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Chromosomal Aberrations in Foreign Body Tumorigenesis of Mice[1,2] (41575)

DOUGLAS RACHKO AND K. GERHARD BRAND[3]

Department of Microbiology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455

Abstract. Sarcomas were induced in 107 male and female isogeneic CBA/H or CBA/H-T6 mice by subcutaneous implantation of double films of unplasticized vinylchloride–acetate copolymer, 15 × 22 × 0.2 mm in size. Tumors were grouped by chromosome number. G-banding was performed on chromosomes of (a) 12 sarcomas, (b) 6 specimens of preneoplastic cells derived from foreign body (FB)-reactive tissues at 4, 6, 9, and 16 months postimplantation, and (c) 11 sarcomas which developed from clonal lines of the preneoplastic cells studied. Karyological analyses lead to the following results and conclusions: (1) Various derangements in chromosome number occurred in preneoplastic cells during early FB reaction at the time of, and possibly in causal relation to carcinogenic initiation. (2) Structural abnormalities of specific chromosomes (insertions, translocations, transpositions, etc.) were found as stable cell markers only during late preneoplasia. They may thus contribute to advanced tumor progression. (3) Ploidy deviations of specific chromosomes (secondary to the early derangements in chromosome number) were most frequently seen in chromosomes 1, 6, 7, 13, 15, 18, and 19; however, these latter aberrations were unstable and inconsistent both *in vivo* and *in vitro*.

Sarcomas of monoclonal origin develop in mice upon subcutaneous implantation of nonbiodegradable materials (1). Unlike carcinogenic chemicals, viruses, or radiation, FB material is not cell invasive. The originator cell type is a microvascular stem cell (2) which at the time of carcinogenic initiation resides in the FB-reactive tissue distant from the implant. Frequency of initiation events correlates with implant size, and hence, with the extent of the FB reaction and the proliferation of FB-reactive cells in general. Conceivably, initiation may be the result of a spontaneous intracellular error in a proliferating cell population.

Virtually all FB sarcomas of mice possess abnormal chromosome numbers. The aberrations occur as early as 4 to 8 weeks following FB implantation, i.e., many months before commencement of neoplastic growth (1). They proved remarkably stable in transplantation experiments and *in vitro* culture (3). Therefore, an etiological connection is suspected.

In the present investigation, we have first surveyed a large number of FB sarcomas to determine the range distribution of abnormal chromosome numbers. And second, by means of chromosome banding we have studied preneoplastic and neoplastic cells at various stages of progression in an attempt to identify specific chromosome changes relative to the tumorigenic process.

Materials and Methods. *Mice.* Isogeneic male and female CBA/H and CBA/H-T6 mice were used as in previous studies (1, 3, 4, 5).

FB implantation, tumor examination, in vitro cultures. The methods have previously been described in detail (3, 5). The preneoplastic cells were karyotyped after the shortest possible time *in vitro* with a minimum number of culture passages. Nevertheless, at least three passages, but no more than five were usually needed to eliminate FB-reactive euploid fibroblasts and to obtain the preneoplastic cells (3). These were identified by clone-specific aneuploidy (3, 5) and pericyte- or endothelial-like morphology (2). The monoclonal aneuploid sarcoma cells were more readily available for karyotyping. They outgrew normal euploid stroma cells within a few days in primary culture.

Tumorigenicity tests of preneoplastic cell lines. Aliquots of 20,000 cells, (a) suspended in 0.2 ml medium, (b) affixed to 11 × 22-mm plastic coverslips (3) were subcutaneously in-

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[2] Abbreviations used: FB, foreign body; BSS, balanced salt solution; GKN, 0.1% glucose, 0.04% KCL, 0.8% NaCl, pH 7.8.

[3] Address reprint requests to K. G. Brand.

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troduced in two mice each. CBA/H-derived cells were tested in CBA/H-T6 recipients and vice versa to confirm the origin of the resulting tumors which appeared after 3 to 16 months in this study. (In contrast, when fully neoplastic cells were transplanted tumors grew within 2 to 3 weeks).

Procedures to identify significant chromosome aberrations and to distinguish them from culture effects. (1) Implants and surrounding tissue capsules were cut in half at various time points during preneoplasia. One half was left in the animals for tumor development and karyotyping. The other half was excised for culturing and karyotyping of the preneoplastic cells. These cells were then implanted in recipient mice for tumorigenicity tests (see above). The resulting homologous (clone-related) tumors were also karyotyped. If specific chromosome aberrations were found common in both the preneoplastic and the homologous tumor cells it would mean that they had persisted throughout the tumorigenic process. They would, therefore, be considered significant.

(2) In addition, a significant aberration was expected to be present in virtually all spreads (at least 80%) of a cell preparation.

(3) From each tissue or tumor, cultures were set up in duplicate and kept separate. A chromosome aberration found present in only one of the two lines was suspected to have arisen during culture.

(4) Numerous control cultures of connective tissue taken from locations distant from implant or tumor sites consistently yielded euploid fibroblasts only which died out within three to five passages.

Preparation of chromosome slides. Confluent cell monolayers in culture dishes were rinsed with GKN, covered with trypsin (0.5% in GKN) (DIFCO, Detroit, Mich.), and incubated at 37°C until the cells began to detach. Then, 3.0 ml medium (McCoy's 5a with L-glutamine and 20% newborn calf serum) was added. The cells were suspended by gentle pipetting and seeded onto five 22 × 22-mm glass coverslips (Clay–Adams, Parsippany, N.J.) contained in 35-mm dishes for culture. After 30 or 46 hr, the culture medium was replaced with medium containing 0.05 or 0.2 μg/ml colcemid (CIBA, Summit, N.J.), followed by 16 or 6 hr incubation, respectively. Then, the coverslips were immersed at room temperature in BSS for 5 min, in hypotonic BSS (BSS:distilled water = 1:5) for 10 min, and in hypotonic medium (medium:distilled water = 1:6) for ten min. One milliliter of fixative (acetic acid:methanol:ethanol = 2:5:1) was then added to the hypotonic medium. After 5 min, the coverslips were transferred to fresh fixative for at least 30 min and then air-dried.

G-banding. Air-dried chromosome coverslips were soaked for 1 hr in 0.3 M NaCl plus 0.03 M sodium citrate, passed briefly through double-distilled water, and then immersed for 90 sec in trypsin (0.05% in 0.9% NaCl, pH 7.2) at room temperature. After rinsing in Sorensen's buffer (0.04 M Na_2HPO_4 and 0.025 M KH_2PO_4, pH 7.0) they were stained with 3% Giemsa (in Sorensen's buffer, pH 7.0) for 10 min, rinsed again, dehydrated through acetone–xylene, and mounted with Eukitt (Calibrated Instruments Inc., Ardsley, N.Y.).

In case the culture fluid contained many free-floating mitotic cells following colcemid treatment, it was centrifuged for 8 min at 1000 rpm. The pellet was resuspended in hypotonic solution and fixed overnight at 4°C with two changes of fixative. The repelleted cells were suspended in a small volume of fixative and dropped from a height of about 3 ft onto clean, cold, methanol-moistened glass slides. After air-drying overnight they were stained and mounted as described above for coverslip cultures.

Chromosome analysis. Whenever possible, 100 metaphase spreads (minimum: 60) were addressed and counted for each preneoplastic or tumor cell preparation. These counts were used to determine the chromosome range, ploidy level, and mode.

G-banded metaphase spreads of superior quality were photographed with a Zeiss microphotography camera at approximately 950× on Kodak High Contrast Copy Film 5069. Whenever possible, 15 spreads (minimum: 10) were photographed for each preneoplastic or tumor cell preparation. The films were developed with Kodak Developer D-76. The negatives were enlarged to about 5225×, and printed on Kodak Kodabrome II RC paper (F-UH or F-EH). The chromosomes were arranged according to the standard G-banded mouse karyotype (6).

Computer analysis of chromosome data. The karyological data of each cell were recorded on single keypunch cards. The cards

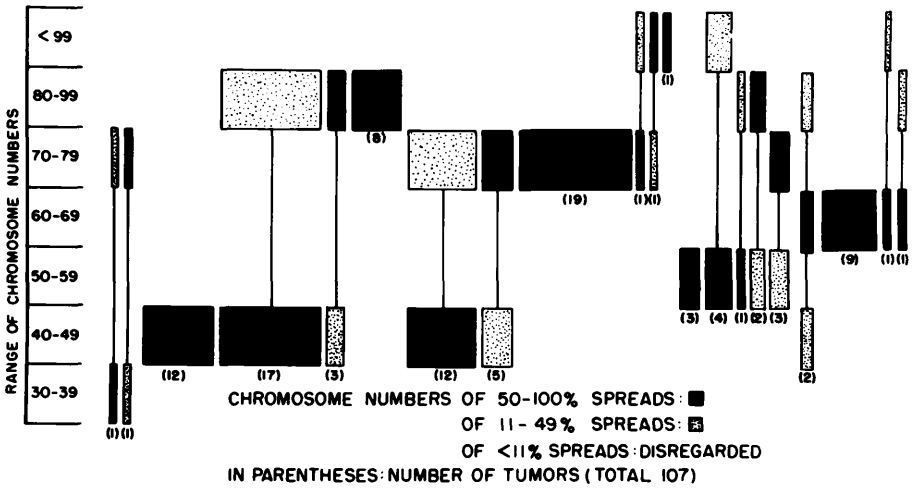

FIG. 1. Ranges of chromosome numbers in 107 foreign body-induced sarcomas of male or female CBA/H or CBA/H-T6 mice.

were read onto a computer file, and the file was stored on magnetic tape. A FORTRAN IV computer program was designed to produce frequency histograms for each of the 40 chromosomes according to predesignated aberration categories. The frequency histograms indicated (a) the chromosomes which were most often abnormal in foreign body-tumor cells, and (b) the type of aberration which predominated for each chromosome. Additional computer programs produced histograms for combinations of abnormal chromosomes, listing frequencies for abnormal chromosome pairs, triplets, etc.

Results. *Ranges of chromosome counts in 107 FB-induced sarcomas.* In Fig. 1, the tumors are categorized according to ranges of chromosome numbers. Solid blocks indicate predominant ranges, representing counts obtained from 50 to 100% of the spreads. Dotted blocks represent ranges obtained from 11 to 49%. Ranges representing fewer than 11% are not included in the chart. Since the ranges were arbitrarily set, some tumors gave overlapping counts. They were assigned to the range which included the modal chromosome number and covered the major portion of the counts.

The following results are depicted in Fig. 1.

(1) A primary reduction of the chromosome number to the range of 30 to 39 occurred very rarely (2 out of 107 tumors).

(2) The most frequent primary chromosomal shift was to the hyperdiploid range (40 to 49). It occurred in 51 tumors. Twelve tumors persisted exclusively in this range suggesting that the hyperdiploid genome provided optimal cell vitality.

In 37 cases, we observed either duplication of the hyperdiploid karyotype to hypertetraploidy (80 to 99 chromosomes), or duplication with subsequent reduction to hypotetraploidy (70 to 79 chromosomes). Occasionally, hypotetraploid tumors underwent further chromosome multiplication into ranges over 99.

Cells at higher ploidy levels equaled or surpassed the hyperdiploid cells with regard to proliferative efficacy. In several cases, the original hyperdiploid cells were entirely outgrown by hypertetraploid cells (8 tumors), by hypotetraploid cells (19 tumors), or by cells with higher ploidy levels (1 tumor).

(3) Only 13 out of 107 tumors showed a primary chromosome shift into the high hyperdiploid range of 50 to 59. Further changes resembled those observed on tumors in the low hyperdiploid range of 40 to 49.

(4) Some tumors shifted to the hypertriploid range of 60 to 69. In 9 out of 13 tumors the hypertriploid cells outgrew cells at other ploidy levels which indicates a high degree of proliferative vitality provided by hypertriploidy.

Search for specific chromosomal aberrations or aberration patterns. Table I presents aberrations detected in specific chromosomes

TABLE I. ABERRATIONS OF INDIVIDUAL CHROMOSOMES IN FB-INDUCED SARCOMAS, PRENEOPLASTIC CELLS, AND CELL LINE-DERIVED TUMORS[a]

	Chromosome number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y
Diploid tumors																					
11234	*TP*	*IN*			*IN*	mo		mo			tr		tr	*FU*	mo				tr		*DI*
11525						*TR*							mo					mo			
11526						tr				tr	mo		va		tr	IN					
11531			*IN*																		
Tetraploid tumors																					
11517	tr						di								pe						*NU*
11519	tr						di			pe	*TR*	pe	tr					di	pe	*DI*	
11524							tr			pe		va	di		pe				*PE*	di	
11527						tr	tr								pe						
11528	tr			pe	pe		di					di		in	*PE*		pe				nu
11530	tr								di	pe										di	
11532				*TL*		*TL*	di	*FU*													
11556	tr													di			fu	fu	pe	di	
Preneoplastic cell samples and cell line-derived tumors																					
a2c/9831 (4 mpi)						tr							mo	mo	mo	tr		tr	va		
tumor 11387											va			mo			va				
tumor 11389		mo		pe		mo				*IN*					mo	va		va			
a1c/9822 (6 mpi)	tr						mo									*DE*		mo	tr	mo	
tumor 11305					va		mo	tr	mo										tr		
a2c/10070 (7 mpi)	tr									*TL*		*TL*		*TL*						*TL*	
tumor 11036			te		mo			mo		*TL*		*TL*		*TL*		mo				*TL*	
a2c/9829 (7 mpi)	*IS*				tr	mo		mo			mo	va				tr				mo	
tumor 11013					pe				pe		mo		mo			va	va	va	*IS*		
tumor 11309i			*IN*		tr			is		mo		pe					pe				
tumor 11310	*IS*									va	mo		mo		mo	is	fu		pe		
tumor 11410i	*IS*																mo				
tumor 11411	*IS*																mo				
tumor 11412	*IS*																		te		
a1c/9833 (9 mpi)	*DE*	*IN*					mo				in		mo			mo					
tumor 10453	de	*IN*					mo				*DI*				*PE*						
a2c2/9808 (9 mpi)	tr	tr					di		tr	*PE*					pe	pe		pe	pe		*NU*

Note. Capital letters: aberrations present in 81 to 100% of the cells examined; *small letters*: aberrations present in 50 to 80% of the cells examined; (aberrations found in less than 50% of the cells were disregarded).

[a] Abbreviations: mpi—months postimplantation, indicating duration of *in vivo* FB reaction before preneoplastic cells were derived; *DE*, de—deletion; *DI*, di—disomy; *FU*, fu—fusion; *IN*, in—isochromosomes; *MO*, mo—monosomy; *NU*, nu—nullisomy; *PE*, pe—pentasomy; *TE*, te—tetrasomy; *TL*, tl—translocation (intrachromosomal); *TR*, tr—trisomy; *VA*, va—variable.

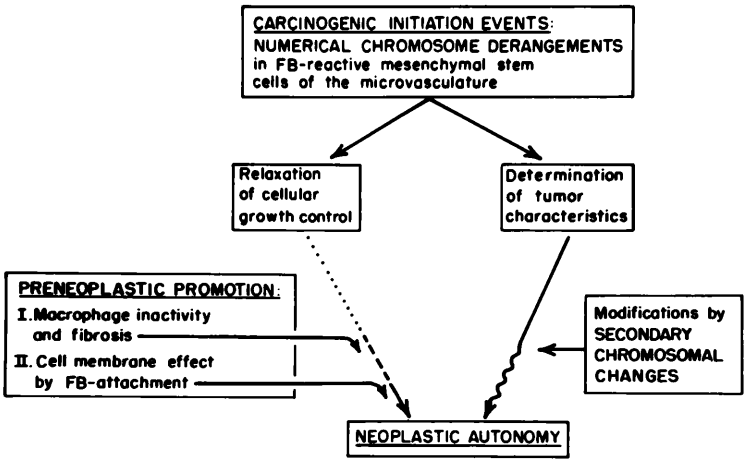

FIG. 2. The possible roles of chromosome aberrations in foreign body tumorigenesis of mice.

of 12 primary FB-induced sarcomas, 6 preneoplastic cell samples, and 11 secondary tumors which developed from the preneoplastic cells. The preneoplastic cells were taken from FB-reactive tissues either before the seventh month postimplantation (samples a2c/9831 and a1c/9822) or later (samples a2c/10070, a2c/9829, a1c/9833, and a2c2/9808).

"Significant" aberrations (as defined in Materials and Methods) comprised almost exclusively certain structural aberrations (insertions, isochromosomes, translocations, and less frequently deletions, nullisomies, and transpositions). However, they were found as persisting markers only in cell samples from advanced FB-reactive capsules and homologous tumors.

In contrast, ploidy deviations of individual chromosomes (mono-, di-, tri-, tetra-, and pentasomies) did not generally meet the criteria of significance. They occurred usually in much less than 80% of the spreads and were inconsistent in duplicate cultures. (Ploidy deviations of individual chromosomes were assessed relative to the general ploidy level of the cell population analyzed. For example, disomy of an individual chromosome in an otherwise tetraploid karyotype was considered aberrant).

Computer analyses showed that structural aberrations occurred with equal probability among individual chromosomes. Ploidy deviations, on the other hand, tended to involve specific chromosomes. Numbers 1, 6, 7, 13, and 18 were most often present in smaller numbers, chromosomes 15 and 19 in larger numbers than expected.

Four of the tumors studied were produced in CBA/H-T6 mice. The T6-marker has resulted from a translocation between chromosomes 14 and 15. Since chromosome 15 is one of the frequently abnormal chromosomes in FB tumorigenesis (Table I) special attention was directed towards chromosome 14 in CBA/H-T6 tumors. Overall, computer analysis showed chromosome 15 abnormal in 58% of the cells. When chromosome 14 was substituted for chromosome 15 in CBA/H-T6 cells, the percentage remained essentially the same (57%).

Discussion. *Primary derangements in chromosome number and the carcinogenic initiation event.* In order to identify specific chromosome aberrations which might be causally related to carcinogenic initiation, prenoeoplastic cells were karyotyped at the earliest possible time following FB implantation. Numerical chromosome derangements are known to occur very early during preneoplasia, often within 4 weeks following FB implantation and in nearly all cases within 8 weeks (1). The deviating chromosome numbers are maintained within narrow ranges throughout preneoplasia. The timing of the numerical derangements coincides with the time during which the predetermination of later tumor characteristics takes place (1). (These include duration of latency, histopathological criteria, degree of anaplasticity and invasiveness, growth behavior *in vivo* and *in vitro*).

In view of these relationships the hypothesis is proposed that the early derangements in chromosome number initiate FB tumorigenesis (Fig. 2). The alteration of the heritable cellular program would explain predetermination of ultimate tumor characteristics. An immediate measurable effect is an increased proliferative drive *in vivo* and *in vitro* [(5) and unpublished observations].

Considering the variety of numerical chromosome derangements in FB tumorigenesis it appears that many different patterns of genetic disorder and imbalance may lead to initiation. This same conclusion has been reached also for certain other cancer induction models (7, 8). There can be no doubt that homeostatic cell proliferation in a normal tissue environment is controlled by a complex regulation system with many functional components and molecular reaction points. A disturbance at any one point, which may be brought about by a multitide of different chromosome derangements, could conceivably affect the whole system. This hypothesis is consistent with observations which indicated nonuniformity of initiation events in FB tumorigenesis (4).

The primary alteration of the chromosome number in a preneoplastic parent cell may be explained by faulty mitosis with unequal chromosome distribution to the daughter cells. This may be the result of an inherent karyotype lability which is characteristically seen in murine mesenchymal stem cells of the microvasculature, the cell type of FB sarcoma origin (1). Other murine cell types such as fibroblasts do not share that property to the same extent (unpublished). In man, the vascular stem cells appear karyologically more stable, and this conforms with the finding that human FB sarcomas are rare (9).

Secondary chromosome aberrations during preneoplastic progression. G-banding revealed a great variety of structural and ploidy aberrations involving virtually every chromosome (and numerous genome loci) of preneoplastic and tumor cells (Table I). These aberrations were unstable and variable during early preneoplasia as shown by samples a2c/9831 and a1c/9822 which were taken before the seventh month postimplantation. Only preneoplastic cells of advanced FB-reactive capsules and their homologous tumor cells had structural chromosome aberrations in common (e.g., the isochromosomes 1 in a2c/9829). Apparently, such aberrations gained stability during advanced stages of preneoplastic progression, possibly by giving affected cells a competitive growth advantage. Thus, they may contribute to tumor promotion and modify the determinants of later tumor characteristics (Fig. 2). (Yet, the major forces in the progression of FB tumorigenesis are known to be connected with tissue–environmental factors involving macrophage inactivity, fibrosis, and interaction between cell membrane and FB surface (1)).

In contrast to structural aberrations, ploidy deviations of individual chromosomes (secondary to the primary derangements in chromosome number) were always markedly unstable. They were often inconsistent in duplicate cultures and rarely present in more than 80% of the cell spreads (Table I). Therefore, they cannot be assumed to exert an essential effect on the tumorigenic process at any stage. More likely, they result from inconsequential mitotic errors during *in vivo* proliferation, if not during culture prior to karyological examination.

Nevertheless, aberrant di-, tri-, tetra-, and pentasomies of individual chromosomes were overall the most frequent abnormalities observed in this study. Individual chromosomes had a strikingly different predisposition for ploidy deviations. This phenomenon may be explained by differences in chromosome stability and/or dispensibility. If a chromosome is of vital importance to the cell it would not only be indispensable, but may actually constitute a "limiting factor of viability" and as such determine the optimum ploidy level. On the other hand, numerical fluctuations of less important or dispensible chromosomes would not reduce cell viability and competitiveness. In this latter category of presumably less important chromosomes we find especially chromosomes 1, 6, 7,13, 15, 18, and 19 which most frequently deviated from the ploidy level of the aberrant karyotype.

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1. Brand KG, Buoen LC, Johnson KH, Brand I. Etiological factors, stages, and the role of the foreign body

in foreign body tumorigenesis: A review. Cancer Res **35**:279–286, 1975.
2. Johnson KH, Buoen LC, Brand I, Brand KG. Light-microscopic morphology of cell types cultured during preneoplasia from foreign body-reactive tissues and films. Cancer Res **37**:3228–3237, 1977.
3. Buoen LC, Brand I, Brand KG. Foreign body tumorigenesis: In vitro isolation and expansion of preneoplastic clonal cell populations. J Nat Cancer Inst **55**:721–723, 1975.
4. Brand KG, Buoen LC, Brand I. Multiphasic incidence of foreign body induced sarcomas. Cancer Res **36**:3681–3683, 1976.
5. Thomassen MJ, Buoen LC, Brand KG. Foreign body tumorigenesis: Number, distribution, and cell density of preneoplastic clones. J Nat Cancer Inst **54**:203–207, 1975.
6. Wurster DH. Mouse chromosomes identified by trypsin–Giemsa (T–G) banding. Cytogenetics **11**:379–387, 1972.
7. Barrett JC. A preneoplastic stage in the spontaneous neoplastic transformation of Syrian hamster embryo cells in culture. Cancer Res **40**:91–94, 1980.
8. Cowell JK. Chromosome abnormalities associated with salivary gland epithelial cell lines transformed in vitro and in vivo with evidence of a role for genetic imbalance in transformation. Cancer Res **41**:1508–1517, 1981.
9. Brand KG, Brand I. Risk assessment of carcinogenesis at implantation sites. Plast Reconstr Surg **66**:591–594, 1980.

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