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Ovarian tumor development: insights from ovarian embryogenesis

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Summary

The study of ovarian embryogenesis can provide important clues about the etiology and development of the different subtypes of ovarian neoplasms. The coelomic epithelium, also called germinal epithelium, was once thought to represent the site of origin of most cellular elements present in the adult ovary. However, recent observations at the morphological, functional, and molecular biological levels strongly suggest that this epithelium plays little or no role in ovarian development. The same observations provide strong support for an important role of the components of the fetal excretory system. These conclusions weaken the hypothesis that the coelomic epithelium is the site of origin of ovarian epithelial tumors. Knowledge of the origin and maturation of germ cells can shed light on several clinico-pathological characteristics of germ cells tumors, including their occasional extra-gonadal origin and differences in the biological behavior of ovarian *versus* testicular lesions. Knowledge of the mechanisms of regulation of mitotic and meiotic activity during ovarian germ cell maturation can provide insights into the molecular genetic determinants of germ cell neoplasms. The elucidation of molecular pathways actively involved in controlling gonadal differentiation may shed further light into our understanding of the relationship between aberrant differentiation and predisposition to gonadal cancers.

Ovarian epithelial tumors are thought to arise from the portion of the coelomic epithelium that overlies the ovarian surface according to a favored hypothesis. The merit of this hypothesis was recently questioned by one of us and arguments were provided favoring an alternate view [1]. This issue is important because a clear understanding of the histogenesis of ovarian epithelial tumors is a key to the identification of ovarian carcinoma precursor lesions, which in turn could facilitate the development of screening protocols for early ovarian cancer detection. The notion that ovarian epithelial tumors are of coelomic origin is, in part, based on the premise that this epithelium plays an important role in ovarian embryological development. It is our desire to review the evidence for such a role that stimulated us to write this article. The study of ovarian embryology also has implications on our understanding of non-epithelial subtypes of ovarian tumors. This article therefore provides an overview of our current knowledge of ovarian embryogenesis, focusing on features relevant to our general understanding of ovarian tumor development including but not limited to an understanding of the role of the coelomic epithelium.

Development of the undifferentiated gonads

The first morphological evidence of gonadal development is noticeable four weeks post-fertilization. Ovary and testis exhibit no morphological differences at this stage of development, and appear as two longitudinal prominences, called genital ridges, which rapidly shorten [2]. Each consists of a thickening of the coelomic epithelium, that becomes 2-3 cell layers by the fifth and sixth week due to increased proliferation. This coelomic epithelium is separated from an underlying mesenchymal thickening by a basement membrane, resulting in a clear demarcation between the outer and inner regions of the developing gonads. By

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the seventh week, cord-like extensions of the coelomic epithelium, called primary sex cords, are seen protruding into the medulla.

The first signs of differentiation of a primitive gonad into an ovary include initiation of meiosis (which does not occur in testes until puberty), formation of primordial follicles, and evidence of sex steroid production [3]. Sex cords are much more prominent in the testes toward the end of the sixth week, providing another mean of distinguishing developing ovaries from testes at the morphological levels at this early stage of development. Other morphological differences include the development of a cortical region clearly distinguished from the surface coelomic epithelium in the ovary during the eighth week and the absence of formation of a thick tunica albuginea, which becomes prominent in the testis during the ninth week.

Anatomical relationship between the undifferentiated gonad and components of the excretory system

An understanding of the anatomical relationships between the developing ovary and components of the excretory system is important to appreciate some of the current controversies in ovarian tumorigenesis, including the importance of the coelomic epithelium. Such relationships are illustrated diagrammatically in Figure 1. The genital ridges develop in the dorsal portion of the coelomic cavity, in an area that is bound laterally by the mesonephros, which is the functioning kidney in the early fetus. The mesonephros eventually degenerates to be replaced by the metanephros, the permanent kidney. However, the mesonephric tubes persist and, in the male, give rise to the genital ducts. The relative contributions of the mesonephros and coelomic epithelium to ovarian histogenesis are discussed below.

Adjacent to the mesonephric ducts and also in close vicinity to the ovary are the paramesonephric (also called mullerian) ducts, which are the precursors of the fallopian tubes, uterus, cervix, and upper part of the vagina (Figure 1). Embryologists have not seriously considered a role for the mullerian ducts in ovarian embryological development to our knowledge. However, given that ovarian epithelial tumors are morphologically indistinguishable from tumors arising in fallopian tubes, uterus, or cervix, the possibility of a relationship between the developing ovary and adjacent mullerian ducts should perhaps be further investigated [1].

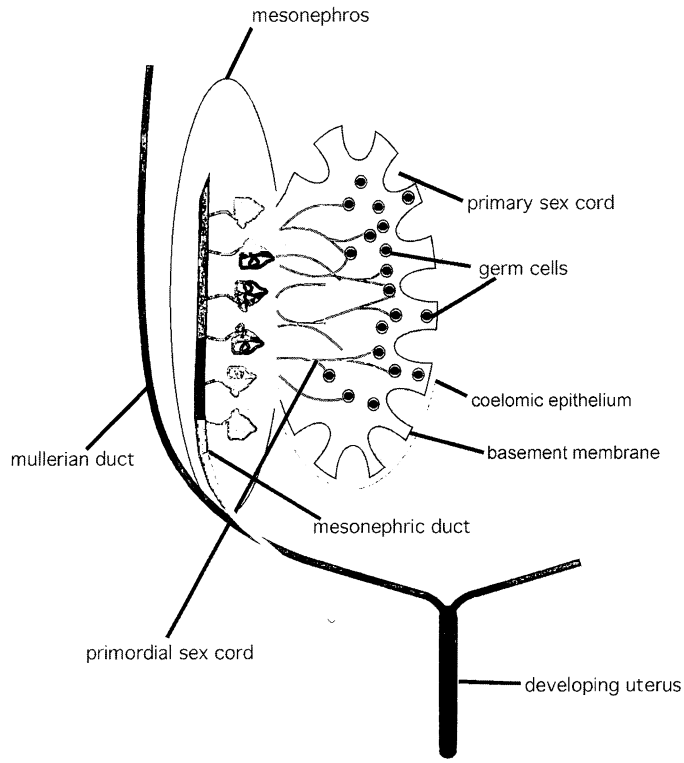

Figure 1. — Relationships between the ovary at nine weeks of gestational age and adjacent mesonephros and metanephros.

Development of primordial sex cords and other ovarian stromal elements

Cord-like structures called primordial sex cords start appearing in the medullary portion of the developing ovary as early as the fifth week of gestation. These cords form branches as they progress toward the ovarian surface (see Figure 1). They are surrounded by a basal lamina that is continuous with the basal lamina surrounding the mesonephric epithelium [4] and that becomes more ill-defined as the cords come closer to the coelomic epithelium [4]. Eventually, cells derived from the primordial sex cords will surround each developing germ cell to give rise to primordial follicles.

From the seventh to the twelfth week post-fertilization, proliferation of the primordial germ cells and of ovarian blastema cells continues. The primordial sex cords and associated primordial germ cells become displaced more peripherally, thus generating a mature ovarian cortex that becomes clearly separated from the medulla. The medullary region is less cellular and characterized primarily by fibro-vascular tissue containing specialized cell types such as hilar cells, which are androgen-producing cells thought to represent the homologues of testicular Leydig cells. The rete ovarii, which is regarded by most embryologists as a mesonephric remnant, is also associated with the ovarian medulla. This structure consists of a series of anastomosing tubules lined by cuboidal or columnar epithelium in the ovarian hilum and medulla.

Does the coelomic epithelium play a role in the histogenesis of primordial sex cords and primordial follicles?

1) Morphological arguments

The initial hypothesis that the primordial sex cords and promordial follicular cells are of coelomic epithelial origin [5] continues to be favored by some authors based on morphological studies [6-8]. Other authors have suggested that the non-epithelial somatic elements of the ovary are derived primarily from condensation of local mesenchyme within the genital ridges [9, 10], or from both the surface epithelial layer and the underlying mesenchyme [11-13]. More recently, several embryologists have suggested that the mesonephros, which is in close relationship with the developing gonad as explained earlier, makes important contributions to ovarian follicular development [3, 4, 14-18].

Cells of mesonephric origin can be readily distinguished ultrastructurally because of their characteristic dark, electron-dense appearance which contrasts with the clear cytoplasm of coelomic epithelial cells [3, 4, 17]. Although the presence of an admixture of dark electron-dense cells and of light staining cells within the developing ovarian stroma has been cited as evidence for a dual mesonephric and coelomic origin of ovarian primordial follicular [17] cells, this conclusion is not supported by a more recent study by Satoh [4]. This author performed a comprehensive histological analysis of serial sections from whole mounts of entire gonads and adjacent tissues over the 5-18 week post-ovulatory period using conditions ensuring high resolution at the light microscopic level. These studies were also complemented with electron microscopic

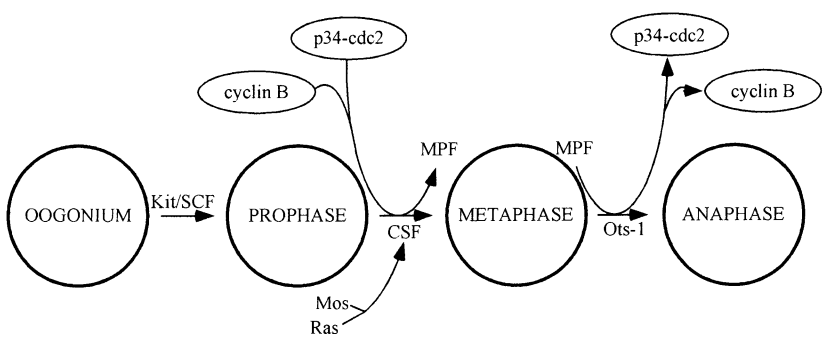

Figure 2. — Molecular components of meiotic regulation.

analyses [4]. He was able to observe, for the first time, budding of mesonephric epithelium at sites where the basal lamina of this epithelium became eliminated, leading to the formation of primordial sex cords [4]. The latter were clearly distinguished from the primary sex cords, which are extensions of the coelomic epithelium as explained earlier. Each primordial sex cord was contiguous to the mesonephros while showing no continuity with the coelomic epithelium [4]. He concluded that coelomic epithelial cells played no role in the formation of primordial sex cords of the ovary (or seminiferous sex cords of the testis).

2) Functional arguments

This study by Satoh [4] provided strong morphological support for the notion that ovarian follicular cells do not originate from the coelomic epithelium, but are derived exclusively from mesonephric structures. This conclusion is further supported by the observations of Byskov [19], who demonstrated that destruction of the rete ovarii during fetal development, while having no effect on the migration of germ cells into the ovaries, prevented the development of primordial follicles. These experiments clearly established an essential role for this structure in follicular development.

3) Molecular genetic arguments

Progress in our understanding of the molecular genetic control of gonadal development, although still rudimentary, led to the identification of several genes or genetic loci essential for such development (see recent article by Lim and Hawkins [20] for a more comprehensive review). The fact that abnormalities in these genes often result not only in gonadal malformations, but also in renal defects further supports the notion that ovarian development is intimately related to or dependent on the development of the excretory system. For example, one of the familial syndromes associated with abnormal gonadal development is the WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation). Affected individuals carry mutations in the *WT1* gene, which is thought to act as a transcription factor [21]. Homozygous knock-out of this gene in mice results in total absence not only of the gonads, but also of the kidneys. Humans with a heterozygous mutation at this locus are affected by Denys-Drash syndrome characterized by congenital nephrotic syndrome, Wilms' tumor, and genital anomalies [22]. The fact that individuals carrying *WT1* mutations show renal as well as gonadal abnormalities strongly supports the idea that the mesonephros plays a role in gonadal development.

Another transcription factor thought to be associated with the development of the gonads, the product of the *LIM1* gene, is likewise also associated with renal development [23]. Although no mutations in this gene have thus far been described in humans, mice lacking a functional *LIM1* gene show absence of gonads as well as of kidneys [23].

4) Concluding remarks on the role of the coelomic epithelium

The above observations provide strong support for the opinion that ovarian primordial follicular cells are of mesonephric origin and that the coelomic epithelium plays a very minor role, if any, in their development. These arguments are summarized in Table 1. This conclusion weakens the merit of the hypothesis that ovarian epithelial tumors are derived from the coelomic epithelium, as this hypothesis is partly based on the notion that this epithelium plays an active role in ovarian embryogenesis.

Table 1. — *Origin of ovarian somatic elements.*

Evidence for an origin from coelomic epithelium	Evidence for a mesonephric origin
Electron-dense cells thought to be precursors of primordial follicular cells have been observed within the coelomic epithelial layer.	Primordial sex cords, which give rise to primordial follicles, are contiguous to the mesonephros and show no continuity with either the coelomic epithelium or primary sex cords.
Cells with a clear cytoplasm characteristic of coelomic epithelial cells have been seen within the fetal ovarian stroma.	The presence of electron-dense cells within the coelomic epithelium can be readily explained by the phenomenon of germ cell exfoliation.
	Mechanical destruction of the rete ovarii prevents the development of primordial follicles.
	Mutations in genes necessary for ovarian development result in renal as well as gonadal abnormalities.

Origin of other ovarian stromal elements

Very little is currently known about the exact origin of ovarian somatic cells other than primordial follicular cells. Progress in this area may provide important clues about the development of ovarian tumors belonging to the sex cord/stromal subgroup. While it is often assumed that granulosa cells are derived from the primordial follicular cells, this hypothesis has not been proven to our knowledge. Although the origin of the theca cells is likewise unclear, our review of the literature suggested to us a possible link to the adrenal cortex, which in turn is derived from the urogenital ridge. SF1, an orphan nuclear hormone receptor that is the product of the *FTZ-F1* gene, is another protein thought to control gonadal development in addition to the transcription factors mentioned in the previous section [24, 25]. This gene product, which has multiple functional domains [26], is first expressed in the urogenital ridge in mouse embryos. As the gonads differentiate, it continues to be expressed in the adrenal cortex as well as in testicular Sertoli cells, but ceases to be expressed in the ovary until adulthood, where it is found in primary follicles. Mice with homozygous disruptions of *FTZ-F1* show defects in the development of the gonads, the adrenal gland, and the hypothalamus. This is interesting in light of a report, published three decades ago by Wong and Warner [27], of eight cases of ovarian thecal metaplasia in the adrenal gland of middle-aged women. Given that the adrenal gland, like the ovary, produces steroid hormones, the idea of a potential embryological link between these two organs is indeed appealing.

Development and maturation of germ cells

Misconceptions about the origin of germ cells were influential in the initial formulation of the hypothesis that the ovary is derived from differentiation of coelomic epithelial cells. Subsequent progress in our understanding of germ cell development and maturation provides important clues about clinical and pathological characteristics of ovarian germ cell tumors.

1) Origin and migration of germ cells

A favored hypothesis during the first half of the 20th century was that germ cells (as well as most ovarian somatic elements) originate from the portion of coelomic epithelium that overlies the ovarian surface. This epithelium was called germinal epithelium for that reason [5]. This hypothesis was ruled out as early as 1948 by Witschi [28], who showed that the germ cells were of extra-embryonic origin. Germ cells are first found three weeks post-fertilization in the endoderm of the dorsal wall of the yolk sac next to the allantois. The nature of the signal responsible for initiating their migration towards the gonads is not known. They may passively acquire an intraembryonic position as the embryo changes from a disc shape to a tubular shape, resulting in incorporation of the yolk sac endoderm into the posterior primitive intestine epithelium [29]. Another hypothesis is that they pass from the epithelium to the underlying mesenchyme as a result of gaps present in the basal lamina [2, 29-31]. Chemotactic factors [32] and/or components of the extracellular matrix [33] may play a role in guiding the active amoeboid movements of the germ cells toward the gonads. Primordial germ cells are first seen entering the ovary at the ovarian hilum during the latter part of the fifth week, at a time when there is still significant proliferation of the coelomic epithelium as well as of the underlying mesenchyme [17]. The germ cells themselves become mitotically active at first, further contributing to the rapid increase in the ovarian size seen at this stage. Although only a few hundred cells leave the yolk sac to migrate to the ovary, their number increases to 6-7 million by the 20th week due to mitotic activity.

It is thought that germ cells that fail to reach the gonads during fetal development are the origin of extragonadal germ cell tumors. This theory has been challenged by some authors, who argue that ectopic germ cells have never been seen in adult humans and that extra-gonadal germ cell tumors show none of the hallmarks of meiosis such as evidence of crossing over or chromosomal homozygosity [34, 35]. These authors favor an alternate view that extra-gonadal germ cell tumors arise from pluripotent somatic cells. However, given that meiosis is not initiated until the germ cells have reached the ovary in normal fetuses, it is perhaps not unexpected that extra-gonadal germ cell tumors would be exclusively premeiotic. In additon, the existence of pluripotent cells capable of differentiating into germ cells in adult tissues has yet to be demonstrated.

2) Germ cell maturation

Meiosis begins in the oogonia at 12-13 weeks post-fertilization [36]. This process is characterized by two successive cell divisions, referred to as the first and second meiotic divisions. A diploid germ cell is duplicated into two daughter cells during the first division, which resembles mitosis in many aspects. The second division takes place without further DNA synthesis, resulting in two haploid gametes. Two important features distinguish the meiotic process in ovaries and testes. First, there is unequal partition of the cytoplasm between the two daughter cells in the ovary. The cells receiving the lesser amount of cytoplasm, known as the first and second polar bodies, eventually degenerate. Thus, each primordial germ cell can only give rise to a single ovum after completion of both meiotic divisions. In addition, while meiosis does not start before puberty in testes, the first meiotic division is initiated during fetal development in the ovary. This first meiosis is not completed immediately, as it stops at the metaphase and does not resume until the initiation of ovulation, which may occur up to 50 years later in some germ cells. This is followed by the second meiotic division, which again arrests in metaphase and is not completed unless fertilization occurs. It is thought that this long period of meiotic arrest that inevitably characterizes oocytes undergoing ovulation toward the end of a women's reproductive years accounts for the higher incidence of meiotic errors, such as non-disjunction, in children born from women approaching menopause.

The fact that ovarian germ cells have already started their first meiotic division by the time of birth is thought to account for the frequently benign nature of ovarian teratomas, in contrast to testicular teratomas which are usually malignant [37]. Indeed, the current evidence suggests that post-meiotic germ cells tend to be associated with a less aggressive biological behavior than pre-meiotic tumors. Only patients with lesions arising from pre-meiotic cells (cells that did not complete their first meiotic division) died of their tumor or underwent recurrences in one series of six immature teratomas [38]. In addition, all six pre-meiotic tumors studied across several case series were aneuploid [38-41]. In contrast, three of four tumors of post-meiotic origin were diploid [38-41]. An association was also suggested between high histological grade, which indicates increased biological aggressiveness, and a pre-meiotic origin [38-41].

3) Molecular determinants of germ cell differentiation

Important insights into the molecular pathogenesis of germ cell tumors are likely to come from progress in our knowledge of the molecular genetic mechanisms involved in the control of germ cell maturation. Our current understanding of the main molecular determinants involved in this process is summarized in Figure 2. There is strong evidence that c-*kit*, a proto-oncogene encoding a transmembrane tyrosine kinase receptor related to platelet-derived growth factor/colony stimulating factor-1 receptor, as well as the c-*kit* ligand, stem cell factor (SCF, also known as Steel), play important roles in regulating germ cell maturation and initiation of meiosis [42]. In the ovary, c-*kit* is predominantly expressed in germ cells whereas SCF is expressed in follicular cells. All stages of follicular development, including the formation of primordial follicles during fetal development as well as primary and secondary follicular formation at the time of ovulation, are thought to be dependent on c-*kit* expression [43]. Reduced SCF expression by follicular cells can result in arrest of follicular development [44].

Normal oocyte maturation also requires a protein complex known as maturation promoting factor (MPF), which is comprised of the cyclin-dependent kinase $p34^{cdc2}$ (also called cdk1) and its regulatory protein cyclin B (Figure 2). This complex drives entry into the first meiotic division. Progression to anaphase, which only occurs at the time of ovulation, coincides with a decrease in MPF expression. This factor rises again as the cell enters the second meiotic division, causing an arrest in metaphase that lasts until ovulation. An important determinant of MPF stability is a protein complex known as cytostatic factor (CSF), which contains the Mos proto-oncogene product [45]. In the absence of Mos, the Ras oncoprotein can also stabilize MPF and induce arrest, suggesting that both proteins act through a downstream target to induce meiotic arrest [46].

Very little is known about the mechanisms leading to MPF degradation, which appears to be necessary for completion of either the first or second meiotic divisions. The existence of a mouse locus on chromosome 6 termed Ots-1 (Figure 2) has been postulated based on findings that genetic predisposition to oocyte parthenogenesis and failure to degrade cyclin B were due to a single dominant trait on this chromosome [47]. The gene encoded by this locus has not yet been isolated and its human counterpart is still unknown. It can be anticipated that further elucidation of the determinants of meiotic as well as mitotic arrest in

ovarian germ cells will form the basis for the development of novel strategies aimed at treating germ cell tumors based on molecular biological interventions.

4) Germ cell degeneration and exfoliation

Another intriguing feature of ovarian germ cell development is that the number of germ cells decreases from about 7 million to 1 million between the 20th week post-fertilization and birth. Their number continues to decrease after birth, although at a reduced rate. The magnitude of the reduction is even greater in certain animals such as mice. Two independent processes, cell degeneration and exfoliation, are thought to account for this phenomenon prenatally. The first process comes in part from the fact that folliculogenesis is inefficient, resulting in a substantial number of oocytes lacking a primordial cell layer that eventually degenerate. Degeneration is not limited to these oocytes, as some germ cells enclosed within a follicular space can also be affected. It has been hypothesized that factors triggering degeneration of intrafollicular germ cells may include the presence of genetic errors or of various metabolic or vascular disturbances [29].

The other process that leads to a reduction in the number of germ cells during the later stages of fetal development is that of germ cell exfoliation from the ovarian surface. Several authors have shown that a substantial number of primordial germ cells migrate to the ovarian surface and can be found resting on the coelomic epithelium in various mammalian species [48-51]. Germ cells involved in this process eventually undergo apoptosis. The surrounding primordial follicular cells become atretic. Rounded holes have been noted on the surface of the ovary and interpreted as representing breaks in the coelomic epithelium through which germ cells have been extruded [29]. It is the mere presence of germ cells undergoing exfoliation within the coelomic epithelium that misled early embryologists to suggest that this epithelium can actually differentiate into germ cells, hence the name germinal epithelium.

Is ovarian differentiation a default pathway in gonadal development?

The realization that individuals such as those with testicular feminization syndrome and others can develop female phenotypic characteristics while carrying XY karyotypes led to the idea that ovarian differentiation is a default pathway which inevitably takes place in the absence of testicular determinants. The fact that abnormalities in genes involved in the control of testicular differentiation, including SRY [52], which codes for the testis-determining factor, and SOX9 [53], a SRY-related gene associated with the compomelic dysplasia syndrome, lead to sex-reversal in XY individuals has been regarded as additional support for this idea. It should be pointed out, however, that developing gonads are invariably in a female hormonal environment due to circulating maternal and placental hormones. It may be that those circulating hormones are sufficient to induce ovarian differentiation. Thus, the notion that ovarian development is a passive, default process and that testicular differentiation depends on the activation of specific hormonal pathways may be inaccurate. In support of this opinion, over-expression of *DAX-1* [54], which is not expressed in testes after induction of *SRY* but continues to be expressed throughout ovarian development, results in ovarian differentiation in XY mice [55]. Recent evidence suggests that this gene is involved in the regulation of steroid hormone signaling [56].

Further progress in our understanding of the mechanisms involved in ovarian and testicular differentiation may provide insights into the reasons why individuals with aberrant gonadal differentiation, such as those with testicular feminization syndrome or with Turner syndrome, are predisposed to certain types of ovarian tumors. Such progress may also lead to a better understanding of the histogenesis of ovarian hilar cells, which are regarded as the equivalent of testicular Leydig cells and may constitute the origin of ovarian Sertoli-Leydig cell tumors.

Conclusions

Knowledge of ovarian embryological development can provide important insights into the mechanisms of development of different subtypes of ovarian tumors. Such knowledge can be particularly relevant to the issue of the origin of ovarian epithelial tumors. We presented arguments in this article favoring the conclusion that there is no substantial evidence for a role of the coelomic epithelium in ovarian development other than providing a mesothelial covering. It is hoped that this will stimulate further thoughts in this area and that alternative sites for the origin of ovarian epithelial tumors, such as intra- and extra-ovarian mullerian

remnants, will receive more serious consideration [1]. Knowledge of the exact site of origin of ovarian epithelial tumors should facilitate the identification of precursor lesions for these tumors, which in turn should accelerate the development of strategies for their early detection in populations at risk.

The other subgroup of ovarian tumors discussed in some details in this article is that of germ cell neoplasms. The extra-gonadal origin of germ cells during embryological development is the most likely explanation for the occurrence of extra-gonadal germ cell tumors. Evidence reviewed in this article suggests that germ cell tumors that develop after initiation of meiosis may have decreased biological aggressiveness. Although speculative at this point, it may be anticipated that further progress in our understanding of ovarian fetal development and embryogenesis will have a significant impact on the clinical management of tumors of germ cell origin. For example, further elucidation of the molecular biological differences between pre- and post-meiotic germ cells may provide a molecular explanation for this decreased biological aggressiveness associated with post-meiotic germ cell tumors and lead to the development of novel prognostic markers for germ cell neoplasms. In addition, knowledge of molecular mechanisms involved in inducing and maintaining mitotic and meiotic arrest during ovarian germ cell maturation may lead to the development of novel strategies, based on molecular biological interventions, for the treatment of such neoplasms.

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