CHROMOSOMES IN HUMAN CANCER

CERVENKA AND KOULISCHER

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PREFACE

AMONG THE manifold challenges of medical science, the etiology of cancer remains the most intriguing for many reasons. Our understanding of the problem has advanced *pari passu* with the many advances in virology, immunology, cell surface research, and an inordinate number of other fields of biomedical research. Cytogenetics in our opinion occupies an important place in this extensive list.

It has been realized that chromosomes are not only involved in transmission of genetic information from generation to generation and from a cell to its daughter cells, but they are also responsible for the ordered release of this information for control of cellular development and function. Data have accumulated concerning systems of gene control and chromosomal activation or inactivation in complex organisms. Quite recently there has been a flurry of investigation of heterochromatins and satellite DNA, not only on the molecular level, but on the level of chromosomal morphology and function.

It seems justified that this line of research has induced considerable enthusiasm among the cytogenetic community and has stimulated Dr. T. C. Hsu to introduce the 9th Annual Somatic Cell Genetics Conference in Galveston (1971) by reminiscing that, "After the great excitement of the late 1950's and early 1960's the field of human cytogenetics has become somewhat dull . . ." and expressing hope that the new staining techniques ". . . should stimulate some interest for at least a few more years."

At this point in time we have decided to gather all available information on chromosomes in human tumors with the fervent hope that they might be used as a source of understanding of the seemingly disordered chromosomal constitution in human cancer cells and of the few rules which may be derived from these data.

We are aware that our attempts come at a time which we consider to be the threshold of a new era of qualitative cytogenetics in man. We hope that our efforts may be exploited by workers involved in clinical research as well as in research of the cancer cell *in vitro,* despite

the fact that we have omitted the subject of chromosomes in established cell lines and in cancers of experimental animals.

It is our sincere desire that this book will contribute to understanding the manifold problems involved in human cancer etiology being a comprehensive and comprehensible overview on the form of genetic material so commonly altered during malignant transformation. Furthermore, it is our desire that this survey may serve a heuristic role in stimulating others to examine these problems.

There is an ancient apothegm that a tome has its incipience in enthusiasm, is continued in anxiety, and reaches fruition in exhaustion. This effort has tested that rule and has found it to be true. After surveying the plethora of papers on this subject, we are effete.

Aiding us in our efforts and to whom we express profound gratitude are Dr. Claude Fievez, Hattie L. Thorn, Marie Anne Cosme, Roberta J. Lensink, Eliane and Christiane Canon, Suzan M. Schwarze, and Bridget A. Stellmacher.

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JAROSLAV CERVENKA
LUCIEN KOULISCHER

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PART I

GENERAL CONSIDERATIONS

Chapter I

MECHANISM OF CHROMOSOMAL REARRANGEMENTS

CHROMOSOME STUDIES in malignancy are, in many respects, quite different from cytogenetic analyses performed in individuals with congenital malformations. In the latter the karyotype remains stable from conception (or from early stages of development) throughout the life of the individual, while in malignancy, important karyotypic changes may occur during a relatively short lapse of time. The approach to chromosome studies in cancer is dynamic, much as the development of the malignant tumor itself with its onset, growth, and metastasis.

It is useful to keep in mind that any chromosome analysis performed on a tumor tissue reflects changes which are present only at a particular time and site, being but one increment in the progression of the malignant process. This is the reason why the cytogenetics of neoplasia may appear at first glance to be confusing. By analogy, it represents but a single photographic enlargement printed from a long cinematographic film. One of the tasks of the cytogeneticist is to interpret the observations in context and to understand the mechanism of transformation of the original karyotype. This chapter deals with some basic concepts of chromosome change in malignancy and with possible ways which may eventuate in an abnormal karyotype in a given tumor.

In 1931, Levine wrote the following:

> Comparatively no phase of the cancer problem has received less attention in the last decade than the cytological phenomena of cancer cells in division. Obviously, this deserves more attention. According to our present knowledge, cancer is directly bound up with the process of nuclear and cell division. Without cell division, there is no cancer.

Today, the reverse situation is actually true: during the last decade, a spectacular amount of work has been devoted to cell division, and

especially to chromosome studies of cancer cells. This has been possible since new cytogenetic techniques have been introduced. These techniques have been used with success both in the study of congenital and malignant chromosome aberrations.

BASIC CONCEPTS

The significance of chromosome abnormalities in malignancy is classically attributed to Boveri (1914). Impressed by the work of von Hansen concerning multipolar mitoses in cancerous tissues (see Turpin and Lejeune, 1965), Boveri suggested that chromosomal change is the primary trigger mechanism in cancerogenesis. Winge (1930) considered the role of chromosome changes in cell selection within tumor tissue. As noted earlier, review of the subject "chromosomes and malignancy," was carried out as early as 1931 by Levine. Since then, numerous works have been devoted to chromosome alterations of spontaneous and induced tumors in animals.

These investigations furnished the basis for the important concept of the *stemline* (Levan and Hauschka, 1953; Makino, 1957; Hsu, 1961): (a) the stemline is mainly responsible for the growth of the tumor, (b) in some neoplasms, one or more stemlines can exist and develop, (c) the cells of a stemline are characterized by a common chromosome set, and (d) cells with other karyotypes represent "sidelines" having small effect on the progression of the tumor. However, depending upon environmental circumstances, a sideline may become one or more stemlines.

In some cases, a *marker chromosome* can be observed. A *marker chromosome* in a tumor is one with a peculiar morphology, easily identified and, as a rule, not seen in the normal karyotype. The presence of a marker in all cells of a malignant neoplasm gives support to the hypothesis of the clonal origin of the tumor: the marker has been transmitted to all tumor cells by a single mutant cell from which they derive (Berger, 1968). Indeed, cytogenetics could be used as well as the study of glucose-6-phosphate dehydrogenase (Linder and Gartler, 1965; Gartler *et al.*, 1966; Beutler *et al.*, 1967; Fialkow *et al.*, 1967) to demonstrate either the single cell or multiple cell origin of a tumor.

In man, reliable cytogenetic studies in malignancy have been possible only since the discovery of the correct number of human chromo-

somes by Tjio and Levan (1956). Progress in this field has been spectacular since the observation of a specific marker in chronic myeloid leukemia by Nowell and Hungerford in 1960.

POSSIBLE MECHANISMS OF CHROMOSOME CHANGES IN MALIGNANCY

Malignant stemlines are often characterized by abnormal chromosome numbers and/or the presence of markers. Some possible mechanisms leading to these karyotype changes are herein described. For a better understanding of the formation of nonspecific or unstable abnormalities such as breaks, gaps, sporadic rings, or isochromosomes, the reader is referred to Rieger *et al.* (1968).

Polyploidy

Polyploidy is defined as the state in which a cell shows three, four, five, or more complete chromosome sets instead of the normal two present in diploid somatic cells (Rieger *et al.*, 1968). A polyploid cell arises from a normal diploid cell when DNA synthesis occurs before division and for reasons unknown, there is failure of the cell to divide. Polyploidy is very often observed in malignant tissues; however, it is also observed in normal tissues such as liver, endometrium, urinary bladder epithelium, megakaryocytes, and in tissue cultures of normal diploid somatic cells. In cancer, polyploid cells may sometimes compose the stemline.

Endoreduplication

This is a special form of polyploidization, taking place within an intact nuclear envelope (Levan and Hauschka, 1953). An endoreduplication is established with certainty only if it is followed by normal mitosis. The chromosomes are seen lying topographically adjacent to one another, two-by-two (or four-by-four). Endoreduplication can lead to high levels of polyploidization and may be responsible for those tumor cells containing several hundreds of chromosomes.

Hyperploidy

This form of aneuploidy is characterized by the presence of one or more supernumerary chromosomes or chromosome segments. Thus

a typical hyperdiploid cell exhibits 47 or more chromosomes, a hypertriploid mitosis 70 chromosomes or more, etc. Hyperploidy may be arrived at through different mechanisms, most frequently being attributed to nondisjunction. At anaphase, the chromosome pair fails to separate and migrates towards the same pole. One daughter cell will thus have a supernumerary chromosome, while the other will lack a chromosome. The cause of nondisjunction is uncertain and has been attributed to aging, viruses, chemicals, or, for example, in maize, to the presence of a mutant gene called "sticky." Indeed, perfect spindle function is also important to avoid nondisjunction. *Selective endoreduplication* of only one or a few chromosomes (Lejeune *et al.*, 1966; de Grouchy *et al.*, 1967) is another possible avenue leading to hyperdiploidy. The nucleolus may be responsible for nondisjunction also, especially in chromosomes carrying the nucleolus organizers (Ohno *et al.*, 1961) since close association during interphase could favor nondisjunction during mitosis.

Hypoploidy

In this condition one or more chromosomes of the diploid set are missing. There are essentially two mechanisms involved. One is *nondisjunction* and the other is *chromosome lagging*. The latter represents loss of one (or more) chromosome during mitosis due to noninclusion in either daughter cell at telophase.

Pseudodiploidy and Marker Chromosomes

In some metaphase spreads, the expected diploid number of chromosomes can be observed. However, the karyotypes may show "odd" or marker chromosomes. Deletions, inversions, translocations, segmental duplications, insertions, formations of ring or isochromosomes can account for the presence of the markers. (A definition of these terms may be found in Rieger *et al.*, 1968.) Such a cell containing the diploid number 46, one or more chromosomes being abnormal, is called *pseudodiploid*. When a structural rearrangement is found in the majority of mitoses of a tumor, we assume that this characteristic stemline is responsible for the tumor propagation. An example of a specific marker in a tumor is the Philadelphia chromosome (a

deleted G group chromosome) found in human chronic granulocytic (myeloid) leukemia. The discovery of a marker chromosome in a malignant tissue is very important since cells with different karyotypes but containing the same marker can be related to a common ancestral cell.

Combined Mechanisms of Aneuploidization

More than one mechanism can be involved in malignant karyotype changes, especially if only one observation has been made. For example, nondisjunction may result in a hyperdiploid cell; translocations may occur between several chromosomes resulting in formation of markers; if chromosome losses then occur (including some markers), the pathways leading to the stemline karyotype actually observed can be completely masked. As said earlier, any one particular observation corresponds to just one single frame of a long movie. Nevertheless, information obtained in such a manner may be useful and worthy of interpretation.

CHROMOSOME EVOLUTION

Serial observations of the same tumor in short time sequences may exhibit important variations of karyotype, thus demonstrating a high degree of chromosome instability of the malignant clone. In some cases these variations are markedly progressive and demonstrate a real "evolution" that has been compared to the evolution of species (Lejeune, 1965 and Chapter V) and described by the term *clonal evolution*. Eventually, the presence of a marker chromosome suggests the common origin of the karyotypes observed. Many examples of clonal evolution are now known (see Table I). One of the principal tasks of the cytogeneticist studying a single tumor is to reconstruct the development of its karyotype from the normal karyotype to obtain insight into the progress of malignant transformation.

Theoretically, any chromosomal change in the cell results in severe genetic imbalance. It is easy to understand that karyotype transformations cannot occur randomly—some genetic disturbances may prove lethal to the cell. Therefore, Lejeune (1965) proposed three "laws" of clonal evolution:

TABLE I
CLONAL EVOLUTION IN A CASE OF ACUTE LEUKEMIA

Chromosome Number	*Karyotype*
47	tris 21
48	tris 21 + 1G
49	tris 21 + 2G
50	tris 21 + 2G + 1D
51	tris 21 + 2G + 2D
52	tris 21 + 2G + 2D + 1C
53	tris 21 + 2G + 2D + 2C
54	tris 21 + 2G + 2D + 2C + 1F
55	tris 21 + 2G + 2D + 2C + 2F

Note: From Lejeune *et al.*, 1963.

1. Clonal evolution is progressive: successive abnormalities finally lead to the observed karyotype of the tumor tissue studied.

2. There is a tendency to duplication of supernumerary chromosomes (Table I).

3. So-called *forbidden combinations* exist. A new karyotype implies a new genetic pattern for the cell. The action of genes carried by "new" chromosomes will most likely alter the cell metabolism. If new requirements cannot be satisfied, the cell will die: these are the forbidden combinations inconsistent with the development of the tumor. Another example of a forbidden combination is the one allowing the host to immunologically reject the tumor clone.

CHROMOSOMES AND PREMALIGNANCY

The cytogenetics of *premalignancy* is indeed interesting and offers an opportunity to better understand the relationship between chromosome aberrations and the origin of cancer. Are chromosomes normal in premalignant lesions? Do some peculiar changes signify transformation from benign to malignant cells and is cytogenetics a useful tool for early detection of cancer? The answers to these questions depend upon the definition of premalignancy.

The concept of premalignancy has stimulated many discussions. For Willis (1967), some tumors are to be considered premalignant since they often transform to malignant types. This is true for localized tumors of the bladder, Bowen's disease of the skin, and familial polyposis of the colon or rectum. Over 10 percent of patients with testicular tumors show ectopy of the testicle; the relationship between Paget's disease of the nipple and cancer is open to discussion, etc. (For detailed analysis see Lynch, 1967). Stewart's (1950) statement: "The female

breast is a precancerous organ" is well-known. In the case of leukemia, many diseases have been called "preleukemic": aplastic anemia, sideroachrestic anemia and other anemias, and thrombocythemia. All myeloproliferative disorders that may transform into chronic myeloid leukemia (Dameshek and Gunz, 1964) are also "preleukemic," as is trisomy 21 syndrome with its high proclivity to develop acute leukemia (Krivit and Good, 1956, 1957; see also Chap. III).

The best cytogenetic approach to this problem seems to be serial chromosomal analysis of nonmalignant tissue, followed by subsequent observation of *the same tissue* when the disease has undergone malignant transformation. Little data based upon these criteria have been published. The most valuable are those concerning CML (chronic myeloid leukemia) because of the presence of a specific marker: the Ph^1 chromosome. In some cases (Kemp *et al.*, 1961, 1964), the Ph^1 chromosome has been observed prior to the appearance of any actual signs of CML. In at least one case, polycythemia vera with Ph^1 chromosome exhibited transformation into CML, thus suggesting that chromosomal aberration occurred *before* clinical onset of the disease. Other cases with a Ph^1 chromosome, but without CML, have been reported in myeloproliferative diseases suggesting future onset of CML.

Less instructive are the data obtained in neoplastic disorders of the cervix uteri (see Chap. XII). Cytogenetic investigations have been made in cases of severe dyplasia of the cervix, carcinoma *in situ,* and invasive carcinoma (Wakonig-Vaartaja and Kirkland, 1965; Wakonig-Vaartaja and Hughes, 1967). The karyotype is, as expected, more frequently normal in dysplasia, and aneuploid (within a wide range) in malignant lesions of the cervix. However, aneuploidy is frequently found in dysplasia. When comparison is made with CML, it may be noted that even a relatively unimportant chromosome change (deletion of the long arms of one of the smallest chromosomes of the set) is sufficient for development of malignancy. In the case of cancer of the cervix, only a statistical analysis is meaningful and, in a single case, even considerable karyotype rearrangements cannot be interpreted as proof of malignancy.

Other examples from each system will be presented later. At this point, however, it must be stressed that severe aneuploidy with marker chromosomes can be observed in nonmalignant tumors and normal

karyotypes can be observed in some cancers. These remarks do not tend to deny the importance of cytogenetics in malignancy, but suggest that interpretation of data must be made with considerable caution.

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