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Differential Expression of a Human Sperm-specific Isozyme in Seminoma Cells Transplanted in Scid-nu^{str} Mice

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An isozyme of human sperm-specific lactate dehydrogenase, LDH-X (C_4) (EC 1.1.1.27), which is expressed only in differentiated germ cells (spermatozoa, spermatids and primary spermatocytes after midpachytene), appeared in the xenografted tumor cells of human seminoma and its metastatic lesions (lymph node and kidney) in scid (severe combined immunodeficiency)-nude^{streaker} double mutant mice, though it was not expressed in the original tumor of the patient. The morphological pattern of seminoma cells also changed in the xenografts and metastatic lesions as in normal spermatogenesis. Thus, the human seminoma cells showed differential expression of the sperm-specific isozyme in parallel with their morphological changes. However, the sperm-specific isozyme disappeared in the mitotically dividing seminoma cells which were newly established from the LDH-X positive xenograft.

Key words: Human seminoma — Seminoma cell line — LDH-X (C_4) — Differentiation — Scid-nu^{str} mouse

A human male germ cell tumor (seminoma) can arise from a primordial germ cell, a spermatogonium or a spermatocyte before meiotic division.[1] Although several analyses on human embryonal carcinomas and teratocarcinomas in cultured cell lines have been performed,[2,3] there is little information about the biological and molecular characteristics of human seminoma cells, simply because of the lack of experimental systems in which to observe the biological behavior of seminoma cells.[4]

Recently, we succeeded in growing a human seminoma in double mutant scid-nude^{streaker} (scid-nu^{str}) mice.[5] A seminoma grew very rapidly in the double mutant mice and metastasized to distant organs, while it grew very slowly in scid mice and it has never grown in athymic nude mice.[6] In the present study, a human sperm-specific isozyme, lactate dehydrogenase LDH-X (EC 1.1.1.27), which first appears in the midpachytene of primary spermatocytes and increases in concentration as spermatogenesis progresses to the spermatids,[7,8] was used as a marker of differentiation. The original tumor from a patient, xenografted tumors and metastatic lesions (lymph nodes and kidney) in the scid-nu^{str} double mutant mice were evaluated for enzyme activity, in addition to morphological and cytogenetic changes, to follow the differentiating potential of germ-line tumor cells. Furthermore, a seminoma cell line was established from the xenograft, and differential expression of LDH-X was examined.

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As shown in Table I, a seminoma was serially transplanted subcutaneously in scid-nu^{str} mice and all of them metastasized spontaneously to distant organs such as lymph node (all mice), lung (two mice) and kidney (one mouse). In the 4th and 5th passages, tumor tissues were transplanted to 18 C.B17-*scid/scid* mice, but only two mice developed large subcutaneous tumors and large metastatic lesions in the lymph node and/or lung. The morphological pattern of seminoma cells changed in the xenografts and metastatic lesions. The original tumor in the patient was composed of large round cells with distinct cell borders. The size and shape of nuclei were homogeneous, and these contained coarse chromatin and one or two prominent nucleoli, showing typical seminoma cells (Fig. 1a). The xenografted tumors were also composed of large round cells with distinct cell borders and prominent nucleoli, but the cells were smaller (like secondary spermatocytes) than those of the original tumor. Furthermore, there were some cells possessing small amounts of cytoplasm and densely stained or pyknotic nuclei (Fig. 1b). Histological patterns in the metastatic lesions were similar to those of xenografts. However, small cells with densely stained nuclei are predominant (Fig. 1c). In the original tumor, xenografts and metastatic lesions, seminoma cells were densely stained with periodic acid Schiff solution, while interstitial and lymphoid cells were not (figures not shown), because seminoma cells are known to contain large amounts of glycogen.[9]

In the original tumor of the patient, the anodal isozymes LDH_1 (B_4), LDH_2 (B_3A_1), and LDH_3 (B_2A_2) were

Table I. Serial Passage of Human Seminoma in Scid-nu^{str} Mice

Passage	Xenograft	Recipient mice	Sites of metastasis	Observation period[a)]
1st	Semi-1	Scid-nu^{str} 224	Lymph node, Lung	32
2nd	Semi-2	Scid-nu^{str} 225	Lymph node, Lung	75
3rd	Semi-3	Scid-nu^{str} 277	Lymph node, Kidney	67
4th	Semi-4	C.B17-scid 465	Lymph node	61
5th	Semi-5[b)]	C.B17-scid 457	Lymph node, Lung	79
5th	Semi-5-2	Scid-nu^{str} 802	Lymph node	98

A testicular seminoma was removed from a 31-year-old patient and about 2 to 3 mm cubed mass of tumor tissue was serially transplanted subcutaneously into the back of scid-nu^{str} mice from the 1st to 3rd and 5th passages, after anesthesia with 0.77% tribromoethanol (Aldrich Chem. Co. Ltd., Milwaukee, Wis.). All of them grew very rapidly. In the 4th and 5th passages, xenografts grew very slowly and only two of 18 C.B17-*scid/scid* mice developed large subcutaneous tumors and large metastatic lesions. These operations were carried out in a specific-pathogen-free (SPF) room. The details for the construction of scid-nu^{str} double mutant mice and a part of the results were given in previous reports.[5, 6)]

a) Days from xenotransplantation to removal of the tumor.

b) Pieces of xenografts were cultivated in Dulbecco's modified Eagle's medium (Nissui Co. Ltd., Tokyo) with 10% fetal bovine serum (Hyclone Lab., Utah) to establish a seminoma cell line.

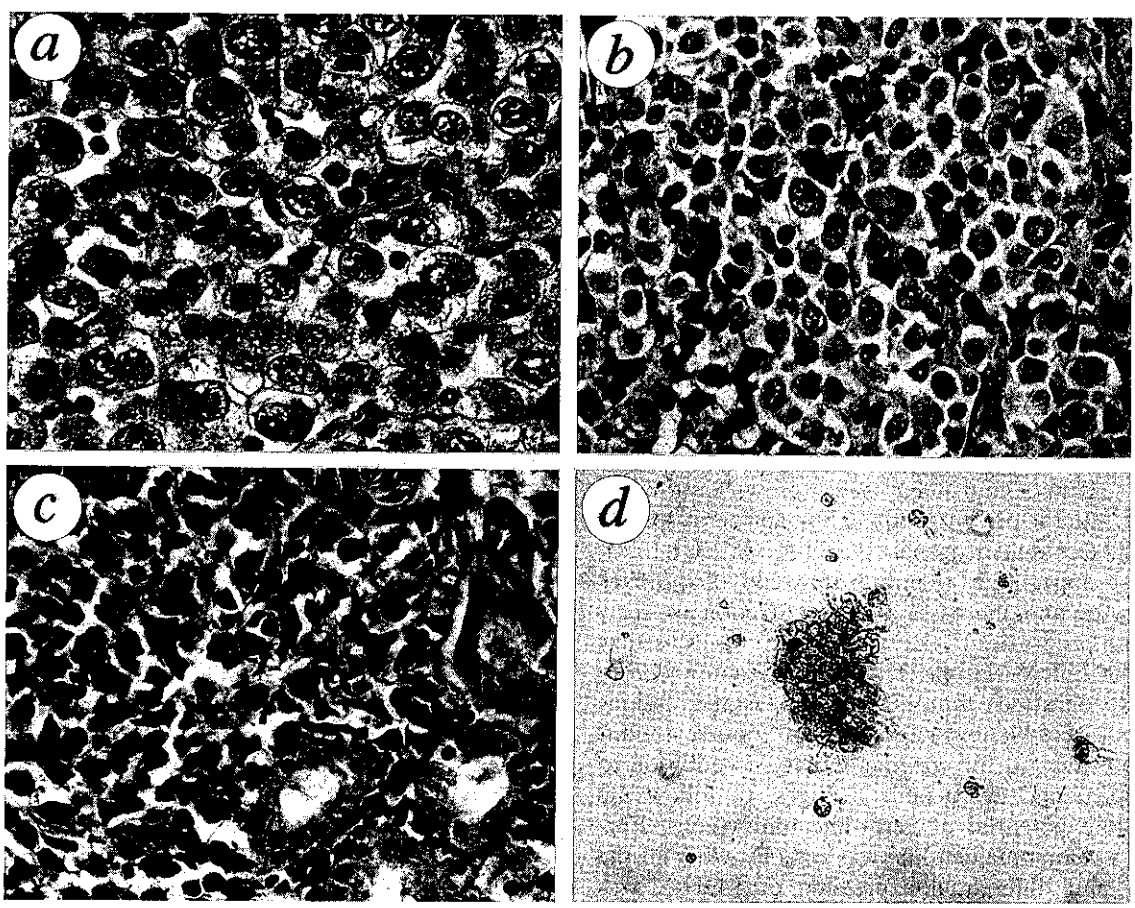

Fig. 1. Microscopic views of seminoma cells of the original tumor in the patient, the xenograft and its metastatic lesion in scid-nu^{str} mice and in culture. a, original tumor (for details, see the text). H & E, ×450. b, subcutaneous xenograft in scid-nu^{str} mice (Semi-2). H & E, ×450. c, metastatic lesion in the kidney (Semi-3). H & E, ×450. d, clusters of cultured seminoma cells. Various sizes of pale cells form spheroids in the culture medium. Multiple spurs are observed on the cell surface. ×180.

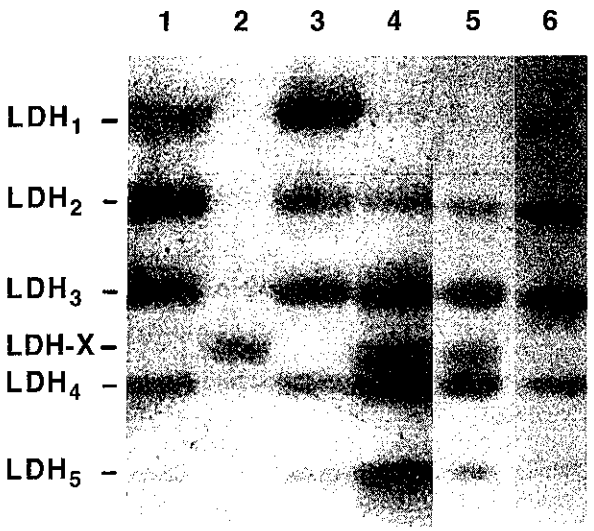

Fig. 2. Electrophoretic analysis of LDH isozymes. Original seminoma, xenografted tumors, metastatic lesions, cultured seminoma cells, and human kidney were homogenized in three volumes of distilled water. The sperm obtained from a normal 40-year-old man were washed three times in 0.9% NaCl solution, then frozen and thawed three times in three volumes of distilled water to acquire the homogenate of the spermatozoa. The homogenate was centrifuged at 15,000*g* for 30 min at 4°C, and 0.3 μl of supernatant was charged on a cellulose acetate plate (Titan III-Lipo, Helena Lab., Texas). Electrophoresis was performed with tris-citrate buffer (pH 8.3) at 120 V for 40 min in the cold chamber (4°C). After electrophoresis, plates were soaked with staining solution and incubated in a dark room at 37°C for 60 s. The final concentration of the staining solution was 0.2 *M* tris HCl, 0.1 *M* DL-sodium lactate, 0.4 mg/ml β-NAD^+, 0.01 *N* sodium cyanate, 0.4 mg/ml nitro-blue tetrazolium and 0.2 mg/ml phenazine methosulfate. Lane 1, human kidney. Lane 2, human spermatozoa. Lane 3, original tumor (seminoma) in the patient. Lane 4, xenografted seminoma (Semi-3) in scid-nu^{str} 277. Lane 5, metastatic lesion (lymph node of Semi-2) in scid-nu^{str} 225. Lane 6, cultured seminoma cells.

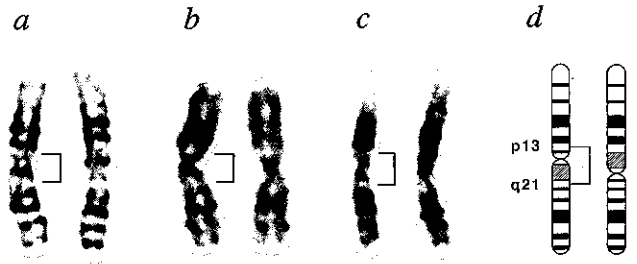

Fig. 3. Inversion between p13 and q21 of chromosome 1 in the patient, xenograft of seminoma and cultured cell line. Chromosomes were prepared from the xenografted seminoma directly or after cultivation, and compared with those of peripheral blood lymphocytes in the patient. Details of chromosome preparation were given in the previous report.[11] a, peripheral blood lymphocytes of the patient. b, xenografted seminoma cells. c, cultured seminoma cells. Inv (1) (p13 q21) was present in all metaphases so far examined. The left indicates normal chromosome 1 and the right, pericentric inversion of the other chromosome 1. d, diagram of chromosome 1 illustrating the proposed breakpoints (left) and the resulting inversion (right).

evident. These isozyme patterns were similar to those of normal human kidney (Fig. 2), indicating that the B subunit of LDH was predominantly synthesized over the A subunit. Human spermatozoa showed a single band, sperm-specific LDH-X (C_4), between LDH_3 and LDH_4 (B_1A_3) (Lane 2 in Fig. 2). LDH-X was not expressed in the original seminoma cells of the patient (Lane 3). However, the xenografts in the scid-nu^{str} mice did express LDH-X in addition to evident LDH_3 (B_2A_2), LDH_4 (B_1A_3) and LDH_5 (A_4) (Lane 4). These results indicated that seminoma cells engrafted into scid-nu^{str} mice initiated synthesis of the C subunit and additional production of the A subunit. Metastatic lesions in the lymph nodes and kidney also showed the same sperm-specific band as the xenografts did (Lane 5). In parallel with the morphological changes, LDH-X appeared in the process of xenograft acceptance and metastatic spread.

The seminoma cell line was newly established from a piece of xenografted seminoma (Semi-5) expressing LDH-X. Seminoma cells formed a cluster in the culture medium and floated as spheroids (Fig. 1d). Cells had pale cytoplasm which was densely stained with periodic acid Schiff solution as in the case of the seminoma cells in the patient and the xenografts. However, cultivated cells had multiple spurs on the cell surface and LDH-X was not expressed, as shown in Fig. 2 (Lane 6). The rapidly dividing or mitotically dividing seminoma cells appear to stop synthesizing C subunits, as is the case in the primordial germ cells and spermatogonia. Alternatively, cells not producing LDH-X might have been selectively cultivated.

Both xenografts and the cultured cell line had the same inversion between p13 and q21 of chromosome 1, which was also found in the peripheral blood lymphocytes of the patient (Fig. 3). Although the inverted region of chromosome 1 is related to aspermiogenesis,[10] the sperm of the patient were morphologically normal and fertile. However, chromosomal instability of this region may have caused abnormal development in a few primordial germ cells, resulting in the malignant transformation of germ cells, and may also have caused unstable forward and reverse differentiation in morphology and gene expression in the different environments.

Xenografts and cell lines of human seminomas will provide important experimental systems to study the

differentiation of male germ cells both morphologically and at the molecular level, in the absence of direct studies on human germ cells.

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