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The New Genetics and Non-Hodgkin Lymphoma

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THE IDENTIFICATION of genes and proteins that are important in the pathogenesis and behaviour of lymphomas has proceeded rapidly in the past decade. These advances are of interest to clinicians, firstly because they may provide a new system of classification based on cytogenetic abnormalities, gene rearrangement and gene expression, and secondly because it may eventually be possible to manipulate these genes as targets for tumour-specific therapy. This article reviews some of the recurrent chromosome translocations found in B-cell non-Hodgkin lymphoma (NHL), how these have led to the molecular cloning of previously unrecognised genes and how these translocations influence the *in vivo* behaviour of the tumour.

RECURRENT CHROMOSOMAL TRANSLOCATIONS

Although lymphoma cells often grow well *in vitro*, obtaining and interpreting high resolution chromosome preparations makes heavy demands on time and experience. Despite these difficulties, many recurrent chromosomal translocations have been recognised in B-cell NHL (Table 1 and Fig. 1). In Table 1, three varieties of translocation have been distinguished. Group 1 DNA sequences are known on either side of the translocation breakpoint. Group 2 DNA sequences are known only on one side of the translocation. These translocations usually involve immunoglobulin on T-cell receptor (TCR) loci which allows rapid molecular cloning of the breakpoint. Group 3 DNA sequences are unknown on both sides of the breakpoint.

Some of these translocations may be extremely common within a given histological subgroup of disease—e.g. t(8;14) (q24.1;q32.1) in Burkitt's lymphoma and t(14;18) (q32.1;q21) in follicular lymphomas. This led to the idea that specific translocations were linked to a specific histological disease with a characteristic clinical behaviour; thus, Burkitt's lymphomas follow an aggressive clinical course, while follicular lymphomas are mostly indolent. In the acute myeloid leukaemias this idea still holds true with certain translocations being associated with a given cytological type of leukaemia, for example t(15;17) in acute promyelocytic leukaemia. However, further analyses have revealed that in B-cell NHL this linkage may not be so "tight"; t(14;18) may be found in up to 30% of diffuse B-cell NHL as well as in rare cases of B-cell acute leukaemia.

The presence of the t(14;18) translocation in cytologically diverse B-cell tumours is a paradigm of the multistep model of tumorigenesis [1, 2]. Follicular lymphomas with t(14;18) as the only cytogenetic abnormality generally grow slowly and respond well to therapy; in contrast, rare tumours with both t(14;18) and t(8;14) translocations are almost invariably of leukaemic phenotype, follow an aggressive clinical course and respond

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poorly to therapy [3]. Diffuse B-cell NHL with the t(14;18) translocation have other cytogenetic abnormalities and are associated with a worse prognosis than diffuse NHL lacking the translocation [4]. Clinically, these are examples of "transformation". They are analogous to chronic myeloid leukaemia, associated with the t(9;22) Philadelphia chromosome which, with the acquisition of further cytogenetic changes, progresses to the phase of "blast crisis". The molecular nature of most transformation events is unknown (see below).

There is, therefore, a spectrum of B-cell NHL in which phenotype and clinical behaviour are linked with cytogenetic abnormalities. Cytogenetic analysis might form the basis of a new means of classification of B-cell NHL, eventually permitting intensification of therapy for specific subgroups of disease, for example diffuse NHL with t(14;18). Care needs to be exercised, however, since the significance of a given cytogenetic abnormality may vary according to the cytological subgroup of disease. Burkitt's lymphoma is characterised by t(8;14) and an aggressive clinical course, whereas the cytogenetically identical translocation in diffuse non-Burkitt NHL may be associated with an indolent course [5].

New, apparently common, recurrent chromosomal translocations are still being described in the B-cell lymphomas. The t(3;22) (q26–28;q11.2) translocation has been reported in up to 10% of a large series of diffuse NHL [6, 7]. This translocation is of interest since breakpoints in the region 3q26–q28 may be seen in up to 20% of NHL; the juxtaposition of this breakpoint with immunoglobulin lambda light-chain sequences at 22q11.2 may permit rapid molecular cloning of this breakpoint. Similarly, Bloomfield and her group have described, in a series of 157 patients, four new recurrent translocations, all of which involve uncharacterised loci [8]. One of these translocations, t(8;9) (q24;p13), appears to be found in conjunction with t(14;18); we have derived two new cell lines from patients with acute B-cell leukaemias with this composite karyotype (E. Nacheva, M.J.S. Dyer, P. Fischer).

Translocations previously thought to be specific for B-cell precursor leukaemias are now also being found in more phenotypically mature B-cell malignancies. The t(4;11) (q21.3;q23) translocation has been associated primarily with undifferentiated leukaemias, often of infants. Most of these leukaemias appear to have made some commitment to the B-cell lineage, although evidence for commitment to T-cell, myeloid and monocytoid lineages may be found. These leukaemias have a poor response to conventional therapy. A cell line with the t(4;11) translocation along with t(14;18) as well as several other chromosomal abnormalities has been derived from a patient with chemotherapy-resistant B-cell NHL [9]. We also have a case of B-cell NHL with the t(1;19) translocation, which again was thought previously to be specific for B-cell precursor leukaemias (T. Khokhar *et al.*).

In conclusion, over the years, a catalogue of chromosome abnormalities in B-cell NHL has been obtained and is of major importance. For the clinician, the occurrence of certain abnormalities in certain histological subgroups of NHL may define clinically important discrete subgroups of disease which might merit different treatment. For the scientist, the major interest is in elucidating the molecular structure of the translocations, since they may play a central role in the genesis of the tumour. The molecular cloning of several translocation breakpoints has defined new DNA sequences of interest; moreover, it has also allowed the subsequent detection of the translocation without recourse to cytogenetic analysis and the development of sensitive assays for residual disease [10]. Nevertheless, cytogenetic analysis remains the "gold standard" against which these other methods should be compared.

Table 1. *Common recurrent chromosomal translocations in B-cell lymphomas*

Chromosomal translocation	Cloned	DNA sequences involved	Tumour types
Group 1			
t(8;14) (q24.1;q32.1) variants: t(2;8) t(8;22)	Yes	Ig heavy or light chains: C-MYC	B-cell ALL/Burkitt's lymphoma, NHL, myeloma, PLL
Group 2			
t(14;18) (q32.1;q21) variants: t(2;18) t(18;22)	Yes	Ig heavy or light chains: BCL-2	Follicular and diffuse NHL, CLL
t(11;14) (q13;q32.1)	Yes	Ig heavy chain: BCL-1	NHL/CLL/PLL, myeloma
t(14;19) (q32.1;q13.1)	Yes	Ig heavy chain: BCL-3	CLL
t(3;22) (q26–q28; q11.2)	No	?:Ig lambda light chain	Diffuse NHL
t(9;14) (p13;q32.1)	Yes	?:Ig heavy chain	NHL
Group 3			
t(4;11) (q21;q23.1)	No	?:?	B-cell precursor ALL/NHL
t(8;9) (q24.1;p13)	No	?:?	Diffuse NHL
t(9;15) (p13;q15)	No	?:?	NHL

ALL = acute lymphodastic leukaemia, PLL = prolymphocytic leukaemia and CLL = chronic lymphocytic leukaemia. ? denotes unknown DNA sequences.

MOLECULAR ANALYSIS OF CHROMOSOME TRANSLOCATIONS

The finding that many translocations in B-cell NHL are specifically targeted to the immunoglobulin (Ig) loci has permitted rapid molecular cloning of the translocation breakpoints with cloned Ig DNA probes to identify rearranged fragments (Table 1). In T-cell malignancies TCR loci are similarly involved [11].

The molecular cloning of such breakpoints has defined previously unrecognised genes that are of importance not only in the pathogenesis of the tumour but also in normal lymphocyte development. An example is the isolation of the BCL-2 gene located at 18q21 by nature of its involvement in the t(14;18) (q32.1;q21) [12, 13]. The BCL-2 gene encodes small (22 and 26 kD) membrane-bound proteins of unknown functions. Despite the fact that translocations involving the BCL-2 gene are found only in phenotypically mature B-cell malignancies, BCL-2 expression is found throughout the early stages of both B-cell and T-cell development but is down-regulated in mature B-cells [14].

DNA sequence analysis of the translocation breakpoints has revealed that t(14;18) may result from an error during physiological Ig rearrangement [15]. This is of considerable interest because it suggests that the progenitor cells of malignancies of mature B-cell phenotype may nevertheless be found among the B-cell precursor population [16]. Any attempts at targeted therapy for these malignancies should therefore aim to eliminate both mature and precursor B-cell populations.

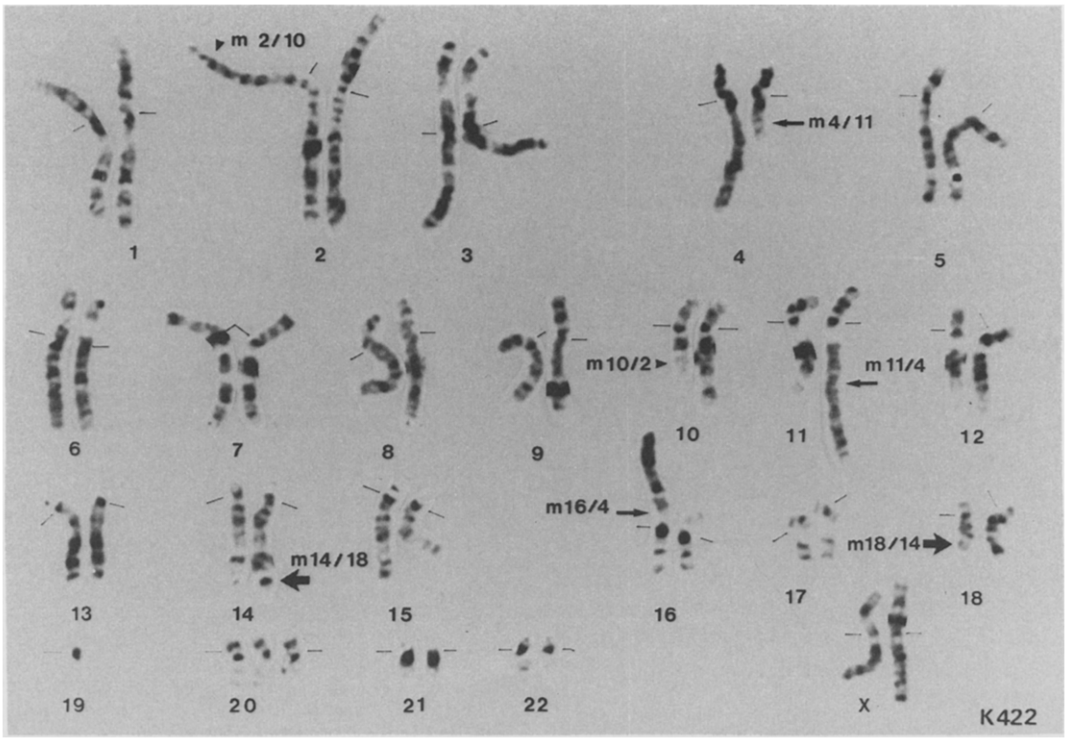

Fig. 1. Karyotype of cell line Karpas K422 [9]. This cell line was derived from patient with intra-abdominal chemotherapy-resistant B-cell NHL. Karyotype illustrates the plethora of abnormalities commonly found in B-cell NHL. Major cell clone is characterised by t(2;10) (p23;q22.1), t(4;11) (q21.3;q23.1), t(4;16) (q21.3;p13.1), t(14;18) (q32.1;q21.3).

BCL-2 translocation results in deregulation and over-expression. In tumours with the t(14;18) translocation, levels of BCL-2 expression may be 100 fold higher than those found in B-cell precursors. The questions now are what are the functions of the normal protein and how does deregulation, over-expression and, perhaps, mutation, result in tumorigenesis? Transgenic mouse and gene transfer experiments have shown that over-expression of BCL-2 results not in transformation but in prolonged survival of B cells: further events are necessary for the acquisition of the full neoplastic phenotype [17, 18].

Some translocations, however, do not appear to involve a transcription unit. An example is the t(11;14) (q13;q32) translocation: several breakpoints derived from a variety of tumours and ranging over 60 kb of DNA have been cloned, but none of the derived probes detects transcripts. Perhaps this is not surprising given that deregulation of C-MYC sequences in the t(8;14) translocations may occur with the C-MYC locus some 600 kb away from the Ig locus.

MOLECULAR EVENTS ASSOCIATED WITH TRANSFORMATION OF NHL

In the multistep model of tumour progression, the t(14;18) translocation allows a low grade proliferation while other changes cause the tumour to become more malignant. One mechanism is the deregulation of other genes through further chromosomal translocations. Deregulation of C-MYC sequences via the t(8;14) translocation in conjunction with t(14;18) generally results in an aggressive leukaemic phenotype. De Jong *et al.* [19] elegantly showed that acquisition of C-MYC rearrangement in a case of transformed follicular lymphoma with the t(14;18) resulted in a change of both the phenotype and clinical behaviour of the lymphoma. Similarly, a synergistic effect between C-MYC and BCL-2 has been shown in gene transfer experiments *in vitro* [20]. The molecular basis for this synergy is, however, unknown.

The combination of t(8;14) and t(14;18) is rare, however, and it is likely that other translocations involving as yet uncharacterised genes are of equal or greater importance than C-MYC. Multiple genomic defects are common in lymphomas. Thus, the K422 cell line has, in addition to the t(14;18) t(4;11), t(2;10), t(4;16) translocation, an interstitial deletion of chromosome 12 and an isochromosome of the long arm of 14 as well as a small acrocentric marker [9]. Molecular characterisation of these other chromosome breakpoints may define new genes of importance not only in transformation of lymphomas but also in normal B-cell development.

Other possible mechanisms of tumour development include mutation of oncogenes and loss or inactivation of "tumour suppressor genes". Both C-MYC and BCL-2 mutations have been demonstrated in cell lines but their contribution to the malignant phenotype is not yet clear. It is interesting to note that fresh cases of follicular NHL with the t(14;18) translocation did not have BCL-2 mutations [21]. So far, the involvement of the "tumour suppressor" genes has not been much investigated in NHL. The retinoblastoma (RBI) gene maps to 13q14 and is inactivated either by point mutation or by allele loss. One case of T-cell ALL has been shown to have an extensive deletion of the 3′ end of the RBI gene [22]. Translocations involving the 13q14 locus have been reported in B-cell CLL [23] whilst chromosome 17 abnormalities, which may involve the p53 locus, are not uncommon in B-cell NHL and are associated with a poor prognosis [24]. Therefore inactivation of these genes may be an important step in B-cell tumour development.

NEW DIAGNOSES, NEW THERAPIES?

The molecular dissection of the genetic events associated with lymphoid neoplasia is proceeding at great pace. The ability to manipulate ever larger fragments of DNA will allow the molecular cloning of chromosome translocation breakpoints for which we currently have no markers. This will hopefully allow the isolation of new genes of importance in B-cell tumour development. Already the combination of immunophenotypic, karyotypic and genotypic analyses is allowing the definition of new subgroups of lymphoma of probable biological significance. It now behoves clinicians who look after patients with lymphoma to ensure that these investigations are undertaken.

As regards possible therapeutic approaches, genes involved in translocations such as BCL-2 are not expressed on the cell surface and it is therefore difficult to envisage targeted therapy using these molecules. It is clear, however, that treatment of lymphoma with certain unconjugated monoclonal antibodies can be successful [25]. A cell surface molecule mutated in some way by the neoplastic process might therefore provide a suitable tumour-specific target for the "magic bullet". An example of this approach is the deletions in the epidermal growth factor receptor of malignant gliomas which have allowed the generation of tumour-specific antibodies [26]. The likelihood of such novel therapies can only increase as we understand more of the biology of these interesting tumours.

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