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# Large Mononuclear Cells in Seminoma
## —An Immunohistochemical Analysis of 21 Cases—

**Nam Hoon Cho, Hee Jeong Ahn and Chanil Park**

*Twenty-one cases of seminoma (including testicular seminoma, ovarian dysgerminoma and extragonadal germinoma) were reviewed for the cell types responsible for the production of alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG). Histologically the cases included seventeen classical seminomas and 4 anaplastic seminomas. The latter had some mononuclear and multinuclear giant cells. All 4 patients with anaplastic seminoma had elevated levels of serum AFP, and each of these cases contained AFP producing tumor cells identified by immunoperoxidase staining. All seminomas of patients with elevated serum levels of HCG were of the classical type but HCG producing tumor cells could not be identified by immunoperoxidase staining. Immunoreactivity to anti-AFP was found in some large mononuclear cells and anaplastic cells. To explain these results, we propose that the large mononuclear cell is a multipotential cell capable of differentiating into a germ cell, yolk sac and embryo, and that the anaplastic seminoma cells might represent a stage on the continuum of cellular differentiation from the large mononuclear cells to germ cells. The multinuclear giant cell does not appear to be essential for the production of either AFP or HCG in seminoma.*

**Key Words:** Seminoma, dysgerminoma, germinoma, germ cell, yolk sac carcinoma, embryonal carcinoma, alpha-fetoprotein, human chorionic gonadotropin.

Since alpha-fetoprotein (AFP) is normally produced in the fetal yolk sac and liver, and human chorionic gonadotropin (HCG) in syncytiotrophoblasts, radioimmunoassays for AFP and HCG have been important tests in the diagnosis and management of nonseminomatous germ cell tumors (Waldmann and McIntire 1974: Lange and Fraley 1977; Scardino *et al.* 1977) containing elements of yolk sac carcinoma, choriocarcinoma and embryonal carcinoma. The significance of these serum markers in patients with seminoma has been a subject of controversy for many years.

HCG is found rarely in association with seminoma (Kurman *et al.* 1977; Javadpour *et al.* 1978; Bosl *et al.* 1981; Lange and Winfield 1987), and the elevated levels of this case are thought to be produced by the characteristic syncytial giant cells sometimes found in seminomas (Heyderman and Neville 1976; Hedinger *et al.* 1979). Elevated levels of AFP are also not commonly associated with pure seminomas, and are usually indicative of the presence of occult nonseminomatous germ cell tumor elements or liver metastasis (Schultz *et al.* 1978; Javadpour 1980; Lange *et al.* 1980). This may explain the occurrence of nonseminomatous metastasis in patients with pure seminoma and the occasional failure of radiation therapy in patients with seminoma (Raghavan *et al.* 1982). However, there have been reports similar to our experiences in which serial sectioning of the primary tumor has failed to demonstrate the nonseminomatous element (Schultz *et al.* 1978; Raghavan *et al.* 1981). The aim of this study was to determine the cell types responsible for the production of AFP and HCG in seminoma using a peroxidase-antiperoxidase technique.

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Department of Pathology, Yonsei University College of Medicine, Seoul, Korea

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Address reprint requests to: Dr. C Park, Department of Pathology, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul, Korea

## PATIENTS AND METHODS

A total of 21 patients with a histological diagnosis of seminoma were chosen from the records of the Department of Yonsei University College of Medicine.

**Table 1. Major clinical features of 21 seminomatous germ cell tumors**

| Case No. | Age/Sex | Histological Dx | Location | AFP | HCG |
|---|---|---|---|---|---|
| 1 | 27/M | Seminoma | Testis | − | − |
| 2 | 35/M | Seminoma | Testis | − | − |
| 3 | 36/M | Seminoma | Testis | − | − |
| 4 | 38/M | Seminoma | Testis | − | − |
| 5 | 40/M | Seminoma | Testis | − | − |
| 6 | 43/M | Seminoma | Testis | − | + |
| 7 | 44/M | Seminoma | Testis | − | + |
| 8 | 12/F | Dysgerminoma | Ovary | − | − |
| 9 | 13/F | Dysgerminoma | Ovary | − | − |
| 10 | 22/F | Dysgerminoma | Ovary | − | − |
| 11 | 25/F | Dysgerminoma | Ovary | − | − |
| 12 | 26/F | Dysgerminoma | Ovary | − | − |
| 13 | 47/F | Dysgerminoma | Ovary | − | − |
| 14* | 62/F | Dysgerminoma | Ovary | + | − |
| 15 | 12/F | Germinoma | Brain | − | − |
| 16* | 45/M | Germinoma* | Retroperitoneum | + | − |
| 17 | 45/M | Germinoma | Mediastinum | − | + |
| 18 | 15/M | Germinoma, 2° | Chest wall | − | − |
| 19* | 21/F | Dysgerminoma, 2° | Bone | + | − |
| 20* | 50/M | Seminoma, 2° | Neck | + | − |
| 21 | 50/M | Seminoma, 2° | Neck | − | + |

*: Anaplastic seminomatous germ cell tumor, 2°: Metastatic

The cases included testicular seminoma, ovarian dysgerminoma and extragonadal germinoma. For convenience, the term seminoma was applied to all of these seminomatous germ cell tumors.

The clinical histories were screened. The serum markers of AFP and HCG had been determined with radioimmunoassay. All tissues had been fixed in 10% formalin and embedded in paraffin. There were 5 to 12 blocks in each case. The hematoxylin-eosin (H-E) stained sections were reviewed for verification of the previous histological diagnoses. For the determination of tissue AFP and HCG, more than 2 consecutive sections were made from each paraffin block, and the peroxidase-antiperoxidase method was applied utilizing anti-AFP and anti-HCG (Histogen kit, Biogenex Lab., San Ramon, CA, USA) as the primary antibodies.

## RESULTS

### Clinical Features

The age and sex of patients, the histological type of tumor with their locations, and the status of serum AFP and HCG are summarized in Table 1. The 21 seminomas included 7 testicular seminomas, 7 ovarian dysgerminomas and 7 extragonadal cases, of which 3 were primary extragonadal germinomas and 4 were metastatic.

Four patients (1 gonadal, 1 extragonadal and 2 metastatic) had elevated levels of serum AFP, and another 4 (2 gonadal, 1 extragonadal and 1 metastatic) had elevated levels of serum HCG. None had elevated levels of both AFP and HCG.

### Pathological Findings

Seventeen seminomas fit the histological criteria for classical seminoma (Fig. 1). They were composed predominantly of broad and diffuse sheets of tumor cells, separated by thin fibrous septa which were infiltrated with lymphocytes. The tumor cells were large, polygonal or round, with a distinct cell membrane and vacuolated cytoplasm. The nuclei tended to be round and vesicular with one or more prominent nucleoli. Mitotic figures were inconspicuous.

The remaining 4 seminomas (cases 14,16,19 and

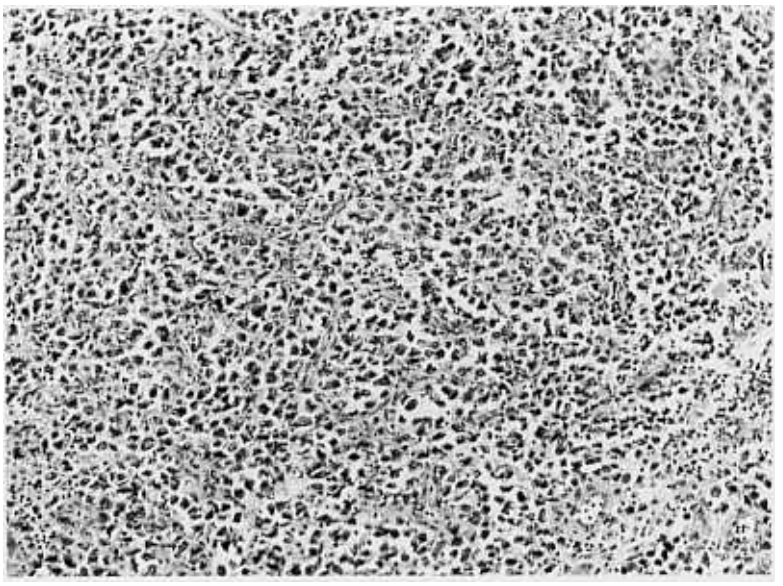

*Fig. 1. Classical seminoma. Tumor cells are set in a lobular pattern divided by delicate fibrovasular connective tissue with lymphocytic infiltration. H-E, ×40.*

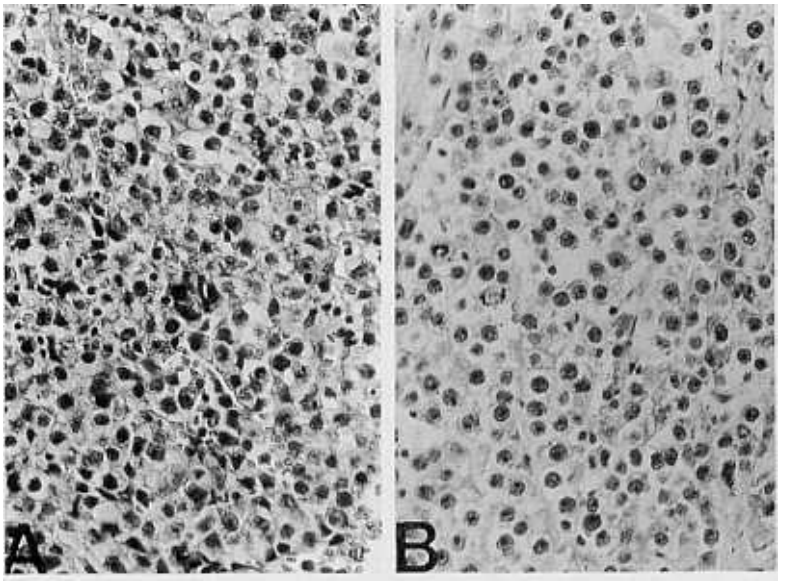


*Fig. 2. Anaplastic seminoma. The architecture is similar to that of the classical seminoma(a). H-E, ×100. Note the cellular anaplasia and mitosis(b). H-E, ×100.*

20) had features of anaplastic seminoma (Fig. 2). The tumor cells were larger with nuclear pleomorphism and a high nuclear-cytoplasmic ratio. They tended to be crowded with frequent overlapping. Mitosis and necrosis were not infrequent. In places, there were some mononuclear or multinuclear giant cells which were two to three times larger than the rest of tumor cells (Fig. 3). They had abundant cytoplasm and open chromatin pattern. All 4 cases had elevated serum levels of AFP, and in each of these cases AFP produc-

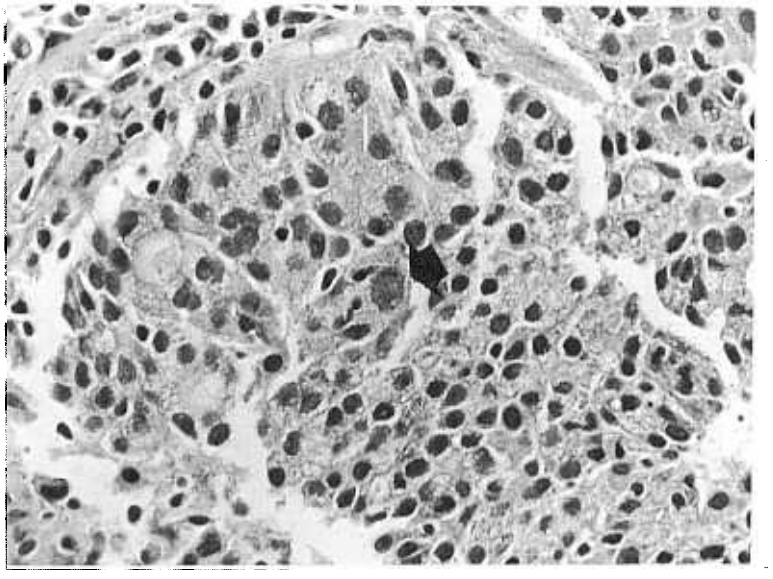

***Fig. 3.*** *Anaplastic seminoma. A single large mononuclear cell is seen (arrow) in this field. H-E, ×200.*

**Table 2. Correlation of the tissue AFP and HCG with their serum status in 21 seminomatous germ cell tumors**

| Case No. | Histological Dx | AFP(S) | AFP(T) | HCG(S) | HCG(T) |
|---|---|---|---|---|---|
| 1 | Seminoma | − | − | − | − |
| 2 | Seminoma | − | − | − | − |
| 3 | Seminoma | − | − | − | − |
| 4 | Seminoma | − | − | − | − |
| 5 | Seminoma | − | − | − | − |
| 6 | Seminoma | − | − | + | − |
| 7 | Seminoma | − | − | + | − |
| 8 | Dysgerminoma | − | − | − | − |
| 9 | Dysgerminoma | − | − | − | − |
| 10 | Dysgerminoma | − | − | − | − |
| 11 | Dysgerminoma | − | − | − | − |
| 12 | Dysgerminoma | − | − | − | − |
| 13 | Dysgerminoma | − | − | − | − |
| 14* | Dysgerminoma | + | + | − | − |
| 15 | Germinoma | − | − | − | − |
| 16* | Germinoma | + | + | − | − |
| 17 | Germinoma | − | − | + | − |
| 18 | Germinoma, 2° | − | − | − | − |
| 19* | Dysgerminoma, 2° | + | + | − | − |
| 20* | Seminoma, 2° | + | + | − | − |
| 21 | Seminoma, 2° | − | − | + | − |

*: Anaplastic seminomatous germ cell tumor, 2°: Metastatic, (S): Serum, (T): Tissue

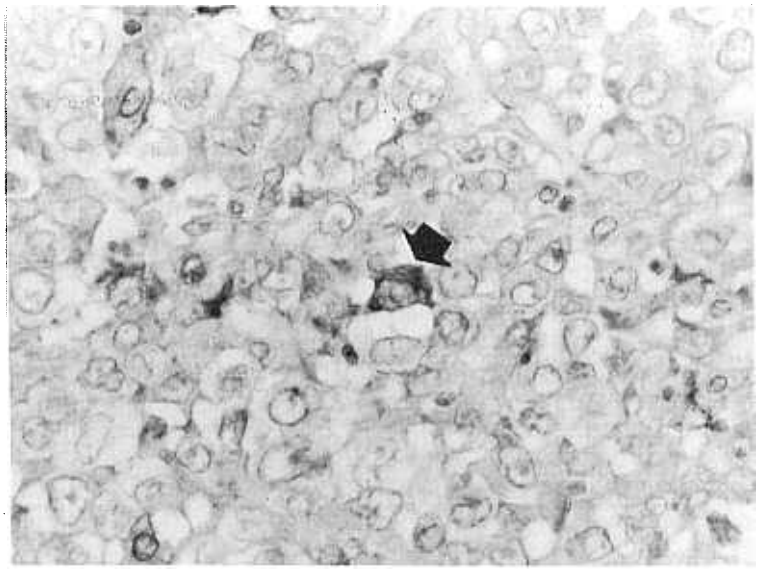

***Fig. 4.*** *Immunoperoxidase staining for AFP. Note a darkly stained anaplastic seminoma cell (arrow). PAP, ×400.*

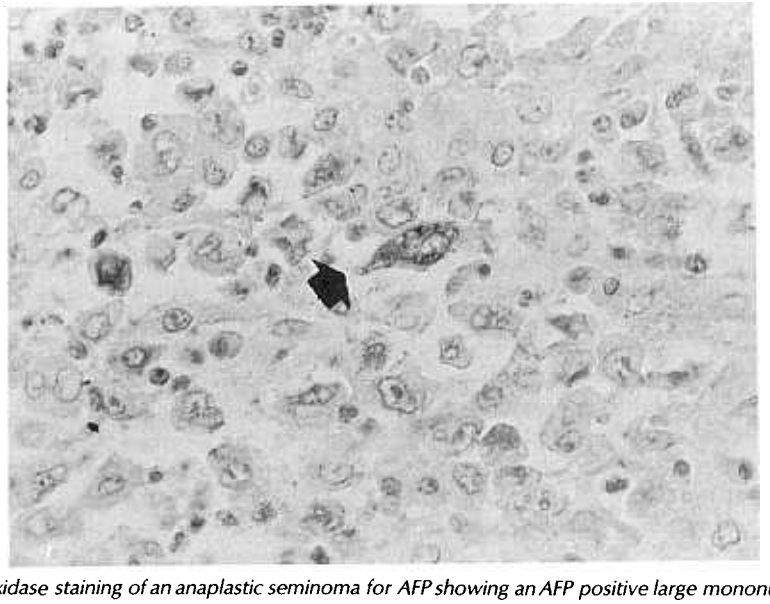

***Fig. 5.*** *Immunoperoxidase staining of an anaplastic seminoma for AFP showing an AFP positive large mononuclear cell (arrow). PAP, ×400.*

ing cells were identified by immunoreactivity to anti-AFP.

**Immunohistochemistry**

Immunoperoxidase staining for the tissue localization of AFP demonstrated immunoreactive cells in each of the 4 anaplastic seminomas (Table 2). None of the 21 seminomas were stained for HCG, even those cases with elevated serum HCG.

The AFP reactive cells appeared to be the anaplastic seminoma cells (Fig. 4) and some mononuclear giant cells (Fig. 5). But not all of the mononuclear giant cells were positively stained. The

multinuclear giant cells did not show any reaction to the anti-AFP or to the anti-HCG.

## DISCUSSION

It is now widely accepted that seminoma and embryonal carcinoma are derived from undifferentiated germ cells; however, the developmental relationship between seminoma and embryonal carcinoma remains unclear, as does the histogenesis of the mixed seminoma and embryonal carcinoma (Kurman *et al.* 1977; Javadpour *et al.* 1978a & b; Raghavan *et al.* 1981 & 1982). If such extraembryonic components, as the yolk sac and trophoblast are derived from the theoretical embryonal stem cells from which embryonal carcinomas are also presumably derived, it would be understandable how the AFP and HCG production in embryonal carcinoma could occur. Furthermore, Raghavan *et al.* (1981&1982) postulated that a continuum of differentiation exists between classical seminoma and extraembryonic yolk sac carcinoma. In turn, it can be speculated that seminoma, embryonal carcinoma and carcinomas of the extraembryonic cell elements are diseases that represent different stages of cellular differentiation.

In fact, seminoma and yolk sac carcinoma have been frequently reported to be associated in several guises. Seminomas have been shown in direct continuity with yolk sac carcinomas (Talerman 1974) and those which have been metastasized by pure yolk sac carcinoma (Teilum *et al.* 1975).

There have also been reports of elevated serum AFP levels in patients with aggressive seminomas (Kurman *et al.* 1977; Javadpour 1980). The seropositivity could, of course, be explained by the existence of occult nonseminomatous components elsewhere in the primary tumor or hidden metastatic foci. We have found in this study, however, that all seminomas with an elevated serum AFP level were histologically anaplastic seminomas with immunological staining for AFP. Of some of the anaplastic cells and some undifferentiated large mononuclear cells, the latter resembled tumor cells of embryonal carcinoma. These findings may suggest that the anaplastic seminomas containing such immunoreactive cells may represent an intermediary form between classical seminomas and embryonal carcinomas.

The large mononuclear cells immunoreactive for AFP seen in the present study might be equivalent to the cells which have been variously described as mononuclear embryonal carcinoma cells, seminoma-like cells, mononuclear giant cells, mononuclear cells, etc. They also resemble the intermediate trophoblasts (Manivel *et al.* 1987; Niehans *et al.* 1987), which were originally proposed by Kurman *et al.* (1984) as transitional cells between syncytiotrophoblasts and cytotrophoblasts. The intermediate trophoblasts were described as being large and having abundant eosinophilic cytoplasm and a single nucleus.

These cells produce HCG, which may explain previous reports of immunoreactivity for HCG in some pure seminomatous germ cell tumors and the elevated serum levels of HCG in the absence of syncytiotrophoblasts in some germ cell tumors (Manivel *et al.* 1987).

There is agreement that germ cell tumors result from neoplastic transformation of germ cells at different stages of maturation. The present study, however, suggests that the anaplastic seminoma cells may represent a transitional stage in the differentiation of large mononuclear cells to germ cells. In this context, we would propose that the large mononuclear cell is the pivotal cell being capable of differentiating into three directions: germ cell, extraembryonic tissue elements and embryo (Fig. 6). When carcinogenesis occurs at the level of the large mononuclear embryonal cell in situ, embryonal carcinoma develops, and when it occurs in the process of differentiating into the germ cell, anaplastic seminoma, classical seminoma and possibly spermatocytic seminoma might develop according to the stage of differentiation. The large mononuclear cells which persist in occasional seminomas of both classical and anaplastic types and the anaplastic seminoma cells may produce AFP and/or HCG.

The HCG and AFP are cytoplasmic or membrane molecules that are secreted into the body fluid. In some cases, the HCG and AFP molecules are incompletely secreted or not secreted at all and remain as tissue markers (Cochran 1976; Perlin *et al.* 1976; Narayana *et al.* 1979; Lange and Winfield 1987). The absence of immunoreactive tissue for HCG in the presence of elevated serum HCG levels in the present study may then be due to complete secretion of HCG. When the large mononuclear cells are either absent or are too few to synthesize AFP or HCG, as is usual in classical seminomas, there will be an absence of these proteins in either the tissue or serum.

The multinuclear giant cells seen in this study were only identified in the anaplastic seminomas. They are histologically similar to the syncytiotrophoblast-like giant cells which are thought to be the source of HCG in some seminomas and embryonal carcinomas. In this study, however, the giant cells did not stain with either anti-AFP or anti-HCG.

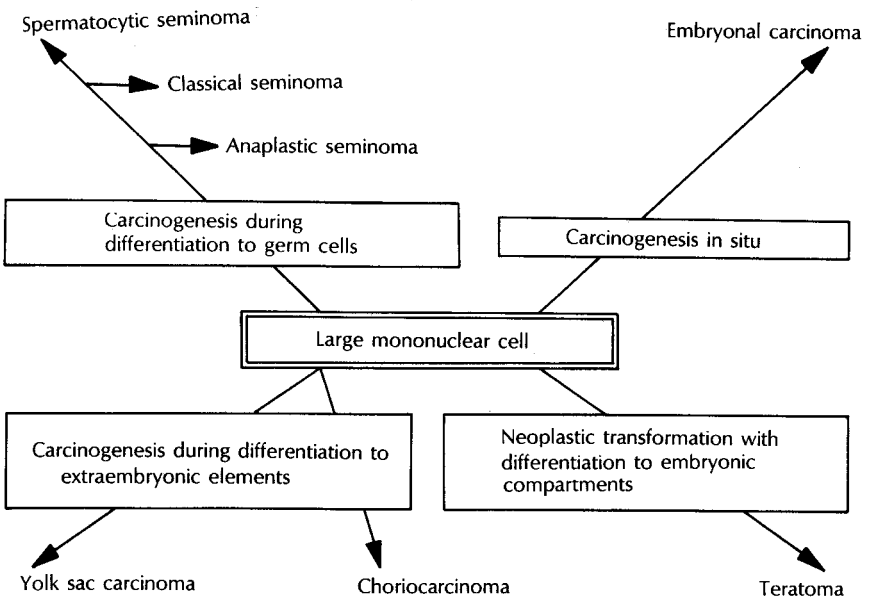


***Fig. 6.*** *Germ cell tumors and their relationship to the large mononuclear cell. In this concept, the large mononuclear cells act as stem cells for all kinds of seminomas, embryonal carcinoma, extraembryonic carcinomas and teratoma.*

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