Immunohistochemical Localization of Murine Stage-Specific Embryonic Antigens in Human Testicular Germ Cell Tumors

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Monoclonal antibodies raised against and/or recognizing stage-specific antigens on preimplantation mouse embryos and stem cells of murine teratocarcinoma were used to localize these antigens immunohistochemically on human testicular germ cell tumors. SSEA-1, the antigen found on mouse embryonal carcinoma (EC) cells and embryonic cells from the 8-cell stage embryo onward, including the fetal primordial germ cells, was detected on yolk sac carcinoma components of human tumors, but not on EC cells. SSEA-3, the antigen found on follicular ova, fertilized eggs, early cleavage stage embryonic cells, and visceral endodermal cells of the mouse embryo, but not on mouse EC cells, was detected on human EC cells. Both antigens were found on the cell surface of fetal testicular germ cells but not in the seminiferous tubules of adult human testes. These data point out differences between human and murine EC cells suggesting that human EC cells correspond developmentally to a less mature embryonic cell than the murine EC cells. The possible histogenesis of human germ cell tumors from primordial and/or fetal germ cells is briefly discussed. (Am J Pathol 1982, 108:225–230)

RECENT DEVELOPMENTS in monoclonal antibody production have considerably advanced the search for tumor-specific markers. Using the technique of Kohler and Milstein,[1] several research groups claim to have identified various antigens that are found exclusively on certain animal or human tumors.[2-4] The possible diagnostic application of monoclonal tumor-specific antibodies in the diagnosis of tumor makes it mandatory that all these claims be critically evaluated and independently confirmed prior to applying each technique to diagnostic human pathology. Cross-reactivity of antibodies raised against one tumor should be checked with other tumors and normal tissues of the same species.[5] Since organ-specific tumors in one species often resemble equivalent tumors in other species, one should study the antigenic complementarity between histologically identical tumors from various species. Such screening may reveal antigens found in other species that could be of diagnostic value in man. In this study we used monoclonal antibodies to compare and point out the similarities and disparities between the murine and human germ cell tumors.

In a previous report, we described a monoclonal antibody, prepared by immunizing mice with a retransplantable murine teratocarcinoma.[6] This antibody binds only to murine embryonic carcinoma (EC) cells, the undifferentiated stem cells of teratocarcinoma, but not to the differentiated descendants of these tumor cells. Since the antigen recognized by this antibody appears at the 8-cell stage of murine embryonic development but is not detectable in the earliest cleavage stages nor the fertilized ovum, it was termed "stage-specific embryonic antigen" (SSEA-1). Absorption and immunohistochemical studies have shown that the same antigenic determinant exists on some fetal, embryonic, and adult tissues of mice, including the ectodermal cells of the early postimplantation stage embryo, the primordial germ cells, ejacu-

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lated sperm, kidney, endometrial cells, and so on.[5-7] On the other hand, mature murine ovary and testis did not contain any detectable SSEA-1.[5]

Thus, although SSEA-1 is obviously not an exclusive marker of EC or embryonic cells, its presence on mouse EC cells has prompted us to speculate that the same antigen might be present on human EC cells as well. Previous studies have shown that human and murine germ cell tumors have many features in common, including some antigenic markers recognized by conventional antibodies raised in rabbits.[8-10] In this paper, we report that SSEA-1 is not found on human ECC but is present on the yolk sac elements of the human germ cell tumors and is detectable in human fetal but not adult testicular tubules.

At the same time, we report that another stage-specific embryonic antigen of the mouse (SSEA-3), which is present on follicular ova, fertilized eggs, and early-cleavage-stage mouse embryos[11] but is not present on mouse EC cells, can be immunohistochemically demonstrated on human EC cells and fetal testicular cells. Our findings point out some important differences between mouse and human embryonic carcinoma cells and provide additional clues for the understanding of the histogenesis of these tumors.

Materials and Methods

Samples were taken from 16 human testicular tumors removed surgically at the Hahnemann Medical College or the University of Minnesota Hospital. The histologic findings pertaining to these tumors are presented in Table 1. All the specimens were freshly frozen in 2-methylbutane, precooled in liquid nitrogen, sectioned immediately on a cryostat, or stored at −70 C until sectioning. In addition, we examined two adult testicles removed at surgery and two fetal testes obtained at autopsy from a 6-month and 8-month premature infant.

Antiserum

The monoclonal antibody that defines SSEA-1 is of the immunoglobulin M isotype with a kappa light chain. It was produced by fusing the spleen cells of a mouse immunized with the nullipotent mouse embryonic carcinoma cell line F9 with cells of the murine myeloma cell line P3X63Ag8. From this fusion, a hybrid cell line was obtained that secreted the anti-SSEA-1 antibody.[6] The monoclonal antibody that defines SSEA-3 was derived by fusing cells of the murine myeloma cell line SP2/0 with the spleen cells of a Fisher rat immunized with 4-8-cell-stage zona-pellucida-free embryos from randomly bred mice.[11] Specific antibody secreted by a hybrid cell line is a rat IgM. Both anti-SSEA-1 and -3 reagents were obtained as ascites fluid from mice given intraperitoneal injections of either anti-SSEA-1- or anti-SSEA-3-secreting hybridoma cells and used at a 1 : 10 dilution in phosphate-buffered saline (PBS) containing 1 mg/ml bovine serum albumin (BSA) and 0.04 mg/ml sodium azide. Ascites fluid (diluted 1 : 10) from a mouse given an intraperitoneal injection of the parent myeloma line of the anti-SSEA-1-secreting hybridoma was substituted for anti-SSEA-1 in the controls. Control ascites for SSEA-3 was from a rat bearing a rat IgM-secreting myeloma cell line.

Immunohistochemistry

Indirect immunofluorescence or immunoperoxidase tests were performed on frozen sections briefly fixed in cold acetone (4 C) for 10 minutes. Slides were incubated with either anti-SSEA-1 or -3 or control ascites for 2 hours at room temperature in a humidified chamber. After rinsing in PBS (4 C), either horseradish-peroxidase-conjugated or fluorescein-conjugated rabbit (IgG fraction) anti-mouse IgM (μ-chain-specific; Cappel Laboratories, Cochranville, Pa) were similarly incubated on the slides for 1 hour. Following the second incubation, the slides were rinsed in PBS (4 C) and either reacted with diaminobenzidine for light-microscopic examination or coverslipped with glycerine for fluorescence-microscopic studies.

Results

Germ Cell Tumors

The results of staining with antibodies to SSEA-1 are summarized in Table 1. No reactivity was de-

Table 1—Histologic Classification of Testicular Tumors and the Reactivity of Their Malignant Components With Monoclonal Antibodies Specifying Murine Stage-Specific Embryonic Antigens (SSEA)

Histologic type	Number of tumors	Reactivity with monoclonal antibodies	
		SSEA-1	SSEA-3
Seminoma	5	(−)	(−)
Teratoma	1	NA	NA
Teratoma + EC	3	−	+ + +
Teratoma + EC + YSC	1	+ + + (YSC)	+ + + (EC)
Embryonal carcinoma	4	−	+ + +
Embryonal carcinoma + YSC	2	+ + + (YSC)	+ + + (EC)
Total number	16	3	10

EC = embryonal carcinoma; YSC = yolk sac carcinoma; NA = not applicable.

tected in seminomas with either antibody. SSEA-3 was found on the plasma surface of embryonal carcinoma cells in 10 tumors: focally in 4 teratocarcinomas, in most cells forming the 4 pure embryonal carcinomas, and in most cells of tumors composed of embryonal carcinoma and yolk sac carcinoma (Figures 1 and 2). SSEA-1 was not present in the solid nests of EC but could be readily visualized on the cells forming the interlacing reticular strands of yolk sac carcinoma in 3 tumors (Figures 3–5). In all teratocarcinomas, there were glandular spaces lined by cuboidal epithelium that focally stained with the antibody to either SSEA-1 or SSEA-3. As has been previously observed (Damjanov et al, unpublished data), polymorphonuclear leukocytes present in the vascular spaces or in foci of necrosis and inflammation reacted with the antibody to SSEA-1.

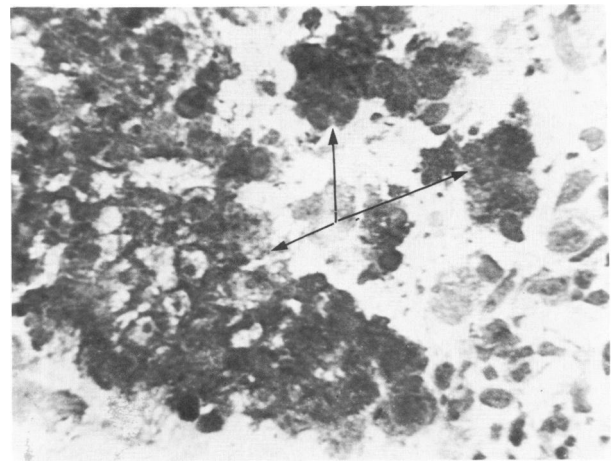

Figure 2—Teratocarcinoma. SSEA-3 is localized in the EC cells, forming nests (*arrows*). Note the unreactive cells surrounding the nests of EC cells. (×320)

Adult Testis

No reactivity with either antibody could be detected in the seminiferous tubules or interstitial tissue of the normal adult testis. On the other hand, the seminiferous tubules of an embryonal carcinoma-bearing testis contained intraluminal carcinoma cells that reacted positively with the antibody to SSEA-3 (Figure 6), but not with the antibody to SSEA-1.

Fetal Testis

Strong reactivity was noticed in the seminiferous tubules of fetal testes reacted with either anti-SSEA-1 or SSEA-3 antibody. Anti-SSEA-1 antibody reacted with intratubular cells but also stained occasional interstitial leukocytes (Figure 7). Anti-SSEA-3 antibody stained not only the intratubular cells (Figure 8) but also the myoepithelial cells in the basement membrane of the tubules.

Discussion

In the present study, we have used two monoclonal antibodies to detect murine embryonic antigens on germ cell tumors of man. In contrast to earlier reports emphasizing antigenic similarities between murine and human EC cells,[9,10] our data show marked differences in the distribution of two stage-specific embryonic antigens. In agreement with recent findings on cultured human EC cells,[12] SSEA-1, the typical marker of murine EC, was not detected on

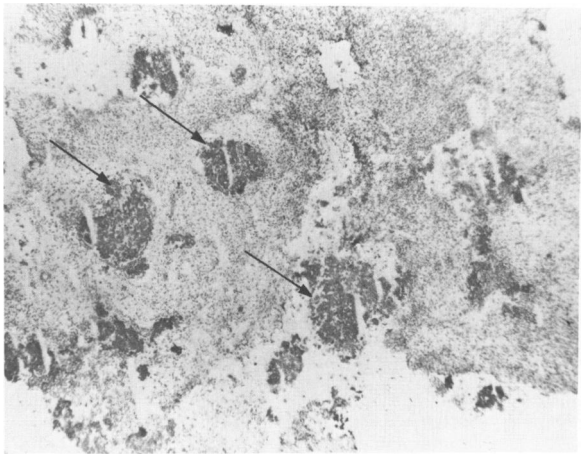

Figure 1—Teratocarcinoma. SSEA-3 is localized in the EC cells forming distinct nests (*arrows*). The tumor tissue surrounding the nests of EC does not stain. (×90)

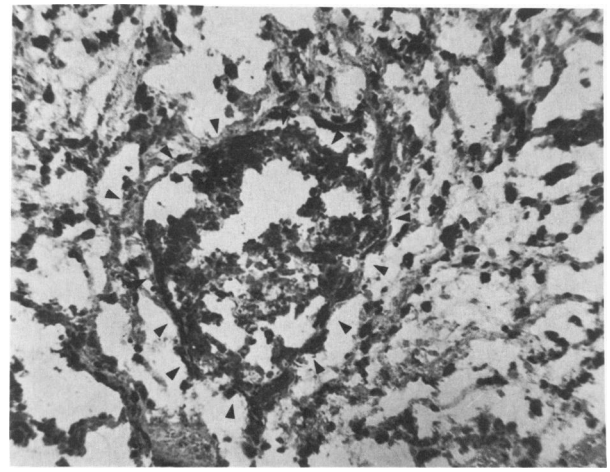

Figure 3—Yolk sac carcinoma. SSEA-1 is localized in most cells forming the anastomosing reticular meshwork and in the centrally located glomeruloid Schiller-Duval body (*arrowheads*). (×180) (With a photographic reduction of 6%)

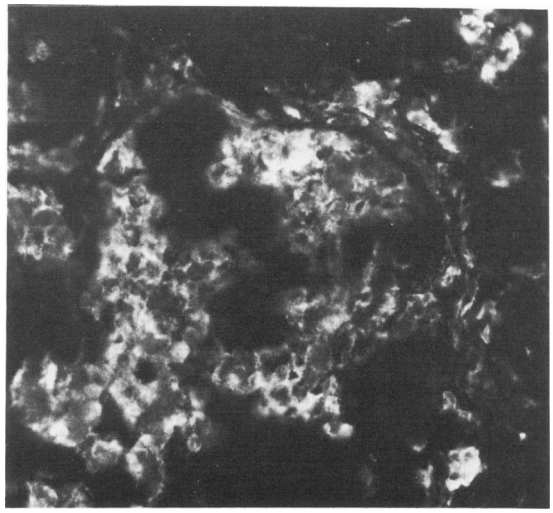

Figure 4—Yolk sac carcinoma. SSEA-1 is seen in the cells forming the Schiller-Duval body and some surrounding cells. (×220)

human EC cells. On the other hand, SSEA-3, an antigen not seen on murine EC, was found to be a characteristic antigenic marker of human EC cells.

SSEA-1, which is present on all testicular, ovarian, and embryo-derived mouse teratocarcinomas tested so far, was not present on EC cells in either human embryonal carcinomas or teratocarcinomas. This finding indicates that the carbohydrate antigenic determinant[13] recognized by anti-SSEA-1 antibody is not expressed on stem cells of human teratocarcinomas. On the other hand, the yolk sac components of human tumors reacted with the antibody to SSEA-1. Since yolk sac elements presumably represent cells that have arisen by differentiation from EC cells,[14] the appearance of SSEA-1 on yolk sac carcinoma cells could be taken as evidence of endodermal (yolk sac) differentiation in the tumor. We have previously shown that postimplantation mouse visceral endoderm also contains SSEA-1.[5] Thus, SSEA-1 could be added to the list of markers for the visceral endodermal cells,[15] both benign and malignant, human or murine.

In contrast to SSEA-1, which is present on mouse EC cells but is not present on human EC cells, SSEA-3 is present on human but not on mouse tumor cells. Embryologic studies using mice indicate that SSEA-3 is an "earlier" stage-specific embryonic antigen than SSEA-1, since it characterizes the earliest stages of embryonic development and actually disappears from the mouse embryonic cells approximately at the time of appearance of SSEA-1. Although we do not know when SSEA-1 or SSEA-3 appears during human embryogenesis, on the basis of data obtained on

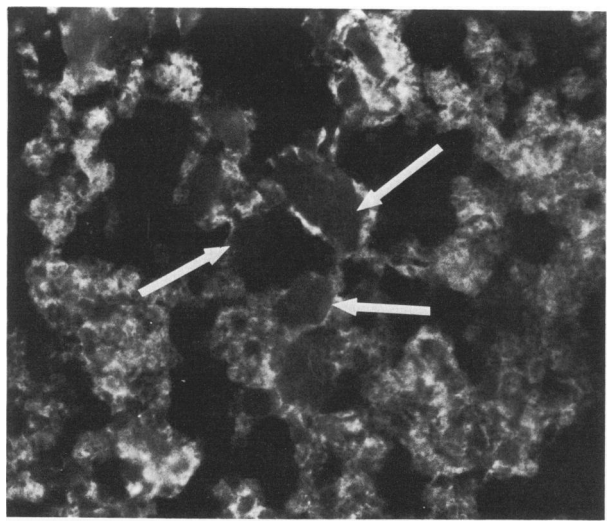

Figure 5—Yolk sac carcinoma. SSEA-1 is seen in the cytoplasm of cells forming the reticular meshwork. Note the nonreactive hyaline globules characteristic of yolk sac carcinoma (*arrows*). (×220)

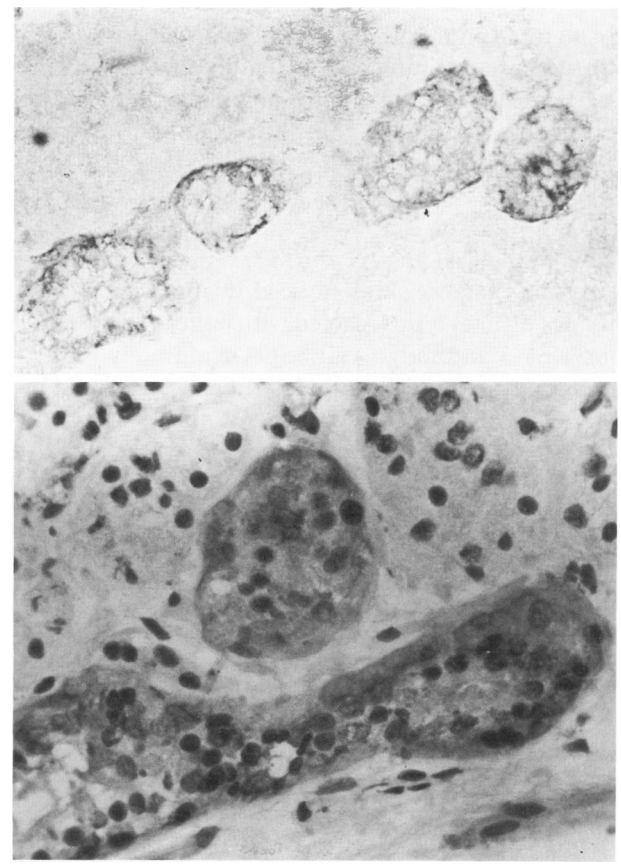

Figure 6—Carcinoma *in situ*. **A**—SSEA-3 is localized on intratubular cells. (×120) **B**—Section reacted with the antibody to SSEA-3 and counterstained with hematoxylin shows the reaction product on most intratubular cells. Note that some of the nuclei are enlarged and hyperchromatic and also displaced toward the lumen of the seminiferous tubules. (×220) (With a photographic reduction of 3%)

mouse embryos, one could speculate that the human EC cells are equivalent to developmentally younger embryonic cells than the mouse EC cells. The occurrence of trophoblastic giant cells in human germ cell tumors and their absence from mouse teratocarcinomas would be in accord with this hypothesis.[8] Trophectoderm develops in the mouse embryo after the morula stage, and the developmental commitment of cells destined to become trophectoderm must occur approximately at the same time the SSEA-1 appears and SSEA-3 disappears from the surface of the embryonic cells. It would thus seem that only SSEA-3–positive cells may give rise to trophectoderm, whereas the appearance of SSEA-1 on embryonic cells signals that these cells have passed the point after which they can no longer differentiate into trophoblastic cells. Since the human germ cell tumors often contain trophoblastic cells, and since the human EC cells express SSEA-3, we postulate that the human EC cells are equivalent to earlier cleavage-stage embryonic cells. Previously, it was suggested that mouse EC cells correspond to undifferentiated embryonic cells from the fourth to seventh day of pregnancy, ie, blastula to egg-cylinder-stage embryo.[8] Thus, mouse EC cells would correspond to developmentally "older" embryonic cells than the human EC cells.

Neither SSEA-1 nor SSEA-3 is an exclusively germ-cell–specific oncodevelopmental antigen. SSEA-1 is present in large amounts in human polymorphonuclear leukocytes, many mucus-producing cells, epididymis, endometrium, and kidney.[5] The distribution of SSEA-3 has not been studied in great detail, but it seems that this antigen is ubiquitously present in erythrocytes and myoepithelial and vascular smooth muscle cells.[11] This is unfortunate, because it will limit the use of monoclonal antibodies to these two antigens in immunodiagnosis of tumors and specific immunodirected chemotherapy.

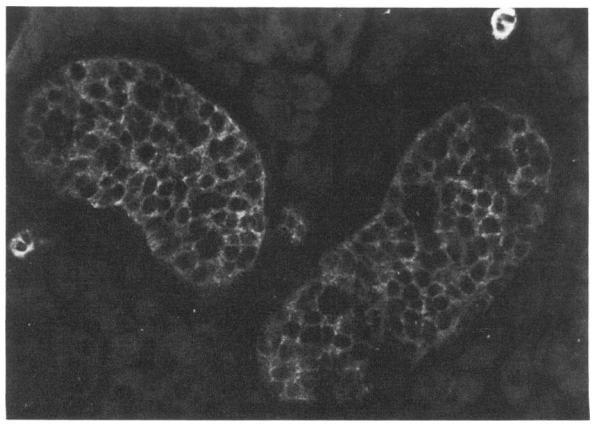

Figure 7—Testis of a 6-month fetus. SSEA-1 is localized in the intratubular cells. Also note the positively stained polymorphonuclear leukocytes. (×220)

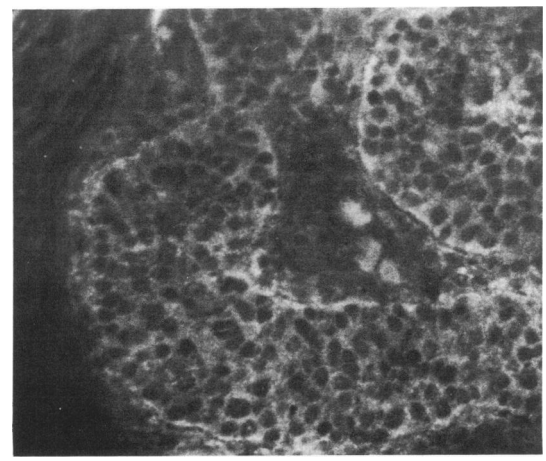

Figure 8—Testis of a 6-month fetus. SSEA-3 is localized on intratubular cells and myoepithelial cells in the wall of the tubules. (×220)

The histogenesis of testicular germ cell tumors in man remains poorly understood.[8,14,16] Although there is no doubt that the tumors originate from germ cells, it is not known whether the tumors arise from undifferentiated primordial germ cells that have remained from earlier stages of development in an undifferentiated form in the seminiferous tubules or from their more differentiated descendants (spermatogonia, spermatids) that have undergone malignant transformation or have dedifferentiated to become the stem cells of tumors. The development of invasive cancer most likely proceeds through a stage of carcinoma *in situ*.[16] *In situ* cancer has also been identified in the seminiferous tubules of tumor-bearing testicles.[17] Our data indicate that these *in situ* tumor cells also express SSEA-3, like the stem cells of the invasive tumor. This again points to the similarities between *in situ* and invasive tumor cells.[18,19] Furthermore, since we have not found any SSEA-3–positive cells in adult testes but have demonstrated SSEA-3 in the seminiferous tubules of the fetal testis, it seems reasonable to postulate that the tumors originate from immature fetal or primordial germ cells rather than adult germ cells. It remains, however, to be determined why the fetal testicular cells are reacting with antibodies to both SSEA-1 and SSEA-3, whereas the malignant cells react only with antibodies to SSEA-3.

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