The Increased Incidence of Seminomas in Cryptorchid Boys and Infertile Males

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The purported incidence of seminoma in males with cryptorchid testes is a contentious issue among physicians. Nonetheless, it has been shown that there is a positive correlation between the susceptibility for developing a seminoma in patients with oligo-astheno-terata-spermia (OAT) syndrome and in males with cryptorchid testes in a high position.

The incidence of testicular tumors in the population is about 0.002% [3]. In males with undescended testes as well as those who are infertile, the risk of a seminoma increases significantly; it ranges from 0.4 to 1.1% [4]. The origin of seminoma cells is still an enigma. One hypothesis favors the persistence of primordial germ cells as the progenitor in that these cells are similar to seminoma cells. They are both replete with glycogen and alkaline phosphatase.

We have determined the incidence of seminomas in a large population of infertile males and cryptorchid boys. We also have followed in a long-term study those patients whose primordial germ cells have persisted through puberty. In addition, we have analyzed the ultrastructural differences that occur in both in the typical carcinomas *in situ* and persistent gonocytes.

Methods

Patients

The biopsies (5201) from 3649 patients were embedded in Epon following typical histological procedures. Semi-thin sections were analyzed first with a light microscope.Those gonads which had atypical germ cells were analyzed further with several special techniques that included fluorometry and electron microscopy. Specific cells and their components were identified with immunohistochemical, ultra-histochemical and ultrastructural analyses.

Testicular biopsies were obtained from 1121 patients with oligo-astheno-terata-spermia (OAT) syndrome. Of these patients, 1101 had bilateral testicular biopsies, 120 were unilateral. Another 218 patients with azoospermia also were included in the study of whom 163 had bilateral biopsies.

Over two thousand (2703) biopsies were analyzed from 2528 boys with cryptorchidism whose ages ranged from just a few days old to 18 years. Only 175 of these boys had bilateral procedures.

Histological procedures

The tissue was fixed in buffered (pH 7.4) paraformaldehyde (3.5% in phosphate-buffered saline, PBS) and was kept at room temperature for several hours. Aldehyde groups were neutralized by incubation in 0.5 M NH_4Cl for 30 minutes; the tissue was washed for 15 minutes in PBS at 20° C (with 3 changes of buffer) and dehydrated through an alcohol series: 50%, 70%, 80%, 95% and 100% for 15 minutes for each step at 4° C. The tissue was then placed in an infiltration solution for one hour. After one change of this solution, the samples were left overnight at 4° C.

The solution was replaced with an embedding mixture which polymerized at 4° C under partial vacuum. Sections were cut at less than one micrometer with a glass knife. Sections were floated on a drop of distilled water, dried at 70° C and fixed on the slide overnight at 40° C. When etching was required, the sections were incubated in xylol for 75 minutes at 20° C (with 5 changes of xylol). This was followed by a 10-minute incubation with hydroxyfuran (HF) at 20° C (with 2 changes of HF), and finally by rehydration in an alcohol series: 100%, 95%, 80%, 70% and 50% (15 minutes in each stage).

For direct immunofluorescence assay, the sections were treated with a microdroplet (50-100 µl) of fluorescein isothiocyanate (FITC)-conjugated polyclonal and monoclonal anti-human antibodies appropriately diluted in 0.35% bovine serum albumin (BSA)-PBS for 60 minutes at 4° C and 60 minutes at 37° C. For indirect immunofluorescence assays, the sections were treated with appropriate dilutions of different specific anti-human antibodies (goat, rabbit, mouse or swine) to which FITC-conjugated antibodies specific for those animals, diluted appropriately, were added following several washes in PBS in the same manner which was described previously. All sections were counterstained with Hoechst 33258 (~1µg/ml in PBS). The sections were mounted in PBS-glycerol (1:1) and sealed with a lacquer. Sections were observed with phase-contrast microscopy in addition to epi-illumination with appropriate excitation and barrier filters for the different fluorochrome. Photomicrographs were taken using Kodak Tri-X pan (400 ASA) film.

Results

Abnormal germ cells characteristically have a large nucleus with a well-differentiated large nucleolus and a large accumulation of glycogen within the cytoplasm. In addition the subplasmalaemal margin of microfilaments occur as a pale peripheral rim. This type of cell was observed in 6 of the 1121 (0.5%) of the patients with OAT-syndrome. In contrast, none of the 218 patients with azoospermia had atypical germ cells. Two of six patients with OAT-syndrome had stage II type of seminoma with interstitial cells that were involved; it was discovered when the testis was examined histologically following orchidectomy. Seminoma *in situ* was observed in four of the 2528 (0.2%) of the patients with cryptorchidism. If the patients with persistent primordial germ cells were included, then 11 of the 2528 (0.4%) of the cryptorchid boys had atypical germ cells.

The persistency of primordial germ cells occurred only in prepubertal cryptorchid boys, and it was not observed in any of the adults with OAT-syndrome, azoospermia or in adolescents with varicocele and testicular atrophy. However, these atypical germ cells are somewhat different as attested by ultrastructural analyses; there was much less glycogen in addition to the absence of the cytoplasmic rim. Nevertheless, the large nucleolus within the pale round nucleus was a prominent feature. These cells were **not** recognized by the M2A mouse monoclonal antibody that is purportedly specific for seminomas and dysgerminomas. This contrasted markedly with the staining pattern by the same monoclonal antibody that was observed on the atypical cells in patients with seminoma *in situ*. During the follow-up period of this long-term study (mean: 10±4 years), not a single boy with persistent primordial germ cells developed seminoma, nor were the serum levels of β-HCG or α-feto-protein outside of the normal range. None of the boys had completed puberty and all of the boys still have their testes and a second biopsy will be obtained after they reach puberty. Three of the 4 boys with seminoma had their testes located intraabdominally.

Discussion

Boys with undescended testes have about a 4-fold increased risk of subsequently developing testicular cancer; intratubular germ neoplasia has been found in about 2-3% of their undescended testes [2]. Carcinoma *in situ* has now been recognized as a precursor to testicular germ cell tumors [2]. Cortes, et al also assessed the incidence of germ cell neoplasia in 843 boys (median age:12.7 years) who had undergone testicular biopsy at orchiopexy [1]. Five cases of testicular germ cell neoplasia were identified including one non-seminoma of the contralateral testis which had been treated before surgery for an undescended testis; and one non-seminoma was found at follow-up, one seminoma and two intratubular germ cell neoplasms. Of these preceding 3 patients, two had abnormal external genitalia, one had an atypical karyotype (45X/46XY).

Although infertile males and boys with cryptorchid testes have nearly the same risk of developing a seminoma, these males have a far greater risk of developing cancer than what is observed in the general population. The common denominator in these two groups is the fact that during the course of embryonic development, the testes are not induced, for some unknown reason, to descend normally; this seemingly poses a unique dilemma for testicular cells: an atypical environment during embryogenesis and later on during early development following birth induces cellular changes that lead to car-

cinogenesis in individuals who have genetic predispositions for such aberrations. Seemingly, genetic predisposition is an important factor in mitigating the induction of normal epididymal and testicular descent; pleiotropic effects of maldescent are congruent with carcinomas *in situ*.

References

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