

The Different Origin of Primary and Secondary Chromosome Aberrations in Cancer

G. Levan and F. Mitelman

A. Abstract

We have proposed a hypothetical model to explain the role of chromosomal aberrations in malignant development. In this model we postulate two kinds of chromosomal changes: (1) *primary, active changes* caused by direct interaction between the oncogenic agent and the hereditary material of the host cell. These changes are mainly somatic mutations, but may also be associated with directed structural changes visible in the microscope; and (2) *secondary, passive changes* arising randomly by nondisjunction and structural rearrangements. They are followed by selection of cells with changes that amplify the primary change and thus appear as nonrandom chromosome patterns.

This hypothesis is discussed in the light of 1827 cases of human malignancy in which we have recently surveyed and systematized chromosomal aberrations. Special support for the idea of somatic mutations as the initiator of malignant development comes from work of Knudson and collaborators in human retinoblastoma. The Ph¹ chromosome, predominant during the chronic phase of chronic myeloid leukemia (CML), is proposed as an instance of a primary change, whereas the chromosome changes during the blastic crisis of CML will illustrate the secondary changes. The most common of these secondary changes is actually the doubling of the Ph¹ and thus an amplification of the primary change. The increase in number of copies of a specific chromosome reported by Green and collaborators demonstrates that this kind of amplification can result in direct response to the need for a specific gene located in that chromosome.

B. Introduction

Towards the end of the nineteenth century it became known from the work of pathologists that malignant cells were often liable to chromosome aberrations. Boveri in 1914 hypothesized that these aberrations actually were the cause of the malignant growth. At that time, technical difficulties in the study of mammalian chromosomes effectively put a stop to any thorough investigation of chromosome aberrations in tumors. Improvements in methodology in the early 1950s made detailed studies feasible, but only after the introduction of chromosome banding in the early 1970s could chromosomal and subchromosomal changes in tumor materials be analyzed with great precision. The results from both experimental and human tumors were significant: clear nonrandom patterns of aberrations became discernible. There were, however, considerable difficulties in interpreting the findings. Firstly, there were usually many different types of changes even within a clinically well defined group of tumors. Secondly, there was a variable degree of "background noise", i.e., chromosome changes that did not fall into the nonrandom pattern. Thirdly, there were cases of malignant tumors in which even refined chromosome banding did not reveal any aberrations.

C. Chromosome Aberrations in Human Malignancies

We have attempted to collect data as completely as possible from all human tumors that have been studied carefully by chromosome banding techniques. The bulk of the material

Myelo-proliferative disorders	Chronic myeloid leukemia (CML)	
	t(9;22) and other aberrations	361
	Aberrant translocations	94
	Acute myeloid leukemia (AML)	496
	Polycythemia vera (PV)	86
	Myelodyscrasia (MD)	278
Lympho-proliferative disorders	Malignant lymphomas (ML)	105
	Burkitt's lymphoma (BL)	24
	Acute lymphocytic leukemia (ALL)	156
	Chronic lymphocytic leukemia (CLL)	30
	Monoclonal gammopathies (MG)	23
Solid tumors	Benign mesenchymal tumors (BMT)	56
	Benign epithelial tumors (BET)	9
	Carcinomas (CA)	87
	Malignant melanomas (MM)	8
	Neurogenic tumors (NT)	7
	Sarcomas (SA)	7

Table 1. Subdivision of 1827 cases of human neoplasms with chromosome aberrations identified by banding techniques

comes from the published cases in the literature. In addition, there are unpublished cases from our own laboratory and from other laboratories, the latter kindly made available by the original investigators. All in all we have collected and systematized 1827 cases of human malignancies exhibiting chromosome aberrations. This figure does not include about 2000 cases of chronic myeloid leukemia that have the Ph¹-translocation as the only aberration. According to the diagnoses the material has been subdivided into 15 classes (Table 1).

The aberrations of each patient with a parti-

cular disease may be scored and surveyed in histograms such as the one in Fig. 1. This diagram represents 496 cases of acute myeloid leukemia. We think it must be obvious to all that the various chromosome types are affected by aberrations in a nonrandom fashion. Clearly chromosomes Nos. 7, 8, 17, and 21 are selectively involved in aberrations. It should be noted, however, that the nonrandom elevation of the aberrations in these chromosomes is blurred by the random aberrations occasionally affecting all chromosomes and causing the rather high background level of aberrations.

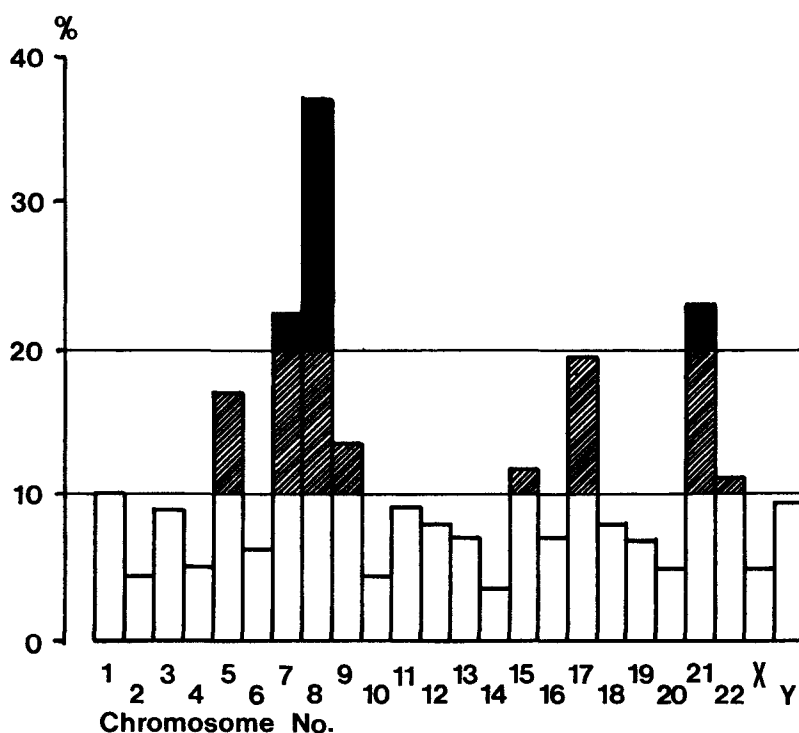


Fig. 1. Histogram of per cent aberrations affecting the 24 human chromosome types in 496 patients with acute myeloid leukemia

Table 2. Selective involvement of chromosome types in 15 classes of human neoplasms

Tumor type ^a	Chromosome No.																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
CML								8	9								17						22	
AML					5		7	8									17					21		
PV	1							8	9												20			
MD					5		7	8													20	21		
ML	1		3						9					14										
BL							7	8						14										
ALL	1													14							21	22		
CLL	1													14			17							
MG	1		3											14										
BMT								8																22
BET								8						14										
CA	1		3		5		7	8																
MM	1								9															
NT	1																							22
SA													13	14										
No. of times involved	8	-	3	-	3	-	4	8	4	-	-	-	1	7	-	-	3	-	-	2	3	4	-	-

^a Key to abbreviations of the tumor types, see Table 1

D. Clustering of Aberrations to Specific Chromosomes

Similar histograms have been prepared for all the different tumor classes listed in Table 1. When the chromosomes affected most commonly in each class are selected, the picture of Table 2 emerges. It is clear from this table that not only are aberrations nonrandom within each tumor class, but there is also a tendency for the aberrations to cluster to specific chromosomes when different classes are compared. Thus, chromosomes 1, 3, 5, 7, 8, 9, 14, 17, 21, and 22 exhibit nonrandom involvement in three or more types of malignancy, whereas 12 chromosome types never show any indication of a selective involvement.

These data from the human tumors are corroborated and strengthened when similar data from experimental tumors are taken into account as well. Thus, sarcomas of the rat, the mouse, and the Chinese hamster all show involvement of specific chromosomes. The same is true of rat leukemias and is especially consistent in mouse leukemias. Virtually 100% of mouse T-cell leukemias exhibit triso-

my No. 15 (Wiener et al. 1978a,b; Chan et al. 1979).

E. The Significance of Chromosome aberrations in Malignancy – A Hypothesis

Thus, banding studies have conclusively demonstrated that chromosome aberrations in tumors are nonrandom. Unfortunately, this knowledge does not immediately throw light on the question what significance this nonrandom involvement has to tumor initiation and development. Levan and Mitelman (1977) proposed a model of interpretation, which may still be useful as a working hypothesis (Fig. 2). According to this model there are two kinds of chromosome changes with different modes of generation: (1) primary or active changes, which arise through direct interaction between the oncogenic agent and the genetic material of the cell, and (2) secondary or passive changes, which arise at random in the proliferating cell population and are subject to subsequent selection. The effect of the primary

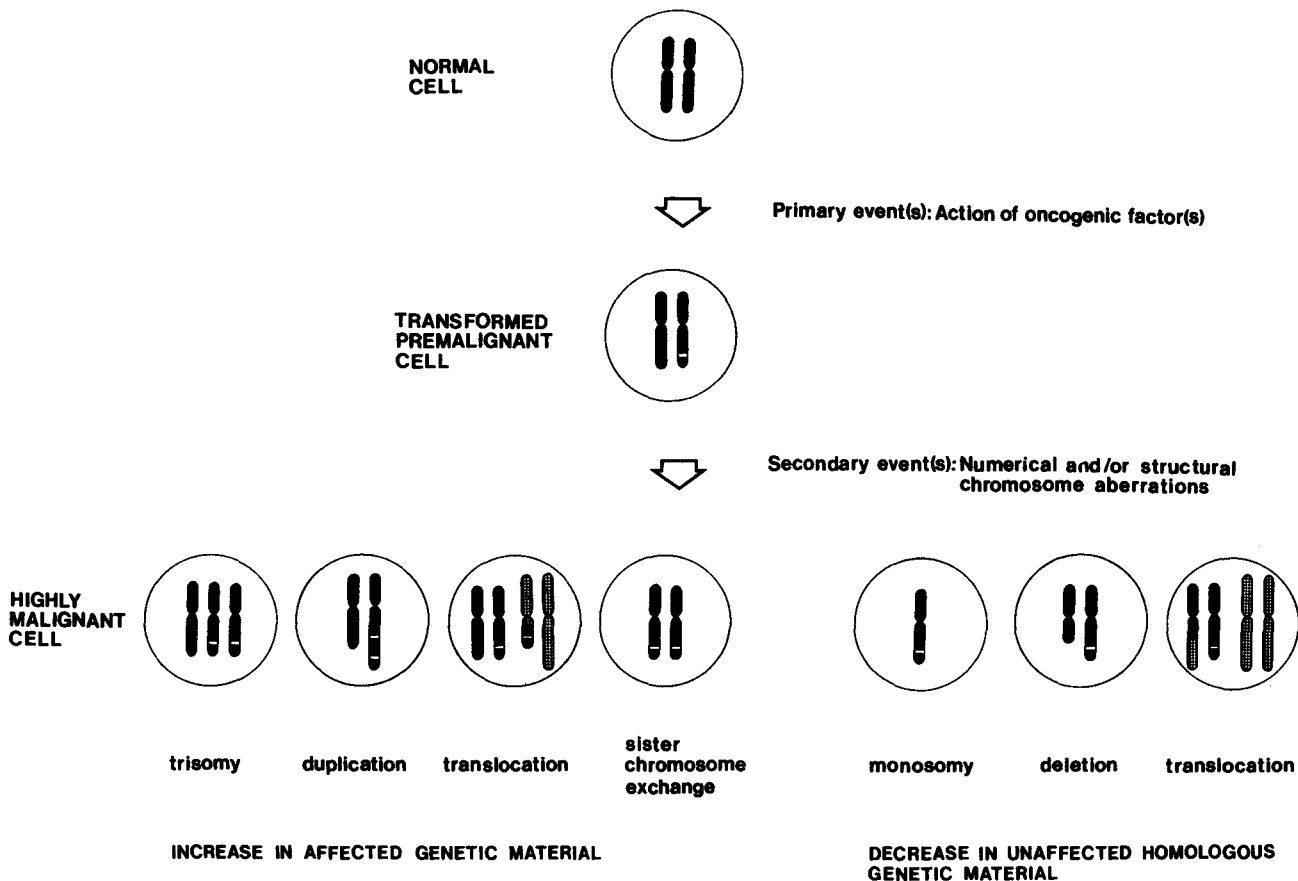


Fig. 2. Hypothetical scheme of chromosomal events in the origin and development of cancer

changes is to transform the cell into an autonomous parasite and the effect of the secondary changes is to stepwise render the parasitic cell population more and more malignant. In the model it is assumed that the primary changes usually are submicroscopic somatic mutations. Secondary changes cause chromosome imbalance to amplify the primary changes and arise from structural rearrangements and nondisjunction.

F. Somatic Mutation in the Initiation of Malignancy

There are still considerable differences in opinion about the role of somatic mutation in cancer. Even though no direct evidence exists that somatic mutation constitutes the initial step in oncogenesis, many facts are compatible with this assumption. Strong support comes from the fact that most carcinogens have proven to be potent mutagens.

The work of Knudson in human hereditary cancers also tends to support the somatic mutation theory (Knudson et al. 1975). In

retinoblastoma, both sporadic and hereditary cases exist. According to Knudson's hypothesis two specific somatic mutations in homologous loci are required for a tumor to develop. This highly unlikely event must be very rare and is the cause of the low incidence of sporadic retinoblastoma. In hereditary retinoblastoma, on the other hand, the first mutation is inherited and thus present in advance in all somatic cells. Due to the great number of cells involved in the development of the retina, the probability is now high that the second mutation will take place in at least one cell, making this cell fully malignant. This hypothetical scheme is supported by the fact that sporadic cases virtually always are unifocal, whereas the majority of the hereditary cases are multifocal.

Recently, some hereditary cases have been studied where one of the mutations apparently is substituted for by small chromosomal deletions (Nove et al. 1979). These deletions all affect chromosome 13 and when different cases are compared the common segment lost is band 13q14. It is well known from genetic studies that expression of a recessive mutant phenotype may be achieved either by a second

mutation in the homologous locus or by a deletion affecting the same locus.

The conclusion from the discussion so far is that one or more somatic mutation is the initiating step in at least some tumors. The somatic mutation may be substituted for by a deletion and possibly also by other specific chromosomal aberrations. It is characteristic of the primary chromosome changes that they are quite specific and directed towards one or more genes probably concerned with growth regulation and differentiation in the affected tissue.

G. The Ph¹ Chromosome – an Example of a Primary Chromosome Aberration

The chromosomes of an ample number of cases of Ph¹-positive human chronic myeloid leukemia (CML) have been studied with modern banding techniques. The total number of cases is not known, since many of them have not been published, but they must amount to several thousands. The great majority of them show one very specific aberration: a translocation between chromosome Nos. 9 and 22. Usually, patients with this aberration alone are clinically in the chronic phase of the disease – when the blastic crisis sets in, secondary chromosome aberrations ordinarily develop.

Thus CML quite nicely follows the scheme: a primary specific change followed by secondary less specific changes, a process which is paralleled by the development of the disease from a less malignant to a highly malignant state. It is, of course, possible to hypothesize that the Ph¹-positive cells arise not through the action of some specific mechanism but through selection from a large number of equally frequent translocations. There is, however, overwhelming evidence against this interpretation. A number of Ph¹-positive leukemias have been detected in which the translocation is not the typical t(9;22) but some other change (Table 3). In all cases one of the translocation partners is the deleted No. 22, but the other is not No. 9 but either another chromosome or the translocation is complex involving more than two chromosomes. There are also three cases where Ph¹ appears to represent a true deletion. All these chromosomal variants are associated with a disease indistinguishable from typical t(9;22) CML. The first conclusion from these data is that the significant change in CML is the deletion of No. 22. The second is that selection from a random population of translocations is excluded. Obviously, the t(9;22) is immensely overrepresented and other translocations leading to del(22) underrepresented. The conclusion must be that t(9;22) in CML is a chromo-

Table 3. Aberrant translocation patterns in 94 cases of Ph¹-positive CML

Translocation	No. of cases	Translocation	No. of cases	Translocation	No. of cases
t(X;22)	1	t(X;9;22)	1	t(X;9;17;22)	1
t(2;22)	2	t(1;9;22)	4	t(1;4;20;22)	1
t(3;22)	1	t(2;9;22)	4	t(3;4;9;22)	1
t(4;22)	1	t(3;9;22)	7	t(4;9;17;22)	1
t(5;22)	1	t(4;9;22)	2	t(7;9;11;22)	1
t(6;22)	2	t(5;9;22)	2	t(9;13;15;22)	1
t(7;22)	2	t(6;9;22)	3	t(9;16;17;22)	1
t(9;22)ab	1	t(7;9;22)	4	t(9;10;15;19;22)	1
t(10;22)	2	t(8;9;22)	1		
t(11;22)	2	t(9;10;22)	2	del (22)	3
t(12;22)	4	t(9;11;22)	3		
t(13;22)	1	t(9;13;22)	2		
t(14;22)	3	t(9;14;22)	3		
t(15;22)	2	t(9;15;22)	1		
t(16;22)	3	t(9;17;22)	2		
t(17;22)	6	t(17;17;22)	1		
t(19;22)	4	t(21;22;22)	1		
t(21;22)	1				
t(22;22)	1				

some change of the primary type, induced by a highly specific mechanism which is slightly error prone and occasionally produces an aberrant translocation.

H. Secondary Chromosome Aberrations Are Due to Random Changes and Subsequent Selection

The cell that has been transformed by a primary change into a premalignant or malignant state begins to divide without being restricted by the homeostasis. Secondary chromosome changes will occur in a random fashion due to the mechanisms frequent in transformed cells, i.e. nondisjunction and chromosome breakage and reunion. Most abnormal cells thus originated will be at selective disadvantage and never attain any importance in the population. If, however, a secondary change occurs which amplifies the effect of the primary change, the cell thus changed will be at selective advantage and divide faster than the surrounding cells. Since cell division is a prerequisite for secondary changes to become manifest, further changes will have an increased chance of arising. In this way increased division rate will generate cells with even more increased division rate in the mode of a vicious circle.

It is this process, we feel, that leads to the nonrandom distribution of secondary chromosome changes mentioned in the beginning of the paper. Conversely, it is possible to deduce from the distribution of aberrations which chromosomes were originally affected by a primary change. Thus, it is significant that the most common secondary change in CML is the duplication by nondisjunction of the Ph¹-chromosome.

It has been long recognized that malignant cell populations give the impression of existing in a condition of perpetual change and selection. Direct evidence that this may involve genetic responses leading to the amplification of specific genes is scarce. Proof that such a mechanism can be at work has been obtained in certain experiments of Green et al. (1971). Growth of human-mouse cell hybrids, selected in the HAT medium, is dependent on the human TK⁺ gene situated on the No. 17 chromosome. Normally, such a hybrid will carry only one No. 17 chromosome. Green and collaborators drastically decreased the thymi-

dine concentration in the HAT medium and then selected clones with the best growth potential. These clones proved to contain both two and three copies of the TK⁺ chromosome. The obvious conclusion of this experiment was that this system selected for randomly occurring nondisjunction products in the cell population and may thus serve as a model for the appearance of secondary changes in malignant development.

I. Is it Reasonable to Postulate That Both Significant and Insignificant Chromosome Aberrations may be Found in Neoplastic Cells?

A fact, which has seemed very difficult to reconcile with the thought of specific and significant chromosome involvement in the origin and early development of tumors, is the presence of a background level of random aberrations in many materials. It does not appear biologically reasonable that both significant and insignificant aberrations should be manifest in tumor cell populations. Recent work by Wiener and collaborators has thrown light on this question. In a series of elegant experiments these workers have shown that mouse T-cell leukemias very specifically exhibit No. 15 trisomy (Wiener et al. 1978a,b). Furthermore, by using four strains of Robertsonian translocation mice ($2n=38$) with rob(1;15), rob(4;15), rob(5;15) and rob(6;15), respectively, they were able to show that T-cell leukemias in these animals display trisomy for the entire translocation chromosome (Spira et al. 1979). Clearly, these neoplasms require trisomy 15 for their development and are not bothered by carrying trisomy for an insignificant chromosome as well, as long as the required No. 15 trisomy is present. We feel that this is a general feature of tumor cell populations that may explain the high level of background aberrations in some tumors.

Acknowledgements

Financial support of this work by grants from the Swedish Cancer Society, the John and Augusta Persson Foundation of Medical Research, and CANCIRCO is gratefully acknowledged.

References

- Chan FHP, Ball JK, Sergovich FR (1979) Trisomy 15 in murine thymomas induced by chemical carcinogens, X-irradiation, and an endogenous murine leukemia virus. *J Natl Cancer Inst* 62:605-610
- Green H, Wang R, Kehinde O, Meuth M (1971) Multiple human TK chromosomes in human-mouse somatic cell hybrids. *Nature (London) New Biol* 234:138-140
- Knudson AG, Heathcote HW, Brown BW (1975) Mutation and childhood cancer: A probabilistic model for the incidence of retinoblastoma. *Proc Natl Acad Sci* 72:5116-5120
- Levan G, Mitelman F (1977) Chromosomes and the etiology of cancer. In: Chapelle A de la, Sorsa M (eds) *Chromosomes today*, vol 6. Elsevier/North-Holland, Amsterdam, pp 363-371
- Nove J, Little JB, Weichselbaum RR, Nichols WW, Hoffman E (1979) Retinoblastoma, chromosome 13, and in vitro cellular radiosensitivity. *Cytogenet Cell Genet* 24:176-184
- Spira J, Wiener F, Ohno S, Klein G (1979) Is trisomy cause or consequence of murine T-cell leukemia development? Studies on Robertsonian translocation mice. *Proc Natl Acad Sci* 76:6619-6621
- Wiener F, Ohno S, Spira J, Haran-Ghera N, Klein G (1978a) Chromosome changes (trisomies #15 and 17) associated with tumor progression in leukemias induced by radiation leukemia virus. *J Natl Cancer Inst* 61:227-237
- Wiener F, Spira J, Ohno S, Haran-Ghera N, Klein G (1978b) Chromosome changes (trisomy 15) in murine T-cell leukemia induced by 7,12-dimethylbenz(α)anthracene (DMBA). *Int J Cancer* 22:447-453