Cytogenetic Analysis of Murine Embryo-derived Tumors

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ABSTRACT

The possible relationship among malignancy, differentiation, and chromosomal constitution of primary embryo-derived tumors was studied. Tumors were induced by transplanting 7-day-old mouse embryos under the kidney capsule of syngeneic BALB/c recipients. Transplantation of 101 embryos resulted in 81 tumor-bearing mice: 36 teratocarcinomas; 18 teratomas; and 27 yolk sac tumors. Some of the yolk sac tumors proved to be retransplantable for several generations.

Cytogenetic investigation of the primary embryo-derived tumors revealed that the majority of teratocarcinomas (82%) were chromosomally normal, whereas almost all (83%) karyotyped teratomas and yolk sac tumors had a highly abnormal chromosomal constitution. Most common aberrations were polyplody; overrepresentation of chromosome 1, 6, 15, or 19; and an underrepresentation of chromosome 2, 4, 14, or a sex chromosome.

INTRODUCTION

In inbred mouse strains teratomas can be obtained experimentally by grafting pre- or postimplantation embryos to extrauterine sites (1–3). The resulting tumors are indistinguishable from spontaneously occurring ovarian and testicular teratomas, and they are referred to as ED tumors. The stem cells of murine teratomas, the EC cells, are pluripotent; they are capable of forming derivatives of all 3 germ layers and can continue to proliferate in the undifferentiated state. Tumors in which all EC cells are differentiated (or have died) are called teratomas, and they are considered benign. Teratocarcinomas are composed of a teratoma component and EC cells, and they are considered malignant. In addition ED tumors may develop along an extraembryonic lineage to form yolk sac tumors (4).

We recently described the histological and biological findings of 101 embryo transplantations (5). In total, 36 ED tumors contained EC cells and were classified as teratocarcinomas. The 45 ED tumors without EC cells could be histologically subdivided into 2 groups: those composed of somatic tissue only and those composed of PYS tumor, most often combined with VYS elements, T, and sometimes Tr elements. Surprisingly some tumors of the latter group proved to be retransplantable for several generations.

The purpose of this study was to investigate whether chromosomal abnormalities are present in primary ED tumors and to determine their possible role in oncogenesis.

MATERIALS AND METHODS

Tumors were induced by transplanting 7-day-old embryos under the kidney capsule of adult male and female BALB/c recipients using the technique described by Damjanov et al. (6). The mice were killed when the tumor reached a size of about 1.5 to 2.5 cm or randomly during the 225- to 365-day period after embryo transplantation. Representative samples of primary ED tumors were taken for retransplantation, histology, and tissue culture in order to analyze the chromosomal constitution of the tumors.

Paraffin-embedded sections of all tumors were made and stained with hematoxylin and eosin. On the basis of the criteria and nomenclature proposed by Stevens and Pierce (7) all tumors were histologically classified as either teratocarcinoma, teratoma, or yolk sac tumor which always contained PYS ± VYS ± T ± Tr (8).

For culture fresh tumor tissue was disaggregated by mincing with scissors and incubating in 0.8% collagenase II (Worthington Diagnostic Systems, Inc., Freehold, NJ). The disaggregated tumor cells were washed once with culture medium (RPMI-1640 [Gibco] supplemented with 15% fetal calf serum [Seralab], 2 mM glutamine [Gibco], 100 units/ml of penicillin [Gibco], and 100 μg/ml of streptomycin [Gibco]), seeded in T75 tissue culture plastic flasks (Corning), and incubated at 37°C in a humidified atmosphere with 5% CO2 in air. The next day the medium was removed and centrifuged, and 50% of it was returned together with 50% fresh medium. Until harvesting, the cultures were maintained by partly changing the medium and, when necessary, by passaging.

Cytogenetic preparations were made by conventional methods, and G banding was performed by a slight modification of the method used by Wang and Fedoroff (8). Chromosomes were classified using descriptions made by Cowell (9) and arranged according to the standard mouse karyotype (10). To describe structural abnormalities the Nesbit and Francke nomenclature (11) was used. According to standard rules, an abnormality is considered clonal if it is found in at least 2 or 3 tumor karyotypes: the first criterion is used for extra copies of a chromosome and structural abnormalities; the second criterion is used for missing copies of a chromosome. To determine numerical abnormalities in polyploid cells, the number of copies present was compared with the expected number of copies (e.g., in a 2N cell, one would expect 2 copies; in a 3N cell, 3 copies, and so on). The ploidy level of a cell was determined using the following arbitrary classes: 30 to 49 chromosomes = 2N; 50 to 69 chromosomes = 3N; 70 to 89 chromosomes = 4N; 90 to 109 chromosomes = 5N; and 110 to 129 chromosomes = 6N.

Flow cytometry was performed on frozen tissue of primary and/or retransplanted ED tumors. Nuclei were isolated using a detergent trypsin method and stained with propidium iodide (12). Measurements were obtained with the FACScan flow cytometer (Becton Dickinson). As an internal standard trout red blood cells were added to the suspensions.

RESULTS

As described 101 embryos were transplanted under the kidney capsule of syngeneic male and female BALB/c recipients (5). All ED tumors were histologically classified as either teratocarcinoma, teratoma, or yolk sac tumor. In total 36 teratocarcinomas, 18 teratomas, and 27 yolk sac tumors were found. Some of the yolk sac tumors proved to be retransplantable for several generations.

Karyotyping was performed after culture of the primary ED tumors, and usually 10 metaphases were examined. The results of cytogenetic analysis are summarized in Table 1. All teratocarcinomas, 18 teratomas, and 27 yolk sac tumors were found. Some of the yolk sac tumors proved to be retransplantable for several generations.
tant teratocarcinoma showed polyploidy. Cytogenetic analysis of teratocarcinomas, induced in female recipients, was more difficult because of poor banding quality and/or a low yield of metaphases. In 5 of 15 teratocarcinomas consistent chromosomal abnormalities were found (Table 2). Loss of a Y chromosome was found in 2 different tumors (E84-207 and E84-455). An extra copy of an autosome was found in 2 other tumors: a trisomy 19 in Tumor E84-166 and 3 copies of chromosome 8 (an isochromosome and a normal copy) in Tumor E84-206. The structurally abnormal chromosome 5 found in another teratocarcinoma (E84-476) is probably the result of a translocation or a duplication.

Teratomas were always small and therefore difficult to culture. Yolk sac tumors were of varying sizes, and only the larger tumors could be cultured. In total, 3 of 18 teratomas and 9 of 27 yolk sac tumors could be karyotyped. Contrary to the teratocarcinomas, almost all (10 of 12) karyotyped primary ED tumors without EC cells were cytogenetically abnormal. The chromosomal abnormalities of the teratomas and yolk sac tumors are summarized in Tables 3 and 4, respectively. All aberrant teratomas (n = 3) and yolk sac tumors (n = 7) were extremely heterogeneous, and polyploidy was a common phenomenon. The ploidy level differed considerably among and within the different tumors (ploidy levels of about 3N, 4N, 5N, and even 6N were observed), making the analysis of numerical abnormalities very difficult. Considering all ploidy levels within a tumor, an overrepresentation of one or more of the following chromosomes (Nos. 1, 6, 15, and 19) was found in 7 tumors, whereas one or more of the following chromosomes (Nos. 2, 4, 14, and a sex chromosome) were underrepresented in 7 tumors. Although other chromosomes were also found to be over- or underrepresented in several tumors, the mentioned ones are, in our view, of special interest because chromosomes 1 and 15 were never found to be clonally underrepresented in a tumor; chromosomes 2, 4, and 14 were never found to be clonally overrepresented in a tumor; and the numerical abnormalities of chromosomes 6, 19, and a sex chromosome were found through all ploidy levels (including 2N cells) and described as aberration in teratocarcinomas.

Structural abnormalities involving one or more of the following chromosomes (Nos. 1, 4, and 13) were found in several tumors. Clonal structural abnormalities of chromosome 1 were found in 2 tumors (one teratoma and one yolk sac tumor; Figs. 1 and 2, respectively); in another 4 tumors, nonclonal abnormalities were found. In all 6 tumors the abnormal chromosome 1 was found in addition to the expected number of copies of chromosome 1. One teratoma and 2 yolk sac tumors had a clonal structural abnormal chromosome 4, with breakpoints in Bands D, E (see Fig. 3), and E2 (see Fig. 2), respectively. In another 2 cases, nonclonal abnormalities were found with breakpoints in Bands D and E2. The presence of an abnormal chromosome 4 actually meant an underrepresentation of the distal half of chromosome 4. Seven tumors missed either a whole copy or parts of chromosome 4. A clonal aberration of chromosome 13 was found in 2 different yolk sac tumors with, in both cases, the break in Band D (Band D, respectively, D2).

Flow cytometry was performed on frozen tissue of primary ED tumors, 34 teratocarcinomas, and only 6 yolk sac tumors. Investigation of all karyotyped primary teratocarcinomas revealed that they all had a diploid DNA content. This is in agreement with the chromosomal findings with one exception (Tumor E86-426) in which karyotypically 2 of 6 cells were tetraploid.

The results of flow cytometry of the yolk sac tumors are ambiguous. Some of the primary yolk sac tumors (E86-874, E86-893, and E86-905), which were karyotypically polyploid,
did not show aneuploidy in the flow pattern. Two yolk sac tumors (E86-580 and E86-875) had, according to the flowcytometric measurement, a cell population with a DNA content of more than 2N. Tumor E86-580 had a DNA index of about 1.32, which is equal to about 53 chromosomes, while all polyploid karyotypes had 87 or more chromosomes. For tumors E86-875 and E86-615, the values of flow cytometry (DNA index = 1.86 and 1.0, respectively) and karyotype analysis were in the same range.

**DISCUSSION**

Chromosomal abnormalities are regularly associated with human and animal malignancies (for review, see Refs. 13 to 15). A number of spontaneous and induced teratocarcinomas have been investigated cytogenetically. Most of them have a near-diploid chromosomal complement. Some of the teratocarcinomas even were chromosomally normal (16–18), although they all were studied after in vivo and/or in vitro passing. Data of 13 different aberrant teratocarcinomas (e.g., EC cell lines and their derivatives) reveal several common chromosomal abnormalities: loss of a sex chromosome; trisomy of chromosomes 6, 8, and 11; a deletion of chromosome 14; and an elongation of chromosome 1 (4, 16, 19–24). All except one of the chromosomally abnormal teratocarcinomas had at least one of the above-mentioned abnormalities.

In this paper we describe the cytogenetic findings of primary ED tumors. In total, 34 of 36 teratocarcinomas, 3 of 18 teratomas, and 9 of 27 yolk sac tumors could be karyotyped.

Contrary to already published results, in this study the majority of the teratocarcinomas were chromosomally normal (82%). Flow cytometry on frozen tissue of primary teratocarcinomas confirmed the karyotypic findings: all had a diploid DNA content. This discrepancy with the literature is probably due to duration of culture time. We karyotyped the primary teratocarcinomas after a short culture time (less than 45 days) while, in the studies cited from the literature, karyotyping was performed on established EC cell lines. So it might be that the chromosomal abnormalities of malignant teratomas described in the literature are associated with in vitro karyotype evolution rather than with teratocarcinogenesis and/or tumor progression. To investigate this possibility we now cytogenetically investigate retransplantation generations of our primary teratocarcinomas. It is of interest that 5 of 6 primary teratocarcinomas with a chromosomal aberration all originated in a female recipient. In 3 of them, one of the above-described common chromosomal abnormalities was found: loss of a Y chromosome in 2 tumors and a trisomy 8 in one tumor.

The cytogenetic findings of the teratomas and yolk sac tumors we karyotyped are in striking contrast to those of the teratocarcinomas. In total, 10 of 12 primary ED tumors without EC cells were chromosomally highly abnormal. All, except one, aberrant tumors were polyploid. To investigate whether polyploidization occurred in vivo or in vitro, flow cytometry was performed on frozen tissue of 6 yolk sac tumors. In 3, the flowcytometric results were in agreement with the karyotypic data, but in the other 3, flow cytometry revealed only a diploid cell population while the karyotypes showed polyploidy, so appar-

<table>
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<th>Tumor</th>
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<th>Recipient</th>
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<th>Harvest time (days)</th>
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**Table 4** Karyotype analysis of primary ED yolk sac tumors with an abnormal chromosomal constitution (7 of 9)

* T, teratoma component; P, parietal yolk sac component; V, visceral yolk sac component. 
* N, ploidy level of cells determined by using arbitrary classes; range, chromosome numbers found in that tumor for that ploidy level; n, number of cells of that ploidy level. 
* Over- and underrepresentation considering all ploidy levels of that tumor; between parentheses are the number of cells in which the abnormality was found; *, the mentioned chromosome clonally over- or underrepresented in more than one copy; S, sex chromosome.
ently here the polyploidization occurred in vitro. Some numerical abnormalities as well as structural abnormalities involving the same chromosome were found in different tumors. In 7 tumors one or more of the following chromosomes (Nos. 1, 6, 15, and 19) were overrepresented. Trisomies of chromosomes 6 and 15 have been described in teratocarcinomas (4, 16, 19, 20, 23), lymphoid tumors, and myeloid leukemias (25). On both, chromosome homeoboxes and oncogenes, known to be important in normal proliferation and differentiation, are located (26, 27). Inappropriate expression of such genes might be important for tumorigenesis. In 7 tumors an underrepresentation of one or more of the following chromosomes (Nos. 2, 4, 14, and a sex chromosome) was found. Loss of a sex chromosome has been described in several animal tumor systems, and it is thought to be a secondary aberration (15, 28). Partial deletion of chromosome 2 has been described for myeloid leukemia (29) and is associated with the genesis of such tumors in mice. Partial deletion of chromosome 14 has been described for teratocarcinomas (20, 21, 23, 24). Although the biological significance of this abnormality remains obscure, it seems to be restricted to germ cell tumors. The possible meaning of the loss of chromosome 4 will be discussed later. In 6 tumors an aberrant chromosome 1 was found in addition to the expected number of copies of chromosome 1. The common part of chromosome 1 that was overrepresented in the only 2 clonal cases is Band 1D to Band 1H. Probably the distal part of chromosome 1 contains genes important for tumor progression and/or oncogenesis. In this respect it is of interest that the oncogene bcl-2 has been mapped to chromosome 1, but the exact location is not known (30). A study by Mock et al. (31) revealed linkage of bcl-2 to the Idh-1/Pep-3 region of murine chromosome 1. This implies that bcl-2 is located in the upper half of chromosome 1 (26). An abnormal chromosome 4 was found in 5 different tumors, in all cases resulting in an underrepresentation of the distal part of chromosome 4 (Band 4E2 to 4qter). In another 2 tumors, whole copies of chromosome 4 were missing. These findings might suggest that a suppressor gene is located on the distal part of chromosome 4. Evans et al. (32) found that suppression of malignancy in hybridoma cells was the result of either the loss of chromosome 4 from the normal fibroblasts or the gain from chromosome 4 of the tumor cells. They concluded that a gene on the normal chromosome 4 is responsible for the suppression of malignancy in a dose-dependent manner. These results are interesting, not only because they showed that different tumors may have a genetic lesion in common, but also because regulation in a dose-dependent man-
Fig. 2. G-banded karyotype of yolk sac tumor (E86-893). Arrows, aberrant chromosomes.

Fig. 3. G-banded karyotype of yolk sac tumor (E86-905). Arrows, aberrant chromosomes.
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REFERENCES

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