Ovarian Tumorigenesis in Irradiated Parabiotic Rats

GILBERT H. FRIEDELL, SHELDON C. SOMMERS, ROSANNA N. CHUTE, AND SHIELDS WARREN

Cancer Research Institute of the New England Deaconess Hospital, Boston, Massachusetts, and the Department of Pathology, Francis Delafield Hospital, New York, New York

Summary

Supralethal total-body X-irradiation of one parabiont rat with its partner shielded was followed by a growth of unilateral or bilateral ovarian Sertoli-cell tumors in about 20% of exposed animals. Granulosa-theca cell ovarian tumors and gynandroblastomas occurred less often. The tumorigenic process began with irradiation, regardless of age, and Sertoli-cell tumors developed earlier and more frequently than in normal single controls. Parabiosis without irradiation did not result in ovarian tumorigenesis.

Introduction

Brambell et al. (2-4) described in detail the ovarian changes which developed during several weeks following exposure of mice at different ages to sterilizing doses of X-rays. The initial atrophy was followed by regeneration and a variable degree of hyperplasia of some stromal elements. Animals were not observed beyond 6 months, however, and no diagnosis of ovarian neoplasm was made.

The association between postirradiation regenerative changes and the development of ovarian tumors was first pointed out by Furth and his colleagues (8, 9). The incidence of ovarian tumors in their irradiated animals was 15 times that seen in control mice. Furth and Butterworth concluded that "the destructive changes in the ovaries of mice which immediately follow irradiation with X-rays are followed by slowly progressive proliferative changes resulting after from 1 to 2 years in the formation of growths with the character of neoplasms" (8). The potent carcinogenic effect of a single dose of X-irradiation on the mouse ovary was revealed and the experimental procedure also emphasized the importance of prolonged observation in such investigations.

At the Cancer Research Institute a different experimental model, namely parabiotic rats, has been employed, but the same principle of prolonged observation of experimental animals after a single dose of irradiation has been the basis of a continuing irradiation and carcinogenesis study carried on for the past 11 years. One rat of a parabiont pair is given a single supralethal dose of whole-body irradiation, while its partner is shielded. Despite the differences in experimental model and irradiation dose, one of the largest groups of tumors encountered in this study, as in the studies of Furth, has comprised ovarian neoplasms. This is a report of these tumors.

Materials and Methods

Young adult female Deaconess-Slonaker rats of the same age were joined in parabiosis. After the parabiosis was well established, the left animal was shielded and the right rat was given 1000 r of whole-body irradiation. The operative technic (5) and irradiation (17) have been described in earlier publications. Following irradiation, the parabiotic animals were fed a standard laboratory diet and water ad libitum, and maintained until their death without any further experimental procedures being performed. As soon as possible after death a complete autopsy was performed on each animal.

During the 1st 8 years (1954-1962) of the project, approximately 1400 pairs of animals were successfully subjected to parabiosis. The right animals in a majority of the 1400 pairs were irradiated. The remaining nonirradiated pairs served as controls and were maintained under the same conditions as the irradiated pairs.

Preliminary microscopic survey by Olive Gates of the organs in 328 irradiated female pairs revealed the presence of possible ovarian tumors in 1 or both animals of 149 parabiont pairs. The histologic sections of the ovaries from these 298 animals were reviewed in the present study, together with sections of 67 nonirradiated pairs and 44 single control animals, in an attempt to classify the tumors and to clarify their pathogenesis.

The sections were studied independently by 2 of us (G. H. F. and S. C. S.). Diagnoses were then compared, and in the event of disagreement in classification of the tumors the slides were reviewed jointly. Reference was also made to the diagnoses 1 of us (S. W.) had made during previous examination of the sections in many of the tumor cases. In a few instances where the disagreement was not resolved by joint review, Arthur T. Hertig served as the gynecopathologic consultant and arbitrator. Sections from various endocrine target organs such as breast, vagina, endometrium, and salivary gland were also reviewed.

Results

The tabulation of the histologically classified tumors is given in Table 1. Most displayed the characteristic features of Sertoli-cell tumors in humans or in various animals (6, 11, 12, 15). Some tumors were composed almost entirely of well-differentiated Sertoli-cell tubules. Characteristically, these tubules contained medium-sized or large cells with an abundant clear or fibrillar cytoplasm, indistinct cell boundaries, and medium-sized, often vesicular, nuclei (Fig. 1). Many, but not all, of the nuclei had a folded or grooved nuclear membrane which Engle (6) noted was "characteristic of the nucleus of the Sertoli cell in man and many

1 This work was supported by U. S. Atomic Energy Commission Contract AT(30-1)-901 with the New England Deaconess Hospital (NY0-901-35).

MARCH 1966
lower animals." Other tumors were made up both of tubules and of cellular elements in an arrangement that suggested primitive sex cords.

A cell intermediate in appearance between indifferent gonadal stroma and the characteristic Sertoli cells that formed tubules was also seen in many tumors. These intermediate cells had medium-sized nuclei and abundant clear cytoplasm. They were found individually or in small clusters within the tumors. Most Sertoli-cell tumors contained either 2 or 3 of the cellular patterns described.

In an occasional tubular tumor there were areas in which the constituent cells had moderately pleomorphic nuclei and relatively little cytoplasm, usually finely granular and eosinophilic (Fig. 2). Despite the anaplasia in these tumors, however, no metastases were seen from these, or any of the other Sertoli-cell tumors.

Aggregates of lipid-rich cells were seen in some of the tumors. Generally these were focal, but in a few cases they were quite extensive. The aggregates appeared to be composed of modified Sertoli cells, and we have therefore preferred to classify tumors composed largely of such cells as atypical Sertoli-cell tumors rather than as luteomas.

Sertoli cells were seen not only in neoplasms but also in ovaries that did not contain tumors (Fig. 3). Typical Sertoli-cell tubules occurred in small numbers in otherwise normal or atrophic ovaries both in irradiated and nonirradiated parabionts. They were also seen in nonirradiated single control rats. In general, the numbers appeared to increase with age. In either irradiated or nonirradiated animals the architecture of the ovary was sometimes obscured but not replaced by foci of 1 or more of the 3 Sertoli cell variants just described. In such instances, the diagnosis made was Sertoli-cell hyperplasia.

Varying numbers of "testis-like tubules" in the rat ovary were first noted by Engle (6) in histologic sections of 34 untreated Wistar rats 900 days of age or older. In each of 3 animals the tubules were so numerous that Engle made the diagnosis of "tubular adenoma." We believe that Sertoli-cell tumor is a better name for these neoplasms since they are histologically quite different from, and should not be confused with, the "tubular adenomas" which appear in mice following the deletion of ova by irradiation or by other means (13).

A few apparent Leydig cells without Reinke crystalloids occurred in some Sertoli-cell tumors in this series (15). One pure Leydig-cell tumor in an irradiated animal contained intracellular Reinke crystalloids. The other pure Leydig-cell tumor in the series, also with Reinke crystalloids, developed in the shielded partner of an irradiated animal that had a unilateral Sertoli-cell tumor (Table 2).

The next largest group of tumors, after Sertoli-cell neoplasms, was composed of granulosa-theca cell tumors, usually with 1 element predominant. In a few instances the tumor was primarily a thecoma, while more often it was largely a granulosa cell tumor. In most of the granulosa cell tumors the follicular or folliculoid pattern with Call-Exner bodies predominated, but other cellular arrangements were also noted (10). In 3 cases with marked anaplasia the tumors were designated as granulosa cell carcinomas, but, as with the other types of tumors, no metastases were found.

Gynandroblastoma has been used to designate tumors in which both Sertoli-cell and granulosa-theca cell components were present (10). In some of the tumors the 2 components were intermixed, but in others the appearance was more like a collision tumor. The histologic appearance of the latter type suggests that the Sertoli-cell component may have originated in 1 part of the ovary and the granulosa-theca cells in another (Fig. 4).

Three tumors were composed of cells that resembled atypical ovarian stroma but could not be classified further, and 1 tumor was composed of cells like those in the rat adrenal cortex. All these tumors, including the Sertoli-cell and granulosa-theca cell types, are considered to have arisen from the ovarian stroma. Only 2 tumors in this series, the papillary cystadenoma and the cystadenocarcinoma, appeared to have originated from the ovarian surface or germinal epithelium.

Almost all tumors developed in the irradiated right partner, and in approximately half of these animals ovarian tumors were bilateral. Generally, the same basic type of neoplasm was present in both ovaries, but in a few cases the tumors differed histologically (Table 1). In 5 pairs of parabionts the shielded, nonirradiated left animals had a unilateral ovarian tumor (Table 2). The irradiated right animal in 4 of these 5 pairs also had 1 or more ovarian tumors. Only 1 nonirradiated animal developed an ovarian tumor when its irradiated partner had none.

In irradiated animals even with small tumors the involved ovary contained no Graafian follicles and few corpora lutea. In irradi-
TABLE 3
Ovarian Tumors in Single Control Female Rats

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No. of animals reviewed</th>
<th>No. with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>400-499</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>500-599</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>600-699</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>700-799</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>800-899</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>over 900</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* Bilateral Sertoli-cell tumors.

TABLE 4
Control Parabionts (Rats) without Ovarian Tumors

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No. of pairs reviewed</th>
</tr>
</thead>
<tbody>
<tr>
<td>up to 199</td>
<td>6</td>
</tr>
<tr>
<td>200-299</td>
<td>6</td>
</tr>
<tr>
<td>300-399</td>
<td>7</td>
</tr>
<tr>
<td>400-499</td>
<td>5</td>
</tr>
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</tr>
<tr>
<td>800-899</td>
<td>5</td>
</tr>
<tr>
<td>over 900</td>
<td>4</td>
</tr>
</tbody>
</table>

TABLE 5
Relationship between Age at Irradiation and Incidence of Ovarian Tumors in Irradiated Parabiont Rats Surviving 200 or More Days Postirradiation

<table>
<thead>
<tr>
<th>Age at Irradiation</th>
<th>No. of parabionts studied</th>
<th>No. of unilateral tumor</th>
<th>No. with bilateral tumor</th>
<th>Total with tumors</th>
<th>% with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-99</td>
<td>154</td>
<td>20</td>
<td>27</td>
<td>47</td>
<td>30</td>
</tr>
<tr>
<td>100-149</td>
<td>82</td>
<td>17</td>
<td>8</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>150-199</td>
<td>48</td>
<td>9</td>
<td>3</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>200-249</td>
<td>15</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>47</td>
</tr>
<tr>
<td>250-299</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>300-349</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>350-399</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All rats used in these studies were 3–5 weeks of age at the time of parabiosis. The age when they were irradiated after parabiosis was apparently not an important factor in the incidence of ovarian tumors. In animals that survived 200 days or more after irradiation the incidence in groups irradiated at different ages was similar and ranged from 25 to 47% (Table 5).

The individual animal's increased risk of ovarian tumor development seems to have begun when irradiation was given, regardless of its age at the time. This is demonstrated in Table 6. With longer postirradiation survival times, the number of animals that developed ovarian tumors increased, through the 699-day group. The percentage of animals with tumors in the 700- to 799-day group is less than in the 600- to 699-day group but is still substantially above 42% in the 500- to 599-day group. The incidence of bilateral ovarian tumors also increased in the groups that survived the longest after irradiation.

In animals with Sertoli-cell tumors the histologic findings in endocrine target organs were usually those associated with androgenic stimulation. These findings included atrophy of endometrium and cervical epithelium, cytoplasmic granules in the eosinophilic ducts of the salivary glands, and atrophy of vaginal epithelium, usually with mucinous change (7). The breast tissue regularly showed either lactation or cystic hyperplasia. Nine % of the irradiated parabionts with ovarian tumors also had malignant breast tumors, but the significance of this finding is unknown since 7% of the entire series of irradiated female parabionts had breast carcinomas.

In animals bearing granulosa cell tumors either alone or associated with Sertoli-cell tumors, histologic evidence of androgenic stimulation was seen less often, and endometrial hyperplasia was more common. The breast tissue in these cases was generally fibrotic. Some animals with granulosa cell tumors alone had histologic evidence of estrogenic stimulation only.

Discussion

The histogenesis of ovarian tumors in our rats appeared to follow the pattern described by Furth in single irradiated mice. Destruction of ova and atrophy of the supporting tissue was followed by proliferation of stromal tissue and some differentiation, chiefly along Sertoli-cell or granulosa-theca cell lines. Hyperplasia and neoplasia of 1 or more of these stromal elements followed the postirradiation phase of regeneration, increasing in frequency with advancing age of the surviving animals. These
pathologic changes in the ovaries are attributed to the effect of sustained production of gonadotropins by pituitary glands no longer receiving the normal cyclic feedback of hormones from ovarian follicular structures (1, 11).

Whether or not the irradiation in our experiments had a tumorigenic effect beyond its role in destroying the ovarian follicles cannot be determined from these studies. The work of Kullander (13) and of Ird (12) suggests that although the most significant etiologic factor is the exposure of ovarian tissue to continual high levels of pituitary gonadotropins, the irradiation may also have had a direct tumor-promoting effect on the surviving stromal elements. Thus, Kullander found that small doses of X-irradiation of ovarian tissue in vitro before autotransplantation into the spleens of castrated rats increased the rate of growth and amount of hormone production by the tumor which subsequently developed. His incidence of tumor development with or without irradiation of the ovarian autotransplant was 100%. Ird, giving larger doses of irradiation in vitro to ovaries autotransplanted i.m. found that the incidence of tumors in the transplanted ovaries was increased over that of control rats receiving nonirradiated autotransplants. Moreover, the latent period was shorter in those rats receiving the irradiated transplants.

The granulosa cell tumors predominated among those which Kullander induced in his experiments, but in many of them there were admixtures of neoplastic Sertoli cells. The induced tumors were histologically similar to the spontaneous tumors he found in 4 of 28 untreated 2-year-old control rats, namely 2 granulosa cell tumors, 1 androblastoma, and 1 mixed granulosa cell tumor and androblastoma. From his illustrations it is apparent that his androblastoma corresponds to our Sertoli-cell tumor and his mixed granulosa cell tumor and androblastoma corresponds to our gynandroblastoma.

In the 44 single control rats in our series only Sertoli-cell tumors were found. This suggests that the high incidence of irradiation-induced Sertoli-cell tumors in our series compared to the type of tumors reported by Kullander reflects a difference in the strain of rat used rather than differences in experimental procedure. It is of some interest that no tumors were observed in our control parabiotic animals. Thus, although it was expected that parabiosis alone would be carcinogenic (16), this would not appear to hold for ovarian tumors. Indeed, insofar as the development of ovarian tumors in nonirradiated rats is concerned, parabiosis may even exert a protective effect. Ovarian tumors were found in 4 of 10 single animals over 800 days of age but none were found in the 18 parabiotic animals (9 pairs) over this age. If ovarian tumorigenesis in the single control animal is considered to be a function of aging, then irradiation in this experimental model may be viewed as either simulating the aging effect, or as accelerating the normal process of aging.

Whether the spontaneous and induced tumors should be considered as benign or malignant neoplasms also remains an unanswered question. Some of them were sufficiently anaplastic to justify the microscopic diagnosis of carcinoma, but no metastases were found in any tumor-bearing animal, and the cause of death could not be attributed directly to the presence of the ovarian tumor in any animal. No transplantation studies have been carried out with these tumors.

The histology of the major target organs suggested that androgenic activity was almost always present in animals bearing Sertoli-cell tumors. When both Sertoli-cell and granulosa-theca cell tumors were present together the target organ histology suggested either a competitive or a synergistic effect of androgenic and estrogenic hormones. Experiments currently in progress may clarify the endocrine activity of these ovarian tumors.

References

4. ———. Changes in the Ovary of the Mouse following Exposure to X-rays. II. Irradiation at or before Birth. Ibid., 101: 95-114, 1927.

Fig. 1. Nuclear folding or grooving is prominent at 3 o'clock in this tubule from a Sertoli-cell tumor. A few cells intermediate in appearance between characteristic Sertoli cells and indifferent gonadal stromal cells are seen alone or in clusters near the tubule. PR 275R. H & E, X 750.

Fig. 2. In this anaplastic Sertoli-cell tumor the tubular cells have less cytoplasm than those in Fig. 1. Nuclei are pleomorphic and mitoses are numerous. PR 244R. H & E, X 600.
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Fig. 3A. Low-power view of normal ovary from nonirradiated parabiont partner. PR 870L. H & E, × 13. B. Follicles, corpora lutea, and medullary tubular structures are seen in this medium-power view of the area outlines in Fig. 3A. H & E, × 125. C. Typical Sertoli-cell tubules are present in this higher magnification of the area outlined in Fig. 3B. Folding of the nuclear membrane is seen in several tubular cells. A solitary nontubular Sertoli cell is seen at the far left. H & E, × 600.

Fig. 4A. Sertoli-cell elements are present in the upper portion and granulosa-theca cell elements are found in the lower portion of this gynandroblastoma. PR 234R. H & E, × 13. B. At the demarcation zone in the lower right of Fig. 4A both upper and lower portions of tumor have tubular patterns. The lower pattern is less well organized and nuclei are smaller with more dense chromatin structure than nuclei in the upper portion. H & E, × 250. C. High-power view of the upper portion of Fig. 4A reveals a characteristic Sertoli-cell pattern. H & E, × 350. D. High-power view of the lower portion of Fig. 4A shows Call-Exner bodies and typical granulosa cells. There are scattered mitotic figures. H & E, × 350.
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