Chromosome Translocations and Human Cancer

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The cytogenetic analysis of human cancer cells by standard and by high resolution banding techniques indicates that more than 90% of human malignancies carry clonal cytogenetic changes (1). The discovery of the Philadelphia chromosome in the neoplastic cells of patients with CML (2) and the subsequent findings that the great majority of human hematopoietic malignancies carry specific chromosomal alterations (3, 4) have suggested that such nonrandom chromosomal changes may be involved in the pathogenesis of human malignancies. This view, however, was not shared by many investigators outside the field of cancer cytogenetics, who regarded such chromosomal alterations as epiphenomena of the neoplastic process.

Recent developments in the analyses of genes involved in the chromosomal rearrangements observed in human leukemias and lymphomas indicate that such rearrangements are the critical steps involved in the pathogenesis of most human leukemias and lymphomas and that they may also play an important role in the pathogenesis of human solid tumors.

The Lesson Learned from the Genetic Analysis of Burkitt's Lymphoma

Perhaps the most striking example of correlation between specific chromosomal translocations and malignancy is the case of Burkitt's lymphoma, an extremely aggressive B-cell neoplasm predominantly affecting children. In this malignancy, Manolov and Manolova detected a marker chromosome 14 (14q+) that was longer than the normal chromosome 14 (5). Subsequently, Zech et al. (6) found that the 14q+ chromosome resulted from a translocation of a small segment of the distal region of chromosome 8 to the distal end of chromosome 14. Subsequently, other investigators in America, Europe, and Japan detected variant chromosome translocations in a minority of human Burkitt's lymphomas (7, 8). In those cases, the same segment of chromosome 8 translocates to either chromosome 22 or 2. In approximately 75% of Burkitt's lymphomas, the distal end of the long arm of chromosome 8 (q24–qter) translocates to the long arm of chromosome 14 at band q32 [a t(8;14)(q24;q32) chromosomal translocation]; 16% carry a t(8;22)(q24;q11) chromosome translocation, and only 9% carry a t(2;8)(p11;q24) chromosome translocation.

Because the distal end of chromosome 8 is involved in translocation with chromosome 14, 22, or 2, each of which carries a locus for human immunoglobulins (9–12), it was speculated that the human immunoglobulin genes might play an important role in the chromosome rearrangements observed in Burkitt's lymphomas and in the pathogenesis of this disease (9). To determine whether the heavy chain locus, which resides on chromosome 14 (9), is directly involved in the chromosomal rearrangement, somatic cell hybrids between mouse myeloma cells and Burkitt's lymphoma cells with the t(8;14) chromosome translocation were produced and analyzed with probes specific for the genes for the variable and constant regions of the human heavy chains (13). The results of this analysis indicated that the human heavy chain locus is split at various sites by the chromosomal translocation and that the genes for the variable regions translocate to the involved chromosome 8 (8q–), while the genes for the constant regions remain on the involved chromosome 14 (14q+). Analysis of the hybrids for the expression of human heavy chains also indicated that the expressed human heavy chain locus in Burkitt's lymphoma resides on the normal chromosome 14 (13). Thus the chromosomal translocation directly involves the unexpressed heavy chain locus. Since the genes for the variable regions (VH) translocate to the 8q– chromosome, while the constant region genes (CH) remain on chromosome 14, it was also clear that the VH genes were more distal than the CH genes at band q32 of chromosome 14 (13). In hybrids between mouse myeloma cells and Burkitt's lymphoma cells with the variant t(8;22) (q24;q11) and the t(2;8)(p11;q24) chromosome translocations it was shown that the loci for the immunoglobulin light chains were also split by the translocations. Since the Vκ and VL genes remain on the involved chromosomes 2 (2p–) and 22 (22q–), respectively, while either the Cκ or the CL light chain gene also resides on the normal chromosome 22 or 2, respectively (14, 15), while the unproductively rearranged λ or κ light chain gene resides on the translocation chromosome (14, 15).

The human homologue, c-myc, of the v-myc oncogene present in avian myelocytomatosis virus resides on chromosome 8 at band q24 and is translocated to the heavy chain locus on the 14q+ chromosome in Burkitt's lymphomas with the t(8;14) chromosome translocation (16). Thus the human c-myc oncogene was thought to play a critical role in the pathogenesis of this malignancy (16, 17). Analysis of Burkitt's lymphoma cells with the t(8;14) chromosome translocations for rearrangements of the c-myc oncogene indicated that the translocated oncogene may be rearranged at its 5' end, so that the 5' end of the oncogene is more proximal than its 3' end at band 8q24 (16–18). Molecular analysis of the c-myc oncogene indicated that it is formed by three exons separated by two introns (19). Since the first exon contains termination codons on all three reading frames, it is noncoding (19). The first ATG signal for initiation of protein synthesis is at the beginning of the second exon and is followed by an open reading frame encoding for a protein of 439 amino acids and a molecular weight of 48,812 (19). The c-myc oncogene has two promoters separated by approximately 160 nucleotides, and its transcripts originate at two initiation sites (20). When the c-myc gene is transactivated by its first exon because of breakpoints within the first c-myc intron, new cryptic promoters are activated within the first c-myc intron, and

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3 The abbreviations used are: CML, chronic myelogenous leukemia; ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; HTLV-1, human T-cell leukemia virus I; bcr, breakpoint cluster region.

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abnormal transcripts initiate from different cryptic transcription sites (21). In these novel transcripts, the first AUG codon for initiation of protein synthesis is the same as that in the normal c-myc transcript. Thus the c-myc product in Burkitt’s lymphomas with or without c-myc gene rearrangement is identical to the normal c-myc gene product.

It appears that most African Burkitt’s lymphomas (Burkitt’s lymphoma is endemic in equatorial Africa) express only cytoplasmic or membrane-bound immunoglobulin, whereas sporadic Burkitt’s lymphomas secrete human immunoglobulins. Thus, endemic and sporadic Burkitt’s lymphomas might involve cells at different stages of B-cell differentiation (22). Interestingly, the great majority of African Burkitt’s lymphomas are characterized by rearrangement of the heavy chain locus in the JH region or 5' to it, whereas the sporadic Burkitt’s lymphoma might have the rearrangement in a heavy chain switch region, Sµ, Sγ, or Sα (22). These switch regions are normally involved in heavy chain isotype switching. In most of the African Burkitt’s lymphomas, the break on chromosome 8 is 5' to the c-myc oncogene, very often more than 30 kilobases 5' to the translocated c-myc gene (17). By contrast, in sporadic Burkitt’s lymphoma, rearrangements often involve the first intron of the c-myc oncogene (17, 23).

We have used somatic cell genetic techniques to show that different heavy chain enhancing elements are involved in c-myc activation (24, 25). The enhancer between JH and Sµ is involved in c-myc oncogene activation in these cases where the rearrangements occur within JH or 5' to the JH segment (24, 25). We have postulated the existence of other enhancing elements within the heavy chain locus which are 3' to the involved switch regions and which seem to activate gene transcription only in more differentiated B-cells (24, 25). Thus at least two different types of enhancers seem to be present within the heavy chain locus. One is located in the region between Sµ and Cµ (23, 24). This enhancer is “promiscuous” and can activate gene transcription in B-cells at different stages of B-cell differentiation, from pre-B to plasma cells (24–26). The other enhancer may be 3' to the Cµ gene and may activate gene transcription in terminally differentiated or nearly terminally differentiated B-cells (24–26). Interestingly, both types of enhancers may activate gene transcription over considerable chromosomal distances (possibly over 100 kilobases of DNA) (24–26).

Analysis of the transcription of the c-myc oncogene involved in the chromosome translocation versus that of the normal c-myc gene on the normal chromosome, using somatic cell hybrids between mouse myeloma cells and Burkitt’s lymphoma cells carrying each of the three different chromosomal translocations (15, 16, 26), revealed that, while the normal c-myc oncogene on normal chromosome 8 is transcriptionally silent, the c-myc gene involved in each of the three different chromosomal translocations is transcribed constitutively at elevated levels. Thus, the juxtaposition of the c-myc oncogene to each of the three immunoglobulin loci leads to the transcriptional deregulation of c-myc (15, 16, 26). Similar analysis with hybrids of other hematopoietic cells, including human lymphoblastoid cells expressing the normal c-myc oncogene on chromosome 8, showed that the normal c-myc oncogene is repressed in a plasma cell background (26). These results are consistent with the interpretation that, while the c-myc oncogene on normal chromosome 8 responds to normal transcriptional control, the c-myc oncogene involved in the reciprocal chromosomal translocations observed in Burkitt’s lymphomas does not and is expressed constitutively at elevated levels in terminally differentiated B-cells (15, 16, 26). Direct analysis of Burkitt’s lymphoma cells for the expression of the translocated c-myc oncogene versus expression of that on normal chromosome 8 also indicated that the gene on the normal chromosome 8 is either transcriptionally silent or expressed at extremely low levels, while the translocated gene is transcribed constitutively at elevated levels (21). Thus, the consequence of the three different chromosomal translocations is a deregulation of the transcription of the c-myc gene involved in the chromosomal translocation (15, 16, 26).

It has been postulated that the deregulation of the c-myc gene is due to alteration of the first exon of the c-myc gene or to its decapitation (18). However, in several Burkitt’s lymphomas, the c-myc oncogene involved in the translocation is perfectly normal, and an altered or decapitated c-myc oncogene derived from Burkitt’s lymphoma is not expressed in B-cells unless it is juxtaposed to an immunoglobulin locus or to an immunoglobulin enhancer (26–28). It seems likely that alterations in the structure of the c-myc oncogene in Burkitt’s lymphoma are secondary and may occur during tumor progression.

Analysis of somatic cell hybrids between Burkitt’s lymphoma cells and cells of different lineages also indicates that the expression of the activated c-myc oncogene involved in the translocation is B-cell specific (26, 27). This finding is consistent with the interpretation that tissue-specific enhancing elements within the immunoglobulin genes are responsible for c-myc deregulation.

Cloning of Genes Involved in B-Cell Neoplasia

Since many human B-cell neoplasms carry translocations involving region 14q32, where the heavy chain locus resides (29), and since the c-myc oncogene in Burkitt’s lymphoma is deregulated because of its proximity to the heavy chain locus (26), we reasoned that it should be possible to isolate human genes involved in the pathogenesis of human B-cell neoplasms by taking advantage of their proximity to the heavy chain locus. Therefore we hybridized mouse plasmacytoma cells with human leukemic cells derived from a patient with CLL of the B-cell type carrying a t(11;14) (q13;q32) chromosome translocation (29). Such a translocation is observed in CLL (30), in diffuse B-cell lymphomas (3), and in multiple myeloma (31). Analysis of the hybrid cells indicated that the heavy chain locus was split by the chromosome translocation (29). We then prepared a DNA library from this case of CLL, cloned the breakpoint between chromosomes 11 and 14, and identified a locus we termed bcl-1 (B-cell lymphoma/leukemia 1), which is involved in the t(11;14) chromosome translocation (32, 33).

Using a similar procedure, we cloned the chromosome breakpoints involved in the t(14;18) (q32;q21) chromosome translocation (34) observed in the most common human hematopoietic malignancy, follicular lymphoma (35). Chromosome 18-specific probes flanking the breakpoints were then used to detect DNA rearrangements in most human follicular lymphomas (34, 35) and to detect transcripts of a gene we called bcl-2, which is involved in the pathogenesis of this human malignancy (35). Interestingly, most of the breakpoints involve the 3' end of the bcl-2 gene or regions distal to it on chromosome 18 and involve one of the JH segments of the heavy chain locus on chromosome 14 (36). Thus the enhancer located between JH and S is always juxtaposed to the involved bcl-2 gene, leading to its deregulation (34–36). Analysis of the structure of the bcl-2 gene revealed that it is formed by two exons separated by an intron of at least 60 kilobases in length. Thus the heavy chain enhancer is at least 65–70 kilobases 3' of the promoters of the bcl-2 gene (36).
sugest that the enhancers within the heavy chain locus activate transcription over considerable chromosome distances (>60 kilobases) (36).

These findings indicate that one can take advantage of specific chromosome rearrangements to isolate genes involved in human B-cell neoplasms. The study of the structure, organization, and function of these genes may provide insight into the mechanisms of control of B-cell proliferation and into the genetic mechanisms involved in the development of B-cell neoplasms.

The Chromosome Translocations Observed in B-Cell Neoplasms Are Catalyzed by the Same Enzymes Involved in Immunoglobulin Gene Rearrangement

DNA sequence analysis of the breakpoints involved in the t(11;14) and in t(14;18) chromosome translocations indicated that these translocations involve the 5' end of one of the J_H segments of the heavy chain locus (33, 37). This region of the J_H segments participates in the D-J rearrangements observed during physiological V-D-J heavy chain joining. Stretches of extranucleotides were also found at joining sites (33, 37), an observation of considerable interest in light of the report of Alt and Baltimore of extranucleotides (N regions) at joining sites in rearranged immunoglobulin genes (38). These extranucleotides are presumably added at joining sites by the enzyme terminal transferase during the pre-B stage of cell differentiation (38). Thus it appeared that the t(11;14) and the t(14;18) chromosome translocations occur during the process of V-D-J joining (33, 37). Since the putative V-D-J joining enzyme requires the presence of signal sequences, i.e., heptamers and nonamers separated by a spacer of 12 nucleotides on one side and of 23 nucleotides on the other side, it was of interest to determine whether such signal sequences were present on chromosomes 11 and 18 near breakpoints. Since the spacer between heptamer and nonamer at the 3' end of the involved J_H segment is 23 nucleotides in length, the spacer on these 2 chromosomes should be 12 nucleotides in length. Indeed, analysis of the DNA sequences of normal chromosomes 11 and 18 in proximity of the chromosome breakpoints indicated the presence of signal sequences for joining with a spacer of 12 nucleotides (33, 37). Thus the chromosomal translocations observed in B-cell neoplasms with the t(11;14) and the t(14;18) chromosome translocations are catalyzed by the same enzyme that is involved in V-D-J joining (33, 37). Presumably the V-D-J joining enzyme mistakenly joins two separated segments of DNA on two different chromosomes instead of on the same chromosome, leading to chromosome translocation. Sequences similar to the signal sequences for joining may be present at many different sites in the human genome. Most likely, the translocations are selected for because of their ability to lead to neoplastic transformation. Similar translocations involving many different sites may go unrecognized because they do not result in malignant transformation. On the contrary those translocations that result in the juxtaposition of a cellular protooncogene to immunoglobulin heavy chain enhancing elements lead to B-cell neoplasia. Thus the chromosomal translocations observed in B-cell tumors provide cancer researchers with the tools to identify and characterize the genes that are directly involved in B-cell neoplasia (22).

While the molecular basis of the chromosomal translocations involving switch regions is not yet clear, it seems likely that they occur during the process of immunoglobulin heavy chain switching.

The Locus for the α-Chain of the T-Cell Receptor Is Involved in the Specific Rearrangements Observed in T-Cell Neoplasms and Deregulates Protooncogene Transcription

The locus for the α-chain of the T-cell receptor maps at band 14q11 (39), a chromosomal region also involved in rearrangements (predominantly translocations and inversions) in most T-cell neoplasms (40, 41). This finding suggested that the α-locus of the T-cell receptor might play a critical role in T-cell leukemias and lymphomas similar to that of the immunoglobulin genes in B-cell malignancies (39). Analysis of hybrids between mouse leukemic T-cells and human leukemic cells from patients with T-cell ALL carrying a t(11;14)(p13;q11) chromosome translocation (42) indicates that the T-cell receptor α-locus is split by the translocation, that the breakpoints occur between the T-cell receptor V_α and C_α genes, and that the V_α genes are proximal to the C_α gene at band 14q11 (42). Thus the locus for the α-chain of the T-cell receptor is directly involved in the chromosome rearrangements observed in most human T-cell neoplasms (42).

Recently we observed two cases of T-cell leukemias carrying a t(8;14) (q24;q11) chromosome translocation (43). In these two leukemias, the breakpoints on chromosome 8 occurred 3' to the involved c-myc oncogene, and the breakpoint on chromosome 14 occurred in joining segments (43). Thus the rearrangements observed in these T-cell leukemias parallel those observed in variant Burkitt's lymphomas (14, 15). We also examined the expression of the c-myc locus involved in the translocation versus that on the normal chromosome 8 in hybrids with mouse leukemic T-cells (43). Interestingly, the hybrids expressed only the c-myc gene involved in the translocation, while the c-myc gene on normal chromosome 8 was completely silent (43). We concluded that the translocation of the locus for the α-chain of the T-cell receptor to a cellular protooncogene leads to its transcriptional deregulation (43).

This observation suggests that the α-locus of the T-cell receptor contains genetic elements capable of activating gene transcription in cis over considerable chromosomal distances. Thus it should be possible to isolate most of the human genes involved in T-cell leukemias and lymphomas by taking advantage of specific chromosomal rearrangements involving band 14q11 and by using "chromosome walking" techniques (33, 35). This approach should enable identification of most of the genes that are directly and primarily involved in the pathogenesis of human B- and T-cell neoplasms.

Presence of Two Specific Chromosome Translocations in Human Leukemias

Some human pre-B-cell leukemias have been reported that carry a t(8;14) and a t(14;18) chromosome translocation in the same cells (44). We thought it unlikely that these two translocations, hallmarks of Burkitt's lymphoma and follicular lymphoma, respectively, occurred at the same time in the same cells (44). Since the phenotype of B-cell neoplasms with a t(14;18) chromosome translocation is that of a low-grade malignancy, while the phenotype of B-cell neoplasms with the t(8;14) chromosome translocation is that of a high-grade malignancy, we speculated that the t(14;18) chromosome translocation precedes the t(8;14) chromosome translocation (3, 44). The t(14;18) translocation probably occurred during V-D-J joining and led to the expansion of a clone of low-grade malignancy. This, in turn, resulted in an increased probability of an additional chromosome translocation during immunoglobulin...
gene rearrangement (35, 36). The t(8;14) translocation then led to a very aggressive B-cell malignancy (44). The same scenario might occur in Burkitt’s lymphoma. In equatorial Africa, where Burkitt’s lymphoma is endemic, children are infected by Epstein-Barr virus and malaria. This could result in a polyclonal expansion of virus-infected cells rearranging their immunoglobulin gene. This, in turn, would increase considerably the probability that a chromosome translocation will occur during immunoglobulin gene rearrangement.

Similarly, leukemias of patients in the region of Japan where HTLV-I is endemic have the same chromosome translocations and inversions that are observed in T-cell leukemias and lymphomas observed in Europe and America in the absence of HTLV-I (45). Thus HTLV-I may just be expanding the pool of proliferating T-cells rearranging T-cell receptor genes and then increasing the probability of a chromosome translocation and consequent T-cell neoplasia (42, 43).

The Lesson Learned from the Analysis of Chronic Myelogenous Leukemia

In most CMLs, a translocation between chromosomes 9 and 22, t(9;22)(q34;q11), has been identified (4). This translocation results in the formation of a small marker chromosome, named Philadelphia chromosome (2). In this translocation, the human homologue of the v-abl oncogene of Abelson leukemia virus, c-abl, is translocated from its normal position on chromosome 9, band q34, to chromosome 22 at band q11 (46). Molecular analysis of the region of chromosome 22 involved in the rearrangements indicated that most breakpoints occur within a short segment (5.8 kilobases in length) of DNA called the ber (46). Analysis of c-abl and ber transcripts in CML cells indicated that these leukemic cells express an aberrant hybrid c-abl-ber RNA that is translated into an aberrant c-abl-ber protein with a molecular weight of 210,000 with protein kinase activity (47, 48). Because of the rearrangement, the c-abl gene product loses its amino terminus, similar to the events in the generation of a transforming v-abl oncogene (47, 48).

Thus the mechanism of oncogene activation in chronic myelogenous leukemia is quite different from that observed in the great majority of human B- and T-cell leukemias and lymphomas. Interestingly, a t(8;22) (q34;q11) chromosome translocation is also observed in approximately 10% of human ALL (49). We have analyzed Philadelphia-positive (Ph) ALL cells for rearrangements of the bcr locus (49) and have found that most Ph-positive childhood ALLs do not show bcr rearrangements and show c-abl and bcr transcripts of normal sizes (49). This result indicates heterogeneity of breakpoints in Ph-positive leukemias and suggests that the genetic mechanism involved in Ph-positive childhood ALL may be different from that observed in CML (49).

Conclusion

Cytogenetic and molecular analysis of human leukemias and lymphomas has shown that at least two different mechanisms are involved in the malignant transformation of hematopoietic cells. In human B- and T-cell neoplasms, the most common mechanisms involve the juxtaposition of cellular protooncogenes and genetic elements capable of activating gene transcription in cis in a tissue-specific fashion over considerable chromosomal distances. This juxtaposition results in high level constitutive expression of the involved protooncogene, which fails to respond to the normal mechanisms of transcriptional control, leading to malignancy. In CML cells the mechanisms of malignant transformation seem to involve the expression of an altered gene product that acquires protein kinase activity. The expression of this altered gene product is associated with a low grade malignancy (CML). The blastic crisis that is observed in CML patients may then be due to additional genetic changes. Additional and consistent chromosomal alterations have in fact been detected in CML cases in blastic crisis.

In the case of most solid tumors, unfortunately, cytogenetic analysis has not yet provided the molecular geneticists with the information necessary to identify the critical genes involved in the malignant process. The molecular mechanisms in the malignant transformation of cells of solid tumors might involve multiple steps, since these cells must escape several levels of different environmental controls and restrictions. Thus, it may be difficult to find common cytogenetic differences in such tumor cells. However, quite likely, such nonrandom chromosomal alterations will be found, at least in some of the solid tumors, and will provide the basis for a rational molecular genetic approach to the pathogenesis of human solid tumors.

References


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