Nonrandom Abnormalities in Chromosome 1 in Human Testicular Cancers

Nancy Wang, Beth Trend, David L. Bronson, and Elwin E. Fraley

ABSTRACT

Tryptsin G banding was performed on metaphase chromosomes from 14 cell lines derived from primary tumors or metastases of 11 patients with testicular cancer. Most of the cell lines, 11 of 14, have a modal number between 51 and 61. All lines have numerical and structural changes involving chromosome 1 with trisomy of the q arm being the common aberration. Break points in chromosome 1 were nonrandom, being concentrated in the regions of q12, q12, p36, and p22, which resulted in morphologically identical marker chromosomes in different cases. These changes probably are not artifacts of cell culture. In one instance, three lines derived from the same patient, one from tissue removed at operation, and two from separate metastases removed at autopsy nearly 3 years later after unsuccessful radiotherapy and chemotherapy had identical chromosome compositions. In another case, lines derived from a primary tumor and a metastasis from the same patient also had identical marker chromosomes. The consistent involvement of chromosome 1 in aberrations may be associated with the highly malignant nature of testicular cancers.

INTRODUCTION

Few analyses have been made of the chromosomes of human testicular tumors (2, 29). Atkin (2) found that the modal chromosome number generally is 50 or more, and in this, testicular tumors are different from tumors at other sites. Martineau (29) performed conventional karyotypic analyses on 25 testicular tumors and reported that seminomas have higher modal chromosome numbers than do other histological types. She also observed marker chromosomes in various kinds of testicular tumors as determined by statistical analysis of total chromosome length and arm ratios. However, it is impossible to identify precisely the origin of these marker chromosomes by conventional analysis.

To obtain information detailing the chromosome constitution of human testicular cancers and to determine whether specific nonrandom chromosome abnormalities exist, we performed trypsin G-banding studies on 14 cell lines derived from 11 patients with testicular cancer. Specific marker chromosomes were identified, and nonrandom abnormalities of chromosome 1 were observed in all of the cell lines.

MATERIALS AND METHODS

Cytogenetic studies were performed on 14 testicular cancer cell lines derived from 11 patients. The cell lines had been given the following designations: 577ML; 577MF; 577MR; 833K; 1156Q; 1218E; 1242B; 1255O; 2044L; 2061H; 2102EP; 2102ER; Tera-1; and Tera-2. The origin of the cell lines and the tissue types of the tumors from which they were derived are summarized in Table 1. Tera-1 and Tera-2 were established in Dr. Jorgen Fogh’s laboratory (8) at the Sloan-Kettering Institute for Cancer Research. The rest of the cell lines were established by Bronson et al.

The cell lines were maintained in Roswell Park Memorial Institute Medium 1640 containing 10% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.), 100 units penicillin per ml, 100 µg streptomycin per ml, and 10 µw 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and subcultured weekly. Cultures were harvested for chromosome studies when 40% confluent, treated with 0.14 µg Colcemid per ml for 2 hr, and trypsinized to obtain a single-cell suspension. The cells were then treated hypotonically with 0.075 M KCl or 0.7% sodium citrate for 10 min and fixed in methanol:glacial acetic acid (3:1; v/v). Slides were prepared by the air-dried method and were treated to obtain trypsin G banding by the method of Wang and Fedoroff (51). Briefly, slides were heated on a 70°C hot plate for 2 min, treated with 0.0725% trypsin (in 0.9% NaCl solution) for 20 to 45 sec, rinsed with 0.9% NaCl solution, and stained with Giemsa for 2 to 5 min. For each cell line, 25 to 30 metaphase figures were analyzed.

RESULTS

577ML, 577MF, and 577MR. These 3 lines were derived from lung, forehead, and retroperitoneal metastases, respectively, from a single patient, and all were classified histologically as teratocarcinoma. 577MR was established from tissue removed at operation while 577MF and 577ML were established from separate metastases removed at autopsy nearly 3 years later after unsuccessful chemotherapy and radiotherapy. The chromosome numbers range from 45 to 58 for 577ML, 50 to 56 for 577MF, and 50 to 54 for 577MR, and the modal chromosome number is 55 for all 3 lines. One normal chromosome 1 and 3 marker chromosomes (M1, M2, and M3) (Fig. 1A) involving chromosome 1 were found in every cell of the 3 cell lines. As shown in Fig. 1A, chromosome M1 results from the addition of a dark band of unknown origin to the distal end of the p arm of chromosome 1. Chromosome M2 is a translocation product of chromosome 18 and the q arm of chromosome 1. Chromosome M3 is a normal chromosome 1 with almost all of the p arm deleted.

1156Q. This cell line was derived from a mixed primary tumor. The chromosome number ranges from 46 to 58, and the modal number is 53. As shown in Fig. 1B, there is one normal chromosome 1 and, of the 11 marker chromosomes, 2 involve chromosome 1 (Fig. 2B). Chromosome M1 results from

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2 To whom requests for reprints should be addressed.

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833K; 1156Q; 1218E; 1242B; 1255O; 2044L; 2061H; 2102EP; 2102ER; Tera-1; and Tera-2. The origin of the cell lines and the tissue types of the tumors from which they were derived are summarized in Table 1. Tera-1 and Tera-2 were established in Dr. Jorgen Fogh’s laboratory (8) at the Sloan-Kettering Institute for Cancer Research. The rest of the cell lines were established by Bronson et al.3

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1156Q. This cell line was derived from a mixed primary tumor. The chromosome number ranges from 46 to 58, and the modal number is 53. As shown in Fig. 1B, there is one normal chromosome 1 and, of the 11 marker chromosomes, 2 involve chromosome 1 (Fig. 2B). Chromosome M1 results from

the addition of a segment containing 3 bands from the distal end of the q arm of chromosome 1 to the distal end of the p arm of another chromosome 1. Chromosome M2, like the chromosome M3 in 577M, is a chromosome 1 with the p arm deleted.

1242B. This cell line was derived from a primary embryonal carcinoma. The chromosome number ranges from 68 to 95, and the modal number is 86. As shown in Fig. 1C, the line is trisomic for chromosome 1. Six marker chromosomes were observed (Fig. 2C), 2 of which involve chromosome 1 and are identical to the Ml and M2 markers in 1156Q (Fig. 1B). However, the cell line differs from 1156Q in its modal chromosome number and its other marker chromosomes (Fig. 2, B and C).

12550. This line was derived from a primary teratocarcinoma with seminoma. The chromosome number ranges from 46 to 61 with a modal number of 51. As shown in Figs. 1D and 2D, there is one normal chromosome 1, and among the 9 marker chromosomes, 4 involve chromosome 1. Chromosome M1 results from an inversion of the terminal segment of chromosome 1 with the break point at the p2 region and the addition of a segment involving 2 dark bands of unknown origin to the distal end of chromosome 1 after the inversion. Chromosome M2 results from the translocation of one 1q arm to the proximal end of the p arm of another chromosome 1. Chromosome M3, identical to the chromosome M1 in 1156Q and in 1242B, results from the addition of a segment containing 3 bands from the distal end of the q arm of chromosome 1 to the distal end of another chromosome 1. Chromosome M4 is the remaining part of chromosome 1 with the entire q arm deleted.

2102EP. This cell line was derived from a primary tumor classified histologically as teratocarcinoma with yolk sac tumor. The chromosome number ranges from 48 to 61 with a modal number of 56. Approximately 40% of the cells are trisomic for chromosome 1. Another 40% contain 2 normal No. 1 chromosomes and a marker chromosome M1 (Fig. 1E) which result from the addition of 2 dark bands of unknown origin to the distal end of the 1p arm. The remaining 20% of the cells contain 2 normal No. 1 chromosomes and 2 identical M1 markers. The M1 is the only marker chromosome found in this cell line (Fig. 2E).

2102ER. This cell line was derived from a retroperitoneal metastasis of the 2102EP tumor and was classified histologically as teratocarcinoma. The chromosome number ranges from 45 to 59 with a modal number of 56. The chromosome composition of the cell line is similar to that of the 2102EP cells having 2 normal No. 1 chromosomes and 2 M1 marker chromosomes with each cell of this line containing 2 identical M1 markers (Figs. 1F and 3).

833K. This cell line was derived from an abdominal metastasis classified histologically as embryonal carcinoma, choriocarcinoma, seminoma, and teratoma. The chromosome number ranges from 58 to 61 with a modal number of 58. Most (90%) of the cells contain 3 copies of normal chromosome 1. The others contain only 2 copies. In addition, 2 marker chromosomes were found in every cell examined. As shown in Fig. 1G, chromosome M1 results from the translocation of a segment involving 4 dark bands from the distal end of the 1q arm to the distal end of the p arm of a chromosome 2. Chromosome M2 is identical to the M3 marker observed in cell line 12550 (Fig. 1D).

1218E. This cell line was derived from a primary tumor classified histologically as embryonal carcinoma and seminoma. The chromosome number ranges from 95 to 117 with a modal number of 115. As shown in Fig. 1H, the chromosome content includes 2 normal No. 1 chromosomes and 3 marker chromosomes, 2 M1 and one M2. Chromosome M2 results from a chromosome 1 with 2 dark bands deleted from the distal end of the q arm. Chromosome M1 probably is derived from a M2 marker by the addition of a chromosome segment identified as 2p to the break point (Fig. 1H).

2044L. This cell line was derived from a retroperitoneal lymph node metastasis classified histologically as embryonal carcinoma. The chromosome number ranges from 50 to 57 with a modal number of 53. Each cell contains 2 normal No. 1 chromosomes and a giant dicentric marker chromosome resulting from the inversion and translocation of the entire 1q arm to the terminal end of the 1p arm of another chromosome 1 (Fig. 1).

2061H. This cell line was derived from a lung metastasis of...
a teratocarcinoma. The chromosome number ranges from 92 to 105 with a modal number of 98. Each cell contains 2 normal No. 1 chromosomes and 4 marker chromosomes (M1 to M4) involving chromosome 1 (Fig. 1J). Chromosome M1 is derived from the breaking off of the short arm of a chromosome 1 at the p22 region and its translocation to the distal end of the p arm of another chromosome 1. Chromosome M2 results from a translocation between a chromosome 12q and 1q. Chromosome M3 is derived from a chromosome with the majority of the p arm broken off at the p22 region. Chromosome M4 is identical to the M3 marker found in line 12550 (Fig. 1D) and the M2 marker found in line 833K (Fig. 1G).

Tera-1. This cell line was derived from a lung metastasis classified histologically as embryonal carcinoma. The chromosome number ranges from 56 to 67 with a modal number of 61. Chromosome abnormalities include trisomy for chromosome 1 and one isochromosome derived from the centromeric fusion of two 1q arms (Fig. 1K).

Tera-2. This cell line was derived from an embryonal carcinoma metastasis to lung. The chromosome number ranges from 59 to 67 with a modal number of 61. The line is trisomic for chromosome 1 and has 2 markers involving chromosome 1. Chromosome M1 results from the centromeric fusion between 1q and 2p with the addition of a segment of 2p15 to 2p25 to the distal end of the 2p arm (Fig. 1L). Chromosome M2 is a chromosome 1 with the p22 to p36 region deleted (Fig. 1L).

In this study, through the G-banding chromosome analysis of 14 testicular tumor lines, the following nonrandom chromosome abnormalities were observed: (a) chromosome 1 is involved in both numerical and structural changes in each of the 14 lines (Table 2); (b) a trisomy state of the q arm of chromosome 1 has been found as the common chromosomal aberration for all the cell lines studied (Fig. 1); (c) the break point of chromosome 1 involved in different structural changes is nonrandom (occurring most frequently at the regions of p12, q12, p22, and p36) with these nonrandom structural changes resulting in morphologically identical marker chromosomes in different cases (Fig. 1); (d) with the exception of 1218E, 1242B, and 2061H, the modal chromosome number of all the cell lines is in the range of 51 to 61, hyperdiploidy (Table 2); and (e) the chromosome compositions of the cell lines established independently from separate sites of the same patient are identical (Fig. 2, A and E).

**DISCUSSION**

Nonrandom chromosome abnormalities have been found in histologically related human cancers. The most convincing are the presence of the Ph1 chromosome in chronic myelocytic leukemia (43), the partial or total deletion of a No. 22 chromosome in meningioma (22, 23, 27, 28, 58), and the translocation with the involvement of chromosome 14 in Burkitt's lymphoma (13, 21, 24, 59). Also, through the analyses of the peripheral blood cells, specific chromosome abnormalities have been identified in the patients of certain cancer types. These include the interstitial deletion of chromosome 13 in retinoblastoma patients (9, 40, 41, 49, 54, 57), the partial deletion of the p arm of chromosome 11 in Wilms' tumor patients (42), and the translocation between chromosomes 3 and 8 in the hereditary renal cell carcinoma cases reported recently (6, 18). The meaning of these nonrandom chromosome abnormalities observed among the tumors of histologically related tissues or organs is not yet clear. However, on the basis of comprehensive cytogenetic studies of chemically and virally induced tumors in rats (1, 20, 30) and mice (52), common chromosome abnormalities may reflect a common etiology of the tumors.

In this communication, nonrandom chromosomal aberration for nonseminomatous testicular cancers is being added to the aforementioned group of human cancers. Fourteen cell lines derived from 11 patients were analyzed, and the consistent involvement of chromosome 1 in both translocation and partial trisomy was found in every case.

The nonrandom chromosome aberrations reported in this study do not seem to be artifacts of in vitro cultivation. Evidence for this is the identical chromosome composition observed in 3 different cell lines (577ML, 577MF, and 577MR) derived from metastases at different locations in a single patient. It is unlikely that artifact would produce 6 identical marker chromosomes (Fig. 2A) and identical modal chromosome numbers (Table 2) in these 3 independent cell lines, one derived from tissue removed at operation and 2 from separate metastases removed at autopsy nearly 3 years later after unsuccessful radiotherapy and chemotherapy. In addition, the primary and metastasized cell lines 21O2EP and 21O2ER from another patient again yield identical chromosome composition with the exception of the appearance of one additional M1 marker chromosome in the metastasized line (Fig. 3).

Chromosome 1 abnormalities are not limited to testicular cancers. They have been occasionally reported for a variety of other human cancers affecting ovary (4, 14, 16), lung (14, 16, 39), bladder (47), breast (7, 15, 19, 36), brain (5), colon (39), and cervix (3), and in lymphoma (21, 24-26), malignant melanoma (17), and hematologic cancers (10-12, 31-35, 37, 38, 44-46, 50, 53, 55, 56, 60). However, a consistent involvement of chromosome 1 in both structural and numerical changes such as that observed in testis cancers has not been reported in any of these tumors. The consistency of this association of

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* Table 2

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<th>Chromosome no. involved</th>
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<th>No. of normal chromosome 1</th>
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* Tera-1 and Tera-2 cells were a gift from Dr. Jergen Fogh, Sloan-Kettering Institute for Cancer Research, New York, N. Y.
chromosome 1 abnormalities with nonseminomatous testicular cancers may reflect the highly malignant nature of the neoplasms. It has been suggested by Atkin and Pickthall (4) that the involvement of chromosome 1 may be a relatively late concomitant of malignant transformation and that its presence can serve as an indication of an unfavorable prognosis or high degree of cancer.

Further cytogenetic studies on primary testicular tumors, both seminomas and nonseminomatous cancers, are required to extend the observations reported herein. These studies, by clarifying the role of chromosome 1 abnormalities in cancer transformation, may be of clinical value in predicting the cancer potential for seminomas. In addition, detailed analysis of the sex chromosome composition of the various testicular cancers may aid in determining the time of germ cell transformation during spermatogenesis.

REFERENCES

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Fig. 1. Chromosome 1 abnormalities in 14 testicular tumor cell lines. Arrows, break points of each translocation. N, normal chromosome 1.
Fig. 2. Marker chromosomes from 8 cell lines derived from 5 patients. Arrows, marker chromosomes involving chromosome 1.

Fig. 3. Chromosome spread from the metastasized cell line 2102ER showing 2 identical M1 chromosomes (arrows).
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