[CANCER RESEARCH 49, 672–676, February 1, 1989]

Chromosomal Changes in Mature Residual Teratomas following Polychemotherapy[1]

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ABSTRACT

A cytogenetic analysis of 13 mature residual teratomas following chemotherapy revealed modal chromosome numbers ranging from 52 to 85, in agreement with the flow cytometric determination of the DNA content of the tumors. At least one copy of an i(12p) was present in 12 tumors. One tumor, however, lacked that marker.

The comparison between the chromosomal abnormalities found in mature residual teratomas following chemotherapy and those from primary testicular nonseminomas suggests that residual teratomas result from selection of clones from the primary tumor with a less abnormal karyotype.

INTRODUCTION

Untreated metastases of nonseminomatous germ cell tumors of the testis rarely consist exclusively of fully differentiated, mature somatic tissues (1–4). However, the residual metastases after polychemotherapy often contain only differentiated teratoma (5–8). The mechanism(s) involved in this therapy-related differentiation are not yet clear (see Reference 9 for review).

Cytogenetic comparison between mature residual teratomas following chemotherapy and primary testicular nonseminomas may shed light on the mechanism(s) of therapy-related differentiation.

Recently, we described our cytogenetic findings in primary testicular nonseminomas.[4] For comparison in Table 1 the modal number of normal chromosomes and i(12p) in 14 primary testicular nonseminomas[4] is given. Here we report on the chromosomal changes in 13 mature teratomas following chemotherapy.

MATERIALS AND METHODS

The tumors were submitted fresh and sterile and were processed for tissue culture and DNA flow cytometry, basically as described (10). For chromosome preparations the tumor cells were harvested either by brief trypsinization (10) or according to the procedures of Gibas (11). The pellets from the cells harvested as described by Gibas were immediately resuspended in fixative. In both methods there was a final centrifugation, after which cells were resuspended and pipetted onto slides and air dried. Chromosomes were GAG and/or GTG banded.

For a statistical evaluation of the chromosomal findings, the number of normal copies per chromosome was analyzed for 13 cases with a two-way analysis of variance. The average number of i(12p) per mature residual teratoma was compared with the average number of i(12p) per primary nonseminoma in our previously reported series[4] using the one-sided Mann-Whitney U test of significance.

RESULTS

Patient age, number of analyzed metaphases, modal chromosome numbers, and DNA index for each case are given in Table 2.

Karyotypes. A representative karyotype of each case is described in Table 3. The numerical abnormalities are given in Table 4. Figs. 1–3 show representative karyotypes of, respectively, Cases 4, 10, and 13.

Statistical Analysis. The number of chromosomes found is partly random, and partly dependent upon differences between persons and differences between chromosomes. These systematic effects account for about 50% of the total variance, and are statistically highly significant when tested with two-way analysis of variance.

To indicate the effect of the relative numbers of each chromosome, Fig. 4 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 7, 12, 21, and X are more frequently found than the normal copies of chromosomes 10, 14, 18, 22, and Y.

The one sided Mann-Whitney U test showed a significantly ($P < 0.05$) lower number of copies of i(12p) in residual teratomas as compared to our series of primary nonseminomas.[4]

DISCUSSION

Untreated metastases of nonseminomatous germ cell tumors usually retain the morphological appearance of the primary tumor (see Reference 12 for review). As is the case in primary nonseminomas, such metastases rarely consist exclusively of fully differentiated mature somatic tissue (1–4). However, after polychemotherapy there is an apparent shift towards higher degrees of differentiation (5–8). This can be achieved by three possible mechanisms: (*a*) Selective destruction of components other than mature teratoma (5, 6, 8, 13); (*b*) Direct induction of differentiation of malignant cells (6, 8, 13, 14); (*c*) Spontaneous differentiation of the malignant cells made possible or facilitated by chemotherapy (13).

Two mechanisms, *a* and *c*, are essentially similar and based on selection: there is selection of already existing mature teratoma in *a* and of cells with an inherent capacity of spontaneous somatic differentiation in *c*. Thus, actually only two basically different mechanisms remain to be considered: induction of differentiation or selection. These two mechanisms are not mutually exclusive.

In myeloid leukemic cells it has been suggested that, irrespective of their chromosomal constitution, the difference between cells that could and cells that could not be induced to

Received 7/5/88; revised 10/7/88; accepted 10/18/88.

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[1] This work was supported by Grants GUKC 84-06 and GUKC 88-10 from the Netherlands Cancer Foundation (K. W. F.), by the Jan Kornelis de Cock Stichting, and by an institutional grant from the Hubrecht Laboratorium, Utrecht, The Netherlands.

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[4] S. M. M. J. Castedo, B. De Jong, J. W. Oosterhuis, *et al.* Chromosomal changes in primary testicular nonseminomas, submitted for publication.

Table 1 *Modal number of normal chromosomes and i(12p) in 14 primary testicular non seminomas*

Case	Modal number of normal copies of chromosomes per tumor																							No. of i(12p)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
1	3	2	3	2	3	2	4	4	2	2	2	4	2	2	3	2	3	2	2	3	4	2	2	1	2
2	2	2	2	2	2	3	4	2	2	2	1	3	2	2	2	2	2	2	2	2	2	2	1	1	0
3	3	3	2	2	2	2	3	2	2	2	2	3	2	1	2	2	2	2	2	1	3	2	2	1	2
4	2	3	3	3	2	3	3	3	2	2	1	3	2	3	2	3	3	2	2	2	3	2	3	1	3
5	1	3	3	2	2	3	3	2	2	2	2	3	2	2	2	3	3	2	2	2	4	3	4	1	3
6	2	2	3	2	2	3	2	2	2	2	3	3	2	2	2	3	3	2	2	2	2	2	1	2	3
7	4	4	5	4	3	3	2	5	3	2	2	4	4	4	4	3	3	4	3	6	4	1	4	2	4
8	2	3	2	2	2	3	2	3	2	2	2	3	2	2	3	2	3	2	2	2	2	2	2	2	3
9	2	2	2	2	3	2	3	3	2	2	1	3	2	3	2	2	3	2	2	2	2	2	2	2	3
10	2	1	3	1	2	2	3	4	2	2	1	3	2	3	2	2	3	2	2	4	5	3	2	0	1
11	1	2	0	2	2	1	2	3	2	2	2	3	2	1	2	3	2	1	2	2	0	1	1	0	0
12	2	3	5	3	3	4	4	3	3	3.5	3.5	5	4	4	2	3	4.5	3	4	5	5	3	3	2	4
13	2	2	3	2	2	2	3	3	2	2	2	3	2	2	2	2	3	2	2	3	3	2	2	1	4
14	4	5	4	3	4	5	5	5	4	4	3	6	5	5	4	4	5	4	4	4	6	4	5	2	2
Total	32	37	40	32	34	38	43	44	32	31.5	27.5	49	35	36	34	36	42.5	32	33	40	45	31	34	18	34

Table 2 *Summary of the patient and cytogenetic data of the 13 cases*

Case	Patient age (yrs)	No. of analyzed metaphases[a]	Modal number[b]	DNA index
1	32	7(7)	78	1.65
2	27	18(18)	60	1.32/1.21[c]
3	50	8(10)	62.5	1.45
4	32	13(13)	58	1.26
5	31	3(3)	62	1.37
6	21	4(10)	63	1.39
7	20	14(14)	53	1.15
8	26	6(6)	57	1.25
9	54	9(11)	52	1.22
10	33	9(9)	57	1.23/1.50[c]
11	24	18(18)	85	1.87
12	30	9(9)	62	1.36
13	24	9(10)	57	1.40

[a] First row, abnormal metaphases; between parentheses, total number of metaphases analyzed.
[b] The modal chromosome number is deduced from the amount of analyzed abnormal metaphases.
[c] Accessory stemline.

differentiate was controlled by the balance between genes that allow (induction of) differentiation and genes that suppress differentiation (15, 16). In cells that could not be induced to differentiate chromosomal changes resulting in a different gene balance were able to suppress malignancy by restoring the ability of the cells to (be induced to) differentiate.

Mature residual teratoma following intensive chemotherapy of nonseminomas might be the result of selection of tumor cells with an abnormal chromosomal constitution that still would allow spontaneous or induced differentiation. The selected cells might either belong to early evolutionary clones (with a low malignant potential and inherent capacity of somatic differentiation) that have been overgrown by more malignant ones, or from later, more malignant clones which obtained through loss and or gain of specific chromosomes the right balance to make possible (induction of) differentiation. Alternatively, the mature residual tumor tissue may be the result of therapy-related induction of differentiation in tumor cells irrespective of their chromosomal pattern and inherent capacity to differentiate.

If mature residual teratomas are the result of differentiation of selected cells with an although abnormal chromosomal pattern, but with a proper balance of genes allowing induction of differentiation one might expect specific differences between the chromosomal constitutions of primary nonseminomas and residual teratomas. However, if induction of differentiation is possible irrespective of the chromosomal pattern, no such differences would be expected.

As was the case in primary nonseminomas, most residual teratomas have between 60 and 64 chromosomes, in agreement with the flow cytometric determination of the DNA content of these tumors (Table 2). However, residual teratomas differ from primary nonseminomas in the chromosomes under- and over-represented. In residual teratomas the underrepresentation of chromosome Y is greater than in primary nonseminomas,[4] whereas the underrepresentation of normal copies of some specific chromosomes (*e.g.*, 9 and 11), as well as the overrepresentation of others (*e.g.*, 12 and X), is smaller than in primary nonseminomas. Similar discrepancies were observed in two different cases where both the primary tumors and residual

Table 3 *Karyotypical description of a representative metaphase from each case*

Case 1 78, X, −Y, +X, +1, +1, +2, +3, +4, +5, +6, +6, +7, +8, +8, +9, +9, +10, +10, +11, +12, +13, +13, +14, +15, +16, +17, +17, +18, +19, +20, +21, +21, +22, +22, +i(12p). (also clonal M(der(18)?)
Case 2 59, XY, +X, +1, +6, +8, +11, +12, +16, +17, +21, +22, +i(12p), +i(12p), +M.
Case 3 62, XY, +X, +1, +3, +5, +6, +7, +9, +10, +12, +16, +17, +19, +21, +i(12p), +i(12p), +del(22)(q12).
Case 4 58, X, −Y, +X, +6, +7, +8, +12, −13, +21, +der(1)t(1;?)(p36;?),+del(2)(q35), +i(12p), +der(17)t(13;17)(q11;q23), +del(22)(q11), +M1, +M2(der(9)?), +M3.
Case 5 62, XY, +X, +3, +6, +7, +8, +9, +12, +16, −17, +21, +21, +22, +der(1)t(1;3)(p32;p21), +der(7)t(5;7)(q13;q22), +i(12p), +i(12p), +i(12p), +der(17)t(17;?)(q25;?).
Case 6 65, XY, +X, +1, +2, +3, +6, +7, +7, +8, +8, −10, +12, +13, +17, +20, +21, +22, +del(1)(q41), +der(9)t(9;?)(p13;?),+i(12p), +del(16)(p13), +M1.
Case 7 53, X, −Y, +X, −5, +7, +8, −10, +12, −18, +del(1)(p34), +der(5)t(3;5)(q21;p15), +der(7)t(7;?)(q22;?), +der(10)t(10;?)(q26;?), +del(17)(p11), +M1(18qter→q11::?), +M2.
Case 8 57, XY, +X, +1, +5, +6, +7, +8, −9, −10, +12, +17, −18, +20, +i(12p), +del(18)(p11), +M1, +M2(9qter→q11::?), +M3.
Case 9 54, XY, +X, +7, +8, −12, +17, +21, +del(1)(p35?), +der(12)t(12;?)(p13;?), +i(12p),+i(12p).
Case 10 57, XY, +X, −1, +6, +7, −8, +12, −14, +17, +21, +21, +der(1)t(1;?)(p34;?), +der(1)t(1;?)(p11;?), +del(8)(p22), +i(12p), +i(12p), +M1, +M2.
Case 11 87, XY, +X, +1, +1, +2, +2, +3, +3, +3, +4, +5, +8, +8, +9, +9, +10, +10, +12, +12, +13, +13, +14, +14, +15, +16, +17, +17, +18, +19, +20, +20, +21, +21, −22, −22, +der(5)t(5;?)(q31;?), inv(7)(p15; p22), +der(7)t(7;7)(p22;q11), +der(7)t(7;?)(p11;?),del(10)(p13), +der(11)t(11;?)(q25;?), +del(12)(q15 q24), +i(12p), +i(12p), +i(12p), +i(22q), +M1(der(7)?), +M2.
Case 12 64, XY, +X, +Y, +1, +2, +3, +7, +7, +8, +9, +12, +13, +17, +20, +21, +21, +i(12p), +M1(5qter→q13::?), +M2. (also clonal:del(7)(q31).
Case 13 57, XY, +2, +3, +5, +7, +8, +12, +16, +17, +der(1)t(1;?)(p36;?), +i(12p), +i(12p).

Table 4 *Modal number of normal copies of chromosomes and of i(12p) per case*

Case	Modal number of normal copies of chromosomes																							No. of i(12p)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
1	3.5	3	3	3	3	4	3	4	3.5	4	3	3	4	3	3	3	4	2	3	2.5	4	3	2	0	1
2	2	2	2	2	2	2.5	2.5	2.5	2	2	3	3	2	2	2	3	3	2	2	2	3	3	2	1	2
3	3	3	2.5	2.5	2.5	3	3	2	2.5	3	2.5	2.5	2	2	2.5	2.5	3	2	2.5	2	3	2	2	1	2.5
4	2	2	2	2	2	3	3	3	2	2	2	3	1	2	2	2	2	2	2	2	3	2	2	0	2
5	2	2	3	2	2	3	2	3	3	2	2	3	2	2	2	3	2	2	2	3	4	2	2	1	3
6	3	3	3	2	2	3	4	4	2	1	2	3	2	2	2	2	3	2	2	3	3	3	2	1	1
7	2	2	2	2	1	2	3	3	2	1	2	3	2	2	2	2	2	1	2	2	2	2	2	0	0
8	3	2	2	2	3	3	3	3	1	1	2	3	2	2	2	2	3	1	2	3	2	2	2	1	2
9	2	2	2	2	2	4	3	2.5	2	2	2	1	2	2	1.5	2	3	2	2	2	2	2	2	1	2
10	1	2	2	2	2	3	3	1	2	2	2	3	2	1	2	2	3	2	2	2	4	2	2	1	2
11	4	4	5	3	3	2	1	4	4	2.5	2	4	4	4	3	3	4	3	3	3.5	4	0	2	1	3
12	2	3	3	2	2	2	4.5	2	3	2	2	3	2	2	2.5	3	2	2	2	3	4	2	2	2	1
13	2	3	3	2	3	2	3	3	2	2	2	3	2	2	2	3	3	2	2	2	2	2	1	1	2

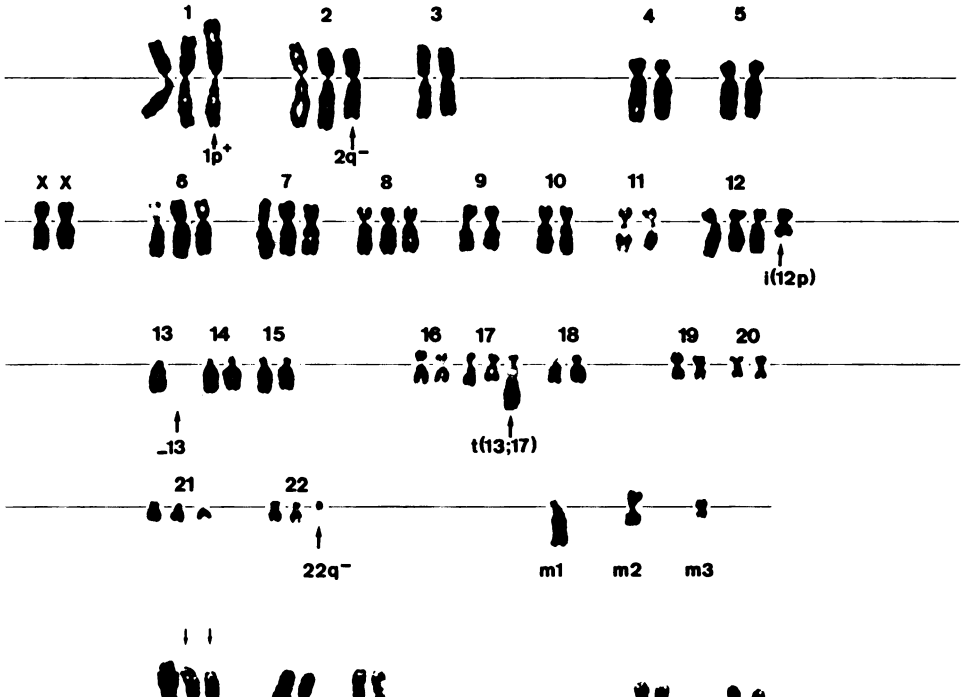

Fig. 1. Representative karyotype of Case 4: 58, X, −Y, +X, +6, +7, +8, +12, −13, +21, +der(1)t(1;?)(p36;?), +del(2)(q35), +i(12p), +der(17)t(13;17) (q11;q23), +del(22)(q11), +M1, +M2(der(9)?), +M3.

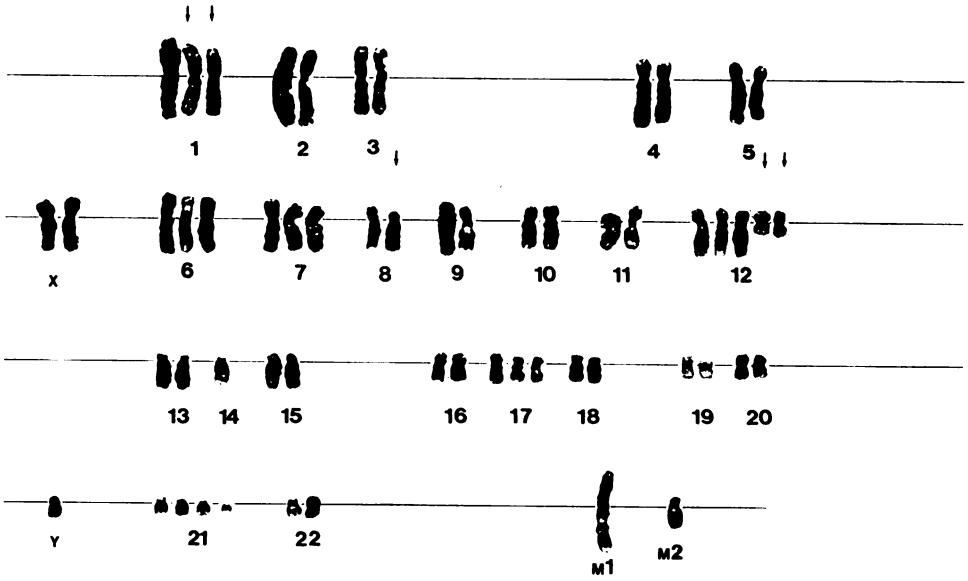

Fig. 2. Representative karyotype of Case 10: 57, XY, +X, −1, +6, +7, −8, +12, −14, +17, +21, +21, +der(1)t(1;?)(p34;?), +der(1)t(1;?)(p11;?), +del(8)(p22), +i(12p), +i(12p), +M1, +M2.

teratomas were karyotyped (17).[5] Although these differences are difficult to interpret in such a small sample, it is conceivable that chromosomes present in residual teratomas in higher numbers than in primary nonseminomas contain genes important for normal differentiation, whereas chromosomes present in higher numbers in primary nonseminomas may contain genes responsible for a more malignant development.

As can be seen in Table 3, chromosome 12 is the chromosome most often involved in structural abnormalities in residual teratomas, as previously noted in seminomas (18)[6] and primary

[5] B. De Jong, *et al.*, unpublished data.

[6] S. M. M. J. Castedo, B. De Jong, J. W. Oosterhuis, R. Seruca, G. J. Te Meerman, A. Dam, and H. S. Koops. Cytogenetical analysis of ten seminomas, submitted for publication.

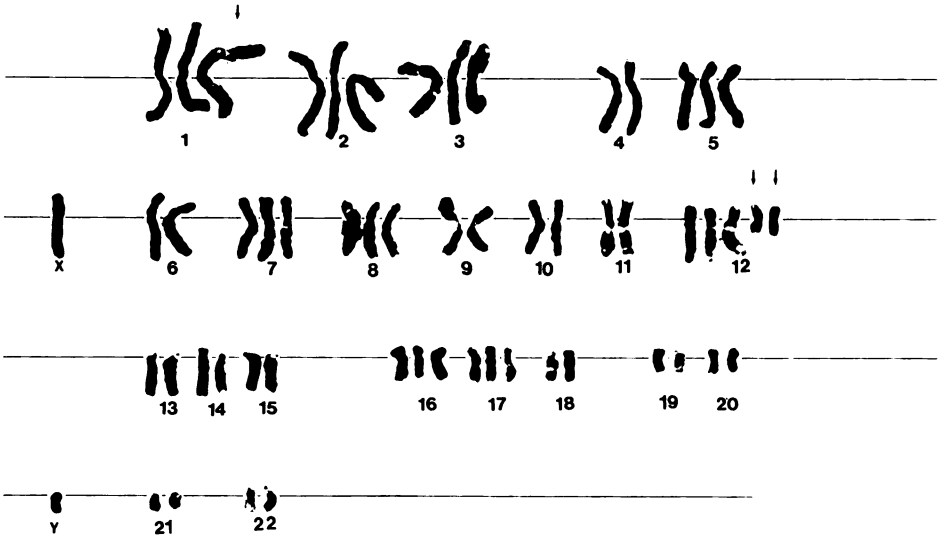

Fig. 3. Representative karyotype of Case 13: 57, XY, +2, +3, +5, +7, +8, +12, +16, +17, +der(1)t(1;?)(p36;?), +i(12p), +i(12p).

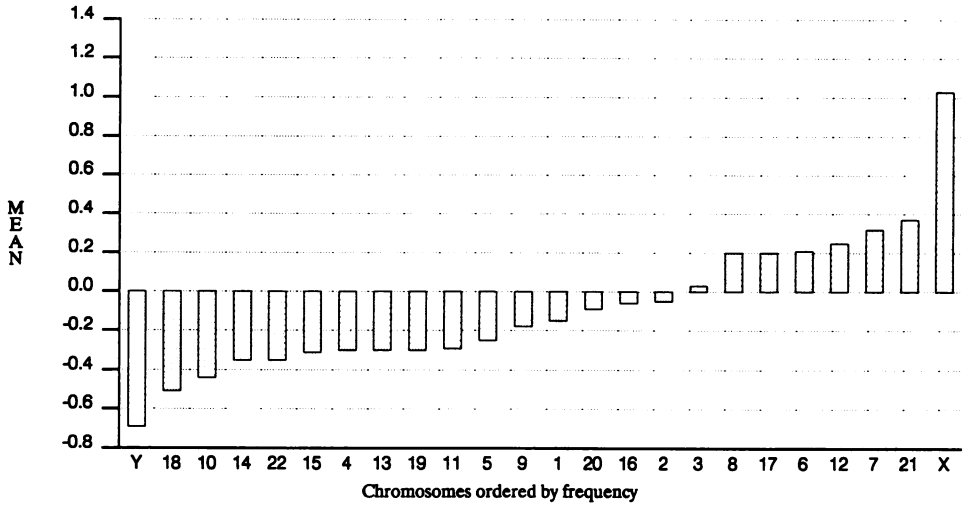

Fig. 4. Average of the standardized number of normal copies of chromosomes per case (compared to 2). Every case was given equal weight in terms of chromosomal counts, which was set arbitrarily to 46.

nonseminomas (19, 20).[4] 12 out of 13 tumors had one or more copies of the i(12p), a specific marker for germ cell tumors of the testis (18–22) and possibly also of the ovary (23, 24). It is of interest that the average number of i(12p) per tumor is significantly smaller in residual teratomas (1.6; $n = 14$) than in primary nonseminomas (2.3; $n = 15$). This finding is in agreement with the contention that the number of copies of i(12p) correlates with an increased malignancy (20).

At variance with our findings in primary nonseminomas[4] and seminomas,[6] only seven out of 13 residual teratomas had rearrangements of chromosome 1 (as opposed to 13 out of 14 primary nonseminomas and eight out of 10 seminomas), and only one with a breakpoint at 1q. Moreover, in the present series of residual teratomas we noted 61 different markers involving 36 different breakpoints (Table 3), as opposed to, respectively, 91, and 73 in our study of primary nonseminomas.[4] It is also remarkable that structural abnormalities, usually considered signs of expression of malignancy, are less frequent in residual teratomas (average, 4.7) than in primary nonseminomas[4] (average, 6.5). On the other hand, 25 out of the 36 different bands involved in structural rearrangements in residual teratomas were also implicated in primary nonseminomas,[4] which stresses the relationship between both groups.

Thus, our findings suggest that residual teratomas following intensive chemotherapy are the result of selection of clones with a less abnormal karyotype and possibly the right balance of genes allowing differentiation.

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