OSE-lined clefts and cysts. To reproduce OSE growth in such confined spaces, several three-dimensional culture systems have been investigated: 1) rat tail tendon-derived collagen gel that is rich in collagen type I and permits differentiation of many cell types; 2) a rat OSE-derived matrix plus collagen gel to produce OSE "organoids"; 3) Matrigel (Collaborative Research, Bedford, MA), a mouse yolk sac tumor-derived basement membrane substitute rich in laminin and other basement membrane components (29, 68); and 4) Spongostan (Health Design Industries, Rochester NY), a pig skin-derived denatured collagenous sponge that provides a rigid skeleton (78). In collagen gel cultures, human OSE cells converted to a mesenchymal form, dispersed in the gel in a manner resembling connective tissue fibroblasts, and then remained stationary and eventually died (29). However, if cocultured with endometrial stromal cells in the presence of 17β-estradiol, OSE were reported to form structures composed of monolayered polarized cells surrounding lumina and expressing markers of endometrial cells. This system may represent an experimental model for OSE-derived endometriosis (130, 131). When cultured on the rat OSE-derived matrix plus collagen gel, the OSE cells again converted to a mesenchymal form and dispersed and then contracted the relatively loose matrix into smaller, denser structures (68). Such contractile function is generally considered as characteristic of fibroblasts in the process of wound healing. On Matrigel, OSE cells aggregated into solid cell clumps. Depending on the lot of Matrigel, the cells showed a varying propensity to lyse the matrix and eventually form monolayers on the underlying plastic (29). This variation may have depended on growth factor contaminants known to occur in Matrigel. In their ability to lyse this matrix, these presumably normal cells resembled cancer cells, which are commonly assumed to be the only cells capable of invading Matrigel. In Spongostan, cells were grown for several weeks until they filled the sponges. In contrast to ovarian cancer cells, which form epithelial linings along the sponge spicules, human OSE cells under these conditions again underwent mesenchymal conversion: they assumed morphological and functional characteristics of stromal cells as they dispersed in intercellular spaces, took on fibroblast-like shapes, and secreted ECM (78). Thus, in all three-dimensional systems except for Matrigel, OSE cells converted to mesenchymal phenotypes.

D. Extension of the life span of surface epithelial cells

One of the problems in human OSE research is the small number and short life span of cells obtained at surgery. To alleviate this problem, "immortalizing" genes such as SV40 large T antigen (Tag) (132) and the HPV genes P6 and P7 (123, 133, 134) have been introduced into OSE. Expression of these genes does not truly immortalize human OSE cell lines in that their population-doubling capacity is greatly extended but not infinite; however, the lines provide sufficiently large cell numbers for molecular studies. One advantage of these lines is that they tend to retain some, although not all, of the tissue-specific properties of the cells from which they are derived. For example, many of these lines retain keratin, and most, if not all of them, continue to express N-cadherin and lack E-cadherin (in common with normal, and in contrast to neoplastic OSE). Although such lines are nontumorigenic in SCID mice (18), their growth controls are profoundly disturbed, which confer on them properties of neoplastic cells such as genetic instability, increased saturation density reduced serum requirements, and variable degrees of anchorage independence. Tag and E6/E7 inactivate the tumor suppressor genes p53 and p105RB (135, 136). Importantly, 30–80% of epithelial ovarian carcinomas have p53 mutations that disrupt controls of the cell cycle, DNA repair, and apoptosis (137). Sometimes, a few cells of such "immortalized" OSE cultures survive crisis and become truly immortal, continuous lines. Recently, we introduced constitutively expressed E-cadherin into an SV40 Tag-immortalized line derived from normal OSE. The resulting phenotype closely resembled neoplastic OSE, and the cells formed adenocarcinomas in SCID mice (17, 18). These adenocarcinomas resembled Mullerian duct-derived epithelia in that they formed papillae and cysts and expressed CA125 and E-cadherin. The line, IOSE-29EC, became not only tumorigenic but also acquired an indefinite, truly immortal growth potential. While the exact relationships between the introduction of T-antigen and E-cadherin to tumorigenicity need to be examined in additional lines, this is the first experimental transformation of normal human OSE to ovarian adenocarcinoma cells and the first direct confirmation that OSE is capable of such a transformation. The results support the hypothesis that E-cadherin may act as an inducer of the Mullerian epithelial differentiation that accompanies neoplastic conversion of OSE (36).

E. Variation in OSE characteristics among species

Important issues that are frequently overlooked in the interpretation of data derived from studies of OSE are the structural and physiological differences among OSE from different species. For extrapolations of results to human OSE, one of the best tissue culture models appears to be bovine OSE because of the relative similarity between the reproductive systems of these two species (138). One example of differences between species, discussed in *Section III.A*, is the constitutive expression of E-cadherin by OSE of rodents and pigs but not humans. Other differences include the dependence of human but not rat OSE on high calcium levels in culture media for growth (122) and the propensity of rat OSE

but not human OSE to undergo spontaneous transformation to immortal cell lines in culture (11). Studies of rabbit OSE have provided some of the earliest and most detailed information on hormonal regulation of OSE. In this species, the responses to hormonal stimulation are associated with morphological changes that differ significantly from those of the human (2, 59), The differences between OSE from different sources are likely related to variations in the reproductive biology of different species and might provide clues for the striking interspecies variation in their propensity to develop epithelial ovarian cancers. Therefore, in order to avoid reporting confusing and irreproducible results, it is mandatory to specify species in discussions of OSE.

F. Culture of OSE from women with family histories of ovarian cancer

One of the pressing problems in ovarian cancer management is the lack of markers for the detection of preneoplastic or early neoplastic changes in the OSE. Our laboratory and others have investigated this problem by studying the properties of overtly normal OSE from women with histories of familial ovarian cancer and, in particular, women with proven predisposing mutations. As stated in *Section IV.B*, the evidence for phenotypic changes in OSE *in situ* of women with these predisposing mutations is controversial. However, it appears that such OSE expresses an altered phenotype in culture that might reveal early changes and, perhaps, be a source of predictive markers for ovarian carcinogenesis (78, 139, 140).

As discussed earlier in this review, normal OSE cells have a tendency to undergo epithelio-mesenchymal conversion in culture. In contrast, ovarian carcinoma cells are nonresponsive to the environmental signals that induce this conversion and remain epithelial in culture indefinitely. The first indication to suggest that overtly normal OSE from women with family histories of ovarian cancer (FH-OSE) differs from the OSE of women with no family history (NFH-OSE) not only genetically but also phenotypically came in 1995, when CA125 in cultured OSE was found to be expressed in more cells and for longer durations in FH-OSE (141). CA125 is an ovarian tumor marker used to monitor the clinical progress of ovarian cancer patients, but it is also an epithelial differentiation marker that is expressed by normal oviductal and endometrial epithelium. The increased expression of CA125 suggested that FH-OSE cells might have a diminished capacity for epithelio-mesenchymal conversion. This hypothesis was supported by subsequent observations that showed an increased tendency of FH-OSE cells to retain an epithelial cellular morphology and growth patterns in two- and three-dimensional culture and to express the epithelial markers keratin and E-cadherin more frequently and over longer periods in culture than NFH-OSE. At the same time, the capacities for sponge contraction and collagen type III secretion, which are mesenchymal markers, were reduced compared with NFH-OSE cultures (36, 78).

Recently, we showed that the Met receptor for hepatocyte growth factor (HGF) was down-regulated in prolonged cultures of NFH-OSE but was stabilized in FH-OSE cultures at all passages, similar to ovarian carcinoma lines. As Met is characteristically expressed by epithelial cells, the presence of this receptor represents yet another epithelial differentiation marker that persists longer in FH-OSE. In view of the capacity of HGF to induce glandular morphogenesis (142), Met expression may enhance the susceptibility of the FH-OSE cells to the aberrant Mullerian differentiation that accompanies ovarian carcinogenesis (139, 143). Our data also revealed concomitant expression of HGF and Met, suggestive of autocrine regulation by HGF-Met in most cases of FH-OSE but rarely in NFH-OSE (Fig. 7).

HGF activated several signaling molecules of the PI3K pathway in NFH-OSE cells. In contrast to NFH-OSE, some of these molecules, including Akt2 and p70 S6 kinase, were constitutively phosphorylated in FH-OSE, perhaps through an autocrine HGF/Met loop (Fig. 8). Similar to other cell types (144), the appearance of both HGF and Met expression in FH-OSE may reflect increased autonomy of differentiation and growth controls that represent an early step in their (pre)neoplastic progression.

Together, these data suggest that some of the factors that enhance the expression of epithelial characteristics, including Met levels, in the malignant progression of ovarian surface epithelial tumors (145–147) may preexist in FH-OSE, and that FH-OSE may have acquired some of the autocrine regulatory mechanisms that characterize malignant cells. Such increased autonomy would indicate an early step or predisposition to neoplastic progression by FH-OSE and would provide a basis for the propensity of such OSE to undergo neoplastic progression.

An additional difference from NFH-OSE was observed in SV-40 large T antigen-immortalized FH-OSE cultures, which were found to exhibit increased telomeric instability and a reduced growth potential indicative of greater proximity to replicative senescence (140). These observations are particularly relevant to the unexplained earlier age of onset that characterizes ovarian cancer in women with hereditary ovarian cancer syndromes (148).

A possible reason why differences between FH-OSE and NFH-OSE were detected mainly in culture may relate to the particular nature of these changes: most of them involve differences in the stability, rather than type, of phenotypic characteristics in culture. Since the response of cells to explantation into culture is thought to mimic their response to injury, the nature of the changes suggests the interesting possibility that FH-OSE may respond abnormally to regen-

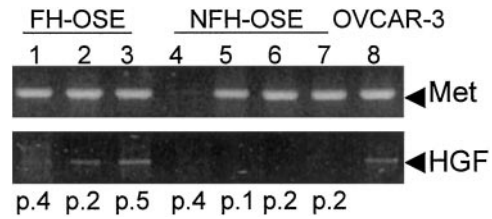

FIG. 7. Representative examples of Met and HGF mRNA expression in cultured human OSE. RT-PCR of Met (*upper panel*) in NFH-OSE, FH-OSE, and ovarian cancer cell lines. Lanes 1–3, FH-OSE; lanes 4–7, NFH-OSE; and lane 8, ovarian cancer cell line OVCAR-3. In lanes 1–7, each lane represents a different case. The passages (p.) of M-CSF *(fms)* cultures are indicated. Note that Met persists to senescence and HGF mRNA (*lower panel*) is detected only in FH-OSE and ovarian cancer cell lines, but not in NFH-OSE cultures.

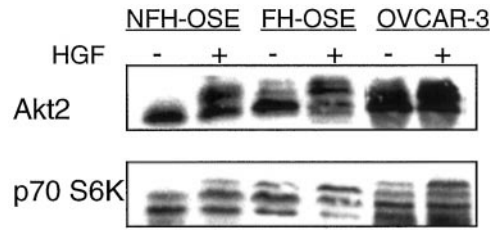

FIG. 8. Effects of HGF stimulation on protein kinase phosphorylation assessed by phosphorylation-induced reductions of kinase mobilities on Western blots. Treatment with 20 ng/ml HGF resulted in apparent phosphorylation of Akt2 and p70 S6K in NFH-OSE, FH-OSE, and the ovarian cancer cell line OVCAR-3. The *bottom band* represents unphosphorylated forms of the kinases, whereas the upper bands represent different phosphorylated forms. Note that phosphorylated forms of Akt2 and p70 S6K are present in FH-OSE and OVCAR-3 even in the absence of HGF stimulation.

erative stimuli. This possibility is particularly intriguing in view of the apparent role of ovulation as a predisposing factor in ovarian carcinogenesis (8, 149).

VI. Regulation by Hormones, Growth Factors, and Cytokines

A. OSE

Normal OSE cells secrete, and have receptors for, agents with growth- and differentiation-regulatory capabilities. Compared with the wealth of information available on the endocrinology of the follicular components of the ovary and on ovarian cancer, research about the roles of such agents in OSE physiology has been limited and, as a result, information on this topic is fragmentary.

1. GnRH and gonadotropins. Recently, we showed that GnRH is an autocrine growth inhibitor for normal OSE. Using RT-PCR and Southern blot analysis, we cloned the GnRH and GnRH receptor in human OSE cells and found that they have sequences identical to those found in the hypothalamus and pituitary, respectively (150). It has been shown that gonadotropins stimulate cell proliferation of normal OSE of several species *in vivo* and *in vitro* (59, 151). Human OSE cells also have receptors for FSH (152). The presence of these receptors lends support to the hypothesis that the high FSH levels in peri- and postmenopausal women may play a promoting role in ovarian carcinogenesis, since this is the age of the peak incidence of epithelial ovarian carcinomas (153). Human and rabbit OSE cells express LH receptors since hCG, which is secreted by human OSE (47), stimulates their proliferation (120, 154) and LH also stimulates rabbit OSE growth in culture (59).

2. Steroids. Receptors for estrogen, progesterone, and androgen were found at the mRNA and/or protein level in rat OSE (12) and human OSE (104, 155). SV-40 large T-immortalized OSE cells expressed ERα but not ERβ (156). No direct effects of these steroids on OSE proliferation have been demonstrated (104), but there is increasing evidence for indirect actions. Expression by OSE of the GnRH receptor appears to be reduced by estrogen (156a), and estrogen also modulates levels of HGF (157) and EGF both of which stimulate OSE growth (see below). Furthermore, in ovarian carcinoma cells, estrogen and progesterone markedly influence the steady state levels of mRNA for the HGF receptor Met (145), and 5α-dihydrotestosterone down-regulates the expression of mRNA for the TGFβ receptors (158), suggesting that these steroids may also have indirect effects on the growth regulation of normal OSE. Although there is no evidence for a direct mitogenic effect of ovarian steroids on OSE, it has been known for a long time that corticosteroids enhance OSE proliferation in culture and that combinations of EGF and hydrocortisone are among the most potent mitogens for cultured OSE (16) (see below). Steroidogenic factor 1, a transcription factor that regulates the differentiation of granulosa cells and inhibits their proliferation, is also growth inhibitory in rat OSE cells (159).

3. EGF family. Among growth factors, those of the EGF family were among the first reported to stimulate human and rabbit OSE proliferation either with or without costimulation by corticosteroids (16, 56, 160, 161). OSE cells express receptors for EGF and for TGFα , which is a structural homolog of EGF also binds to the EGF receptor (162). EGF not only stimulates proliferation of human OSE cells but also profoundly affects their differentiation: within a few days of EGF treatment, the cells convert from an epithelial to the spindle-shaped morphology and lose epithelial differentiation markers such as keratin (16). EGF is not present in large amounts in the plasma (163) but is released from platelets during the clotting process. In the ovary, EGF should therefore be present in increased amounts due to the hemorrhage that occurs during follicular rupture (164). The resulting localized stimulation of the OSE likely contributes to its rapid postovulatory proliferation and perhaps also to epithelio-mesenchymal conversion of OSE cells trapped within the ruptured follicle. EGF has numerous functions in the ovary, which include inhibition of FSH induction of LH receptors (165), inhibition of estrogen production (166) and of theca differentiation (167), and stimulation of progestin biosynthesis (168). TGFα has been demonstrated immunohistochemically in human OSE *in vivo* and *in vitro* and found to stimulate thymidine incorporation by cultured human OSE cells. It was also demonstrated immunohistochemically in human theca cells, suggesting that it plays a role in the reproductive functions of the ovary (169). In OSE cells whose life span has been extended by transfection with SV40 large T antigen, EGF does not enhance proliferation but promotes survival (170). Amphiregulin, another EGF homolog, is also a potent mitogen for OSE cells and appears to control OSE and ovarian cancer cell proliferation in a complex manner (171, 172).

Of particular interest for ovarian cancer are the heregulins, including the heregulin/*neu* differentiation factor, which are a family of ligands that cause phosphorylation of the HER2/neu receptor, a 185-kDs transmembrane protein kinase with extensive homology to the EGF receptor (reviewed in Ref. 173). HER1 (synonymous with EGF receptor), HER2, HER3, and HER4 are members of the type I receptor tyrosine kinase family (RTK I) of epithelial growth factor receptors (174). These receptors interact in multiple ways that modify their influence on a variety of cells (reviewed in Ref. 175). Although normal OSE cells express EGF receptors, they express

little or no HER-2/*neu* (110, 172, 176, 177). However, HER2/neu is amplified and overexpressed in 25–30% of ovarian and breast cancers, and this overexpression is associated with a poor prognosis (110, 173).

4. Other growth factors. Among other growth factors, basic fibroblast growth factor (bFGF), a member of the FGF family of growth factors (178), stimulates the proliferation of rabbit OSE (161) and maintains viability in cultured rat OSE cells. The latter function involves alterations in intracellular calcium levels and can be mimicked by N-cadherin-mediated intercellular adhesion (129, 179). Platelet-derived growth factor (PDGF) also stimulates proliferation of OSE cells (180). Finally, TNFα, produced, for example, by macrophages, induces both proliferation and TNFα expression in OSE cells (57, 58, 181). It is significant that EGF and PDGF, which stimulate OSE growth, are released from platelets during the clotting process that occurs at ovulation. Recently, it was reported that keratinocyte growth factor (KGF) and its ligand, Kit, represent an autocrine mitogenic system for bovine OSE and that KGF/Kit may interact with HGF in the regulation of this system (138).

5. TGFβ family of growth-inhibitory factors. Among agents that inhibit OSE growth are several members of the TGFβ family of growth factors (182), which affect and/or are produced by OSE. TGFβ itself, a widely distributed growth factor with multiple modes of action, acts as an autocrine growth inhibitor for cultured human OSE (74) and also counteracts the growth-stimulatory effect of EGF (183). In contrast to some other inhibitory factors, TGFβ does not induce apoptosis in OSE cells (184). TGFβ inhibits growth of rabbit OSE (161) and regulates Kit ligand expression in immortalized rat OSE (185). A detailed examination by immunohistochemistry and *in situ* hybridization of TGFβ subtypes, the related protein endoglin, TGFβ receptors, and TGFβ-binding protein demonstrated the presence of all of these in human OSE and, with the exception of the binding protein, levels were lower than in ovarian cancers (186). Interestingly, 5α-dihydrotestosterone down-regulates the expression of mRNA for the TGFβ receptors I and II in ovarian carcinoma lines (158), suggesting that it might also counteract growth-inhibitory effects of TGFβ in normal OSE. Welt *et al.* (187) investigated the TGFβ-related factors, activin, inhibin, and follistatin, in normal and neoplastic ovarian epithelia. OSE, immediately after removal from the ovary, expressed mRNA for follistatin 288 and 315, for the activin receptors IA, IB, II, and IIB, as well as for the α-subunit and (weakly) the β-subunit of the ligands. At the protein level, OSE produced inhibin only. After 1 month in culture, the α-subunit was undetectable while the βA-subunit became abundant. Another member of the TGFβ family, anti-Mullerian hormone (AMH), which causes regression of the Mullerian ducts in male fetuses, is produced at low levels by granulosa cells throughout the reproductive life of women (188). In view of the close developmental relationship between the Mullerian ducts and OSE, it might be expected that AMH should affect OSE cells; however, no information on this topic seems to be available.

6. HGF. A growth factor with pleiotropic effects, which has attracted increasing attention in recent years, is HGF and its receptor, Met. HGF is produced primarily by mesenchymal and stromal cells and acts on epithelial cells by a paracrine mechanism through its receptor tyrosine kinase encoded by the c-*met* protooncogene (189, 190). During mouse development, HGF is produced by the mesenchyme at the urogenital region in the vicinity of Met-expressing epithelia, suggesting that the development and morphogenesis of urogenital organs, including ovary, depend on a paracrine regulation of HGF-Met (191). In the adult ovary, including human, the expression of Met persists in the OSE, granulosa cells, and Mullerian epithelia (145–147, 192, 193). Extraovarian mesothelial cells, which share a common embryological origin and anatomical environment with OSE, lack HGF and Met (194). This suggests that expression of the Met receptor might be a feature characteristic of celomic epithelial derivatives at the urogenital ridge through local differentiation. Immunohistochemical studies have localized expression of HGF to bovine, rat, and human OSE (195, 196), but the mRNA was not found in OSE of the mouse by *in situ* hybridization and Northern blot analysis (197). There are two possible explanations for this discrepancy. First, there may be species differences among human, bovine, rat, and mouse OSE. Second, the detected HGF protein could have been produced by adjacent mesenchymal cells and bound to the Met receptor on OSE. The physiological influence of HGF on OSE depends on the presence or absence of basement membrane components. For example, HGF decreases N-cadherin-mediated cell contacts, increases intracellular calcium concentration, and ultimately induces apoptosis *in vitro* if these cells are cultured on plastic (129). On the other hand, HGF is mitogenic when OSE cells are plated on a fibronectin-like ECM (RGD peptide) (154). *In vivo*, these modulations may regulate the contributions of OSE to follicular rupture before ovulation and to postovulatory repair. HGF levels are transcriptionally regulated by a variety of steroid hormones, cytokines, and growth factors, including estrogen and gonadotropins. Estrogen increases the expression of HGF in the ovary, but not in other organs such as kidney and liver, suggesting that this may be a crucial part of the mechanism through which estrogen mediates cell growth and differentiation in the ovary (157). hCG has also been shown to stimulate OSE cell growth, and this ability is mediated by upregulating the expression of HGF (154). The serum levels of HGF change during the menstrual cycle, which supports the possibility that HGF secretion is regulated by steroid hormones and/or gonadotropins. The level of HGF is lowest at ovulation and is highest in the late follicular phase and during the luteal phase, suggesting that apoptosis and mitotic activity of OSE before and after ovulation might be regulated via HGF (193). Together, these findings illustrate the role of HGF in normal OSE physiology and show that both cell-ECM interaction and hormonal regulation during the menstrual cycle determine the outcomes. In culture, HGF is mitogenic for both bovine (196) and human (139) OSE.

7. Cytokines. Cultured human OSE also secretes bioactive cytokines, including IL-1, IL-6, macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF). These agents have regulatory effects on

follicular growth and differentiation, ovulation, and the distribution of intraovarian cells of the immune system (55), and IL-1 enhances OSE proliferation (181). Little is known about the regulation of cytokine expression in OSE, but it may be relevant that ovarian steroid hormones regulate GM-CSF production by uterine epithelial cells, which are developmentally related to OSE (198).

B. Ovarian carcinomas

Ovarian carcinomas also secrete and have receptors for agents with growth-regulatory capabilities. The potential roles of peptide hormones, sex steroids, and growth factors in ovarian cancer are discussed below.

1. Peptide hormones.

a. GnRH. GnRH acts as a key hormone in the regulation of the pituitary gonadal axis (199, 200). In addition to its well documented role in gonadotropin biosynthesis and secretion in the pituitary, an autocrine/paracrine role for GnRH has also been suggested in tumors of the ovary, breast, prostate, and endometrium (201–206). This concept is based on the detection of binding sites for GnRH, as well as the expression of GnRH and its receptor gene transcripts in these tumors. Especially noteworthy is the finding that GnRH and its receptor are expressed in normal and neoplastic OSE cells (Fig. 9). GnRH receptors were detected in approximately 80% of human ovarian epithelial tumors and in numerous ovarian cancer cell lines such as EFO-21, EFO-27, and OV-1063 (201, 207, 208). GnRH and its analogs have been shown to be efficient in treatment of the sex steroid-responsive tumors of ovary, breast, and endometrium *in vivo* and *in vitro* (201–206, 209, 210). *In vivo*, long acting GnRH agonists are thought to act by desensitizing or down-regulating the GnRH receptors in the pituitary, resulting in a subsequent decline in gonadotropins that serve as tumor growth factors. The suppression of endogenous LH and FSH secretion by GnRH-agonist treatment results in growth inhibition of heterotransplanted ovarian cancers in animal models (211). *In vitro,* GnRH and its analogs have been shown to inhibit the growth of a number of GnRH receptor-bearing ovarian cancer cell lines. For instance, Emons *et al.* (201) reported a time- and dose-dependent inhibition on the growth of two ovarian cancer cell lines, EFO-21 and EFO-27, by the GnRH agonist [D-Trp^6]LHRH. In other studies, growth inhibition of the ovarian cancer cell line, OVCAR-3, was observed by the administration of GnRH agonists such as [D-Trp^6]LHRH and Lupron-SR (211, 212). Another GnRH agonist, buserelin, suppressed FSH-induced proliferation of the DMBA-OC-1 cell line (213). Interestingly, an antagonistic analog of GnRH, SB75, also inhibited the proliferation of OV-1063 cells in a dose-dependent manner, as indicated by the reduction in cell number and DNA synthesis (214). In a clinical trial, the combined treatment with the GnRH agonist, [D-Trp^6]LHRH, and cisplatin has been shown to improve the positive outcome as compared with patients on chemotherapy alone (215). To improve the therapeutic efficiency of GnRH analogs against cancer cells and reduce cytotoxicity against normal cells, targeted chemotherapy based on the GnRH receptor has been developed recently (reviewed in Ref. 216). Targeted cytotoxic peptide conjugates consist of a peptide that binds to receptors in tumors and a cytotoxic chemical. Cytotoxic analogs of GnRH—AN-152 in which a cytotoxic chemical, doxorubicin (DOX), is linked to a peptide, [D-Lys^6]GnRH, and AN-207, which consists of 2-pyrrolino-DOX (AN-201) coupled to the same peptide—have been developed. Preliminary studies have demonstrated that these cytotoxic analogs of GnRH showed high-affinity binding for GnRH receptor in tumor cells and were less toxic and more effective than their respective radicals in inhibiting the growth of GnRH receptor-positive human ovarian, mammary, or prostatic cancer cells (217, 218). AN-152 given intraperitoneally was more effective and less toxic than equimolar doses of DOX in reducing the growth of GnRH receptor-positive OV-1063 human ovarian cancers in nude mice (208). In the same study, AN-152 did not inhibit the growth of GnRH receptor-negative UCI-107 human ovarian carcinoma, indicating a targeted cytotoxic effect of the GnRH conjugate. In a recent study, another cytotoxic analog of GnRH (AN-207) also inhibited the growth of ovarian tumor cells, OV-1063, in nude mice with less toxicity than equimolar doses of its radical 2-pyrrolino-DOX (AN201) (219). AN-152 and AN-207 have also been shown to inhibit the growth of estrogen-independent MXT mouse mammalian tumor cells (220) and PC-82 human prostate cancer cells in nude mice (221).

The exact mechanism underlying the growth-inhibitory effect of GnRH analogs remains to be elucidated. At the ovarian GnRH receptor level, the putative endogenous ligand may stimulate the proliferation of the cells through the receptor, which might be down-regulated by continuous treatment with a potent GnRH agonist. The finding that continuous treatment with GnRH agonists, which is thought to induce receptor down-regulation, inhibited ovarian cancer cell growth, and that this effect was abolished by cotreatment with a specific GnRH antagonist, corroborated this view (150, 222). Alternatively, the ovarian GnRH receptor might mediate direct antiproliferative effects of GnRH analogs. However, this notion is not corroborated by the observation that both antagonistic and agonistic analogs have been reported to induce growth inhibition of ovarian cancer cells (214). Recently, it has been suggested that the well established GnRH receptor signaling mechanism mediated by phospholipase C (PLC) and protein kinase C (PKC) is likely not involved in the antiproliferative effects of GnRH in tumor cells (223). Rather, GnRH binding in cancer cells could activate a downstream phosphotyrosine phosphatase (PTP) in GnRH receptor-bearing tumors, thereby counteracting the effects of growth factors that function through receptor tyrosine kinase (224, 225). It has been reported that analogs of GnRH reverse the growth-stimulatory effect of EGF and insulin-like growth factor (IGF) in cancer cells including carcinomas of the ovary (226–228), possibly by down-regulating their receptor numbers and/or mRNA levels. In addition, there is evidence that the GnRH receptor is coupled to $G_{i\alpha}$ in reproductive tract tumors (229, 230). In prostate tumor cells, the GnRH receptor is coupled to $G_{i\alpha}$ which, by the inhibition of cAMP accumulation, may mediate the growth-inhibitory action of GnRH (230). At the ovarian cell level, it has been demonstrated that GnRH analogs reduce cell proliferation by increasing the portion of cells in the resting phase, G_0-G_1

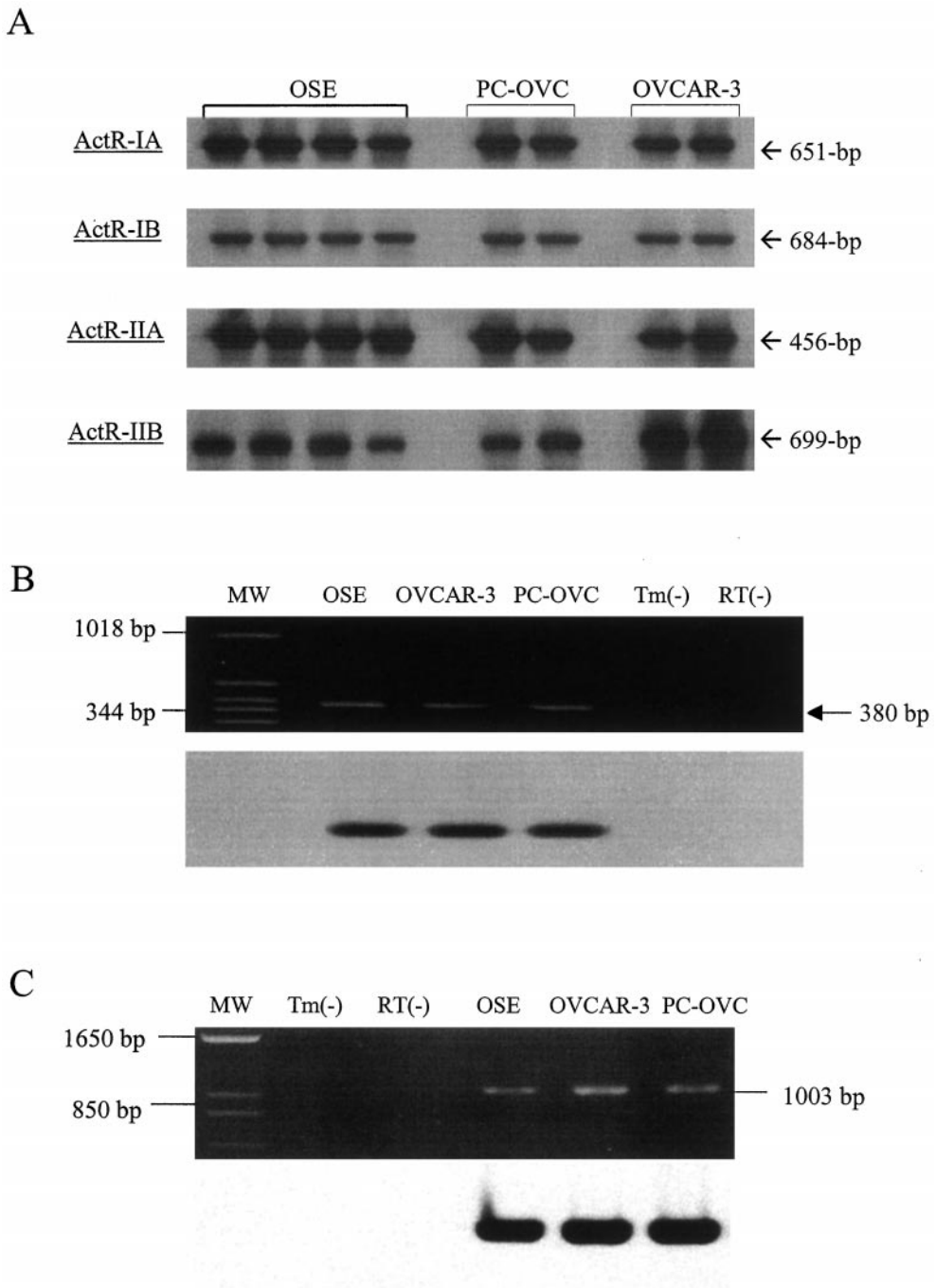

FIG. 9. Expression of activin receptors (A), GnRH (B), and GnRH receptor (C) in normal OSE, primary cultured ovarian cancer (PC-OVC), and OVCAR-3 cells. Total RNA was extracted and cDNA was synthesized from total RNA by reverse transcription (RT). The synthesized cDNA was used as template for PCR amplification. The primers for each activin receptor were employed in intracellular domain. The 651-bp, 684-bp, 456-bp, and 699-bp PCR products were obtained in these cells and confirmed as activin receptor IA, IB, IIA, and IIB using Southern blot hybridization, respectively. The PCR products amplified were subcloned and sequenced and found to be 100% identical to published sequences of activin receptors (data not shown). The PCR products of GnRH and GnRH receptor were observed on an ethidium bromide-stained gel (B and C, *top panels*, respectively). No PCR products were observed or detected in negative controls (without template [Tm(−)] and without reverse transcriptase [RT(−)] in the reaction) by ethidium bromide staining and Southern blot analysis. Sequence analysis revealed that GnRH and GnRH receptor mRNAs from human OSE, PC-OVC, and OVCAR-3 cell lines had a nucleotide sequence identical to those found in the hypothalamus and pituitary, respectively (data not shown). [Adapted with permission from S. K. Kang *et al.*: *Endocrinology* 141:72–80, 2000 (150). © The Endocrine Society.]

(222), and inducing cell death or apoptosis (231, 232). Treatment of ovarian cancer cells with GnRH analogs may induce apoptosis mediated by the Fas ligand-Fas system, which has been shown to trigger apoptosis in a variety of cell types (233). Recently, it has been demonstrated that a GnRH analog may modulate ovarian cancer cell growth by inhibiting te-

lomerase activity without altering the RNA component of telomerase expression (234).

b. Gonadotropins. The involvement of gonadotropins in ovarian epithelial cancer development is supported by several observations. A number of epidemiological studies have demonstrated an increased occurrence of ovarian cancer with exposure to high levels of gonadotropins during menopause or infertility therapy (235–237). Clinically, administration of human menopausal gonadotropin (hMG) for ovulation induction may increase the risk of epithelial ovarian tumors (237). Reduced risk of ovarian cancer is associated with multiple pregnancy, breast feeding, and oral contraceptive use, which results in lower level and reduced exposure to gonadotropins (235, 236, 238, 239). Receptors for FSH and LH/CG were demonstrated to be present in normal OSE and ovarian tumors (152, 240–242). As in normal OSE cells, FSH and LH/CG stimulated the growth of some ovarian cancer cells in a dose- and time-dependent manner *in vitro* (243, 244). Elevated levels of gonadotropins may promote the growth of human ovarian carcinoma by induction of tumor angiogenesis *in vivo* (245). Despite these observations, the roles that elevated levels and prolonged exposure to gonadotropins play in ovarian tumorigenesis remain to be elucidated. For instance, in other reports, increased risk of ovarian cancer development has not been demonstrated in women undergoing ovulation induction for *in vitro* fertilization (246, 247). The mechanism by which gonadotropins increase ovarian cancer cell growth is unclear. It has been shown that hCG induced estradiol production in a dose-dependent manner, whereas FSH had no such effect in primary cultures of epithelial ovarian cancer cells (248). The combined treatment of hCG with estradiol may regulate the growth response of epithelial ovarian cancer cells through IGF-I and EGF pathway (249). hCG treatment has been demonstrated to suppress cisplatin-induced apoptosis by 58% in the ovarian carcinoma cell line, OVCAR-3 (250), suggesting that gonadotropins may play a role in preventing apoptosis. Taken together, gonadotropins may be a contributing factor in ovarian tumorigenesis, presumably by enhancing cell proliferation and/or inhibiting apoptosis.

c. Activin/inhibin. Activin and inhibin are members of the TGFβ superfamily (251–253). Activin is a dimeric protein composed of two β-subunits, βA-βA (activin A), βB-βB (activin B), or βA-βB (activin AB) (252). Inhibin is composed of an α- and one of two β-subunits, α-βA (inhibin A) or α-βB (inhibin B). The main function of these gonadal peptides is to regulate FSH secretion from the anterior pituitary gland (254, 255). However, since activin and inhibin are produced in the ovary (256), it has been hypothesized that they may act via an autocrine/paracrine mechanism to regulate ovarian function (256, 257). Activin mediates its cellular effects through heterodimeric complexes of type I and II activin serine/threonine kinase receptors (258), which are expressed in normal and neoplastic OSE cells (Fig. 9).

It has been demonstrated that recombinant activin has no mitogenic effect on normal OSE that also expresses activin receptors (187, 258a). Interestingly, activin may function to support cell survival and stimulate the proliferation of epithelial ovarian carcinoma cell lines, including OVCAR-3, CaOV-3, CaOV-4, and SW-626 (259, 260), whereas follistatin, an activin-binding protein, inhibits this action (187, 260). Most primary epithelial ovarian tumors (96%) synthesize and secrete activin *in vitro,* and serum levels of activin are frequently elevated in women with epithelial ovarian cancer (187). These findings suggested that, in epithelial ovarian cancer 1) βA-subunit mRNA is expressed; 2) activin is secreted more frequently than inhibin; and 3) βA-subunit mRNA expression is greater in neoplastic and normal epithelium after culture. Thus, activin may act as an autocrine/paracrine regulator of epithelial ovarian tumors, but its exact role in tumorigenesis has yet to be defined (187). Inhibin α-subunit, which was expressed in 47% cases of normal OSE, was not found in the epithelial component of ovarian cystadenomas, tumors of low malignant potential (LMP), or carcinomas. βA-subunit was expressed in 93% cases of OSE, in the epithelial component of all cystadenomas, in 81% cases of LMP tumors, and in 72% cases of carcinomas. These observations suggest that an imbalanced expression of inhibin and activin subunits in OSE may represent an early event that leads to epithelial proliferation (261).

Serum inhibin levels are elevated in most postmenopausal women with mucinous cystadenocarcinomas and mucinous borderline cystic types of epithelial ovarian tumors (262, 263), whereas immunoreactive inhibin is undetectable or present at low levels in normal postmenopausal subjects. α-Inhibin has been proposed to be a serum marker for epithelial ovarian cancer in postmenopausal women (264). Ovarian neoplasms may produce a variety of peptides related to the inhibin. It has been shown that inhibin B is detected in more ovarian cancers than inhibin A (265). The majority of granulosa cell tumors appear to secrete significant amounts of dimeric inhibin-A, whereas mucinous tumors secrete predominantly other forms of inhibin, presumably related to the α-subunit (266, 267). Serous tumors may also secrete inhibin-related peptides but not dimeric inhibin-A (266). The expression of inhibin subunit genes in granulosa cell tumors and in mucinous or serous epithelial ovarian tumors revealed that these tumors are the source of the increased immunoreactive inhibin observed in the serum of patients with ovarian tumors (268). On the contrary, it has also been reported that ovarian carcinomatous epithelial cells do not secrete inhibin and that serum inhibin levels detected in patients with epithelial ovarian carcinoma may reflect an ovarian stromal response to the ovarian carcinoma (269). Thus, the role of inhibin in ovarian cancer remains to be elucidated.

2. Sex steroids. Both epidemiological and experimental observations have implicated sex steroids in the pathogenesis and growth regulation of carcinomas arising from the ovary (270–274). A number of studies have suggested that the risk of developing ovarian cancer increases with the usage and duration of hormone replacement therapy (275, 276). Estrogens taken as oral contraceptives during premenopausal years are protective but, when used in postmenopausal years as hormone replacement therapy, may increase the risk of ovarian cancer (235, 239, 275–277). Breast feeding, which appears to offer protection in a number of studies (278), is associated with reduced serum concentrations of estradiol. In addition to estrogens, other ovarian steroids such as andro-

stenedione, testosterone, and progestins have also been implicated as risk factors for ovarian cancer (235, 239, 277). In patients with ovarian cancer, elevated plasma levels of 17β-estradiol, estrone, progesterone, 20α-hydroxyprogesterone, dehydroepiandrosterone sulfate, androstenedione, and testosterone have been observed and shown to correlate with tumor volume (279–283). Elevated levels of sex steroid hormones are thought to be produced by ovarian tumor cells. This notion is supported by the increased levels of sex steroids in the ovarian vein draining the tumor-bearing ovary, as compared with the contralateral ovarian vein and the peripheral blood (284–286). Exogenous estrogen stimulated the growth of several ER-positive ovarian carcinoma cell lines *in vitro* (272–274).

The classical estrogen receptor (ER), now referred to as ERα, and the progesterone receptor (PR) were found in less than 50% of ovarian tumors, whereas androgen receptor (AR) was detected in the majority of cases reported (>80%) (235, 239, 277). In malignant epithelial ovarian tumors, the concentration of ER is generally higher, while the concentration of PR is generally lower in malignant lesions as compared with that of benign tumors or normal ovaries (287–292). Also, the presence of a second isoform of estrogen receptor (ERβ) has been reported in normal and malignant ovarian cells in primary cultures or ovarian cancer cell lines (155, 156). Nevertheless, the relationship between receptor content and prognostic factors such as histology, stage, and grade is unclear. Several authors found no correlation between ER content and histological type or grade of differentiation (293–297). Others reported that endometrioid tumors more frequently express PR, while serous tumors were more frequently found to be to ER positive (296–298). Some investigators observed that ER positivity was correlated with poor differentiation (298, 299), whereas others found that well differentiated tumors more frequently express ER (300, 301) or both ER and PR (302, 303). PR status was found to be of significant prognostic value in advanced epithelial ovarian cancer (304). However, in other studies, no clinical significance of ER and PR status in epithelial ovarian carcinomas was reported when correlated with age, parity, race, smoking, surgical stage, histological type, histological grade, progression-free interval, or patient survival (305). Also, no correlation between the presence of AR and tumor histology was found (306, 307). The apparent discrepancy of these observations may be explained by differences in the assay methods, the criteria for positivity for steroid receptors, and/or heterogeneity of tumor cell populations with respect to steroid receptor contents (307). The ERα mRNA mutation with a 32-bp deletion in exon 1 was found in the SKOV-3 cell line, which is insensitive to E_2 with respect to cell proliferation and induction of gene expression (155). This may provide an explanation for the lack of responsiveness and resistance to E_2 in some ovarian cancers.

Endocrine therapy for the management of ovarian cancer is only applied after failure of first and second line chemotherapy or in the case of recurrent disease. In a study on the use of progestins in patients with advanced ovarian cancer, objective response was reported in about 15% of the patients, with an additional 10% of patients showing stabilization of the disease (308). Progestins have also been used in combination with estrogen, antiestrogens, and chemotherapeutic drugs (309, 310). Freedman *et al.* (309) studied the effect of combination treatment with medroxyprogesterone acetate (MPA) and ethinylestradiol in 65 patients with refractory epithelial ovarian carcinoma and reported that 14% and 20% of patients responded and had stabilized disease, respectively. However, no objective responses were observed in a phase I study of cyclic therapy with MPA and tamoxifen (310). The synthetic antiestrogen tamoxifen has been used as a single agent therapy in the treatment of ovarian cancer with considerable variation in the reported response rates (311–313). In a prospective randomized study of 100 ovarian cancer patients in advanced stages, no beneficial effect of combined treatment with tamoxifen and cytotoxic chemicals, cisplatin and adriamycin, was reported (314). A dose-dependent inhibitory effect of antiandrogens and epostane was observed in ovarian cancer cell lines with AR, suggesting that blockage of androgen action or synthesis may have therapeutic value in ovarian cancer (315).

The exact mechanism of action of steroid hormones in ovarian cancer remains unclear. Induction of c-myc oncoprotein has been shown to mediate the mitogenic response to growth stimuli (272). Depending on the levels of ER, up-regulation of c-myc protein by estrogen has been shown to mediate estrogen-induced ovarian cancer cell growth. It has been demonstrated that estrogen interacts with other growth factors in the normal ovary and ovarian cancer cells. In the ovarian cancer cell line, PE01, the estrogen-mediated growth-stimulatory effects were reversed by an EGF receptor-targeted antibody (316). In addition, estrogen induced a significant increase in TGFα protein concentration in media and regulated EGF receptor expression in those cells. These results suggest that estrogen may act through increasing production of TGFα and regulation of the EGF receptor. Estrogen produced a concentration-related potentiation in the growth response to IGF-I and EGF under conditions in which the growth responses to EGF and IGF-I were submaximal (249). Estrogen has been shown to exert its enhancement of EGF- and IGF-I-mediated growth through increased binding affinity for EGF receptor and IGF-I receptor number (249). In other studies, estrogen caused a marked decrease in insulin-like growth factor binding protein-3 (IGFBP-3) mRNA, but increased IGFBP-5 mRNA levels, suggesting that IGFBP expression can be regulated in estrogen-responsive ovarian cancer by E_2 (317).

As discussed above, germline mutations in the BRCA1 gene are associated with increased cancer risk in breast, ovary, and prostate, but not in other tissues. The obvious implication, that BRCA1 mutations therefore affect neoplastic transformation in conjunction with hormonal factors, is supported by recent reports that showed that estrogen and PRL stimulate proliferation of ovarian and breast carcinoma cells and concurrently up-regulate BRCA1 mRNA and protein (92, 94). Subsequently, Fan *et al.* (93) demonstrated that, in breast and prostate cancer cells, BRCA1 inhibits signaling by ligand-activated ER-α and blocks its transcriptional activation function. Together, these data suggest that BRCA1 functions as a negative feedback inhibitor of growth induced by estrogen and PRL. It is important to note that some ovar-

ian carcinoma cells proliferate in response to estrogen (156a, 318) while normal OSE cells do not (104, 156a).

3. Growth factors. Trends in the expression and response to growth regulators include the secretion of, and responses to, factors also found in the normal OSE (56) as well as factors that may be typical for ovarian malignancies (319, 320). The former includes growth inhibition by TGFβ (74) and growth stimulation by bFGF (321), EGF, and TGFα (176).

a. TGFβ. TGFβ is a multifunctional peptide that is involved in cell growth regulation, tissue remodeling, immune suppression, and other crucial cellular functions through both autocrine and paracrine mechanisms (322). Three mammalian TGFβ isoforms (TGFβ1, TGFβ2, and TGFβ3) that are encoded by different genes have been identified (323). The peptides share extensive homology in amino acid sequence (70–80%) and exist as homodimeric chains of between 111 and 113 amino acids, with molecular masses of 25 kDa. Three types of receptors for TGFβ (TβRI, TβRII, TβRIII) that belong to the family of serine/threonine kinase membrane receptors have been identified (324, 325). TGFβ binds to a type II TGFβ receptor (TβRII), which recruits and phosphorylates a type I TGFβ receptor (TβRI) (326–328). TβRIII, also known as betaglycan, has no known signaling motif (327, 328) and appears to bind and present TGFβ to TβRII (329–331). The expression of TGFβ has been demonstrated in ovarian tumors, suggesting an autocrine and/or paracrine role of TGFβ (332–334). TGFβ inhibited the proliferation of monolayers of normal human ovarian epithelial cells by 40–70% (74) and by 95% in primary epithelial ovarian cancer cell cultures obtained directly from ascites (335). Daniel *et al*. (336) reported that TGFβ inhibited colony formation of seven of nine fresh ovarian cancers in soft agar. In contrast, epithelial ovarian cancer cell lines are found to be relatively resistant to the growth inhibition of exogenous TGFβ treatment (74, 337). These data suggest that TGFβ may act as a growth inhibitor that prevents inappropriate proliferation of normal OSE cells, while loss of this autocrine inhibitory pathway may lead to cancer development *in vivo* and/or immortalization of cells *in vitro*. Several possible mechanisms have been proposed to explain the loss of responsiveness to TGFβ in primary culture of ovarian carcinomas and/or ovarian cancer lines. Some cells may become resistant to the effects of endogenous TGFβ because they cannot produce and/or activate secreted latent TGFβ. In this regard, it has been shown that normal ovarian epithelial cells can produce and activate TGFβ1 and -2, whereas production or activation does not occur in several ovarian cancer cell lines (74). As in other cells, defective ligand binding to the cell surface caused by absence of TβRII or expression of truncated form or splice variant of TβRII may account for the resistance to activated TGFβ in ovarian cancer cells (328, 338–341). It is also possible that alterations in signal transduction pathways may account for the development of resistance to TGFβ during the transformation process. In this regard, the binding of TGFβ to its cell surface receptors has shown to down-regulate c-myc, a DNA-binding protein whose expression is induced by growth factors that stimulate proliferation (342). The loss of TGFβ responsiveness has been associated with the inability of TGFβ to down-regulate c-myc in some, but not all, cases of ovarian tumors (343). It has been suggested that inactivation of the p53 or Rb tumor suppressing gene products due to deletion, mutation, or binding of viral oncoproteins may be responsible for the loss of TGFβ responsiveness (344). However, in most ovarian cancers, it is thought that mutation and overexpression of p53 frequently occur, but this may not lead to the development of resistance to TGFβ (335, 345, 346).

The molecular mechanisms that mediate the growth-inhibitory effect of TGFβ are poorly understood (325). Binding of TGFβ to its receptors initiates a cascade of molecular events that are thought to decrease activity of cyclin-dependent kinase (CIP1/WAF1/p21), resulting in arrest of cell cycle from G_1 into S phase of DNA synthesis in normal and neoplastic ovarian cells (325). In addition to the cell cycle inhibition, it has been shown that TGFβ can induce apoptosis in both normal and malignant cells under certain circumstances (184, 347). It is reported that malignant ovarian cells are more susceptible to apoptosis in response to TGFβ than their normal nontransformed counterparts (184).

b. EGF and TGFα. The EGF receptor (also known as c-erbB1/HER1) is a membrane tyrosine kinase that forms homodimers after binding to either EGF or TGFα (348). Homodimerization activates tyrosine kinase activity and autophosphorylates several tyrosine moieties in the cytoplasmic domain of the receptor, thereby transmitting the growth-stimulatory signal to the nucleus (348). The presence of EGF receptor has been shown in 33–75% of ovarian tumors using ligand binding, immunohistochemistry, or Northern blot analysis (162, 176, 177, 349–353). The level of EGF receptor has been demonstrated to be higher in malignant ovarian tumors than in benign tumors or the normal ovary (354, 355), implicating its prognostic importance. The contribution of a TGFα/EGF receptor autocrine loop to the growth of epithelial ovarian cancer cells is corroborated by several studies. TGFα levels in the normal ovary increase after menopause, *i.e.*, at the peak incidence of ovarian neoplasms (177, 356). Exogenous treatment with TGFα promotes the growth of several ovarian cancer cell lines *in vitro* and enhances direct clonogenic growth of ovarian tumor cells (357–359). Coexpression of EGF receptor with TGFα, but not EGF, in primary ovarian tumors was reported (352). Neutralizing antibodies against either TGFα or the EGF receptor induced growth inhibition in primary ovarian cancer cell cultures (169, 352).

The amplification and/or overexpression of the c-erbB-2 (HER2/neu) oncogene product ($p185^{c\text{-}erbB\text{-}2}$), frequently observed in different types of tumors, was seen in 30–70% of human ovarian cancers (360, 361), but in only 5–10% of normal ovarian cells (362). At the mRNA level, c-erbB-2 has extensive homology with EGF receptor, c-erbB-3, and c-erbB-4 (363–365). Immunohistochemically, increased expression of c-erbB-3 and c-erbB-4 proteins has been demonstrated in malignant ovarian tumors as compared with benign ones (366). In spite of marked sequence homology between the EGF receptor and HER2, EGF and TGFα do not bind to HER2 (348). It has been demonstrated that HER2 can be transactivated by EGF through heterodimerization with EGF receptors (348, 367) or by heregulin through heterodimerization with HER-3 or HER-4 receptors (368–370). In addition to cell proliferation, activation of EGFR and

$p185^{c\text{-}erbB\text{-}2}$ has been shown to play an important role in cell motility (371), which is mediated *in vitro* by several polypeptide growth factors, including HGF and EGF (372, 373). In this regard, overproduction of proteinases of the plasminogen activator (PA) and matrix metalloproteinase (MMP) families have previously been reported in ovarian cancer cells and tissues (374). *In vitro*, EGF-dependent stimulation of migration, and induction of MMP-9 (gelatinase B) were observed in two ovarian cancer cell lines (OVEA6 and OVCA429) (375). These findings suggest that the EGF- or the $p185^{c\text{-}erbB\text{-}2}$-dependent enhancement of cell motility may contribute to peritoneal spread and invasion of tumor cells, resulting in tumor metastasis.

Clinical studies indicate that overexpression of the c-erbB-2 (HER2/neu) gene correlates with poor prognosis (376, 377). No correlation between the presence of EGF receptor mRNA and pathological subtype was reported in the majority of studies, even though some authors observed higher expression of EGF receptor mRNA in the serous form of ovarian tumor (352, 378). The presence of EGF receptor mRNA was correlated with an advanced stage of ovarian tumors in some studies. Serum level of TGFα can be used as a tumor marker to distinguish malignant ovarian tumors from benign ones (379). The observations of overexpression of the EGF receptor and c-erbB-2 (HER2/neu) in ovarian tumors have stimulated preclinical investigations targeting growth inhibition of HER2-expressing ovarian tumor cells as novel cancer therapies (380–382). Treatment of an ovarian cancer cell line with a human-mouse chimeric anti-EGF receptor monoclonal antibody (mAb) or an anti-HER2 mAb resulted in growth inhibition (383). Concurrent treatment with two mAbs resulted in augmentation of inhibition. TGFα-stimulated growth of ovarian cancer cell lines was completely inhibited by treatment with an EGF receptor-specific tyrosine kinase inhibitor, ZM252868, suggesting that blocking of receptor activation may have therapeutic value (384). Antisense molecules that are designed to specifically block encoded genetic information from sense DNA have been developed for targeting the c-erbB-2 oncogene. Wiechen and Dietel (385) and Wu *et al.* (386) have shown the ability of c-erbB-2 antisense oligonucleotide to reduce $p185^{c\text{-}erbB\text{-}2}$ levels and thereby inhibit growth of an ovarian cancer cell line. Single-chain immunoglobulin (scFv) molecules that retain antigen-binding specificity but lack other functional domains have been designed to modulate the expression levels of oncogenes and the intracellular mobilization and function of oncoproteins. A gene encoding an anti-erbB-2-scFV with a signal peptide sequence that directs its localization to endoplasmic reticulum has been constructed and transfected into the ovarian cancer cell line, SKOV3, which overexpresses erb-B2 (387). Introduction of anti-erbB-2-scFV resulted in down-regulation of cell surface erbB-2 gene expression and marked inhibition of cellular proliferation (387). In addition, scFV-mediated erbB-2 ablation caused phenotypic alteration in tumors cells, including increased sensitivity of cells to chemotherapy and radiotherapy.

c. HGF. The HGF/Met system is considered to be a principal paracrine mediator of normal mesenchymal-epithelial interaction (388) and is also involved in the growth and spread of tumors (144). The Met/HGF receptor was overexpressed in a significant proportion of well differentiated ovarian carcinomas (145–147). Although little is known about the regulation of HGF and Met expression in ovarian tumors, the level of Met may be regulated by gonadotropin, steroids, certain cytokines and growth factors *in vivo,* and in various cell lines (145, 155, 389). HGF itself has been shown to autoregulate c-met mRNA levels (145, 390). High levels of HGF are found in cystic fluids or ascites of ovarian cancer patients compared with the peritoneal fluid of normal women (391). Recombinant HGF increased migration and proliferation of ovarian cancer cell lines that express high levels of Met protein (392, 393). Thus, high levels of Met expression in ovarian cancer cells may facilitate HGF-mediated tumor growth and dissemination (392).

d. IGFs. IGF affects the growth and differentiation in normal and neoplastic cells (394–396). IGF-RI mRNA was detected in ovarian cancer cell lines and primary or metastatic ovarian cancer tissues, suggesting a role of the IGF system in neoplastic ovarian cells (397–399). Expression of IGF-I, its receptor, and IGFBPs in epithelial ovarian cancer cells and its mitogenic effect on these cells *in vitro* implicate a role for IGF-I in the regulation of human ovarian cancer (397, 400, 401). IGF-II is also expressed in both normal ovary and ovarian cancer, and the expression level of IGF-II is elevated in ovarian cancer (402). The treatment of OVCAR-3 cells with hCG suppressed cisplatin-induced apoptosis via up-regulation of IGF-I expression, suggesting that LH/hCG may influence the chemosensitivity of ovarian cancer cells (250). In addition, the overexpression of IGF receptor-I transformed ovarian mesothelial cells to become resistant to apoptosis caused by down-regulation of Fas expression (403). These results support the notion that the IGF system plays a role in tumor growth and apoptosis of ovarian cancer.

IGFBPs appear to bind to IGFs and deliver them to target organs. A limited number of studies (404–406) have implicated the involvement of IGFBPs in ovarian cancer. IGFBP-2, a major binding protein in benign and malignant ovarian cancers, is highly expressed in malignant as compared with benign neoplasms (404, 405), suggesting that IGFBP-2 may serve as a marker for ovarian cancer. Further, IGFBP-2 correlated positively with the serum tumor marker, CA 125. By contrast, the serum IGFBP-3 level was decreased in patients with ovarian cancer as shown by RIA and Western ligand blotting (405). Treatment with estradiol induced a marked decrease in IGFBP-3, but IGFBP-5 levels were enhanced by estradiol, indicating that IGFBP expression is differentially regulated by estradiol in estrogen-responsive ovarian cancer (406).

Considering that IGFs induce cell growth and mitogenesis mediated with IGF receptors in ovarian cancer, antisense or antibody therapy against IGFs and/or IGF receptors can be considered as a potential management strategy of ovarian cancer patients. Treatment of cells with antisense IGF-I receptor oligonucleotides markedly inhibited cell proliferation (407, 408). Further, the effects of antisense oligonucleotide to IGF-II to induce apoptosis in human ovarian cancer cells

were evaluated, suggesting that IGF-II may also be a potential target in the therapeutic approach of ovarian cancer (409).

e. Vascular endothelial growth factor. Angiogenesis is a critical phenomenon in the growth, progression, and metastasis of solid tumors. Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) is a 34- to 50-kDa dimeric, disulfide-linked glycoprotein synthesized by normal and neoplastic cells (410–413). Through binding to the specific membrane tyrosine kinase receptors that are expressed in vascular endothelial cells (414), VEGF has been shown to be an important regulator of tumor angiogenesis. Abundant levels of VPF have been identified in the malignant effusions of ovarian tumors (415–417), indicating that VPF may be an important mediator of ascites formation and tumor metastasis observed in the neoplastic ovary. The expression of VEGF mRNA and protein (416–418) has been demonstrated in ovarian carcinoma, suggesting that neoplastic OSE is one source of VEGF production. *In vitro*, the conditioned medium from VEGF-positive ovarian cancer cell lines has been shown to stimulate DNA synthesis of vascular endothelium (416). *In vivo*, treatment of mice carrying tumor engraftment with a function-blocking VEGF antibody (A4.6.1) specific for human VEGF significantly inhibited subcutaneous SKOV-3 tumor growth as compared with controls (419). In mice bearing intraperitoneal tumors, ascites production and intraperitoneal carcinomatosis were completely inhibited by treatment with a VEGF antibody (419). These results suggest that neutralization of VEGF activity may have clinical application in inhibiting malignant ascites formation in ovarian cancer. Angiogenesis has been correlated with prognosis in patients with ovarian cancer. Higher positive immunostaining for VEGF and serum VEGF levels was observed in ovarian carcinoma compared with that in LMP tumors and benign cystadenoma (420). High VEGF expression in epithelial ovarian carcinomas was found to be associated with poor overall survival (421). Serum VEGF levels decreased after surgical removal of tumor in ovarian cancer patients, suggesting that serum VEGF could be used as a marker for monitoring tumor progression and ascites formation (422–425).

f. Other growth factors and cytokines. PDGF is a dimeric protein composed of two related A- and B-chain polypeptides encoded by separate genes. Two distinct receptors for PDGF have been found according to affinity (PDGF-Rα and PDGF-Rβ). A functional role of PDGF via autocrine growth stimulation has been suggested. Expression of PDGF and PDGF-Rα in ovarian tumor cells is related to progression of malignant ovarian tumors, suggesting an independent role for PDGF-Rα as a prognostic factor (426). However, there was a contradictory report that many ovarian carcinomas lose the PDGF receptors, while PDGF stimulates growth of normal OSE in culture and the cells have both α- and β-receptors (180). The loss of PDGF-Rα and PDGF-Rβ may be indicative of independence from hormonal influences to cell growth. Platelet-derived endothelial cell growth factor (PD-ECGF) is associated with angiogenesis and the progression of human ovarian cancer. The levels of PD-ECGF and its mRNA were higher in ovarian cancers than in normal ovaries, suggesting that PD-ECGF might be related to advanced stages of ovarian cancers associated with neovascularization (427). Thus, prevention of angiogenic activity of PD-ECGF may have a potential role in ovarian tumor therapeutics (428).

bFGF and other members of the FGF family share several biological properties that have the potential to mediate neoplastic cell growth. It has been shown that ovarian cancer cell lines produce and respond to bFGF and other members of the FGF family (429). The bFGF and its receptor are also expressed in epithelial ovarian tumors (430). In advanced primary ovarian tumors, the levels of bFGF mRNA and protein were significantly higher regardless of histological types (431), indicating that this growth factor may contribute to growth, invasion, and metastasis with neovascularization. It is hypothesized that bFGF may induce a fibroblastic response, which causes tumors with a high bFGF to be less aggressive than those with less stromal tissues (432).

While the secretion of cytokines is a normal OSE function (55), their recruitment into autocrine loops may be important during neoplastic progression. Cytokines produced by and growth stimulatory for ovarian carcinomas include M-CSF (433), GM-CSF (434), IL-1 and IL-6 (435, 436), and TNFα (57, 58, 181, 437, 438). High levels of M-CSF and IL-6 in blood and ascitic fluid correlate with a poor prognosis in ovarian cancer, as does overexpression of the M-CSF receptor *fms* (433), which has also been associated with increased invasiveness in endometrial and breast cancer (439, 440). Interestingly, *fms* is expressed by many ovarian cancers but not by benign ovarian tumors (433) or normal OSE (56). Thus, M-CSF, when secreted by normal OSE, acts in a paracrine manner but becomes an autocrine-regulatory factor with malignant progression. GM-CSF is a regulatory glycoprotein that stimulates the production of granulocytes and macrophages. Recombinant human GM-CSF stimulates colony formation in human ovarian cancer cell lines, IGROV-1, A2774, ME-180, Pa-1, and A2780 (434).

IL-1 and IL-6 enhance tumor cell motility and metastasis (435) and cause changes in gene expression including the induction of TNFα, which is mitogenic for OSE cells but growth inhibitory for ovarian cancer cells (181). Proliferation of OSE cells was stimulated by IL-1 and TNFα (181). Stimulation of proliferation by IL-1β could be partially blocked by an antibody against TNFα or by a soluble TNFα receptor (58). Thus, TNFα may function as an autocrine/paracrine growth factor in normal and malignant ovarian epithelial cells. Epithelial ovarian cancer cells produce IL-6, a multifunctional cytokine with diverse biological effects, in both ovarian cancer cell lines and primary ovarian tumor cultures (441). IL-6 may be a useful tumor marker in some patients with epithelial ovarian cancer, as it correlates with the tumor burden, clinical disease status, and survival (442). Inhibition of IL-6 gene expression by exposure to IL-6 antisense oligonucleotides resulted in greatly decreased cellular proliferation (443). However, the addition of exogenous IL-6 failed to restore the proliferation of the antisense-treated cells, and antibodies to IL-6 did not consistently inhibit cell growth (441), suggesting that IL-6 is not an autocrine growth factor for these established ovarian tumor cell lines. As the majority of epithelial ovarian cancers produce IL-6, the direct specific

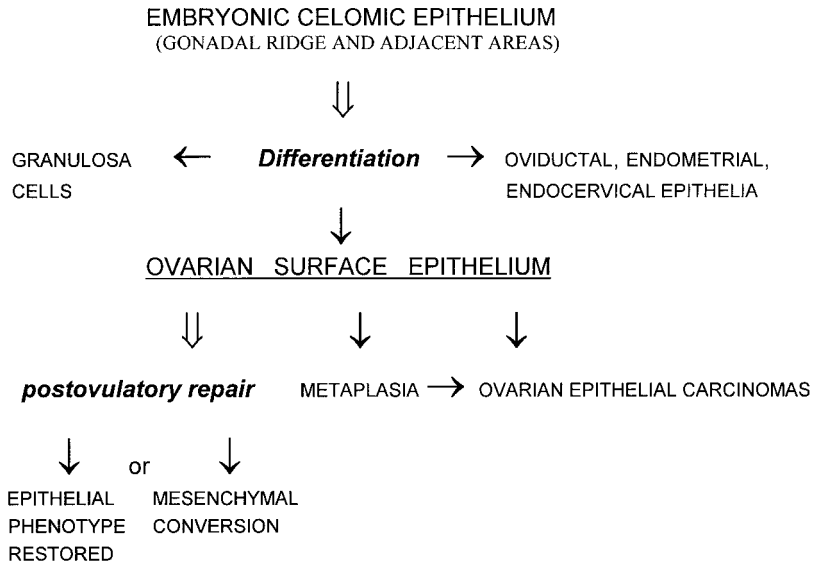

FIG. 10. Origin and fate of the ovarian surface epithelium.

inhibition of IL-6 gene expression may be of potential therapeutic value (443). Many of these agents are produced normally by various ovarian cell types and by cells of the immune system that reside in the ovary. Factors from these sources may contribute to the metaplastic and neoplastic changes in the OSE.

Interferon-γ (IFNγ) is known to modulate many cellular functions. A clinical relevance of IFNγ has been suggested because IFNγ has an antiproliferative activity on the majority of the established human ovarian carcinoma cell lines (444). It has been shown that IFNγ decreases constitutive tyrosine phosphorylation of erbB-2 and inhibits erbB-2 kinase activity in an ovarian cancer cell line, SKOV3 cells, which overexpress erbB-2 (445). The elevated expression of tumor-associated antigens and major histocompatibility complex (MHC) antigens by IFNγ may improve immunogenicity of ovarian tumor cells and explain the therapeutic effects observed in IFN therapy of ovarian cancer (444).

A potent growth-stimulatory factor from ascites of ovarian cancer patients has been purified and characterized as ovarian cancer-activating factor (OCAF), which plays a role in ovarian tumorigenesis both *in vitro* and *in vivo* (446, 447). In addition, this purified OCAF induced a proliferation of ovarian cancer cells. OCAF is composed of various species of lysophosphatidic acid (LPA), including LPAs with polyunsaturated fatty acyl chains (linoleic, arachidonic, and docosahexaenoic acids) (446). LPA is a bioactive phospholipid with mitogenic and growth factor-like activities that acts via specific cell-surface receptors present in many normal and transformed cell types. LPA has been implicated as a growth factor present in ascites of ovarian cancer patients (448).

As reviewed above, multiple factors including peptide hormones, sex steroids, growth factors, and cytokines have been implicated as stimulatory or inhibitory growth regulators in ovarian cancer. These regulators appear to exert their actions through specific receptors in an endocrine, paracrine, or autocrine manner. A better understanding of the potential cross-talk between these regulator pathways in normal and neoplastic OSE cells will be a necessary first step in understanding ovarian tumorigenesis.

VII. Concluding Remarks

The observations summarized in this review (Fig. 10) demonstrate that, contrary to its unassuming appearance and limited functional significance, OSE in adult women has the capacity to participate in ovulation-related functions in a variety of ways that are regulated by a complex set of hormone/growth factor responses. OSE can lyse and synthesize ECM and it can contract connective tissues. These properties allow the OSE to contribute to ovulation-related changes in the tunica albuginea and the ovarian cortex and to the major alterations in ovarian contours that occur with pregnancies and aging. It is tempting to speculate that the posttranscriptional regulation and shape-dependent expression of E-cadherin by OSE are adaptations that permit rapid modifications in intercellular adhesion in response to changes in ovarian contours. The physiological significance of the secretion by OSE of several growth factors and cytokines is presently unknown, as are the roles of most of the steroids and peptide hormones for which OSE has receptors. In addition to ovary-related functions, it is likely that OSE, in common with the extraovarian pelvic peritoneum, maintains the homeostasis of the pelvic cavity. However, in contrast to extraovarian mesothelium, OSE has retained properties of relatively uncommitted pleuripotential cells as reflected by its growth potential, its capacity to modulate phenotypically in response to environmental variables, and its ability to differentiate along several pathways. This immature state may be responsible, in part, for the propensity of OSE to undergo neoplastic transformation, a process during which the cells acquire characteristics of Mullerian epithelial phenotypes. Changes in overtly normal OSE from women with histories of hereditary ovarian cancer indicate that an increased commitment to epithelial phenotypes and/or reduced responsiveness to environmental signals may be among the earliest changes in the process of ovarian carcinogenesis. Normal OSE and ovarian carcinomas secrete and have specific receptors for hormones

TABLE 2. Summary of properties of OSE and epithelial ovarian carcinomas

	OSE	Epithelial ovarian Cancer	Refs.
General			
Developmental stage	Multipotential	Determined	1, 2
Responsiveness to environmental signals	High	Low	27, 78
Epithelio-mesenchymal conversion	Normal physiologic response	Partial, in late-stage invasive tumors	78
Autocrine regulatory mechanisms	Few	Many	56, 58, 138, 181 397–399
Specific features			
Morphology	Simple epithelium	Variable, complex, with features of oviductal, endometrial, and endocervical differentiation	1, 4, 5
Extracellular matrix			
Basement membrane	Present	Variable	27
Stromal collagens	Present in culture	?	27
Protease secretion	At ovulation, in culture	Associated with invasion	29, 30
Adhesion			
Integrins	α-β1, α6β4, and αvβ3	α-β1, varying levels of αvβ3, decreased levels of α6β4 and α3β1	29, 30
Cadherins	N-cadherin	N, E, and/or P-cadherin	31, 32, 34, 36, 40
Other	Desmosomes	Variable	
Cytoskeleton			
Intermediate filaments	Keratin, vimentin	Keratin, vimentin	27, 78
Secretory products			
Hormones	GnRH, inhibin	GnRH, activin	150, 187
Growth factors	TGFβ, TNFα	LPA, EGF, IGF, VEGF, bFGF, TNFα	57, 58, 332, 397, 416, 429, 447
Cytokines	IL-1, IL-6, M-CSF, G-CSF, GM-CSF	IL-1, IL-6, M-CSF, G-CSF, GM-CSF	55, 181, 433–438,
Miscellaneous	Mucin (MUC1)	CA125, mucin (MUC1, MUC2, MUC3, MUC4)	19, 20, 28
Receptors to:			
Hormones	GnRH, activin, FSH, LH	GnRH, activin, FSH and LH (variable)	150, 187, 240–242
Steroids	Estrogen, progesterone, corticosteroids, androgen	Estrogen, progesterone, and androgen	155, 156, 235, 239, 277
Growth factors	EGF, TGFβ, TNFα, HGF, PDGF	EGF, TGFβ, TNFα, HGF, VEGF, PDGF, bFGF	145–147, 177, 180, 403, 414
Heregulin/neu	Absent	Present	360, 361
M-CSF (*fms*)	Absent	Present	56, 433

and growth factors, indicating the role of these factors in normal OSE physiology and in the transformation and progression of ovarian cancers. In particular, overexpression of several receptors such as HER2/neu and fms in ovarian tumors emphasizes the importance of these factors in neoplastic transformation of normal OSE and as prognostic indicators. OSE-derived epithelial ovarian carcinomas encompass a diverse, biologically complex group of malignant neoplasms with a dismal clinical prognosis. A comparison of the properties of these neoplasms with normal OSE is summarized in Table 2. It should be emphasized that this table represents a major simplification and, in its selection of information, reflects the bias of the authors. There is an urgent need for a better understanding of regulatory mechanisms that control growth and differentiation of their source, the OSE, for better means to therapeutically exploit the hormone/growth factor responsiveness and dependence of ovarian carcinomas, and for the identification of new, clinically useful detection markers.

Acknowledgments

We wish to thank members of the Department of Obstetrics and Gynecology, University of British Columbia, for their cooperation in providing surgical specimens of normal and neoplastic OSE, and Dr. Steven Pelech, University of British Columbia, for his collaboration in the kinase activation studies.

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