Molecular Analysis of Rearrangements in Philadelphia (Ph^1) Chromosome-Positive Leukemia

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A. Introduction

The close association of specific chromosome abnormalities with particular types of human cancer has been established by a number of investigators during the past decade [1–6]. A few of the genes involved in consistent chromosome rearrangements, notably translocations, have already been identified, and it is likely that the identity of most of the genes affected by these aberrations will be determined within the next decade. Moreover, for several of the rearrangements, some of the changes in gene structure and function have been defined. Therefore, some general principles that may be applicable to many chromosome rearrangements in human malignant disease are beginning to emerge. Chronic myeloid leukemia (CML) provides one of the clearest examples of our progress in first identifying a recurring chromosome abnormality and then cloning the genes involved in the abnormality. The analysis of these genes and their alteration as a result of the chromosome change is the subject of this lecture.

B. Cytogenetic and Clinical Features of Chronic Myeloid Leukemia

Chronic myeloid leukemia is important because it was the first human cancer in which a consistent chromosome abnormality was identified. The abnormality is the Philadelphia or Ph^1 chromosome [7], which was shown with banding to involve No. 22 (22q−). The correct chromosome defect was shown to be a translocation involving Nos. 9 and 22; this was the first consistent translocation specifically associated with any human or animal disease (Fig. 1) [8]. The reciprocal nature of the translocation was established only recently, when the Abelson protooncogene, *ABL*, normally on No. 9, was identified on the Ph^1 chromosome [9]. Other studies with fluorescent markers or chromosome polymorphisms have shown that, in a particular patient, the same No. 9 and No. 22 are involved in each cell. The Ph^1 chromosome is present in granulocytic, erythroid, and megakaryocytic cells, in some B cells, and probably in a few T cells. The karyotypes of many Ph^1+ patients with CML have been examined with banding techniques by a number of investigators; in a review of 1129 Ph^1+ patients, the 9;22 translocation was identified in 1036 (92%) [4]. Variant translocations have been discovered, however, in addition to the typical t(9;22). Until very recently, these were thought to be of two kinds; one appeared to be a simple translocation involving No. 22 and some chromosome other than No. 9 (about 4%), and the other was a complex translocation involving three or more different chromosomes, two of which were No. 9 and No. 22 (about 4%). Recent data clearly demonstrate that No. 9 is affected in the simple as well as the complex translocations, and that its involvement had been overlooked [10]. Virtually all chromosomes have been involved in these variant translocations, but No. 17 is affected

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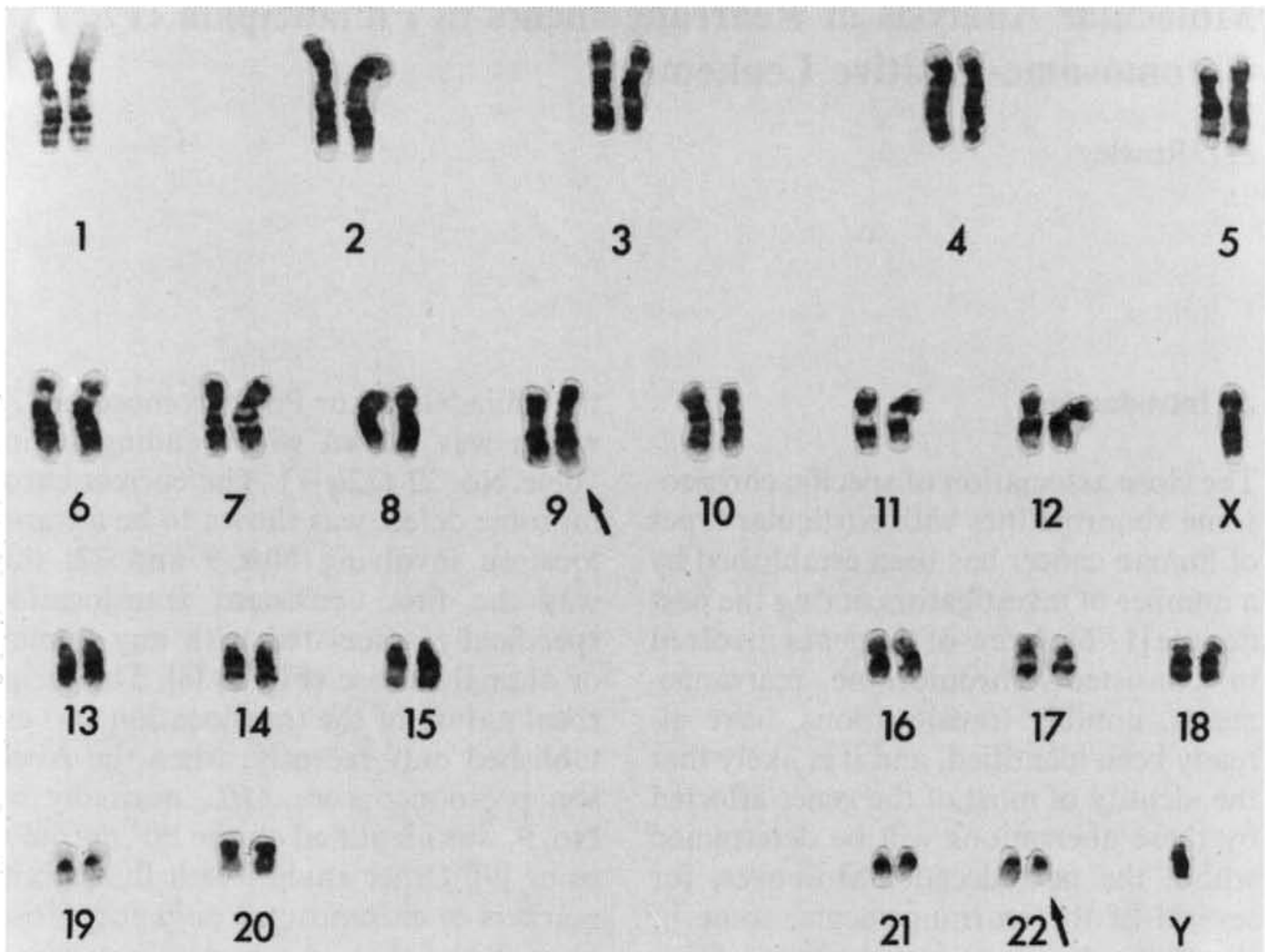

Fig. 1. Trypsin-Giemsa-stained karyotype of a metaphase cell from a bone marrow aspirate obtained from an untreated male with CML illustrating the t(9;22) (q34;q11). The Philadelphia chromosome (Ph^1) is the chromosome on the right in pair 22 (↑). The material missing from the long arm of this chromosome (22q−) is translocated to the long arm of chromosome 9 (9q+) (↑), and is the additional pale band that is not present on the normal chromosome 9

more often than are other chromosomes. The genetic consequences of the standard t(9;22) or the complex translocation involving at least three chromosomes is to move the *ABL* protooncogene on No. 9 next to a gene on No. 22, called *BCR*, whose function is currently unknown (Fig. 2).

Chronic myeloid leukemia usually terminates in an acute leukemia in which the blast cells have either lymphoid or myeloid morphology. In the acute phase, about 10%–20% appear to retain the 46, Ph^1+ cell line unchanged, whereas most patients show additional chromosome abnormalities resulting in cells with modal chromosome numbers of 47 to 50 [4]. Different abnormal chromosomes occur singly or in combination in a distinctly nonrandom pattern. In patients who have only a single new chromosome change, this most commonly involves a second Ph^1, an isochromosome for the long arm of No. 17 [i(17q)], or a +8, in descending order of frequency. Chromosome loss occurs only rarely; that most often seen is −7, which occurs in 3% of patients.

Early cases of acute leukemia in which the Ph^1 chromosome was present were classified as CML presenting in blast transformation; at present, patients who have no prior history suggestive of CML are classified as Ph^1+ acute leukemia. In fact, some of the patients with Ph^1+ ALL have a different breakpoint in the *BCR* gene on No. 22. In blast crisis, some blasts have intracytoplasmic IgM, which is characteristic of pre-B cells, and these cells have an immunoglobulin gene rearrangement [11].

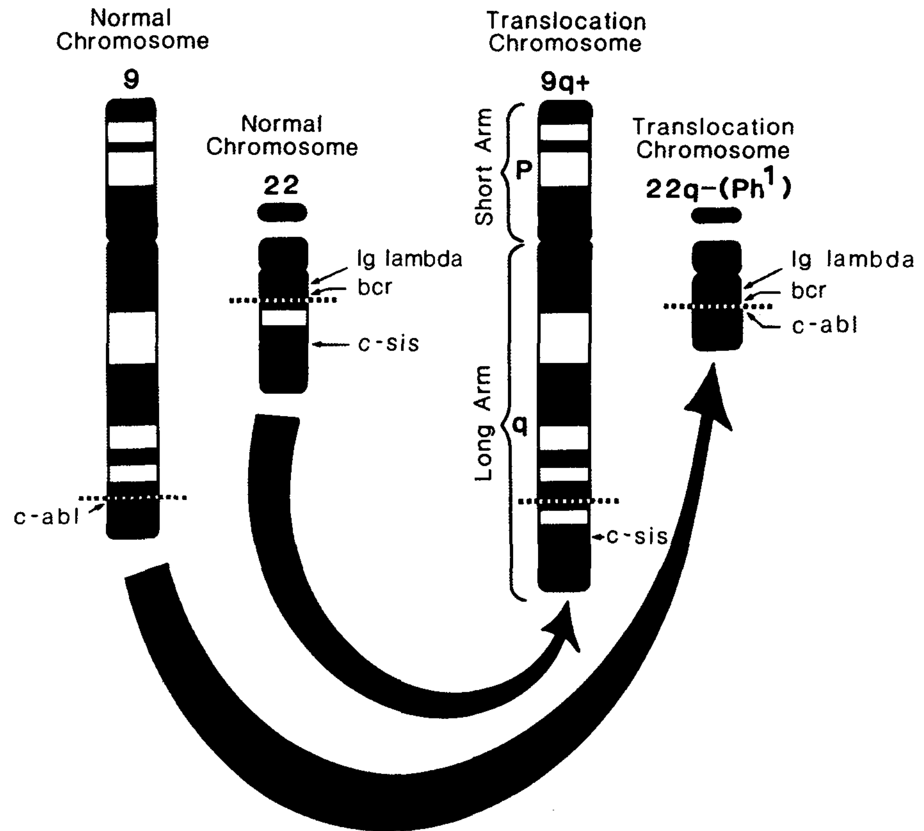

Fig. 2. Schematic drawing of chromosome No. 9 and No. 22 illustrating the chromosome translocation that produces the 9q+ and 22q− (Ph[1]) chromosomes. One protooncogene, *ABL*, is moved to No. 22 adjacent to a gene of unknown function called *BCR*; the break in No. 22 is distal to the IG lambda locus which is not involved in the translocation. The *SIS* protooncogene is moved to the 9q+ chromosome. It is located at some distance from the breakpoint on No. 22 and there is no evidence that it is altered as the result of the translocation

Marrow cells from some patients appear to lack a Ph[1] chromosome. The majority of these patients had a normal karyotype. Somewhat surprisingly, the survival of these patients was substantially shorter than those whose cells were Ph[1]+ [12]. Our recent review of the histology of 25 Ph[1]− patients showed that most of them did not have CML but they had some type of myelodysplasia, most commonly chronic myelomonocytic leukemia or refractory anemia with excess blasts [13]. However, the situation has become more complex because it has been shown recently that some patients with clinically typical CML who lack a Ph[1] chromosome cytogenetically have evidence of the insertion of *ABL* sequences into the *BCR* gene [14, 15]. Thus, it can be proposed that the sine qua non of CML is the juxtaposition of *BCR* and *ABL*.

C. Molecular Analysis of the 9;22 Translocation

Investigators are now in the process of unraveling the mystery of the Ph[1] translocation in CML and ALL. In the t(9;22) in CML and ALL, the Abelson protooncogene (*ABL*) is translocated to the Ph[1] chromosome [9]. The *ABL* gene was first identified because of its homology to the viral oncogene that had been isolated from a mouse pre-B-cell leukemia. The breakpoint junction in CML was cloned and the site on the Ph[1] was called *bcr*, for breakpoint cluster region, [16] since the majority of breaks cluster in a small 5.8-kilobase (kb) region. The gene in which this cluster is located has also been cloned. It is a very large gene greater than 100 kb and it is presently also called *BCR*, which leads to a great deal of confusion. In this lecture, *bcr* is used to denote the CML breakpoint region and *BCR* to identify the whole gene. In contrast to *bcr*, the breaks in *ABL* on No. 9 occur over an incredible distance of more than 200 kb. We have used pulse-

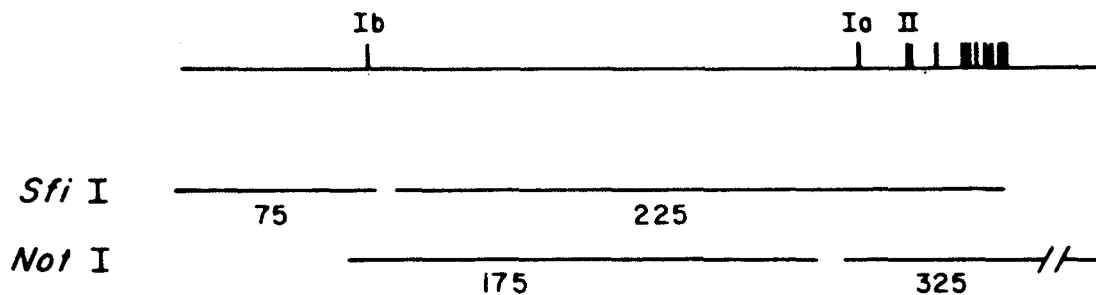

Fig. 3. Map of the *ABL* gene showing the position of the two alternative exons Ib and Ia relative to exon II. Exon Ib is the most 5′ exon, whereas Ia is less than 20 kb from exon II which is the common splice acceptor site. The *vertical bars* above the *horizontal line* represent the more 3′ exons which are homologous to the v-*abl* sequences. *Sfi* I and *Not* I are the enzymes used to determine the relative positions of exons Ia, Ib, and II. (Figure adapted from [17])

field gel electrophoresis (PFGE) to great advantage in the study of the *ABL* protooncogene. Southern blotting with standard gel electrophoresis leads to separation of DNA fragments in the size range of 2 to about 25 kb. Since the *ABL* gene is larger than 200 kb, mapping it in 10- to 20-kb pieces is a formidable task. In contrast, by using PFGE one can separate fragments more than 1000 kb in size, and this technique is also very effective in the 100- to 600-kb range. A normal chromosome band contains roughly 5000–10 000 kb and, thus, several very large, overlapping fragments could contain a single band. Using many probes for *ABL* provided by various investigators, Drs. Westbrook and Rubin have constructed a map of the normal *ABL* gene [17]. This is a very complex gene that normally uses one of two alternative beginnings, exon Ia or Ib. During transcription, either of these can be spliced at the same point on the remainder of the gene, which is called the common splice acceptor site or exon II (Fig. 3). One of their first discoveries was that the type Ib exon mapped more than 200 kb upstream from exon II. As a result, a very large segment of the RNA transcript is removed or spliced out to form the mature mRNA. This is a remarkable feat, not identified before in biological systems. The breakpoints in the chromosomes of various CML patients and cell lines occur in many locations upstream (5′) of exon II. However, the same size (8.5 kb) mRNA is found in all CML patients; this occurs because the *BCR* exons are spliced to *ABL* exon II, resulting in a chimeric mRNA which is translated into a chimeric protein ($p210^{BCR\text{-}ABL}$) [18, 19].

With regard to Ph^1-positive ALL, it has always been an enigma why the typical Ph^1 translocation is seen in ALL and in fact is the most common translocation in adults with ALL [20]. One relatively trivial explanation would be that the patients really had CML in lymphoid blast crisis with an undiagnosed chronic phase, and this may occur in some patients. However, analysis of DNA from some Ph^1-positive ALL cells indicates that the breakpoint in No. 22 is outside the *bcr* region. In one study, the majority of adult patients (13 of 17) appeared to have the same *bcr* rearrangement that is seen in CML whereas it has not been found in any of 7 children, who presumably had a more 5′ breakpoint in the *BCR* gene [21] (Table 1). Data from our

Table 1. Ph^1-positive leukemia

Diagnosis	Number of patients	Number with *bcr* rearrangements [a]
CML	135	133
ALL–Adults	32	17
ALL–Children	8	0

[a] *bcr* rearrangement in CML breakpoint cluster region

laboratory as well as others indicate that the breakpoints on No. 22 are greater than 50 kb proximal to the CML break but that they still are within the *BCR* gene [22]. The breakpoints on No. 9 are similar to those in CML. Several investigators have shown that these Ph¹+ ALL patients have an abnormal size chimeric BCR-ABL mRNA (7.0–7.4 kb) and ABL protein ($p185^{BCR\text{-}ABL}$) [23, 24].

These discoveries and the development of DNA probes that can detect rearrangements in the *BCR* and *ABL* genes have been applied very rapidly for use in diagnosis and monitoring of patients thought to have CML or Ph¹+ ALL. The results of the diagnostic use of the *bcr* probe are summarized in Table 1. Equally important is the ability to check for the recurrence of a Ph¹+ clone in CML patients who have undergone bone marrow transplantation or in Ph¹+ ALL patients in remission. These screening procedures have become even more sensitive with the use of the polymerase chain reaction to detect the *bcr-ABL* junction in leukemic cells.

We have recently studied seven patients with Ph¹+ ALL to determine whether the translocation breakpoints all occur within the *BCR* gene [25]. With PFGE we could show that every patient had a rearrangement within the *BCR* gene either in the 5′ portion of *BCR* in the first intron (five patients) or in *bcr* (two patients). Moreover *ABL* was fused with *BCR* in each patient. Of the seven patients, two were children, one of whom, age 12 years, had a *bcr* rearrangement. Further studies with additional patients will allow more precise correlations of the clinical features of the leukemias with the molecular abnormalities that underlie them.

In the future, we will understand the role of the BCR and ABL proteins in normal cells and that of the two different chimeric BCR-ABL proteins in CML and in ALL. Thus, the genetic analysis of what appeared to be a simple chromosome change, namely the 9;22 translocation, has revealed unexpected complexity. I am sure that, in the future, an understanding of the altered function of the ABL protein will be central to the development of more specific and more effective forms of therapy.

D. Biological Significance of Chromosomal Rearrangements

One of the most surprising revelations in the recent past has involved the cellular oncogenes and their chromosome location (Fig. 4). Much of the excitement derives from the observation that many protooncogenes are located in the bands that are involved in consistent translocations [3, 6]. There is a remarkable specificity of certain chromosome rearrangements for particular subtypes of tumors especially leukemia or lymphoma. The mechanism or mechanisms by which this specificity is achieved are unknown; however, a number of investigators have shown that certain proteins required for promotion of gene expression are synthesized in a very cell-type-specific manner [26]. These proteins are only present in the appropriate cell type and therefore the particular gene is activated only in that cell type. The chromosome rearrangements affecting *MYC* in B-cell [27, 28] and T-cell [29, 30] tumors strongly support the interpretation that the specificity resides in the gene that is uniquely active in the particular cell type. Thus the immunoglobulin genes are highly regulated in B cells and they can therefore serve as the switch or activator mechanism for *MYC* in B cells; on the other hand, the alpha chain of the T-cell receptor (*TCRA*) is an active gene in T cells with a strong enhancer/promotor and it clearly is an activator for *MYC* in T cells. A reasonable paradigm is that translocations bring together, in an inappropriate manner, a growth factor or growth factor receptor gene (the protooncogene in the examples defined to date) adjacent to an active cell-specific gene.

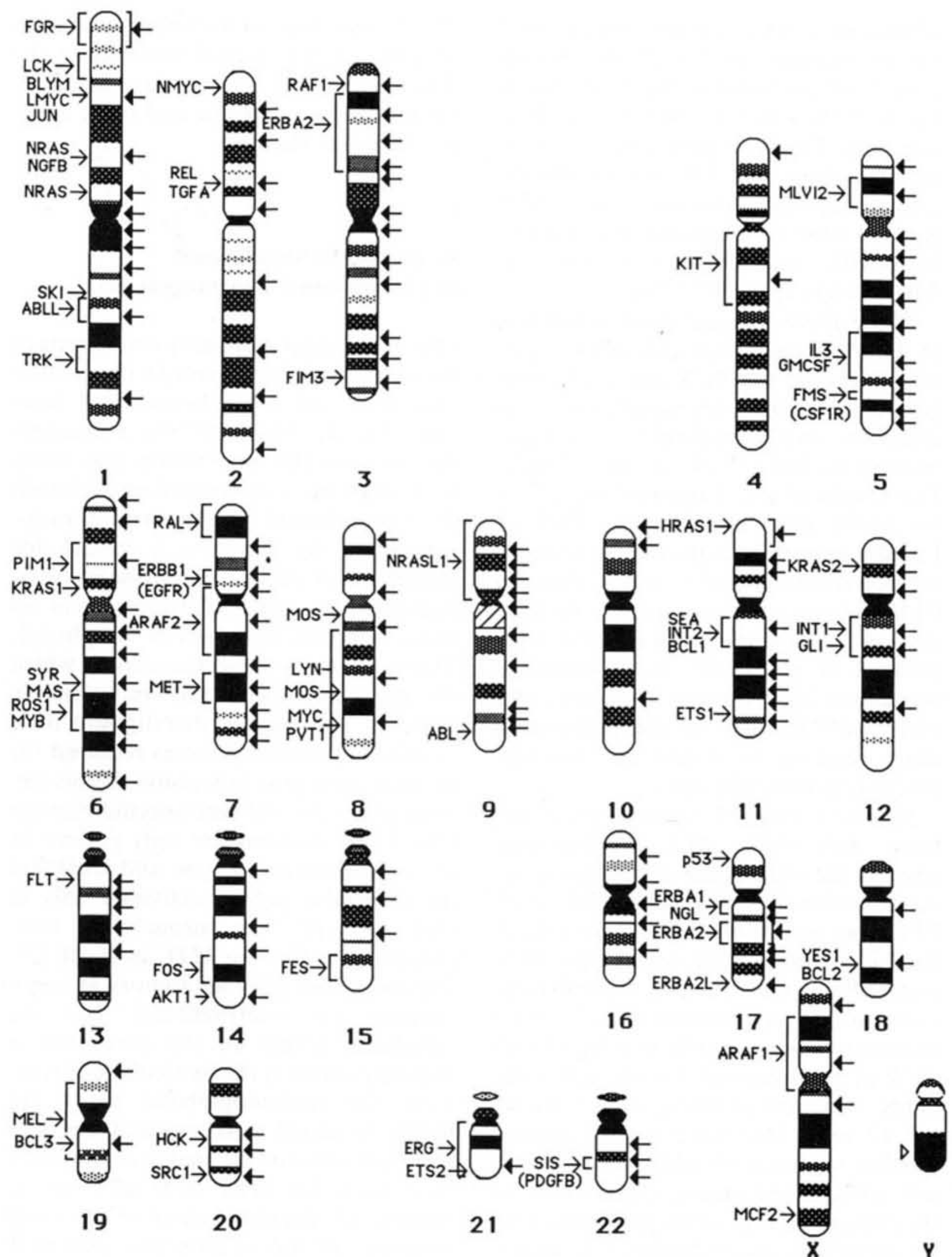

Fig. 4. Map of the chromosome location of protooncogenes or of genes with transforming properties and the breakpoints observed in recurring chromosome abnormalities in human leukemia, lymphoma, and solid tumors. The protooncogenes and their locations are placed to the left of the appropriate chromosome band (*arrow*) or region (*indicated by a bracket*). The breakpoints in recurring translocations, inversions, deletions, etc., are indicated with an *arrow to the right* of the affected chromosome band. The locations of the cancer specific breakpoints are based on the Human Gene Mapping 9 report [5]

It should be emphasized that many of the protooncogenes were identified in viruses that cause tumors. However, these genes have not been conserved through evolution from yeast and *Drosophila* to the chicken, mouse, and man to cause cancer! Where we have any insight into the function of these genes in normal cells, they are growth factors or growth factor receptors. It is not unexpected that the genes which a virus might coopt if it developed into a tumor-producing virus would be genes that control proliferation, genes which under viral regulation would function abnormally with regard to cell growth. Further support for the concept that oncogenes are growth factors gone wrong is provided by studies at the Hall Institute in Melbourne. There, investigators inserted the cloned gene for granulocyte-macrophage colony-stimulating factor into a viral vector, transfected mouse myeloid cells with this gene, and then injected the cells into mice which developed leukemia [31]. The term "oncogene" is too short and easy for it to be discarded, but it really refers to respectable genes for growth factors or their receptors.

The analysis of various tumors for alterations in protooncogenes has revealed that a number are abnormal as a result of translocations, amplification, or mutations [32]. In some situations the relationship of the change in the protooncogene to the multistage process of malignant transformation is unclear [33]. Such ambiguity is not a problem with chromosome translocations; the evidence is overwhelming that the t(8;14) in Burkitt's lymphoma and the t(9;22) in CML are an integral component of the cascade of events leading to the transformation of a normal to a malignant cell. The everincreasing number of translocations reviewed in this chapter provide a potential gold mine for identifying new genes that are unequivocally related to the malignant phenotype of the affected cell. The challenge is to isolate these translocation breakpoint junctions, to identify the genes that are located at these breakpoints, and then to determine the change in gene function that occurs as a consequence of the translocation. The ultimate measure of success, however, will be in the application of these new insights in the development of new, more effective treatments for cancer. In the future, each particular subtype of tumor will be treated in a uniquely defined way that is most appropriate for the specific genetic defect present in that tumor. This should lead to a new era of cancer therapy that is both more effective and less toxic.

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