Chromosomal Changes in Human Primary Testicular Nonseminomatous Germ Cell Tumors

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ABSTRACT

A cytogenetic analysis of 14 primary testicular nonseminomatous germ cell tumors has been carried out after short term tissue culture. The modal chromosome numbers ranged from 53 to 113, in agreement with flow cytometric determination of the DNA content of the tumors. At least one copy of an i(12p) was present in 12 tumors. Two tumors, however, lacked that marker. Some chromosomes are apparently overrepresented, whereas others are underrepresented, although some differences between seminomas and nonseminomas were noticed.

INTRODUCTION

Testicular germ cell tumors of adults can be divided both clinically and morphologically in two distinct entities, seminoma and nonseminomatous germ cell tumor (1–3). The latter may have one or more of the following histological subtypes: embryonal carcinoma; teratoma; yolk sac tumor; and choriocarcinoma. In the British classification (4) tumors with a seminoma and a NSGCT component are classified as combined tumors; in the WHO classification they are classified as NSGCT (5). In about 20% of germ cell tumors seminoma and NSGCT coexist (4, 5). In general, seminomas are less aggressive than NSGCT, although the aggressiveness of the latter depends on the histological subtype, in particular the presence of embryonal carcinoma, yolk sac tumor, and/or choriocarcinoma.

From the different theories on the pathogenetic relationship between seminomas and NSGCT (6, 7) two main concepts emerge. One model updates the former hypotheses of Pierce and Abell (8) and assumes that seminomas and NSGCT independently derive from transformed (dysplastic) intratubular germ cells via carcinoma in situ (2, 10, 11). Another model suggests that all testicular germ cell tumors (with the possible exception of spermatocytic seminoma) have a single origin in carcinoma in situ and progress through a seminoma stage (9, 12). This hypothesis represents a further development of the theories of Ewing (6) and Friedman (7).

Cytogenetic studies of seminomas and NSGCT, as well as of the seminoma and NSGCT components of combined germ cell tumors of the testis, may clarify the possible relationship between the different subtypes of testicular germ cell tumors.

Recently, we presented our cytogenetic findings in seminomas (13, 14, 15, 18, 19, 22, and Y) and in a combined germ cell tumor of the testis (14). Now we report the results of the cytogenetic analysis and DNA flow cytometry of 14 primary NSGCT.

MATERIALS AND METHODS

The tumors were submitted fresh and sterile at the time of diagnosis. Tissue culture and DNA flow cytometry were carried out basically as described (5). For chromosome preparations the tumor cells were harvested after short term tissue culture (on an average of up to 7 days), either by brief trypsinization or according to the procedures described by Gibas et al. (16). Colcemid (0.05–0.5 μg/ml culture medium) was added 2 to 5 h before harvesting. After harvesting, the cells were centrifuged for 5 min at 240 × g. The pellets from the trypsinized cells were resuspended in 0.06 M KCl, incubated at 37°C for 15 min, centrifuged, resuspended in a mixture of methanol:acetic acid (3:1), centrifuged, resuspended, and left in the tubes for 20 min. The pellets from the cells harvested as described by Gibas et al. were immediately resuspended in fixative. In both methods there was a final centrifugation, after which the cells were resuspended and pipeted onto slides. Air dried chromosome preparations were GAG and/or GTG banded.

Clinical staging was carried out as described by Peckham et al. (18).

RESULTS

A summary of the clinical, histopathological, and cytogenetic data of all cases is given in Table 1.

Karyotypes. The karyotype of one metaphase from each case, representative for the clonal structural abnormalities observed, is described in Table 2. An overview of the numerical abnormalities found in all cases is presented in Table 3. Figs. 1–4 show karyotypes of, respectively, cases 3, 9, 10, and 14. Case 12, cases 2 and 11, and case 7 have been described previously (14, 19, 20).

Statistical Analysis. The analysis of variance showed that all effects are highly significant, indicating that copies of normal chromosomes are present in different numbers and that different tumors have different total numbers of chromosomes. The interaction term, which is used as error term, is numerically rather small. The variability is best described by saying that some tumors have more chromosomes than others and that some chromosomes are found in higher numbers than others. The distribution of the numbers of copies of chromosomes does not vary very much depending on the person whose cells are analyzed.

Fig. 5 shows the mean chromosome counts combined for all cases, after standardizing the total number of normal chromosomes to the arbitrary number of 46. Multiple comparison using the Newman-Keuls method shows that the normal copies of chromosomes 12 and X are more frequently found than the numbers of copies of normal chromosomes 1, 2, 5, 9, 10, 11, 13, 14, 15, 18, 19, 22, and Y.

The modal chromosome numbers of NSGCT are significantly lower than those of seminomas (P < 0.05).
Table 1  Summary of the clinical, histopathological, and cytogenetic data of the 14 cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Patient age (yr)</th>
<th>Clinical stage</th>
<th>Histology of Primary NSGCT</th>
<th>DNA index</th>
<th>No. of metaphases analyzed</th>
<th>Modal no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>I</td>
<td>EC/TI/TT/CHO*</td>
<td>1.45</td>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>I</td>
<td>EC/TI/TT</td>
<td>1.27</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>IV</td>
<td>EC/YS</td>
<td>2.27 (1.26)*</td>
<td>7</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>I</td>
<td>TD</td>
<td>1.45</td>
<td>15</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>I</td>
<td>EC/TI/TT</td>
<td>1.59 (1.42)</td>
<td>7</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>I</td>
<td>EC/TDD/YS</td>
<td>1.27</td>
<td>5</td>
<td>59</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>IV</td>
<td>EC/TI/TT/YS</td>
<td>2.26</td>
<td>24</td>
<td>102</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>II</td>
<td>TD</td>
<td>1.28</td>
<td>9</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>IIC</td>
<td>EC</td>
<td>1.28</td>
<td>9</td>
<td>59</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>I</td>
<td>EC/TD</td>
<td>1.26</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>11</td>
<td>64</td>
<td>IIC</td>
<td>SE/EC/TD/TI</td>
<td>SE:1.68, NSE:1.33</td>
<td>9</td>
<td>53*</td>
</tr>
<tr>
<td>12</td>
<td>24</td>
<td>I</td>
<td>SE/EC</td>
<td>SE:2.51, NSE:2</td>
<td>7</td>
<td>101</td>
</tr>
<tr>
<td>13</td>
<td>24</td>
<td>IV</td>
<td>EC/TD/TT/YS</td>
<td>1.29</td>
<td>11</td>
<td>62</td>
</tr>
<tr>
<td>14</td>
<td>31</td>
<td>I</td>
<td>EC/TI/TT/YS</td>
<td>2.43 (1.47)</td>
<td>13</td>
<td>113</td>
</tr>
</tbody>
</table>

* At presentation [according to the report of Peckham et al. (18)].

Numbers refer only to abnormal metaphases.

EC, embryonal cell carcinoma; CHO, choriocarcinoma; TI, immature teratoma; TD, mature teratoma; YS, yolk sac tumor; SE, seminoma; NSE, nonseminomatous component. The SE and NSE components of the tumors 11 and 12 were separately processed for DNA flow cytometry.

DISCUSSION

Numerical Abnormalities. Cytogenetic studies of testicular germ cell tumors (17, 21–32) have demonstrated the presence of a generally hyperdiploid to hypotriploid chromosome complement, with higher modal chromosome numbers in seminomas than in NSGCT. Similar results were obtained by measuring the DNA content of the different subtypes of testicular germ cell tumors (33).

Plotting the number of reported primary NSGCT (21–28,
30, 31) against their modal ranges (Fig. 6), it is clear that most NSGCT have between 60 and 64 chromosomes, and only very few are not peritriploid. At variance, although most seminomas have between 65 and 69 chromosomes, higher numbers are not unusual in these tumors (13).

Several hypotheses have been proposed on the mechanism of origination of aneuploidy in testicular germ cell tumors: successive nondisjunctions of a diploid cell; polyploidization or cell fusion; followed and/or preceded by chromosomal gain and/or loss (see Ref. 34 for review). If successive nondisjunction of a diploid cell were a mechanism of oncogenesis of NSGCT, one would expect a higher frequency of near diploid NSGCT and lower frequencies in higher classes. Since the opposite is observed, there should be other modes of origin of aneuploidy.
The clear peak in the hypotriploid range is in keeping with the measured DNA content of testicular germ cell tumors (33) and can be explained by loss of chromosomes starting from a triploid or tetraploid cell. This would be in keeping with the model of pathogenesis of testicular germ cell tumors proposed by Ewing (6) and Friedman (7). According to this model most testicular germ cell tumors, irrespective of their histology, would originate in a carcinoma in situ cell and progress through a seminoma stage. In disagreement with the tumor progression model proposed by Nowell (35, 36), according to which the clonal evolution of a tumor cell population goes from diploid to higher chromosome numbers, the progression of testicular germ cell tumors apparently goes from high to lower numbers of chromosomes, i.e., is accompanied by a net loss of chromosomal material. This decrease is probably the end result of the loss of specific chromosomes, the development of structural
abnormalities, and the gain of some other chromosomes (or part of chromosomes).

It has been suggested that loss of certain chromosomes or (loss of heterozygosity for) some chromosomal regions is important for the development of malignancy, presumably because of loss of genes with tumor suppressing and differentiation regulating properties (37-45).

If loss of chromosomes in testicular germ cell tumors is related to loss of genes crucial for normal cell differentiation, different chromosomes should be underrepresented in NSGCT as compared to seminomas. However, since both are germ cell tumors, it should not be surprising that some chromosomes are underrepresented in both subtypes. Tables 2 and 3 and Fig. 5 give an idea of which chromosomes and part of chromosomes are over- or underrepresented in NSGCT. As can be seen, chromosomes 4, 5, 9, 10, 11, 13, 14, 15, 18, 22, and Y are usually underrepresented, whereas chromosomes 7, 8, 12 (especially its short arm), and X are usually overrepresented. It is of interest that chromosome 15 is overrepresented in seminomas (13) and underrepresented in NSGCT, while chromosome 17 is underrepresented in the former, but not in the latter. It might be speculated that chromosome 15 contains genes important for sperm cell differentiation. Chromosomes more frequently represented in NSGCT than in seminomas (e.g. chromosome 17) may contain genes responsible for a more malignant development.

The rare occurrence of NSGCT with chromosome numbers higher than 69 can also be fitted into this model. If loss of heterozygosity for a certain chromosome region plays an important role in the progression of a cell from the seminoma to the NSGCT stage (which remains to be proved), it will be in the triploid range that chromosome loss will be most critical. As a matter of fact, if a cell contains three copies of a certain autosome, e.g., two paternal and one maternal, the probability of becoming homozygous for the paternal chromosome through random loss of a single chromosome is 1:3, whereas for a tetraploid cell two random events would be necessary (probability, 1:6). Accordingly, the higher the chromosome number in a seminoma stage cell, the lower is the probability of obtaining a NSGCT.

Structural Abnormalities. As can be seen in Table 2, in primary NSGCT chromosome 12 is very often involved in structural abnormalities, which was also noted by Gibas et al. (30) and DeLozier-Blanchet et al. (32). Twelve of 14 tumors had 1 or more copies of i(12p), a specific marker for germ cell tumors of the testis (28-30, 32) and possibly also of the ovary (46, 47). Two tumors, however, lacked that marker. These i(12p) negative testicular germ cell tumors may represent a different group of tumors with a different clinical evolution (19).

Besides the i(12p) we did not find any structural abnormality in common with different tumors.

As is the case for seminomas (13, 16), chromosome 1 is also frequently involved in structural abnormalities in NSGCT (Table 2). In 4 of 6 primary NSGCT reported by Gibas et al. (30), and in the case described by Saikevych et al. (31), there were also structural abnormalities of chromosome 1. In cell lines...
derived from testicular germ cell tumors abnormalities of chromosome 1 are also very common (49–51). Wang et al. (48) found a nonrandom involvement of chromosome 1 in structural and numerical abnormalities in each of 14 NSGCT-like tumor cell lines. The breakpoints most often found were at Ipl2, and numerical abnormalities in each of 14 NSGCT-like tumor in the present series. Chromosome 1 rearrangements, however, have been found in a variety of other solid tumors and in many hematological malignancies (see Ref. 52 for review). Yet, as our results show, chromosome 1 is much more frequently involved in testicular germ cell tumors than in any other tumor.

In the present series we noted structural abnormalities involving 73 different breakpoints (Table 2), 22 of which had been reported by Gibas et al. (30) and Saievych et al. (31) in their studies of NSGCT. Since many breakpoints described (e.g., in chromosomes 1, 4, 8, and 11) are also found in other kinds of malignancies (see Ref. 52 for review), they should probably be considered proliferation specific rather than differentiation associated breakpoints (53).

REFERENCES

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