On the site of origin of epithelial tumors of the ovary

A. I. Karseladze, *Ph.D., D.Sc.*

Department of Pathology, Cancer Research Centre of the Russian Academy of Medical Sciences, Moscow (Russia)

Summary

Ovaries removed at 1,050 autopsies (accidental deaths) and from 300 patients with various benign gynaecological diseases were studied in search of the incipient benign epithelial tumors. One percent of the ovaries contained incipient mucinous tumors, 1.1% - Brenner tumors, 0.5 % - endometrioid tumors. The exact percentage of the serous tumors was difficult to establish because of the absence of morphological criteria that distinguish these tumors from tumor-like conditions (inclusion cysts). The mucinous and Brenner tumors, as well as some serous tumors were located deep in the medullary or hilar regions of the ovary and were not connected to the covering of the ovary. The theory of incessant ovulation that links ovulatory damage of the ovarian surface with the initiation of neoplastic growth does not explain the genesis of all epithelial tumors. It is more likely that the latter two types arise in other parts of the female gonad. The process of morphogenesis of epithelial benign tumors is closely related to stromal alterations, specific for each histogenetic entity.

Key words: Ovarian tumors; Incipient ovarian tumors; Epithelial tumors of the ovary; Genesis of ovarian tumors.

Introduction

The histogenesis of ovarian epithelial tumors is one of the most complex problems of modern oncopathology [1-5]. Its complexity is partly due to the fact that the normal ovary is not composed of structures which could be considered morphologic (and physiologic) counterparts of most epithelial neoplasms. Currently, pathologists assume that ovarian epithelial tumors arise from surface epithelium that is capable of undergoing various types of differentiation (mucinous, endometrioid, etc.) [6-16] and subsequent malignant transformation, although not all authors support this view [17]. The repeated healing of the surface epithelium that occurs after each ovulation is considered a main factor in initiating malignant changes (theory of "incessant ovulation") [18, 19].

The above-mentioned theory has several weak points. In spite of rare reports describing a connection between the surface epithelial cells and Brenner tumors [15, 16] or the presence of an endocervical type of lining in inclusion cysts [20], there are no convincing morphological data reproducing all steps of transition from surface epithelium to the other types of epithelium except serous. On the other hand, there are reports of small mucinous and Brenner tumors in regions of the ovary which are distant from the surface and therefore not related to healing of postovulatory wounds [21-26]. This raises a question of whether the role of postovulatory damage and metaplastic potential of the surface epithelium in the histogenesis of ovarian epithelial tumors is overestimated.

The present study was conducted in search of small epithelial tumors in different parts of the ovary and to determine whether the ovarian surface epithelium is the source of all ovarian epithelial neoplasms.

Revised manuscript accepted for publication November 15, 2000

Materials and Methods

The ovaries of 1,050 females between the ages of 16 and 95 obtained during autopsies after accidental deaths and those from 300 patients from our surgical material were studied. We also investigated the ovaries of 70 patients with uterine myomas or other benign conditions with scanning electron microscopy (SEM). Macroscopically the female gonads in all cases were unremarkable.

The ovaries were fixed in 10% buffered-formalin, paraffin-embedded, and stained with hematoxylin and eosin, PAS reaction, or Alcian blue. Each ovary was cut so that all regions of the organ, especially the hilar region and sites adjacent to the mesovarium were available for investigation. The blocks were cut serially at 6 μ.

The specimens for SEM were washed to remove contaminants with Hanks' solution, fixed in 2% glutaraldehyde in 0.15M phosphate buffer (pH=7.2) for 24 hours at 4°C and dehydrated in aceton. Critical point dried material was coated with gold and examined on a SEM (Cambridge Stereoscan 250 MK).

Results

A small incipient epithelial tumor was defined as a structure that did not exceed 0.5 mm in diameter and displayed all or some principal stages of morphogenesis.

Serous tumors

The histogenesis of serous tumors seems more or less defined. There is convincing evidence of their connection with the surface epithelial cells of the ovary. In many reactive and hyperplastic processes the surface epithelium of the female gonad spontaneously differentiates towards tubal-type cells. The morphogenesis of serous benign tumors may proceed in two patterns. Both of them according to our data include an activation phase of the surface cells that can be better demonstrated by SEM (Figure 1, A-C). Flat covering cells of the ovary whose apices are covered by microvilli and rarely by a single cilium are usually transformed into round cuboidal and even columnar cells. Gradually the process of ciliogene-

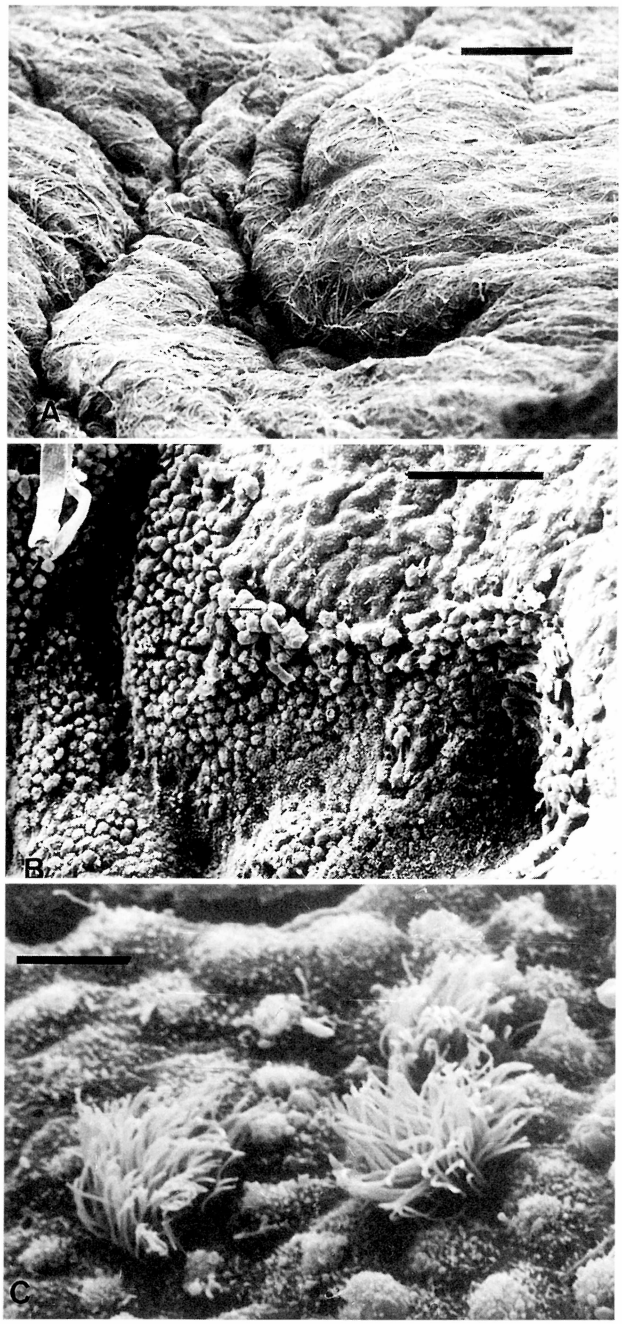

Figure 1. — Scanning electron microscopic images showing succesive stages of formation of inclusion cysts. The surface of the ovary: (A) The pore of the invagination; (B) The invaginating cells are increased in size (bar 40 μM); Cells with prominent cilia (C) in the same field (bar 4 μM).

sis intensifies and the cells aquire the appearance of tubal-type epithelium. On light microscopic examination the ovarian stroma becomes loosely arranged underneath such "islands" of activated cells due to the appearance of thin-walled blood vessels. After activation the surface epithelium either invaginates into channel-like infoldings or crypts, or forms papillary excrescences. At a certain stage, the isthmic parts of these crypts cohere, the invaginated material is detached from the surface, displaced deeper to the ovarian cortex and aquire the cystic shape - so called inclusion cysts (Figure 2).

At the early stages of the reverse process, surface papillomatosis, the surface epithelium is detached from the underlying stroma by a narrow rim of edema. The same phenomenon occurs repeatedly in the growing papillae (Figure 3). The zone of edema and detachment suggests, at first glance, mechanical damage caused by infolding and subsequent tissue distention following cell proliferation. However, we encountered the edematous rim even at the stage when the surface epithelium was almost intact and smooth. Usually, a zone of a delicate network of small blood vessels is observed adjacent to this rim, - the presumable source of fibroblasts which later colonize the core of the papillae and establish a dense stroma. The covering cells also gradually acquire the cytological characteristics of the serous type epithelium. It is noteworthy that the above-mentioned process does not necessarily result in formation of surface papillomatosis or in serous tumors. For some unknown reason, the cells may begin to proliferate after a latent period. We saw many papillary excrescences of the surface epithelium covered with

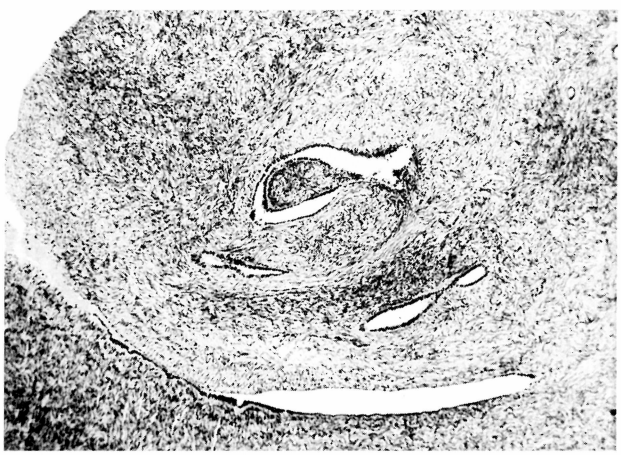

Figure 2. — Fully invaginated epithelium. Some of the cells lost their connection with the surface (H&E x 60).

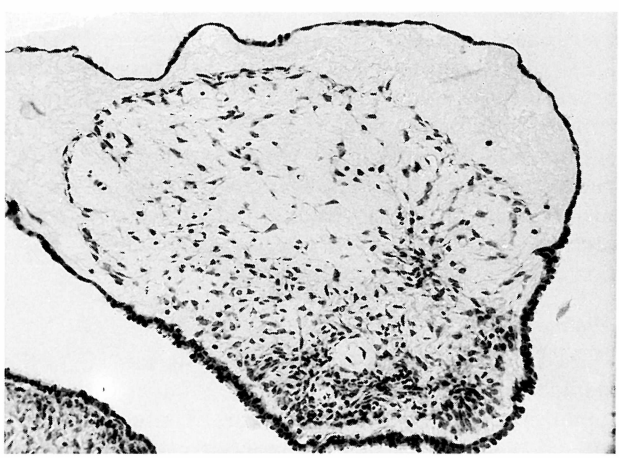

Figure 3. — Peripheral rim of edema in the papillae of the serous papillomatosis (H&E x160).

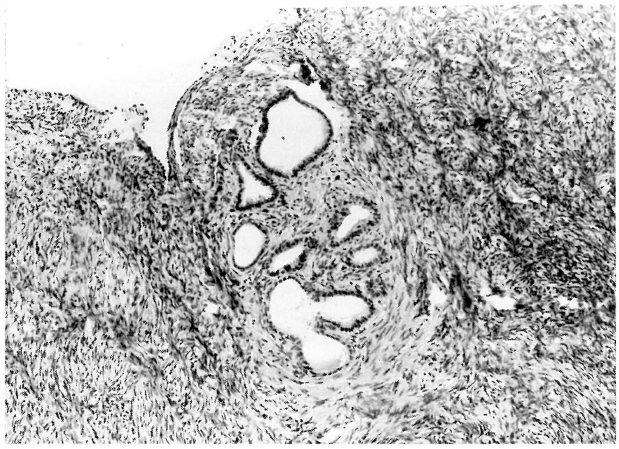

Figure 4. — Small inclusion cysts with fibrosis of the surrounding stroma (H&E x100).

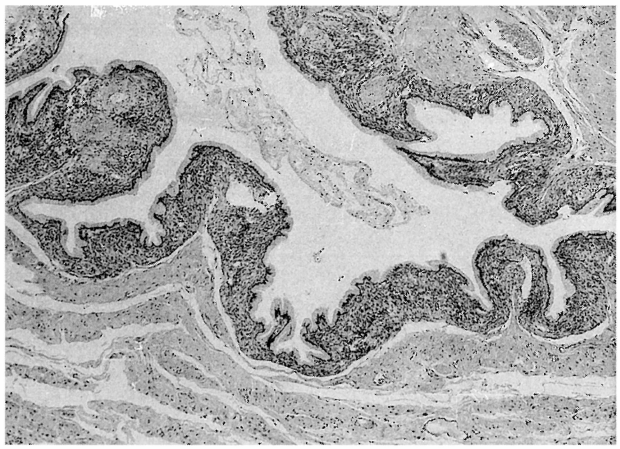

Figure 5. — Small mucinous cyst in the hilus of the ovary (H&E x40).

small, inconspicuous cells and inclusion cysts with indifferent lining in the ovaries of women 90-95 years old.

The inclusion cysts may be surrounded either by ovarian-type or sclerotic stroma (Figure 4). In the latter type structures we observed more pronounced cellular proliferation, dysplastic changes and expansive growth.

In addition to the convex region of the ovary, inclusion cysts can develop in the hilar region or more external areas adjacent to the mesovarium. We stress this point because it is believed that these sites are covered by simple mesothelium which differs from the modified surface mesothelium of the ovary. Furthermore, in the above-mentioned regions we often encountered cysts with branching papillary patterns, which probably represents the insertion of tubal mucosa rather than invagination of the ovarian surface cells.

Mucinous tumors

The frequency of small mucinous cysts was 1% of the studied population. The age of the women with these tumors ranged from 17 to 62 years. These cysts were located at the border of the hilar region with the medullary stroma and were partly or completely surrounded by smooth muscle (Figure 5).

The predominant structures were small cysts, although some tubules and acini could be detected as well. The shape of the cysts was even. Sometimes the cysts had intracystic branching and small papillae. Usually, the lumen of these cysts contained either eosinophilic or amphophilic liquid. In one case, the cyst was surrounded by the annular deposits of calcium. The lining of the cysts varied from flattened, indifferent, to columnar cells. As the cells accumulate mucin the centrally located vesicular nuclei become displaced to the basal part of the cytoplasm and the cells acquire an endocervical-like appearance (Figure 6).

In the cysts with bilayer lining, the inner layer often contained goblet cells.

In two cases, we encountered cysts that were lined with columnar epithelium with large nuclei located parabasally which did not resemble the endocervical mucin-secreting cells. However, a special staining revealed large amounts of intracellular mucin.

As a rule, the underlying stroma was either sclerotic or somewhat loosely arranged with angiomatoid features and resembled the stroma of cervical mucosa or that of the large mucinous tumors. This transformed zone of stroma was often infiltrated with leukocytes, mainly lymphocytes. A similar infiltrate was observed in the epithelial walls of the cysts. There were many hilus cells scattered throughout the adjacent ovarian tissue and even in the cyst walls.

In one case, foci of mucus-secreting cells were found in ovarian endometriosis, probably as the result of metaplasia of endometrioid-type epithelium. Of particular interest are cases in which surface epithelium was transformed into mucus secreting cells. Such foci were observed only in three cases in the transitional zone from the ovarian to the pure mesothelial covering of the mesovarian region. The ovaries of these patients did not contain any small tumors.

Brenner tumors

Incipient Brenner tumors were detected in 1.1% of all cases. The age of women ranged from 36 to 57 years. The small Brenner tumors were located at the hilus margin with the medullary stroma like the mucinous tumors.

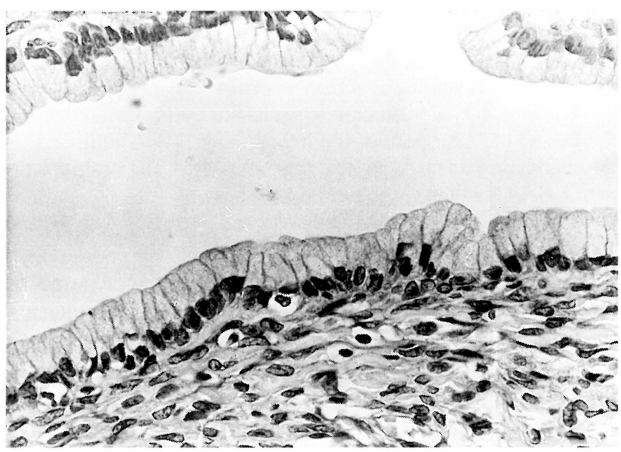

Figure 6. — The lining of the mucinous cyst displaying endocervical type epithelium (H&E x 400).

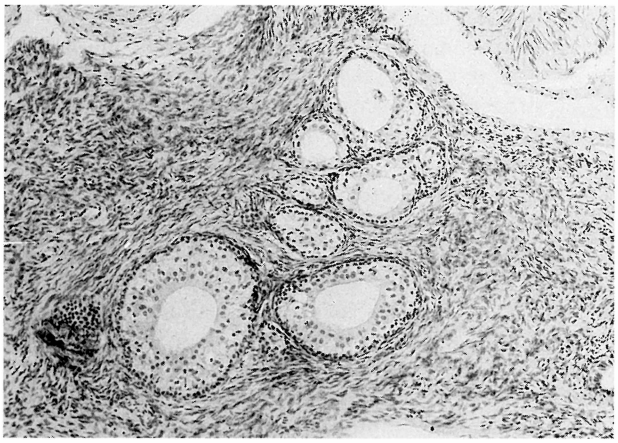

Figure 7. — Small Brenner tumor in the hilar region of the ovary (H&E, x60).

These tumors were surrounded by a narrow rim of ovarian-type stroma which extended from the cortex in a peninsular fashion. Usually, the tumors were composed of 6-7 small nests. The surrounding stroma displayed gradual concentric-type sclerosis (Figure 7). Small cysts lined with a single or double layer of epithelium probably represent the initial steps of morphogenesis. The external layer consists of flattened cells with small round nuclei. The inner layer consists of cuboidal cells with central pale nuclei displaying longitudinal grooves which give the cells a coffee-bean appearance. The proliferating cells become columnar and gradually fill the lumen of the cystic space. They acquire a polygonal shape with well defined cell borders. Faintly eosinophilic and optically translucent cytoplasm sometimes contains acidophilic granular droplets.

At a certain stage, when the cysts still contain a lumen, the cell agglomeration resembles transitional epithelium. In well defined Brenner-like nests the lumen is often surrounded by columnar cells with marked apical borders and opaque eosinophilic cytoplasm. In one case, there was a small focus consisting of a mixture of mucinous and Brenner tumors. Both components originated from a cyst lined by undifferentiated flattened epithelium.

Endometrioid tumors

Identification of the incipient forms of endometrioid neoplasms is much more difficult especially in the absence of endometrioid stroma . The cells of endometrioid glands do not display any specific structural peculiarities. Their specifity is determined by their capacity to respond to the cyclic hormonal changes in the female organism.

As a marker of endometrioid differentiation we initially searched for evidence of hormonal sensitivity e.g., subnuclear vacuoles. They are frequently found in inclusion cysts, but in our material their presence did not correlate with the secretory phase of the menstrual cycle, and they were often present in the inclusion cysts of elderly women (80-87 years.). So we looked for the initial steps of morphogenesis of endometrioid tumors in foci where the cells were surrounded by specific endometrial-type stroma.

In our material small endometrioid benign tumors appeared as adenomatous nodules in foci of endometriosis (8A) and the early stages of their malignant morphogenesis were associated with gradual regression of the stromal component and progressive dysplasia in the epithelium of the glands.

Proliferating epithelial cells formed glandular structures with epidermisation. The specific endometrioid stroma gradually disappeared and was substituted by spindle-shaped cells (Figure 8B). The fields with marked regression of the endometrioid type stroma contained glands with more expressed dysplastic changes. The same picture was once encountered in a fallopian tube, where the diminishing of specific stroma in the foci of endometriosis paralleled the step-by-step formation of endometrioid cancer with epidermisation.

We often saw dysplasia in glands and the monolayer lining of cysts, but did not see a focus of cancer in endometriosis with well preserved specific stroma. We are not referring to a focus of endometriosis close to invasive endometrioid cancer. Some endometriotic lesions with prominent cellular atypicality in patients with a previous spontaneous or artificial abortion were interpretated as a type of Arias-Stella reaction. The frequency of incipient benign endometrioid tumors (0.5%) was lower than that of mucinous and Brenner tumors.

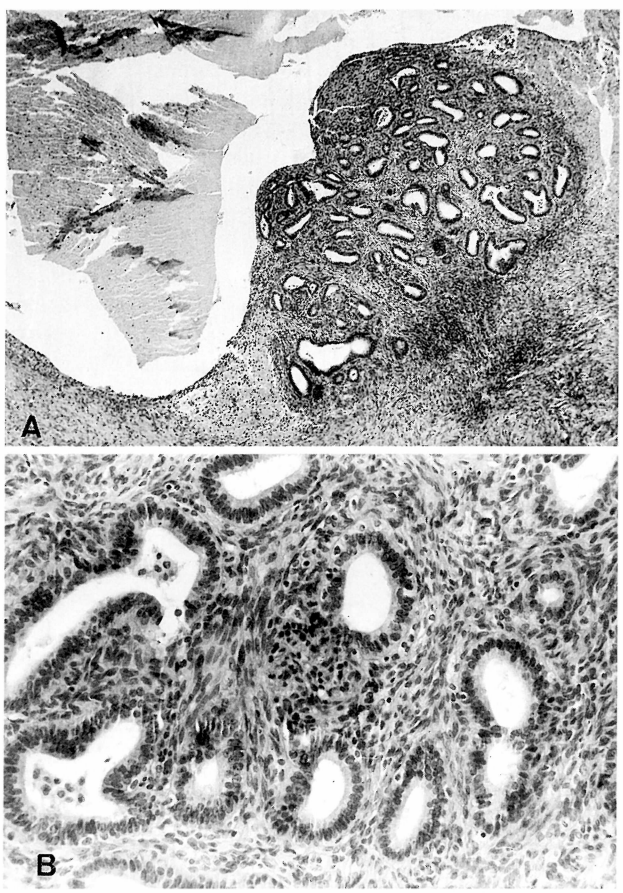

Figure 8. — A) Endometriosis of the ovary in a 43-year-old patient (H&E, x40); B) Higher magnification of the same figure; endometrioid stroma is replaced by spindle-shaped cells (H&E x250).

Discussion

The results of our investigations show that the theory of a single genesis of ovarian epithelial tumors from the surface epithelium of the female gonad does not explain many of the findings that have been made by pathologists. The well documented presence of the Brenner tumor [21-26] in the hilar region, and our similar findings in cases of mucinous tumors indicate that the source of certain epithelial neoplasms may be different. Some epithelial tumors are connected with the covering ovarian epithelium, while others originate in the medulla of the ovary and adjacent mesovarium. We can assume that the latter neoplasms arise from tubular structures persisting in this region after degeneration of the mesonephros [27]. Small remnants of efferent ducts are seen in the broad ligament in the postnatal period at the junction with the mesovarium [28], although the nature of a variety of tubules in that site in the adult is not always clear [29]. Probably the functional and morphological properties of most of these structures are considerably altered in postnatal life. Nevertheless, a Wolffian origin of Brenner tumors remains possible [30].

With regard to Brenner tumors, Walthard nests have been considered to have some relation to Brenner tumors because of their striking resemblance. These nests, however, appear as inert agglomerations of the cells without any evidence of proliferation or the divergent differentiation characteristic of Brenner tumors.

It is more appropriate to conclude that ovarian epithelial tumors are derived from several sources and differentiate towards mullerian derivates possibly under the influence of the distinctive hormonal milieu of the ovary. Our findings, as well as the recent data [5] challenge the universal role of postovulatory wound healing in the genesis even of serous carcinoma. In some well documented cases serous tumors arise from the ovarian surface in sites close to the mesovarium adjacent to smooth muscle sites that are never involved in the ovulatory process. The mesothelial covering of these sites seems to differ functionally from the ovarian surface epithelium [31, 32], although it responds to estrogen stimulation by proliferation.

Our observations confirm the co-existance of stromal changes in the early phase of development of epithelial ovarian neoplasms, as was also suggested by others [33, 34], some of whom consider hyperplasia of the ovarian stroma as a cause of inclusion cyst formation [34]. It is difficult to say, however, whether the epithelium after switching intracellular mechanisms of differentiation induces stromal alterations specific to a given type of tumor, or vice versa initially the stroma under the action of some unknown factors is transformed and induces the epithelium to enter a cascade of proliferative processes. The same problem in interpretation exists with the stromal-epithelial relationship in foci of endometriosis. The disappearance of specific stroma, which plays an important role in the proper functioning of the receptor activity of the epithelium, may facilitate damage to its cellular genome.

Stromal edema in papillomatous serous tumors may be involved in some more important mechanisms than it seems. It may be the result of a local paracrine effect of hyperestrogenemia since estrogens cause a rise of mitotic index in the surface epithelium [35] and possibly can promote its neoplastic transformation. Experimental data on the induction of serous tumors in animals by administration of estrogens favors this assumption [36].

As most of our cases were studied at autopsy they are unsuitable for elaborate molecular-genetic investigations. In view of our findings such studies in the future should focus not only on the ovarian surface epithelium as the only source of the epithelial tumors, but should include other ovarian sites as well. Application of recent methodical innovations of molecular biology (microdisection of tissue slides, in situ hybridization, etc.) should allow localization of the genomic damage to small microscopic structures such as those that have been described in this communication.

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Address reprint requests to:
APOLLON I. KARSELADZE, M.D., Ph.D. D.Sc.
Department of Pathology,
Cancer Research Centre of the Russian Academy
of Medical Sciences 115478
Kashirskoe sh. 24
Moscow (Russia)