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# The Implications of Nonrandom Chromosome Changes for Malignant Transformation*

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The role of chromosome abnormalities in malignant transformation has been debated for more than 60 years (Boveri 1914). The evidence that chromosome changes play a fundamental role and that they perhaps may even be the ultimate transformation event in some malignancies is becoming ever more compelling. This view is contrary to that held by most investigators even a few years ago. The change in attitude is the result of new technologic advances which allow cytogeneticists to identify precisely each human chromosome and parts of chromosomes as well. Application of the same chromosome banding techniques to the cytogenetic study of animal cancers has provided data that confirm observations of specific nonrandom chromosome abnormalities in virtually every human tumor that has been adequately studied (Mitelman and Levan 1978; Rowley 1978; Sandberg 1980). The evidence obtained from the study of human leukemias is summarized in chapter four.

These nonrandom chromosome changes consist of gains or losses of part or all of certain specific chromosomes and of structural abnormalities, which are most frequently relatively consistent translocations and are presumed to be reciprocal. The nonrandom translocations that we observe in malignant cells would represent those that provide a particular cell type with a selective advantage vis-a-vis the cells with a normal karyotype. There is very strong evidence that many malignancies, for example, chronic myelogenous leukemia and Burkitt lymphoma, are of clonal origin. This means that a particular translocation in a single cell gives rise to the tumor or to the leukemia that ultimately overwhelms the host. Other rearrangements may be neutral, and the cells therefore will survive but will not proliferate differentially; still others may be lethal and thus would be eliminated. In such a model, the chromosome change is fundamental to malignant transformation.

Two questions are raised by these observations. First, how do such chromosome changes occur, and second, why do they occur? There is very little experimental evidence that is helpful in answering either of these fundamental questions. They clearly provide a focus for future research.

## A. Production of Consistent Translocations

The mechanism for the production of specific, consistent reciprocal translocations is unknown. Chromosome breaks and rearrangements may occur continuously at random and with a low frequency, and only those with a selective advantage will be observed (Nowell 1976; Rowley 1977). Alternatively, certain chromosome regions may be especially vulnerable to breaks and, therefore, to rearrangements. In the rat, Sugiyama (1971) showed that a particular region on No. 2 was broken when bone marrow cells from animals given DMBA were examined. In man, however, trisomy for lq is not necessarily related to

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fragile sites (Rowley 1977). Thus, a comparison of the break points seen in hematologic disorders that involve balanced reciprocal translocations with those leading to trisomy lq revealed a clear difference in preferential break points, depending on whether the rearrangement resulted in a balanced or an unbalanced aberration.

Other possible explanations depend on either (1) chromosomal proximity, since translocations may occur more frequently when two chromosomes are close together, or (2) regions of homologous DNA that might pair preferentially and then be involved in rearrangements. Many of the affected human chromosomes, e.g., Nos. 1, 9, 14, 15, 21, and 22, are involved in nucleolar organization which would lead to a close physical association. All partial trisomies which result from a break in the centromere of No. 1 involve translocations of lq to the nucleolar organizing region of other chromosomes, specifically Nos. 9, 13, 15, and 22 (Rowley 1977). In the mouse, chromosome No. 15 also contains ribosomal cistrons (rRNA) (Henderson et al. 1974). Sugiyama et al. (1978) noted that in rat malignancies aneuploidies frequently involve chromosomes with late-replicating DNA or those which have rDNA and late-replicating DNA. They have suggested that nucleolus-associated late-replicating DNA rather than rDNA is involved in the origin of nonrandom chromosome abnormalities.

On the other hand, if chromosome proximity or homologous DNA sequences were the mechanism, this should lead to an increased frequency of rearrangements such as t(9q+;22q−) or t(8q−;14q+) in patients with constitutional abnormalities, but this has not been observed. It is possible that either or both of these mechanisms are subject to selection; a translocation might occur because the chromosomes are close together, but only certain specific rearrangements might have a proliferative advantage which results in malignancy and thus allows them to be detected.

One other possible mechanism which should be considered concerns transposable genetic elements that can cause large-scale rearrangements of adjacent DNA sequences (Rowley 1977). Not only do these elements exert control over adjacent sequences, but the type of control, that is, an increase or a decrease in gene product, is related to their position and orientation in the gene locus. Whereas they can cause nonrandom chromosomal deletions adjacent to themselves, these controlling elements can also move to another chromosomal location, and they may transpose some of the adjacent chromosomal material with them. The evidence for the presence of transposable elements in mammalian cells is tenuous, but a more precisely defined gene map is required for the detection of such nonhomologous recombinations.

## B. Function of Nonrandom Changes

Our ignorance of how nonrandom changes occur is matched by our ignorance as to why they occur. The question to be examined now relates to the kinds of gene loci that can provide a proliferative advantage.

### I. Host Genome

First, two points should be emphasized; one concerns the genetic heterogeneity of the human population, and the second, the variety of cells involved in malignancy. There is convincing evidence from animal experiments that the genetic constitution of an inbred strain of rats or mice plays a critical role in the frequency and type of malignancies that develop. Some of the factors controlling the differential susceptibility of mice to leukemia not only have been identified but also have been mapped to particular chromosomes, and their behavior as typical Mendelian genes has been demonstrated (Ihle et al. 1979; Lilly and Pincus 1973; Rowe 1973; Rowe and Kozak 1979). These genes have been shown to be viral sequences that are integrated into particular sites on chromosomes; these sites vary for different inbred mouse strains and for different murine leukemia viruses.

Certain genetic traits in man predispose to cancer, such as Bloom syndrome, Fanconi anemia, and ataxia-telangiectasia (German 1972). We recognize the existence of cancer-prone families, of inherited genetic susceptibility to specific types of malignancy, such as retinoblastoma and breast cancer, and of the inheritance of lesions which have a high propensity for becoming malignant, such as familial colonic polyps (Mulvihill et al. 1977). How many gene loci are there in man which in some way control resistance or susceptibility to a particular malignancy? We have no way of

knowing at present. These genes may influence the types of chromosome changes that are present in malignant cells.

The second factor affecting the karyotypic pattern relates to the different cells that are at risk of becoming malignant and the varying states of maturation of these cells. The catalog of the nonrandom changes in various tumors maintained by Mitelman and Levan (1978) provides clear evidence that the same chromosomes, for example, Nos. 1 and 8, may be affected in a variety of tumors. On the other hand, some chromosomes seem to be involved in neoplasia affecting a particular tissue; the involvement of No. 14 in lymphoid malignancies and the loss of No. 7 in myeloid abnormalities might be suitable examples. All of the consistent translocations are relatively restricted to a particular cell lineage. Given the great genetic diversity, the number of different cell types that might become malignant, and the variety of carcinogens to which these cells are exposed, it is surprising that nonrandom karyotypic changes can be detected at all.

## II. Chromosome Changes Related to Gene Dosage

Gains or losses of chromosomes directly affect the number of functioning structural gene copies and therefore alter the amount of gene product in the cell. Although this is only speculative at present, the action of translocations may be to modify the regulation of gene function and therefore to alter the amount of gene product in the cell. There is ample evidence that as cells evolve to a more malignant state many of them gain one or more extra copies of particular chromosomes which must carry genes that provide a proliferative advantage. In some instances, particularly in secondary leukemias, chromosome material is lost; this may allow putative recessive transforming genes to alter the cell (Comings 1973). Alternatively, the loss may shift the balance between genes for the expression and those for the suppression of malignancy (Sachs 1978).

### *1. Homogeneously Staining Regions and Double Minute Chromosomes*

One of the most rapidly moving areas of current investigation involves chromosomes of unusual morphology such as homogeneously staining regions (HSR), reiteration of apparently identical sequences of dark-light bands, double minutes (DM), and selective gene amplification. Homogeneously staining regions (HSR) were first described by Biedler and Spengler (1976) in drug-resistant Chinese hamster cell lines and in human neuroblastoma cell lines. Within the HSR, replication was synchronous and rapid and was completed before the midpoint of the DNA synthesis period. Similar HSR regions have been described in other human neuroblastoma cell lines by Balaban-Malenbaum and Gilbert (1977). Some animal tumor cells also have HSR (Levan et al. 1977).

Double minutes are small, paired DNA-containing structures that are palestaining with various banding techniques. They appear to lack a centromere and are apparently carried through cell division by attaching themselves to remnants of nucleolar material or by "hitchhiking" on the ends of chromosomes (Levan and Levan 1978). Variable numbers up to 100 DM per cell are found in malignant cells from a number of human tumors as well as those induced in experimental animals.

Neither HSR nor DM are commonly described in hematologic malignancies, for reasons that are not clear. I have seen DM in only five patients with various types of acute leukemia in more than 150 cases studied, and I have never seen them in any form of CML. The apparent absence of HSR may be explained by their altered appearance in hematologic malignancies. One patient in the leukemic phase of histiocytic lymphoma had several unusual marker chromosomes that contained multiple repeats of alternating dark and light bands (Brynes et al. 1978). Biedler and Spengler (1977) have recently reported what may be an analogous phenomenon in drug-resistant Chinese hamster cell lines. Evidence relating HSR and DM has recently been obtained from a human neuroblastoma (Balaban-Malenbaum and Gilbert 1977) in which about one-half of the cells contained a long HSR in 5q, whereas the other one-half of the cells contained two normal No. 5s and DM. The HSR and DM were never seen in the same cell, although there was clear cytogenetic evidence that both subpopulations had a common precursor.

### *2. Evidence for Gene Amplification*

The function of HSR and DM within the cells is largely unknown. Structure and function have been correlated in an elegant fashion in the

drug-resistant Chinese hamster cell lines described by Biedler et al. (1976). When cells from these lines were exposed to methotrexate or methasquin, they developed extraordinarily high levels of dihydrofolate reductase (EC1.5.1.3-DHFR) in association with their drug resistance. Of the 13 independently derived drug-resistant cell lines, only those with greater than 100-fold increases in enzyme activity contained HSR-bearing chromosomes. In some cell lines, HSR represented as much as 6% of the chromosome complement.

In studies of various methotrexate-resistant mouse cell lines, Alt et al. (1978) have shown that the relative number of DHFR gene copies is proportional to the cellular level of DHFR and DHFR mRNA sequences. Giemsa banding studies of a methotrexate-resistant murine lymphoblastoid cell line (Dolnick et al. 1979) showed a large HSR on chromosome No. 2. Molecular hybridization studies in situ indicate that the DHFR genes are localized in this HSR. Similar observations have been made in a methotrexate-resistant Chinese hamster ovary cell line (Nunberg et al. 1978). Evidence from other tumors suggests that amplification of genes coding for 18S and 28S ribosomal RNA may occur (Miller et al. 1979).

All of these data taken together indicate that HSR and DM provide the chromosomal evidence of gene amplification which, in the case of these particular drug-resistant lines, represents amplification for the DHFR gene. The nature of the genes that are amplified in human neuroblastomas, in other human tumors, and in animal tumors is unknown. Recent technical advances provide the tools with which these significant questions can be answered.

## C. Conclusion

The relatively consistent chromosome changes, especially specific translocations, that are closely associated with particular neoplasms provide convincing evidence for the fundamental role of these changes in the transformation of a normal cell to a malignant cell. In some tumors these changes may be too small to be detected, and the cells would appear with present techniques to have a normal karyotype.

When one considers the number of nonrandom changes that are seen in a malignancy such as ANLL, it is clear that not just one gene but rather a class of genes is involved. Our knowledge of the human gene map has developed concurrently with our understanding of the consistent chromosome changes in malignancy (McKusick and Ruddle 1977). It is now possible to try to correlate the chromosomes that are affected with the genes that they carry. Clearly, these efforts are preliminary, since relatively few genes have been mapped, and since some of the chromosomes that are most frequently abnormal have few genetic markers. In such a preliminary attempt in 1977 (Rowley 1977), I observed that chromosomes carrying genes related to nucleic acid biosynthesis and also the specific chromosome region associated with these genes were frequently involved in rearrangements associated with hematologic malignancies. More recently, Owerbach et al. (1978) reported that a gene for the large external transformation-sensitive (LETS) protein is located on chromosome No. 8. They noted that because LETS protein has been implicated in tumorigenicity and cellular transformation, its localization to a human chromosome associated with malignancies may prove to be a significant observation.

In the future we will be able to determine the break points in translocations very precisely, to measure the function of genes at these break points, and to compare the activity of these genes in cells with translocations with their activity in normal cells. In other types of abnormalities such as HSR or DM we will be able to identify the genes involved in this process of gene amplification. Such information will be the basis for understanding how chromosome changes provide selected cells in certain individuals with a growth advantage that results in malignancy.

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