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Trisomy of Chromosome 15 in Spontaneous Leukemia of AKR Mice

(mouse thymomas/karyotype analysis/trypsin–Giemsa banding)

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ABSTRACT **Karyotypes of spontaneous thymomas of AKR mice were determined by trypsin–Giemsa banding methods. Trisomy of chromosome 15 occurred in 10 of 11 leukemic mice. Seven of the thymomas were predominantly trisomic for chromosome 15, one was trisomic for chromosome 12, and one exhibited multiple trisomies of chromosomes 3, 12, 15, and 17. Trisomy was not found in the normal AKR tissues examined.**

The Ak mouse strain, initiated by Furth in 1933 (1), has played a central role in leukemia research. Greater than 80% of mice of this strain develop leukemia of lymphoid type by 10 months of age. Several key findings have come from the study of Ak mice and the AKR substrain, e.g., recognition that the thymus is essential for leukemia development (2), identification of the first mammalian leukemia virus (3), evidence that the leukemia virus is integrated in the host genome and is transmitted as a Mendelian characteristic (4), etc. Although much has been learned about genetic and environmental factors that influence expression of the leukemia virus genome and the development of leukemia, the critical events whereby infected lymphoid cells are transformed into leukemic cells are unknown. Immunological analysis has identified a series of antigens related to the leukemia virus (5). As these are found in infected "normal" cells as well as in leukemia cells, none of the antigens discovered thus far is transformation-specific, i.e., found only in or on leukemia cells. Biochemical analysis has similarly failed to disclose qualitative differences between normal and leukemia cells.

Chromosome analysis of AKR leukemia performed by Stich and his colleagues in 1959 revealed that a proportion of the leukemia cells had an extra chromosome (6). This remarkable observation, which was confirmed by others (7, 8), could not be extended at that time because of the well-known difficulty of distinguishing mouse chromosomes one from another. The advent of new fluorescence and Giemsa staining methods (9–11) whereby chromosomes may be individualized by differential banding patterns along their lengths now allows the investigator to identify the 20 chromosome pairs in the mouse (12, 13) and to assign, by means of translocation stocks, linkage groups to particular chromosomes (14, 15). These technical advances prompted us to reexamine the karyotype of AKR leukemias to see whether a consistent chromosomal abnormality might accompany leukemogenesis in this strain.

Abbreviation: PBS, phosphate buffered saline.

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MATERIALS AND METHODS

Mice. AKR/J females were obtained from the Jackson Laboratory, Bar Harbor, Me., and were maintained in our laboratory until they developed leukemia.

Karyotype Analysis. For the preparation of metaphase cells, mice were injected intraperitoneally with 0.3 ml of 0.001% colchicine. After 30 min, the thymus was finely minced in phosphate-buffered saline (Dulbecco formulation) without added calcium and magnesium. Cells were pelleted and resuspended in hypotonic 0.56% KCl for 8 min at 37°. Methanol/acetic acid (3:1) fixative was added slowly to the cell suspension. Cells were rinsed with three changes of fixative, spread onto wet glass slides, and air-dried. Metaphase preparations were stained by a modification of the method of Seabright (11). Slides were treated with 0.2% trypsin in phosphate-buffered saline (pH 7.3), rinsed twice in this buffer, and stained with Gurr's Giemsa R66 diluted 1:50 in phosphate–citrate buffer (pH 6.8) for 25 min. Karyotypes were prepared according to a standardized system for the mouse, *Mus musculus* (15).

RESULTS

Chromosome Number of AKR Thymomas. From a total of 39 primary lymphomas described in earlier studies (7, 8, 16), analysis of neoplastic cells from thymus, spleen, or lymph nodes revealed modal chromosome numbers of 40 for 61% of mice, 41 for 36%, and 43 for one mouse. Some "diploid" leukemias also comprised cells with 41 or 42 chromosomes or with structurally abnormal (marker) chromosomes. This distribution is essentially what we found in an initial survey of 35 individual AKR thymomas, with the use of conventional staining techniques for determination of chromosome number, and indicated that at least one-third of our AKR stock with thymomas would be apt to yield cells with 41 chromosomes.

Karyotypes of AKR Thymomas. Results of karyotype analysis by trypsin-Giemsa banding procedures are summarized in Table 1. Of the 11 thymomas examined, 10 showed trisomy of chromosome 15 in some proportion of cells (Fig. 1). Seven tumors (mouse nos. 1, 2, 3, 10, 11, 20, and 25) were predominantly trisomic for chromosome 15. One thymoma (mouse no. 14) was characterized by trisomy of chromosome 12, while another (mouse no. 21), which was partially trisomic for chromosome 15, was consistently trisomic for chromosome 12. This thymoma was also characterized by trisomy of chromosome 17 and, to a lesser extent, chromosome 3. For mouse

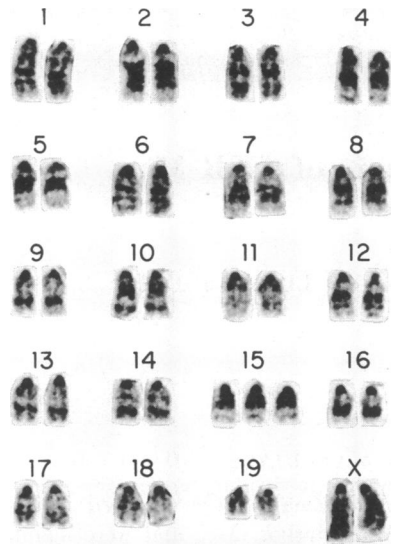

FIG. 1. Trisomy of chromosome 15 in a metaphase cell from thymoma of mouse no. 2.

no. 7, where the modal chromosome number was 39, all cells examined were lacking one X chromosome; two cells were trisomic for chromosome 15. Three metaphases from this thymoma showed a translocation to the distal end of chromosome 14; the origin of the translocated segment could not be identified. One tumor (mouse no. 5) had a modal chromosome number of 40; however, one of the cells was trisomic for chromosome 15 and another for chromosome 10. Seven cells of this thymoma had apparently normal karyotypes. In the total of 97 cells analyzed, chromosomes 7, 9, 14, and 18 were present only once in a trisomic state.

Occurrence of trisomy and/or subsequent selection of a cell with a particular trisomic condition were seemingly not random events; addition of chromosomes 1, 2, 4, 5, 6, 8, 11, 13, 16, and X was not found.

Most of the thymomas examined by chromosome banding methods had modal chromosome numbers of 41; this is in contrast to previous findings with conventional techniques where chromosome modes of 40 were found in approximately 60% of leukemias. At present we have no explanation for this discrepancy.

Karyotype of Normal AKR Thymus and of AKR Embryo Cells. Two sources of nonleukemic AKR cells were examined: thymus from 13 female AKR mice aged 1–3 months and primary cultures of pooled AKR embryos obtained late in the gestation period. No chromosomal trisomy was observed.

DISCUSSION

Our study shows that chromosome 15 is frequently present in a trisomic state in spontaneous lymphomas of AKR mice. There are several well-documented instances of chromosomal trisomy in viral and chemically induced tumors of rats and hamsters. The first was observed by Sugiyama *et al.* in 7,12-dimethylbenz[*a*]anthracene-induced leukemias of rats, where one identifiable chromosome was found to be trisomic (17). Subsequently Mitelman *et al.* reported identical chromosomal trisomy in rat sarcomas induced by the same agent (18). Nonrandom trisomy was observed also in a large number of rat tumors induced by Rous sarcoma virus (19), but in these tumors the extra chromosome was not the same as that found in the chemically induced leukemias and sarcomas. In another species, chromosomal trisomy was present in a substantial proportion of virus-induced (20) and chemically induced (21) sarcomas of an inbred Chinese hamster strain; once again, the trisomy involved different sets of chromosomes depending on the oncogenic agent.

Three conclusions can be drawn from these studies of rodent tumors: (*1*) reproducible and specific chromosomal changes are associated with tumor development; (*2*) different etiologic agents induce different chromosomal changes, as Mitelman *et al.* have suggested (22); and (*3*) trisomy of specific chromosomes is the most frequent and consistent concomitant of these karotypic changes.

How often will the trisomy 15 of AKR leukemias be found in other spontaneous and induced tumors of the mouse? The answer will undoubtedly be shortly forthcoming, now that mouse chromosomes can be distinguished by banding procedures. We do know that many tumors of the mouse have chromosome numbers in the diploid range. For example, radiation-induced leukemias of C57BL/6 (23, 24) and chemically-induced leukemias of Swiss mice (25), as well as sarcomas induced in various mouse strains by carcinogenic hydrocarbons (26, 27) or by Rous sarcoma virus (28), display to some extent chromosome modes of 40–42 or contain a substantial number of near-diploid cells.

What is the link between chromosomal trisomy and origin of the malignant phenotype? With regard to AKR leukemias,

TABLE 1. *Distribution of chromosome numbers and incidence of chromosomal trisomy in thymomas of AKR mice*

Mouse no.	No. of chromosomes per cell:								Modal chromosome no.	No. of cells with trisomy of chromosome no.:						No. of cells analyzed
	⩽38	39	40	41	42	43	44	>44		3	10	12	15	17	19	
1	1			8					41				8			9
2	1		2	5					41				7			8
3		1	5	5					40, 41				10			11
5		2	7	1					40		1		1			10
7		8	1						39				2			9
10	1	1	1	4					41				7			7
11				5					41				5			5
14		1		7					41			7				8
20				5			2		41	1	1	3	7	1	2	7
21					6		3	4	42	7	2	13	7	12		13
25				10					41			1	10			10

does chromosome 15 have a special relationship to the endogenous leukemia virus of this mouse strain? Several AKR genes have now been identified that control murine leukemia virus production, e.g., *Akv-1* and *Akv-2* (4), and expression of murine leukemia virus structural and cellular antigens (5). None of those with assignment to known linkage groups resides on chromosome 15. However, the *Akv-2* gene has not been mapped, and it will be of interest to determine whether it might be found in linkage group VI (chromosome 15). Another possibility relating trisomy 15 to leukemogenesis is that regulatory genes for lymphocyte proliferation are located on this chromosome and that imbalance in gene dosage gives the cell a selective advantage.

What is the origin of trisomy 15? Two mechanisms for occurrence of chromosomal trisomy are selective endoreduplication and mitotic nondisjunction, with nondisjunction being more frequently implicated. It has been proposed that chromosomes associated with organization of nucleoli are prone to nondisjunction (17, 29), and, in species where it can be clearly demonstrated, such chromosomes show secondary constrictions. In the mouse there are approximately five pairs of chromosomes with secondary constrictions (14, 15, 30); chromosome 15 and chromosome 12 are among those exhibiting this constriction. Clearly, the relation between chromosomal trisomy of malignant cells and abnormalities in nucleolar development and function needs further study.

Is trisomy characteristic of any human cancer? The highly consistent abnormality of chronic myelocytic leukemia, the Philadelphia chromosome (31), does not apparently involve trisomy; the abnormality has been recognized recently as a translocation between the longer arms of chromosomes 22 and 9 (32, 33). Occurrence of trisomy 8 in bone marrow of patients with myeloproliferative disorders or acute myeloblastic leukemia (34, 35) and trisomy 9 in acute myelomonoblastic leukemia (36) have been reported for the small number of C-group trisomies so far investigated by chromosome banding procedures. An isochromosome of the long arm of 17, resulting in effective trisomy of that arm, has been described for several cases of chronic myelocytic leukemia during blastic crisis (31, 37). In one investigation an additional terminal band on chromosome 14 was found in 10 out of 12 cases of Burkitt's lymphoma (38). Another instance of specific chromosomal abnormality is manifested as chromosome loss rather than either translocation or gain, i.e., deletion or loss of chromosome 22 as documented in a recent survey (39) of 105 meningiomas. However, at least from studies to date, most human cancers have not shown the consistent chromosomal patterns that have emerged from the analysis of primary rat, hamster, and mouse tumors.

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