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Are Nonrandom Karyotypic Changes Related to Etiologic Agents?

Janet D. Rowley

Department of Medicine, The University of Chicago, Chicago, Illinois 60637; and The Franklin McLean Memorial Research Institute, Chicago, Illinois 60637

At the present time, there is not one shred of evidence to support the hypothesis that, in human tumors, specific chromosomal abnormalities might be related to particular etiologic agents. Nevertheless, I believe that this hypothesis merits consideration for two reasons. First, there is evidence from animal tumors that supports this notion, and second, the concept can be tested with techniques that are currently available.

The hypothesis was proposed (34) as a possible explanation for nonrandom chromosomal changes that have been observed in a variety of human neoplasms, some malignant, others benign. Some of the evidence was obtained prior to the application of the new banding techniques; with these techniques, however, more precise information has been obtained. This chapter covers three areas: first, the evidence for nonrandom changes; second, the evidence in animals relating nonrandom changes to specific etiologic agents; and finally, the human neoplasms that, in my view, offer the most promising opportunities for the investigation of this question in humans.

NONRANDOM CHROMOSOMAL ABNORMALITIES IN HEMATOLOGIC DISORDERS

This subject has been reviewed briefly by Harnden (12). Additional data regarding nonrandom chromosomal changes in both chronic myelogenous leukemia and acute leukemia are available and should be presented.

Chronic Myelogenous Leukemia (CML)

Chronic Phase

It is now accepted that the Ph¹ chromosome, which is found in patients with CML, represents a translocation rather than a deletion (33). The karyotypes of 187 Ph¹-positive patients have been examined with banding techniques (see ref. 36 for review). The original report on the translocation and a number of reports confirming the observation noted that in 173 of these patients the translocation occurred only between chromosomes 9 and 22. Measurement of the DNA content of the Ph¹ and the 9 with the additional material (9q+) demonstrates no detectable loss of chromosomal material when these chromosomes are compared to their normal homologs (23). Recently, 14 cases with either unusual or complex translocations, accounting for 7.5% of all Ph¹-positive patients, were detected. In eight of these, the translocation involved No. 22 and some other chromosome, namely, Nos. 2, 13, 16, 17, 19, 21, or an unidentified chromosome (7,10,13,15,26,36). In one patient, the translocation occurred between an abnormal 9 and a 22 (13). Five cases have also been reported in which the rearrangement involved three chromosomes; in all these cases, two of the chromosomes were 9 and 22 with breaks in the usual band (13,18,30). Thus, among the 187 Ph¹-positive patients whose cells have been examined with banding, there appear to be 14 who showed an unusual chromosomal rearrangement; No. 9, with a break in band q34, was involved in six of these 14; therefore, only eight of 187 Ph¹-positive patients had a normal No. 9. The explanation for the great specificity of the translocation involving 9 and 22 remains an enigma.

Acute Phase

The complex karyotypic changes that occur in the acute phase of CML can now be determined with the use of banding. Bone marrow chromosomes from 63 patients with Ph¹-positive CML, who were in the acute phase, have been analyzed with banding techniques (13,36). Twelve showed no change in their karyotype, whereas 51 patients showed additional chromosomal abnormalities. The gains or, more rarely, losses of particular chromosomes observed in 46 patients who had relatively complete analyses are summarized in Table 1.

The table shows that the single most common change is the addition of a second Ph¹ (22q—) chromosome, an event that occurred in more than 50% of the patients. Prior to banding, the most commonly observed abnormality was an additional C group chromosome. The use of banding reveals that, although different additional C group chromosomes are present, one pair, No. 8, is involved much more frequently than the remainder. Of 25 patients whose cells contained additional C's, 19 had an additional No. 8 (Table 1). In 12 patients, this was the only abnormality involving a C, whereas in seven others a second additional C was contributed by some other pair. The i(17q) appears to be the second most common structural rearrangement, after the 9;22 translocation, as it was observed in 12 patients.

TABLE 1. *Chromosom*

	3	4	5	6	7	8
No. of patients with gain	1	2	1	3	2	19
No. of patients with loss					1	

[a]Updated from ref. 36.
[b]Includes two dicentric Ph¹ chrc

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TABLE 1. *Chromosome changes in 46 patients in acute phase of CML*[a]

	Chromosome no.																							
	3	4	5	6	7	8	9	10	11	12	X	C	13	14	16	17	i(17q)	18	19	21	22	22q-[b]	Mar
No. of patients with gain	1	2	1	3	2	19	3	5	4	4	2	6	2	4	2	2	12	3	10	4	1	27	8
No. of patients with loss					1		1		1	1	1			1	2				1				

[a] Updated from ref. 36.
[b] Includes two dicentric Ph[1] chromosomes.

Except in four cases, it was found to be associated with an additional No. 8 (four times) or an additional unidentified C (four times). Without exception, the additional F noted in 10 patients was a No. 19.

Acute Leukemia

Prior to banding, the chromosomal pattern in acute leukemia, both myelogenous and lymphocytic, appeared to be so variable that chromosomal changes were assumed to be an epiphenomenon. Furthermore, abnormal karyotypes were observed in only 30 to 50% of patients (review, ref. 36).

I have recently completed a study of the karyotype (with banding) of 50 patients with acute nonlymphocytic leukemia (37). Twenty-five patients showed an abnormal karyotype in the initial examination; although there was considerable variability, some patterns tended to recur. The presence of nonrandom patterns becomes much more apparent if published data on chromosomal abnormalities determined with banding techniques are added to my own.

The data from the 23 patients in my series whose cells could be analyzed completely, together with the published data from 37 patients (36), provide information on the banding patterns in 60 patients. There is a surprisingly narrow range of modal chromosome numbers in this group, with 22 individuals having 45 chromosomes, 15 having 46, and 14 having 47; five patients had 42 to 44 and four patients had 48 to 50 chromosomes. The chromosomal abnormalities can be grouped into three major types: gain of one autosome, loss of one autosome, and balanced translocations.

Prior to banding, the frequent involvement of C and G group chromosomes and the absence of F group involvement had been noted. The use of banding confirms these observations and demonstrates that there is a nonrandom pattern within both the C and G groups as to the particular chromosomes involved (Fig. 1). Thirteen patients had one extra autosome, identified as No. 8 in 10 cases. Nine patients showed loss of one autosome, which was identified as No. 7 in six. The other

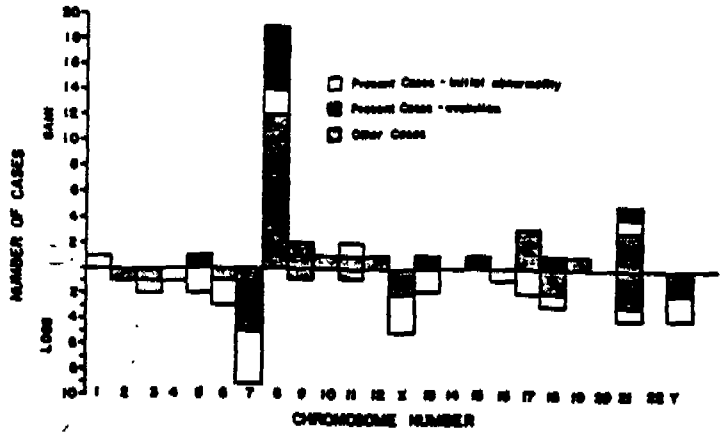

FIG. 1. Summary of gains and losses of whole chromosomes observed in patients with acute leukemia. (From ref. 37, with permission.)

chromosomal changes that occurred in more than a few patients were the loss of an X chromosome in five, the gain or loss of a No. 21 in five and four patients, respectively, and the loss of the Y chromosome in four patients. Balanced translocations involving No. 8 (with or without loss of sex chromosomes) were seen in six patients, and balanced translocations of other chromosomes were noted in nine others, giving a total of 15.

The evidence for nonrandom involvement of particular chromosomes in other tumors has been summarized by Harnden (12) and others (21,34,35). It appears that only certain chromosomes are abnormal in a particular tumor, and that these same chromosomes may be aneuploid in a variety of tumors (21,35).

EXPERIMENTAL EVIDENCE RELATING NONRANDOM CHANGES TO ETIOLOGIC AGENTS

In my view, the evidence for nonrandom changes is very convincing, and we must now search for mechanisms producing these changes. We must also look for some plausible explanation for the malignancy of these chromosomally abnormal cells. One possible mechanism, namely, that specific karyotypic changes are related to specific agents, is explored more fully in this chapter. An alternative hypothesis, which states that malignancy represents an imbalance between genes concerned with the expression or suppression of malignancy (2,17,45), has received much attention; it is outside the scope of this presentation and therefore is not discussed.

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Chromosomal Patterns in Specific Tumors

Nonrandom chromosomal abnormalities occur in some animals exposed to chemical and viral mutagenic agents. For example (Fig. 2), when leukemia (32,43,44), sarcomas (27), and epithelial tumors (1) were induced by 7,12-dimethylbenz[a]anthracene (DMBA) and 6,8,12- and 7,8,12-trimethylbenz[a]anthracene in rats, trisomy for the largest telocentric chromosome (A2) was observed; trisomy for a metacentric chromosome was less frequent (32,43). An analysis of the banding pattern of DMBA-induced sarcomas has shown that additional material of chromosome A2 was present in 12 of 13 tumors (19). A different, but very consistent karyotypic pattern was seen in sarcomas induced in the rat by Rous sarcoma virus (RSV) (29). Some Rous sarcomas in the rat may show a normal karyotype initially. On independent serial propagation of these tumors, however, the same nonrandom karyotypic changes are noted in the different passages (25). The pattern of karyotypic evolution involved additions of one medium-sized telocentric chromosome, followed by one medium and one small subtelocentric chromosome. Chromosomes most frequently abnormal in DMBA-induced tumors were rarely abnormal in RSV-induced tumors, and vice versa. *The sarcomas produced by DMBA and RSV were histologically indistinguishable.*

A similar phenomenon was observed in the Chinese hamster. One or more additional chromosomes belonging to three pairs (Nos. 5, 6, or 10) were found in most sarcomas induced by RSV (16), whereas No. 11 was the most frequent addition in cells from DMBA-induced sarcomas (28) in the same inbred Chinese hamster strain.

These observations led Mitelman et al. (29) to propose that the specific chromosomal abnormalities are associated with particular etiologic agents, and that the chromosomal change is independent of the type of tumor produced. Further research has shown that this is a somewhat oversimplified view, because the karyotypic changes in sarcomas produced by other polycyclic hydrocarbons,

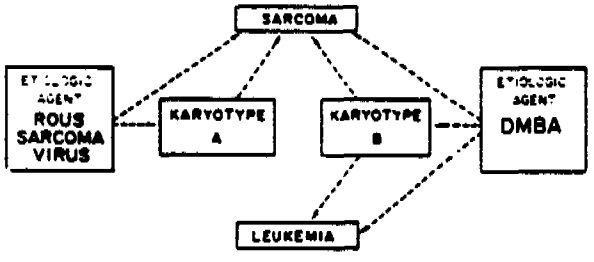

FIG. 2. Although histologically identical, sarcomas induced in rats by *different* agents have *different* chromosomal abnormalities; whereas, two different malignancies induced by the *same* agent have *identical* karyotypes. (From Rowley, J. D. (1975): Nonrandom chromosomal abnormalities in hematologic disorders of man. *Proc. Natl. Acad. Sci. USA*, 72:152–156, *with permission*).

such as methylcholanthrene (MC) or benzo(a)pyrene (BP), although still nonrandom and frequently involving chromosome A2, are more complex and show greater variability (20).

Even in experimental animals, some chromosomal variability is present in tumors induced with a single, known oncogenic agent. It may be significant, however, that chromosomal variability is lower in inbred (20 to 30%) than in random-bred (approximately 50%) animals.

It should be acknowledged that not all investigators have observed nonrandom changes. Some of the data are presented in a recent review by DiPaolo (4).

Mechanisms for Agent-Chromosome Specificity

One mechanism that could account for the specificity of the chromosomal abnormalities observed with different oncogenic agents is suggested by Sugiyama (42). He noted that two regions on the long telocentric chromosome (A2), which is frequently present in a trisomic state in DMBA-induced rat leukemia, were specifically vulnerable to the chromosome-damaging action of DMBA. These same regions were late-replicating and heterochromatic. It has been known for some time that such regions show preferential susceptibility to mutagenic agents (40). In addition, Levan and Levan (20) remarked on the very interesting relationship between chromosomal changes and carcinogenicity. In the rat, the order of carcinogenic potency is DMBA > MC > BP (31); the frequency of nonrandom changes shows the same order, with DMBA producing the most consistent and BP the least consistent karyotypic changes. Although the significance of this relationship is unknown, Levan and Levan have suggested that, if a given mutagen is more potent as a carcinogen, it may attack certain genes more selectively in the induction of malignancy (20).

HUMAN TUMORS WITH A KNOWN ETIOLOGY

Whereas there is no evidence in man at the present time that links any specific chromosomal abnormality with a particular carcinogen, we needn't remain so ignorant. There are a number of patients in whom this hypothesis could be tested.

Human Tumors with a Known Environmental Cause

Present Knowledge

A number of human tumors are associated with known environmental carcinogens. Some of these associations have been recognized for many years; they include skin cancer and exposure to soots, tars, and oils; lung cancer and exposure to asbestos or benzo(a)pyrene and other polycyclic aromatic hydrocarbons in industry, in cigarette smoke, and polluted city air; and urinary bladder cancer from

benzidine and β-naphthylamine (see ref. 24 for review). More recently, it has been recognized that girls whose mothers received diethylstilbestrol (DES) in early pregnancy have vaginal adenosis and a high risk of developing adenocarcinoma of the vagina and cervix (14); individuals who received X-ray therapy for enlarged tonsils, adenoids, or thymus, or for acne are prone to carcinoma of the thyroid (6). Workers exposed to vinyl chloride show an increased incidence of hepatic angiosarcomas (3), and those exposed to chloromethyl methyl ether (CMME) have an increased incidence of lung cancer, usually the oat cell type (8).

Many of these tumors are otherwise rare, and, to my knowledge, none has been studied with cytogenetic techniques. The nearest approach to such a study was a report on the DNA content of cells from DES-induced vaginal adenosis (three patients) and adenocarcinoma (three patients), which showed that the DNA content of the former was in the diploid–tetraploid range, whereas cells from the latter showed an aneuploid amount of DNA (22). The authors concluded that the "diploid" (and therefore "normal") state of the adenosis cells indicated that these cells were unlikely to be the source for the adenocarcinoma. However, Granberg et al. (11) studied a uterine adenocarcinoma and found a consistent chromosomal abnormality (46,XX,+3,−D) in cells with a diploid amount of nuclear DNA. Thus, measurements of DNA cannot reveal consistent chromosomal changes that are the result of reciprocal translocations, nor do they detect gains and losses of chromosomes of relatively similar size.

Areas for Future Research

For an answer to the question whether there are nonrandom chromosomal changes in environmentally induced human tumors, the following four criteria should be met with regard to the tumors selected.

1. There should be a relatively specific association between the tumor and the carcinogenic agent.
2. Relatively few tumors should occur in the same site without a known exposure to the agent; alternatively, exposed and nonexposed groups should be clearly distinguishable on the basis of clinical history or the specific histologic appearance of the tumor.
3. The tumors should be relatively frequent (preferably at least 10 to 20 cases per year) in the United States.
4. Surgical specimens should be available for cytogenetic analysis.

The tumors that appear to be among the most promising candidates for investigation at the present time are included in Table 2.

Workers exposed to asbestos dust provide a particularly important population for study because they show an increased incidence of several types of tumors and therefore may provide evidence in man that could be compared with that obtained for DMBA-induced tumors in rats. The tumors observed in asbestos workers include not only lung cancer, but mesotheliomas of the pleura and peritoneum,

TABLE 2.

Tumors[a]	Population at risk
1. Thyroid carcinoma (6)	Patients who received X-rays for enlarged tonsils, adenoids, thymus, or for acne
2. Lung cancer, mesotheliomas of pleura and peritoneum, and gastric cancer (24,38,39)	Workers exposed to asbestos
3. Oat cell cancers of lung (8)	Workers exposed to halo ethers
4. Hepatic angiosarcoma (3)	Workers exposed to vinyl chloride
5. Clear cell adenocarcinoma of the vagina (14)	Daughters of mothers who received diethylstilbestrol early in pregnancy
6. Urinary bladder carcinoma (24)	Workers exposed to polycyclic aromatic compounds

[a]References appear in parentheses.

and gastric and colon cancer as well. These individuals also show pulmonary fibrosis and pleural thickening (asbestosis), so that abnormal, possibly premalignant, tissue is also available for chromosomal analysis. Present evidence indicates that the increased risk of bronchogenic carcinoma in workers exposed to asbestos dust is confined to smokers (39). One could thus compare the karyotypes of lung cancers from these two populations to determine whether the chromosomal changes are similar or different.

Although there is no information on the karyotype of any of these environmentally induced tumors, there is evidence that some of the agents discussed previously cause chromosomal damage. An increased incidence of chromosomal aberrations has been detected in cultured lymphocytes obtained from workers exposed to vinyl chloride (9). It has also been reported recently that Chinese hamster cells cultured with asbestos fibers show an increased frequency of polyploids, fragments, and other structural rearrangements (41). These changes were not observed in control cells exposed to fiber glass or glass powder. It has also been reported that carcinogenic substances produce banding of mammalian chromosomes, whereas noncarcinogenic substances do not (5).

As we are unwittingly exposed to an increasing variety of environmental pollutants, some of which are carcinogenic, information regarding the relationship of etiologic agent and tumor formation becomes critically important.

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Discussion

Nance: In acute leukemia, do the karyotypes correlate in any way with the clinical features, such as survival, response to treatment, etc.?

Rowley: As Dr. Sandberg found [*Cancer*, 33:1548–1557 (1974)] and we confirmed, patients with an abnormal karyotype respond more poorly to chemotherapy and have a shorter median survival than do patients with normal karyotypes. This is particularly true in acute myeloblastic leukemias as compared with acute myelomonoblastic. We and others are looking into the significance of the possible presence of the gene for glutathione reductase on chromosome 8.

Pogosianz: Maybe it would interest this audience to know our Russian data on some of the human hematologic neoplasias. I should say, for those who are not cytogeneticists, that all sorts of geneticists were very excited with the data that came from the Levans' laboratory and Dr. Rowley's work involving nonrandom distribution of chromosomal changes in malignancy. So, it is interesting to have data from different laboratories in different countries.

In our laboratory, Drs. E. E. Prigogina and E. V. Fleishman carried out chromosome studies in 54 cases of human hemoblastoses [*Humangenetik*, 30:109–119 (1975)]. Out of 19 cases of acute leukemia studied with G-banding technique, 11 had normal karyotypes, and 8 were aneuploid. Among the aneuploid were two cases each with chromosomes 7, 8, and 21 involved.

All 21 patients with chronic myelogenous leukemia had 9;22 translocation. This was the only abnormality in six cases; whereas eight also had involvement of chromosome 17 (iso 17q). Trisomy 8 and 19 were added to the i(17q) marker in a progressive fashion, especially during blast crisis. Half of the cases had these patterns and half had a different pattern. So, we agree with Dr. Rowley that not all chronic myelogenous leukemias are alike. Whether the differences reflect different etiologic agents, no one knows.

Finally, we have six cases of chronic lymphatic leukemias (two with chromosomal abnormalities, one involving chromosome 14, one a 14q+), and eight cases of lymphosarcoma (four with abnormalities, three involving chromosome 14, one with chromosome 14q+). Altogether, aneuploidy was seen in six of these 14 cases of lymphoreticular malignancy, and four had an abnormality of chromosome 14.

Hirschhorn: Dr. Pogosianz, did you test for Epstein–Barr virus in your patients, in particular in the two cases with 14q+ in the lymphoma group?

Pogosianz: We do not have data on Epstein–Barr viral antibodies. If you are implying that the two cases have Burkitt's lymphoma, I can say there are no known cases of typical Burkitt's lymphoma in our country. It could be that 14q+ is found in lymphomas other than Burkitt's or that our patients had atypical Burkitt's lymphoma.

Rowley: It is nice to get confirmation from another country, the Soviet Union. I say this because Ford and Gunz from Australia have recently reported that the most consistent abnormality they see in acute leukemia is an additional chromosome 9 and a missing chromosome 8 [*J. Natl. Cancer Inst.*, 55:761–765, (1975)]. This contrasts with our findings and raises the question of a different climate or etiologic agent. Alternatively, I might suggest that it is difficult to distinguish chromosome 8 from 9 in leukemic cells using Giesma banding alone as Ford and Gunz did. A further reason for uncertainty is their observation that the chromosomal abnormality persisted even in remission. Most other laboratories have reported that patients in good clinical remission lose their chromosomally abnormal cell line, so perhaps the Australian patients were not in remission.

Hecht: Dr. Hirschhorn's question concerning the Soviet experience with 14q+, lymphoid tumors and the Epstein–Barr virus is another way of getting back to Dr. Rowley's hypothesis relating the pattern of chromosomal abnormalities to the etiologic agent. I am afraid that such simple thinking is not going to get us far in man because there is overwhelming evidence, I think, that the type of chromosomal aberration pertains to the *cell type* of the malignancy and not to its *etiology*. Rowley and others have clearly shown the nonrandom association of erythromyeloid malignancy with abnormalities of chromosomes 8, 22, and 9; whereas lymphoid tumors seem to involve preferentially chromosome 14 as reported from the Soviet Union, Great Britain, Sweden, Japan, and the United States. The chromosomal pattern looks quite cell-specific, although it does not rule out the idea that it is also agent-specific. For example, not all 14q+ tumors are positive for Epstein–Barr virus; we have some that are negative.

German: In the Levans' report cited by Dr. Rowley to support her hypothesis, I was impressed that the karyotypes of tumors induced by certain chemicals were far less specific than, for example, the 14q12 band in Burkitt's lymphoma in humans. Rather, certain morphologically similar chromosomes had the tendency to become trisomic or monosomic following treatment with certain agents.

Rowley: To the contrary, the Levans did careful Giesma banding on the tumors induced by polycyclic hydrocarbons. They felt they sometimes observed reliably consistent patterns even to the point of identifying trisomy of particular regions of the rat chromosome A2. Certainly, some tumors had greater variability than others.

Riccardi: Concerning malignancy in trisomy 8, I have two observations. First, of some 65 patients with *congenital* trisomy 8 known to me from a worldwide survey, one has developed a hematologic malignancy [*Clin. Genet.*, 9:134–142 (1976)]. Second, we studied a man in Denver who had acute myelogenous leukemia. His peripheral lymphocytes showed a reciprocal balanced translocation (7p;20p), which was also found in his brother, sister, and nephew. His bone marrow showed leukemic cells with trisomy 8. Skin fibroblasts showed the translocation, as anticipated, and, to our surprise, trisomy 8 as a mosaic line [*Am. J. Hum. Genet.*, 27:76a (1975)]. Both these observations caution us about drawing premature conclusions on the etiologic significance of the extra chromosome 8.

Rowley: I do not mean to imply that an additional chromosome 8 causes the leukemia. I do say there is an *association* that seems to be very important, although its significance is not presently understood. Furth., one has to distinguish between trisomy 8 as a constitutional abnormality and the abnormalities we see in bone marrows that appear to be *somatic* mutations. In our trisomy 8 patients, we have not looked at skin fibroblast cultures, but phytohemagglutinin-stimulated peripheral lymphocytes from many cultures have had no additional chromosome 8 at 72 hr.