CHAPTER 1

A New Approach to an Old Problem

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The role of genetic changes in neoplasia has been a matter of debate for more than 100 years. The earliest systematic study of cell division in malignant tumors was made in 1890 by the German pathologist David von Hansemann. He drew attention to the frequent occurrence of aberrant mitoses in carcinoma biopsies and suggested that this phenomenon could be used as a criterion for diagnosing the malignant state. His investigations as well as other studies associating nuclear abnormalities with neoplastic growth were, a quarter of a century later, forged into a systematic somatic mutation theory of cancer, which was presented in 1914 by Theodor Boveri in his famous book Zur Frage der Entstehung maligner Tumoren. According to Boveri’s hypothesis, chromosome abnormalities were the cellular changes causing the transition from normal to malignant proliferation.

For a long time, Boveri’s remarkably prescient idea, the concept that neoplasia is brought about by an acquired genetic change, could not be tested. The study of sectioned material yielded only inconclusive results and was clearly insufficient for the examination of chromosome morphology. Technical difficulties thus prevented reliable visualization of mammalian chromosomes, in both normal and neoplastic cells, throughout the entire first half of the twentieth century.

During these “dark ages” of mammalian cytogenetics (Hsu, 1979), plant cytogeneticists made spectacular progress, very much through their use of squash and smear preparations. These techniques had from 1920 onward greatly facilitated studies of the genetic material in plants and insects, disclosing chromosome structures more reliably and with greater clarity than had been possible in tissue sections. Around 1950 it was discovered that some experimental tumors in mammals, in particular the Ehrlich ascites tumor of the mouse, could also be examined using the same squash and smear approach. These methods were then rapidly tried with other tissues as well, and in general mammalian chromosomes were found to be just as amenable to detailed analysis as the most suitable plant materials.

Simultaneously, tissue culturing became more widespread and successful, one effect of which was that the cytogeneticists now had at their disposal a stable source of in vitro grown cells. Of crucial importance in this context was also the discovery that colchicine pretreatment resulted in mitotic arrest and dissolution of the spindle apparatus and that treatment of arrested cells with a hypotonic salt solution greatly improved the quality of
metaphase spreads. Individual chromosomes could now be counted and analyzed. The many methodological improvements ushered in a period of vivid expansion in mammalian cytogenetics, culminating in the description of the correct chromosome number of man by Tjio and Levan (1956) and, shortly afterward, the discovery of the major constitutional human chromosomal syndromes. Two technical breakthroughs around the turn of the decade were of particular importance: the finding that phytohemagglutinin (PHA) has a mitogenic effect on lymphocytes (Nowell, 1960) and the development of a reliable method for short-term culturing of peripheral blood cells (Moorhead et al., 1960).

Cytogenetic studies of animal ascites tumors during the early 1950s, followed soon by investigations of malignant exudates in humans (Fig. 1.1), uncovered many of the general principles of karyotypic patterns in highly advanced, malignant cell populations: the apparently ubiquitous chromosomal variability within the tumor surmised by pathologists since the 1890s; the stemline concept, first defined by Winge (1930); and the competition between stemlines resulting in labile chromosomal equilibria responsive to environmental alterations. The behavior of malignant cell populations could now be described in Darwinian terms: by selective pressures, a dynamic equilibrium is maintained, but any environmental change may upset the balance, causing shifts of the stemline karyotype. Evolution thus occurs in tumor cell populations in much the same manner as in populations of organisms: chromosomal aberrations generate genetic diversity, and the relative "fitness" imparted by the various changes decides which subclones will prevail.

FIGURE 1.1   Camera lucida drawing of tumor cell mitosis from one of the first (early 1950s) human cancerous effusions submitted to detailed chromosome analysis. The modal number was 75. The stemline also contained numerous abnormal chromosome shapes (Courtesy of Prof. Albert Levan, 1985).
The elucidation of these evolutionary principles in numerous studies by a number of investigators, for example, Hauschka (1953), Levan (1956), and Makino (1956), paved the way for the new and growing understanding of the role of karyotypic changes in neoplasia and laid the foundation of modern cancer cytogenetics. In humans as well as in other mammals, the results strongly indicated that the chromosomal abnormalities observed were an integral part of tumor development and evolution (see, e.g., Levan, 1967; Koller, 1972; Hsu, 1979; Sandberg, 1980, for review of the early data). It should be kept in mind, however, that the object of these early investigations was always metastatic tumors, often effusions, that is, highly malignant cell populations. Hence, few, if any, conclusions could be drawn from them as to the role of chromosomal abnormalities in early tumor stages.

Interest in cancer cytogenetics influenced human cytogenetics much more profoundly than is currently appreciated. For example, the main goal behind the study that eventually led to the description of the correct chromosome number in man (Tjio and Levan, 1956) was to identify what distinguished a cancer cell. The motivation was not primarily an interest in the normal chromosome constitution, which at that time had no obvious implications, but the hope that such knowledge would help answer the basic question of whether chromosome changes lay behind the transformation of a normal to a cancer cell.

The first spectacular success in cancer cytogenetics came when Nowell and Hungerford (1960) discovered that a small karyotypic marker (Fig. 1.2), the Philadelphia (Ph)

![FIGURE 1.2](image)

**FIGURE 1.2** Unbanded metaphase cell from a bone marrow culture established from a patient with chronic myeloid leukemia. The arrow indicates the Ph chromosome (previously called Ph1). The superscript number 1 indicated that this was the first cancer-specific aberration detected in Philadelphia. This naming practice was later abandoned, but the abbreviation Ph has been retained for sentimental reasons, since it was the first consistent chromosome abnormality detected in a human malignancy.
chromosome, replaced one of the four smallest autosomes (the G-group chromosomes according to the nomenclature at the time) in the bone marrow cells of seven patients with chronic myeloid leukemia (CML). This was the first consistent chromosome abnormality in a human cancer, and its detection seemed to provide conclusive verification of Boveri’s idea. It was reasonable to assume that the acquired chromosomal abnormality—a perfect example of a somatic mutation in a hematopoietic stem cell—was the direct cause of the neoplastic state.

Nowell and Hungerford’s discovery greatly stimulated interest in cancer cytogenetics in the early 1960s, but for several reasons the Ph chromosome long remained an exceptional finding. The confusing plethora of karyotypic aberrations encountered in other malignancies suggested that the changes were epiphenomena incurred during tumor progression rather than essential early pathogenetic factors. The enthusiasm for tumor cytogenetics as a result gradually faded. With this change of mood, the perceived significance of the Ph chromosome also changed, and the very uniqueness of the marker came to be regarded as a perplexing oddity. Why should there be such a simple association between a chromosomal trait and one particular malignant disease when more and more data from other neoplasms showed either no chromosome aberrations at all or a confusing mixture of apparently meaningless abnormalities?

That an orderly pattern existed in what had hitherto been seen as chaos was suggested independently in the mid-1960s by Levan (1966) and van Steenis (1966). Surveying chromosomal data available in the literature, mainly on ascitic forms of gastric, mammary, uterine, and ovarian carcinomas, they found clear evidence that certain chromosome types tended to increase and others to decrease in number in the tumors. Soon afterward, the nonrandomness of karyotypic changes was also demonstrated beyond doubt in specific types of human hematologic disorders and solid tumors, for example, deletion of an F-group chromosome in polycythemia vera (Kay et al., 1966), loss of a G chromosome in meningioma (Zang and Singer, 1967), and a C–G translocation in acute myeloid leukemia (Kamada et al., 1968). The results of comprehensive cytogenetic studies of experimental tumors, including more than 200 primary sarcomas induced by Rous sarcoma virus in four species of animals, supported the same conclusion (Mitelman, 1974). In both humans and animals, the karyotypic abnormalities seemed to be of two essentially different kinds: nonrandom changes preferentially involving particular chromosomes and a frequently more massive random or background variation affecting all chromosomes. To differentiate between the two could be exceedingly difficult, however. As a consequence, in spite of painstaking efforts, little progress was made in cancer cytogenetics during this period.

The situation changed dramatically in 1970 with the introduction of chromosome banding techniques by Caspersson and Zech (Caspersson et al., 1970). The new methodology completely revolutionized cytogenetic analyses. Each chromosome could now be precisely identified on the basis of its unique banding pattern; whereas formerly identification was restricted to chromosome groups, all descriptions of chromosome deviations immediately became more precise and the conclusions based on them more stringent. The first neoplasia-associated chromosome abnormalities characterized by banding were published in 1972: monosomy 22 in meningioma (Mark et al., 1972; Zankl and Zang, 1972), trisomy 8 in acute myeloid leukemia (de la Chapelle et al., 1972), a 14q+ marker chromosome in Burkitt lymphoma (Manolov and Manolova, 1972), and deletion of the long arm of chromosome 20 (20q−) in polycythemia vera (Reeves et al., 1972). The following year, Rowley (1973) showed that the Ph chromosome in CML was the result of a translocation between chromosomes 9 and 22, not a deletion of chromosome 22 as was
previously thought. The 9;22-translocation thus became the first example of an acquired balanced rearrangement in neoplasia, but soon afterward similar consistent and even specific cancer-associated chromosome aberrations were disclosed in a wide variety of neoplastic entities among the hematologic malignancies and during the following decade also in solid tumors.

The advent of molecular genetics in the 1980s and the development of a range of powerful molecular cytogenetic technologies during the last two decades, such as fluorescence in situ hybridization (FISH), multicolor FISH, and chromosomal and array-based comparative genomic hybridization (CGH) (Kearney and Horsley, 2005; Pinkel and Albertson, 2005; Speicher and Carter, 2005), combined with rapid progress also in other areas of cell and tumor biology, have dramatically widened our knowledge and understanding of the molecular mechanisms that are operative in neoplastic initiation and progression. The new techniques have enabled researchers to investigate tumor cells at the level of individual genes, even at the level of single base pairs, and the molecular consequences of an ever-increasing number of cancer-associated chromosome aberrations have thus been laid bare.

The newly reached molecular insights into two of the first and most distinctive cancer-specific chromosomal translocations—the t(9;22)(q34;q11) of CML that fuses the BCR and ABL1 genes, and the t(8;14)(q24;q32) of Burkitt lymphoma that juxtaposes the MYC oncogene with the immunoglobulin heavy-chain gene (IGH)—stimulated an enormous interest in cancer cytogenetics as a powerful means to pinpoint the locations of cancer-initiating genes (Heim and Mitelman, 1987). As a consequence, the information on chromosome aberrations in neoplasia has steadily increased over the past two decades, and the total number of tumor cases in which clonal cytogenetic abnormalities have been reported now exceeds 55,000, published in more than 12,000 articles (Mitelman et al., 2008). To date, more than 400 genes in the breakpoints have been found to be rearranged and/or deregulated as a consequence of a chromosomal change in neoplasia (Mitelman et al., 2007).

It is obvious that cross-fertilization between cytogenetics and molecular genetics has led to conceptually new advances and insights into the fundamental cell biology mechanisms that are disrupted when neoplastic transformation occurs. At the same time, the clinical usefulness of cytogenetic abnormalities as diagnostic and prognostic aids in cancer medicine has been increasingly appreciated. The ultimate goal is to arrive at specific therapies individualized to counter those molecular mechanisms that have gone awry in each patient’s cancerous disease. The development of imatinib (Druker, 2004) as a therapeutic agent for CML—the first example of a targeted therapy against a specific fusion gene in cancer—is a wonderful example of how progress in cytogenetics and molecular biology has led to a qualitatively new treatment approach: the discovery of the Ph chromosome, the finding that the Ph chromosome results from a reciprocal translocation, the identification of the two genes in the breakpoints of the translocation, and the subsequent characterization of the fusion gene and its protein product. We are convinced that many similar success stories are unfolding as we write; cancer genetic research helps obtain more effective and less toxic treatments for malignant diseases. Thus, in the little over 100 years since von Hansemann’s initial report, cancer cytogenetics has come of age. It is no longer a purely descriptive discipline but one that attempts to synthesize information from several investigative approaches. Cancer cytogenetics has become both a central methodology in basic cancer research and an important clinical tool in oncology.
REFERENCES

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