Malignant Germ Cell Tumors of the Ovary and Testis
An Immunohistologic Study of 69 Cases

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ABSTRACT

An immunohistologic study of 21 patients with germ cell tumors of the testis with measured serum levels of human chorionic gonadotropin (HCG) and alpha fetoprotein (AFP) was undertaken to correlate the various types of neoplasms with the presence of these tumor markers in the tissue and serum. The same immunoperoxidase technique was applied to 48 patients with malignant germ cell tumors of the ovary in whom serum was not available.

In the testicular tumor group, AFP was demonstrated in mononuclear embryonal cells within embryonal carcinoma and endodermal sinus tumor. HCG was identified within syncytiotrophoblastic giant cells frequently in association with embryonal carcinoma, and rarely with endodermal sinus tumor and seminoma, as well as in the syncytiotrophoblastic component of choriocarcinoma. Eighteen of the 21 patients (86 percent) had elevated tumor markers in their serum; serum HCG alone was elevated in 5 (24 percent), AFP alone in 5 (24 percent) and both were elevated in 8 (38 percent). There was tissue localization of HCG in 12 of 13 patients (92 percent) with elevated serum HCG while AFP was identified in the tumor in eight of the 13 patients (53 percent) with elevated serum AFP levels.

In the ovarian tumor group, all 15 endodermal sinus tumors examined were positive for AFP and negative for HCG. Seven of 10 embryonal carcinomas were positive for AFP and all 10 were positive for HCG. The two cases of choriocarcinoma were both positive for HCG and negative for AFP. In contrast, the 11 dysgerminomas and 10 teratomas were negative for both AFP and HCG. The results parallel those for malignant germ cell tumors of the testis, affording additional evidence of the analogous nature of germ cell tumors of the gonads. Based on these findings a tentative immunohistologic classification of germ cell tumors utilizing AFP and HCG is proposed. Thus, embryonal carcinoma is frequently associated with both AFP and HCG, endodermal sinus tumor with AFP and choriocarcinoma with HCG, whereas pure seminoma, dysgerminoma and teratoma are unlikely to be associated with either marker.

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Introduction

The value of alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) as tumor markers in the diagnosis and management of germ cell tumors of the testis is now well established. Clinically, determination of these markers has merit for several reasons as follows:

1. Successful treatment of patients with germ cell tumors depends on accurate clinical staging. Occult metastasis not detectable by lymphangiography can be identified by the presence of elevated levels of AFP and/or HCG in the serum using highly sensitive and specific radioimmunoassays (RIA).

2. Chemotherapy is most effective when used for the eradication of microscopic rather than gross disease. The RIA for AFP and HCG has been shown to be effective in detecting early "occult" recurrence before tumor can be identified by clinical examination and can therefore be used to direct chemotherapy.

3. Since certain tumor markers are associated with particular histologic types, determination of these markers may have prognostic import.

4. Similarly, tumor markers, by identifying specific tumor types, may be significant in the selection of appropriate therapeutic agents.

For these reasons it is apparent that a correlation of the histologic type with the tumor markers is vital for a rational approach to accurate diagnosis and management of ovarian and testicular germ cell tumors. Also, a correlative histopathologic and tumor marker study might be helpful in shedding light on the histogenesis of this poorly understood group of neoplasms. Factors that must be considered in this type of study and problems encountered in the past are the following:

1. In view of their rarity, much of the confusion regarding germ cell tumors has resulted from a lack of a uniform terminology. Neoplasms with distinctive histologic patterns and biologic behavior have been confused because terms such as embryonal carcinoma, teratocarcinoma, malignant teratoma, teratoblastoma, endodermal sinus tumor, yolk sac tumor, embryonal carcinoma of the infant testis have been used interchangeably.

2. Until recently, histopathologic studies of germ cell tumors have not paralleled the advances in tumor immunology. Consequently, a contemporary hypothesis of AFP and HCG synthesis by germ cell tumors is compromised by outmoded concepts of histopathology. For example, the presence of HCG in the serum of patients with germ cell tumors has been regarded as evidence of the presence of choriocarcinoma. However, the rarity of choriocarcinoma arising in the male testis (only 18 cases of pure choriocarcinoma among 6,000 testis tumors on file in the American Testicular Tumor Registry at the Armed Forces Institute of Pathology and only 5 percent of mixed germ cell tumors contain choriocarcinoma) is inconsistent with the fact that approximately 70 percent of nonseminomatous testicular germ cell tumors are associated with elevated serum levels of HCG as measured by RIA.

3. Finally, since 40 percent of testicular germ cell tumors and almost 10 percent of ovarian germ cell tumors are composed of combinations of two or more histologic patterns, it is manifestly impossible to determine which element in the tumor is responsible for the synthesis of a particular tumor marker in the serum using orthodox histologic techniques. In view of this limitation an investigation was undertaken to identify the specific cellular components responsible for the synthesis of AFP and HCG using an immunohistologic method.

Methods

The analysis of testicular tumors was based on 21 patients for whom paraffin blocks of tissue were available. Serum determinations of AFP and HCG were performed either prior to or within 30 days of
tissue acquisition, thereby permitting a correlation of the various histologic patterns together with the cellular localization of AFP and HCG and the levels of these markers in the serum. An indirect immunoperoxidase technique for the localization of AFP and HCG, previously reported by Kurman and Norris, was selected since this method can be used in a retrospective fashion to study tumors in paraffin blocks, frozen tissue not being necessary. The tumors were classified in accordance with the classification of testicular neoplasms proposed by Mostofi and Price.

A double antibody RIA developed by Vaitukaitis and associates, specific for the β subunit, was utilized for the quantitation of the serum level of HCG and a double antibody RIA developed by Waldmann and McIntire was used for quantitation of AFP in the serum.

Serum samples were not available for the patients with ovarian tumors. Consequently, this part of the study was limited to an immunohistologic analysis of 48 pure malignant germ cell tumors using an immunoperoxidase technique identical to that utilized for the testicular study. The ovarian neoplasms were classified in accordance with the WHO classification for ovarian tumors adopted in 1973.

Results

Testicular Germ Cell Tumors (21 cases)

Areas of typical seminoma were found in seven instances. Areas of teratoma containing elements derived from all three germ cell layers were encountered in eight instances. Foci of primitive neuroectoderm and mesoderm were present in five instances. Embryonal carcinoma was found in 15 instances and was characterized by sheets of large pleomorphic cells arranged in solid, papillary and acinar patterns. Endodermal sinus tumor (yolk sac carcinoma) or embryonal carcinoma, infantile type, characterized by a distinctive reticular-papillary pattern was found in four instances.

Trophoblast was present in two forms in this group of tumors. One type, corresponding to choriocarcinoma, contained an intimate admixture of cytotrophoblast and syncytiotrophoblast and was found in two cases. The other form was comprised of syncytiotrophoblastic giant cells exclusively. These were typically multinucleated with abundant eosinophilic or amphophilic cytoplasm containing one or more vacuoles of varying size. These cells were found in association with areas of embryonal carcinoma in 11 instances, with endodermal sinus tumor in one case and with seminoma in another.

With the immunoperoxidase technique, HCG was found only in the syncytiotrophoblastic element of choriocarcinoma (two cases) or in syncytiotrophoblastic giant cells (13 cases).

AFP was identified in the mononuclear embryonal carcinoma cells of embryonal carcinoma in seven of the eleven cases (47 percent) and within similar type cells in two of the four areas of endodermal sinus tumor (50 percent). Cells in which HCG was localized did not contain AFP and similarly those in which AFP was localized did not contain HCG. Thus, it appears that AFP and HCG are found in and probably synthesized by two distinctly different cell lines. The cellular constituents of seminoma, teratoma, and the cytotrophoblastic element of choriocarcinoma were negative for both AFP and HCG.

Elevated serum levels of AFP alone were found in five patients (24 percent), HCG alone in five (24 percent) and both were found elevated in eight patients (38 percent). There was almost complete correlation of elevated levels of HCG in the serum with the tissue localization (92 percent) while AFP was identified in the tissue in 53 percent of patients with elevated serum levels.
OVARian GERM CELL tumors (48 cases)

The histologic characteristics of the ovarian germ cell tumors closely approximated those in the testicular neoplasms. The results of the immunocytologic localization are summarized in table I.

Discussion

In recent years there has been a growing awareness of the homologous nature of germ cell tumors of the ovary and testis. The similarity of pattern for AFP and HCG distribution in both testicular and ovarian germ cell tumors further corroborates their analogous nature.

The immunohistochemical identification of HCG within syncytiotrophoblastic giant cells indicates a relationship between choriocarcinoma and embryonal carcinoma, while a relationship between endodermal sinus tumor (yolk sac tumors) and embryonal carcinoma can be illustrated by the presence of AFP within embryonal carcinoma cells in both these neoplasms. Taken in conjunction with the ultrastructural studies of Pierce and his associates showing the common origin of all germ cell tumors and the experimental production of teratomas using embryonal carcinoma explants by Stevens and his co-workers, the immunohistochemical findings lend support to the concept that embryonal carcinoma may be the neoplastic progenitor of the other germ cell tumors. This is further substantiated by the in vivo and in vitro synthesis of AFP by these transplantable murine embryonal carcinomas.

In figure 1 is illustrated a tentative histogenic and immunohistologic classification for germ cell tumors that incorporates AFP and HCG tumor markers. Thus, embryonal carcinoma is frequently associated with both AFP and HCG, endodermal sinus tumor with AFP and choriocarcinoma with HCG, whereas pure seminoma, dysgerminoma and teratoma are unlikely to be associated with either marker. It is important that the presence of syncytiotrophoblastic giant cells which were associated with embryonal carcinoma, seminoma and endodermal sinus tumor be specifically noted in the histologic diagnosis, since unlike choriocarcinoma, it is not well recognized that these cells are responsible for elevated serum levels of HCG. Terms such as "teratocarcinoma" or "mixed germ cell tumor" should be amplified to include an enumeration of the various germ cell tumor components so that a more accurate correlation of tumor histology and tumor markers might be achieved.

The importance of this approach lies in the fact that it provides a basis for a combined immunologic and histologic classification of these tumors, thereby clarifying some of the clinical aspects that have
been observed. Discordance of AFP and HCG can occur after treatment, and either AFP alone, HCG alone or both markers may rise with the development of recurrent tumor depending on which component of the tumor recurs.\textsuperscript{3,11} As further investigation identifies additional markers associated with germ cell tumors, it may become necessary to modify this classification.

Thus, tumor histopathology stands at the threshold of a new era. The limits of morphologic characterization have now been realized and the functional aspects of tumors must be considered. In the future, both a morphologic and functional classification of neoplastic disease should be established.

References