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CHAPTER 1

How it all began: cancer cytogenetics before sequencing

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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 20th 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

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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).

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 (Figure 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.

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 (Figure 1.2), the Philadelphia (Ph) 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

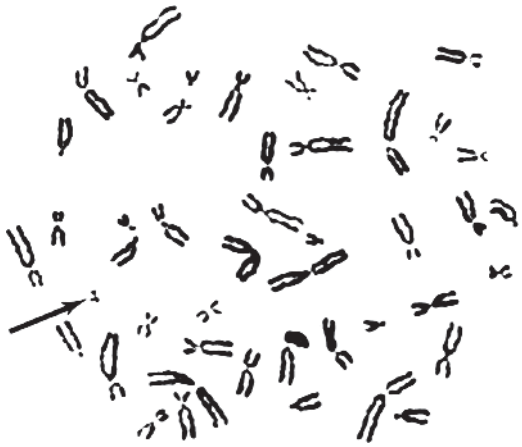

Figure 1.2 Unbanded metaphase cell from a bone marrow culture established from a patient with chronic myeloid leukemia. The arrow indicates the Ph chromosome (previously called Ph^1); the superscript indicated that this was the first cancer-specific aberration detected in Philadelphia. This naming practice was later abandoned, but the abbreviation Ph has for sentimental reasons been retained, since it was the first consistent chromosome abnormality detected in a human malignancy.

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, trisomy of a C chromosome in acute myeloid leukemia (Hungerford and Nowell, 1962), 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 the Rous sarcoma virus in mice, rats, and the Chinese hamster, 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 by Caspersson and Zech of chromosome banding techniques (Caspersson et al., 1970a). 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. As a consequence, a steadily increasing number of cancer cases, initially predominantly malignant hematologic disorders, were investigated with the new techniques, and a number of characteristic, specific, sometimes even pathognomonic changes were soon discovered (Table 1.1). Caspersson et al. (1970b) first used banding in this context and identified the Ph chromosome as a deleted chromosome 22, and in 1972, three of the nonrandom aberrations described in the 1960s were clarified: the additional C-group

Table 1.1 Characteristic neoplasia-associated cytogenetic aberrations detected by banding analyses 1970–1979

Year	*Disease*	*Aberration*	*References*
1970	Chronic myeloid leukemia	del(22q)	Caspersson et al. (1970b)
1972	Acute myeloid leukemia	+8	de la Chapelle et al. (1972)
	Burkitt lymphoma	14q+	Manolov and Manolova (1972)
	Meningioma	–22	Mark et al. (1972) and Zankl and Zang (1972)
	Polycythemia vera	del(20q)	Reeves et al. (1972)
1973	Acute myeloid leukemia	t(8;21)(q22;q22)	Rowley (1973a)
	Acute myeloid leukemia	i(17)(q10)	Mitelman et al. (1973)
	Acute myeloid leukemia	–7/del(7q)	Petit et al. (1973) and Rowley (1973c)
	Chronic myeloid leukemia	t(9;22)(q34;q11)	Rowley (1973b)
	Acute myeloid leukemia/ Myeloproliferative disorders	+9	Davidson and Knight (1973), Rowley (1973d), and Rutten et al. (1973)
1974	Acute myeloid leukemia	+21	Mitelman and Brandt (1974)
	Refractory anemia	del (5q)	van den Berghe et al. (1974)
1975	Myeloproliferative disease	t(11;20)(p15;q11)	Berger (1975)
1976	Acute myeloid leukemia	t(6;9)(p23;q34)	Rowley and Potter (1976)
	Burkitt lymphoma	t(8;14)(q24;q32)	Zech et al. (1976)
1977	Acute lymphoblastic leukemia	t(4;11)(q21;q23)	Oshimura et al. (1977)
	Acute promyelocytic leukemia	t(15;17)(q22;q21)	Rowley et al. (1977)
	Neuroblastoma	del(1p)	Brodeur et al. (1977)
1978	Acute monocytic leukemia	t(8;16)(p11;p13)	Mitelman et al. (1978)
	Acute myeloid leukemia	ins(3;3)(q21;q21q26)	Golomb et al. (1978)
1979	Acute lymphoblastic leukemia	t(8;14)(q24;q32)	Berger et al. (1979a) and Mitelman et al. (1979)
	Burkitt lymphoma	t(2;8)(p12;q24)	Miyoshi et al. (1979) and van den Berghe et al. (1979)
	Burkitt lymphoma	t(8;22)(q24;q11)	Berger et al. (1979b)
	Chronic lymphocytic leukemia	+12	Autio et al. (1979)
	Follicular lymphoma	t(14;18)(q32;q21)	Fukuhara et al. (1979)
	Mouse plasmacytoma	t(6;15), t(12;15)	Ohno et al. (1979)

chromosome in acute myeloid leukemia was identified as trisomy 8 (de la Chapelle et al., 1972), the lost G-group chromosome in meningioma corresponded to monosomy 22 (Mark et al., 1972; Zankl and Zang, 1972), and the deleted F-group chromosome in polycythemia vera was a del(20q) (Reeves et al., 1972). A previously unrecognized recurrent abnormality, a 14q + marker chromosome in Burkitt lymphoma (BL), was also described the very same year (Manolov and Manolova, 1972). The first recurrent balanced rearrangements were reported shortly afterward: a reciprocal translocation between chromosomes 8 and 21, that is, t(8;21)(q22;q22), was found in the bone marrow cells of some patients with acute myeloid leukemia (Rowley, 1973a), and the Ph chromosome of CML was demonstrated to stem from a t(9;22)(q34;q11), not a deletion of chromosome 22 as was previously thought (Rowley, 1973b). Among other important translocations also soon identified were t(8;14)(q24;q32), t(2;8)(p12;q34), and t(8;22)(q24;q11) in BL (Zech et al., 1976; Berger et al., 1979b; Miyoshi et al., 1979; van den Berghe et al., 1979), t(15;17)(q22;q21) in acute promyelocytic leukemia (Rowley et al., 1977), t(4;11)(q21;q23) in acute lymphoblastic leukemia (Oshimura et al., 1977), and t(14;18)(q32;q21) in follicular lymphoma (Fukuhara et al., 1979). Ohno et al. (1979) identified two characteristic translocations—t(6;15) and t(12;15)—in mouse plasmacytomas (MPC), the first specific rearrangements in experimental neoplasms and, as it turned out (see below), the perfect equivalents of the characteristic translocations in human BL. In total, more than 1200 neoplasms with clonal abnormalities were reported during this first decade of banding cytogenetics,

Table 1.2 Characteristic cytogenetic aberrations detected by banding analyses of solid tumors 1980–1989

Year	*Tumor type*	*Aberration*	*References*
1980	Salivary gland adenoma	t(3;8)(p21;q12)	Mark et al. (1980)
1982	Germ cell tumors	i(12)(p10)	Atkin and Baker (1982)
	Lung cancer	del(3)(p14p23)	Whang-Peng et al. (1982)
	Retinoblastoma	i(6)(p10)/del(13q)	Balaban et al. (1982) and Kusnetsova et al. (1982)
	Rhabdomyosarcoma (alveolar)	t(2;13)(q36;q14)	Seidal et al. (1982)
1983	Ewing sarcoma	t(11;22)(q24;q12)	Aurias et al. (1983) and Turc-Carel et al. (1983)
	Salivary gland adenoma	der(12)(q13–15)	Stenman and Mark (1983)
	Wilms' tumor	der(16)t(1;16)(q21;q13)	Kaneko et al. (1983)
1985	Chondrosarcoma (myxoid)	t(9;22)(q31;q12)	Hinrichs et al. (1985)
1986	Kidney cancer	t(X;1)(p11;q21)	de Jong et al. (1986)
	Lipoma	t(3;12)(q27;q13)	Heim et al. (1986) and Turc-Carel et al. (1986)
	Liposarcoma (myxoid)	t(12;16)(q13;p11)	Limon et al. (1986a)
	Salivary gland carcinoma	t(6;9)(q23;p23)	Stenman et al. (1986)
	Synovial sarcoma	t(X;18)(p11;q11)	Limon et al. (1986b)
1987	Kidney cancer	del(3p)/der(3)t(3;5)(p13;q22)	Kovacs et al. (1987)
	Lipoma	Ring chromosome(s)	Heim et al. (1987)
	Lipoma	der(12)(q13–15)	Mandahl et al. (1987)
1988	Primitive neuroectodermal tumor	i(17)(q10)	Griffin et al. (1988)
	Salivary gland cystadenolymphoma	t(11;19)(q21;p13)	Bullerdiek et al. (1988)
	Uterine leiomyoma	del(7)(q22q31)	Boghosian et al. (1988)
	Uterine leiomyoma	t(12;14)(q14;q24)	Heim et al. (1988), Mark et al. (1988), and Turc-Carel et al. (1988)
1989	Infantile fibrosarcoma	+8,+11,+20	Mandahl et al. (1989) and Speleman et al. (1989)
	Lipoma	der(6)(p21)	Sait et al. (1989)
	Ovarian cancer	add(19)(p13)	Pejovic et al. (1989)

and more than 60 recurrent chromosomal aberrations were identified.

The following decade saw a rush of data coming from studies of solid tumors, initially in particular mesenchymal neoplasms. The chromosome abnormalities of more than 2000 solid tumors were reported between 1980 and 1989, and almost 200 recurrent structural changes were identified. Several of them were no less specific than those previously found among hematologic disorders (Table 1.2), for example, t(2;13)(q36;q14) in alveolar rhabdomyosarcoma (Seidal et al., 1982), t(11;22)(q24;q12) in Ewing sarcoma (Aurias et al., 1983; Turc-Carel et al., 1983), and t(12;16)(q13;p11) in myxoid liposarcoma (Limon et al., 1986a). At this time, it also became clear that many benign tumors carried characteristic aberrations, including reciprocal translocations, for example, t(3;8)(p21;q12) in salivary gland adenoma (Mark et al., 1980), t(3;12)(q27;q13) in lipoma (Heim et al., 1986; Turc-Carel et al., 1986), and t(12;14)(q14;q24) in uterine leiomyoma (Heim et al., 1988; Mark et al., 1988; Turc-Carel et al., 1988).

The identification of specific cytogenetic aberrations enabled meaningful clinical–cytogenetic association studies, the most important of which were the International Workshops on Chromosomes in Leukemia established in the late 1970s. The workshops provided an arena for a fruitful and at the time unique collaboration among cytogeneticists, clinicians, and pathologists who shared their data and insights in order to find diagnostically and prognostically interesting associations between cytogenetic aberrations and clinical characteristics in various hematologic disorders. The results obtained by this collaborative study group

over a 10-year period showed that cytogenetics could subdivide phenotypically identical leukemias and lymphomas into distinct subgroups on the basis of specific abnormalities and that this classification had important clinical implications. For example, the workshop collaborators demonstrated that the diagnostic karyotype in childhood acute lymphoblastic leukemia was of greater prognostic importance than any hitherto known risk factor, such as patient age, white blood cell count, or immunophenotype (Bloomfield et al., 1986). The studies performed by the Workshops on well-characterized patient materials from different parts of the world were thus instrumental in consolidating cytogenetics as clinically well-nigh indispensable in hematology. A similar collaborative study group dedicated to the genetic analysis of mesenchymal tumors—the Chromosomes and Morphology (CHAMP) study group—was formed a decade later and has identified several important clinical–cytogenetic associations among different bone and soft tissue tumors (e.g., Mertens et al., 1998).

Technological advances at the same time made it possible to supplement cytogenetic investigations by molecular genetic studies of the same tumor types. Analyses in the early 1980s of the specific translocations in MPC, BL, and CML proved particularly pivotal for our understanding of how chromosome aberrations contribute to neoplastic transformation not only in these specific disorders but also generally (Mitelman et al., 2007). The picture to emerge was that reciprocal translocations exert their effects by one of two main alternative mechanisms: deregulation, usually resulting in overexpression, of a seemingly normal gene in one of the breakpoints (the BL scenario) or the creation of a hybrid, chimeric gene through fusion of parts of two genes, one in each breakpoint (the CML scenario). Deregulation of an oncogene by juxtaposition to a constitutively active gene region was predicted by Klein already in 1981, and the principle was soon demonstrated in MPC (Adams et al., 1982; Harris et al., 1982; Kirsch et al., 1982) and human BL (Dalla Favera et al, 1982; Taub et al., 1982; Croce et al., 1983; Erikson et al., 1983). The breakpoints of the characteristic translocations in mice and humans were found to be located within or close to the *MYC* oncogene and one of the immunoglobulin heavy- or light-chain genes (*IGH*, *IGK*, or *IGL*). As a consequence of the translocations, the entire coding part of *MYC* is juxtaposed to one of the immunoglobulin genes, resulting in deregulation of *MYC* because the gene is now driven by regulatory elements of the immunoglobulin genes. The alternative mechanism—the creation of a fusion gene—was documented at the same time in CML with the demonstration that the Ph chromosome, that is, the der(22)t(9;22)(q34;q11), contains a fusion in which the 3′ part of the *ABL* oncogene from 9q34 has become juxtaposed with the 5′ part of a gene from 22q11 called the *BCR* gene, resulting in the creation of an in-frame BCR–ABL fusion transcript (de Klein et al., 1982; Heisterkamp et al., 1983; Groffen et al., 1984; Shtivelman et al., 1985).

These and similar molecular insights into how cancer-specific chromosomal abnormalities act pathogenetically sparked an enormous interest in cytogenetics as a powerful means to pinpoint the locations of genes important in tumorigenesis (Heim and Mitelman, 1987). An impressive amount of information has been accumulated through these efforts. More than 65 000 neoplasms with at least one clonal cytogenetic change have been identified, and more than 700 gene fusions have been found by genomic characterization of breakpoints in cytogenetically identified aberrations in various leukemias, lymphomas, and solid tumors (Mitelman et al., 2015). We now know that practically all acquired balanced rearrangements lead to in principle the same consequences as the ones originally elucidated in BL and CML, that is, deregulation of a seemingly normal gene or the creation of a hybrid gene. In addition to oncogene activation via translocations and other balanced rearrangements (inversions, insertions), gene fusions may also be produced by unbalanced changes such as deletions leading to fusion of genes in the deletion edges.

The advent of molecular genetics in the 1980s and the development of a range of powerful molecular cytogenetic technologies during the last three decades, such as fluorescence *in situ* hybridization (FISH), multicolor FISH, comparative genomic hybridization (CGH), various array-based genotyping technologies, and DNA and RNA sequencing (Lander, 2011; Ozsolak and Milos, 2011;

Le Scouarnac and Gribble, 2012; Mwenifumbo and Marra, 2013; Mertens and Tayebwa, 2014), 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 genomic aberrations have thus been laid bare (Vogelstein et al., 2013).

It is obvious that the 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, 2008) 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. Similar targeted therapies are presently being developed against a number of fusion genes, and some have already turned out to be successful, for example, crizotinib targeting the *EML4–ALK* fusion gene generated by an inversion on the short arm of chromosome 2 in a subset of patients with non-small cell lung cancer (Shaw and Engelman, 2013). While it took 40 years from the discovery of the Ph chromosome to the development of imatinib, it only took a few years from the description of the *EML4–ALK* fusion in lung cancer to the development of crizotinib. 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 100 years since Boveri first postulated that chromosome change may initiate the carcinogenic process, 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.

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