Karyotypic and molecular abnormalities in chronic lymphocytic leukaemia

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Introduction
B cell chronic lymphocytic leukaemia (B-CLL) is the commonest type of adult leukaemia in the western world, accounting for over 40% of all adult patients with leukaemia. About 50% of patients present with early stage, often asymptomatic disease. The disease may remain stable or slowly progress but at least half of all patients die of a cause unrelated to CLL. Age, sex, Binet stage, and karyotype status have all been identified as important independent prognostic factors. Clonal karyotypic abnormalities have been detected in approximately 50% of all patients with a complex karyotype, a high percentage of abnormal metaphases, trisomy 12, and abnormalities of chromosome 14, which are all associated with a poor prognosis. The molecular changes responsible for the development and progression of CLL have not been elucidated fully, but both karyotypic and molecular studies have been hampered by the inclusion of patients with prolymphocytic leukaemia (PLL), splenic lymphoma with villous lymphocytes (SLVL), hairy cell leukaemia, and non-Hodgkin’s lymphoma (follicular and mantle cell) in leukaemic phase. Unfortunately, no single test is diagnostic of CLL and a combination of morphology and immunophenotyping is required. The situation is confused further by the recent observation that subtypes of CLL exist with atypical features (and often atypical behaviour!).

The aim of this review is therefore to try to clarify what is known about the karyotypic and molecular abnormalities in both typical and atypical CLL and to suggest future studies which may shed further light on this complex disease.

Karyotypic abnormalities in CLL
Successful karyotyping of CLL cells was first described in the late 1970s and since then data from many single and multicentre studies have been published. Karyotypic abnormalities may be single (29–32%), double (11–12%), triple or more (12–15%), with chromosomes 6, 11, 12, 13, and 14 being the most commonly affected. Chromosome 11 Initial studies showed that abnormalities of chromosome 11 occurred in up to 10% of patients with CLL. The commonest abnormality is t(11;14)(q13;q32), which is found characteristically in mantle cell lymphoma and PLL. It has recently been shown to be associated with atypical CLL in which cells are CD23 negative, surface immunoglobulin strongly staining and karyotypic evolution not uncommon.

Indeed, these studies confirmed earlier reports of a worse prognosis in patients with this abnormality. As in mantle cell lymphoma, the t(11;14)(q13;q32) translocation results in rearrangements of the bcl-1 locus on 11q13 with the immunoglobulin heavy chain gene on 14q32. However, these early studies include many patients with features more in keeping with non-Hodgkin’s lymphoma rather than CLL (for example, CD5 or CD23 negative, FMC7 positive) which has led some authors to conclude that bcl-1 rearrangements do not occur in CLL, although this is disputed. Deletions of 11q were identified in some of the earliest studies of CLL but were thought to be relatively rare. Several recent studies, however, have shown that del (11q) is one of the commonest abnormalities in patients with typical CLL (14–27%) and may be associated with advanced progressive disease and karyotypic evolution.

Studies using fluorescence in situ hybridisation (FISH) and YAC (yeast artificial chromosome) probes have revealed a genomic region of 7–9 Mbp that is located proximal to the MLL gene on 11q23. These studies indicate the likely presence of a previously unreco-

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ised tumour suppressor gene in the region of 11q23 which is important in the pathogenesis of CLL. Interestingly, a recent study revealed a novel fusion between the promyelocytic leukaemic zinc finger gene (on 11q23) and the retinoic acid receptor α gene (on 17q21) in five of 11 patients with CLL.16

**CHROMOSOME 12**

Early studies showed that trisomy 12 is one of the commonest chromosomal abnormalities in CLL, occurring in about 20% of all patients and associated with a poor prognosis.7 8 10 11 It arises from duplication of one chromosome 12 with retention of the other copy rather than the loss of one chromosome 12 and triplication of the other.17 18 The use of FISH permits detection of trisomy 12 in patients with a normal karyotype.19 21 This is partly because CLL metaphases are not easy to obtain, but also because trisomy 12 usually exists as a subclone.22 Using FISH, as few as 2% of cells with trisomy 12 can be detected, but it is not known whether patients with FISH only detectable trisomy 12 also have a poor prognosis. Indeed, it is difficult to imagine that a subclone consisting of only 2% of malignant cells can affect survival! Sequential FISH studies accurately monitoring the trisomy 12 clone are needed.

Three recent reports have shown that trisomy 12 is uncommon in typical CLL and is found in only 2–7% of patients compared with 45–57% of atypical cases.34 43 44

Trisomy 12 cells often have a slightly abnormal immunophenotype with strong FMC7, CD20 and SmIg and CD5 negativity.45 46 There are, as yet, no studies with multivariate analysis to indicate whether it is the atypical morphology or the trisomy 12 which contributes to a poor prognosis. Karyotypic studies have shown that trisomy 12 is usually detectable early in the disease and rarely acquired later; however, studies using a combination of FISH with either Southern blotting or APAAP suggest that trisomy 12 is not the initiating event in the pathogenesis of CLL and occurs as a secondary event in an established neoplastic B cell clone.6 14 15 47 51

This apparent contradiction is difficult to reconcile. Either the malignant transforming CLL clone is able to lose the extra chromosome 12, which is extremely rare in sequential karyotypic studies and casts doubt on the importance of trisomy 12 as an independent prognostic factor. Alternatively, patients with trisomy 12 have undergone a long preclinical course which only manifests as a clinical disease once trisomy 12 appears. In almost 50% of patients with trisomy 12 it occurs as part of a more complex karyotype with these additional abnormalities adversely affecting prognosis.6 10 11 The complex karyotypes may either be apparent at diagnosis or evolve clonally in cells which previously had trisomy 12 alone.50 51 Interestingly, in karyotypic studies trisomy 12 virtually never occurs in association with deletion of 13q14, which is one of the commonest abnormalities in typical CLL (see later); however, molecular studies using the retinoblastoma gene (Rb-1; situated at 13q14) have shown that 6% of patients with CLL have both trisomy 12 and deleted Rb-1 genes.57

Other structural abnormalities—for example, translocations/deletions of chromosome 12, have been described involving, amongst others, p11, p12, p13, q13, and q24, but these occur in less than 3% of patients.2 10

Several oncogenes reside on chromosome 12 but none seem to be important in the pathogenesis of CLL.3 The murine double-minute-2 gene (MDM-2) is located at 12q13-14 and encodes in 90 kDa protein, which complexes with both wild type and mutated p53 and inhibits its growth regulating properties.34 MDM-2 can overcome the growth suppressing activity of p53 with a similar functional consequence to that of p53 mutation.39 In view of the possible role of p53 mutations in the pathogenesis of CLL (see later) several studies looking at MDM-2 concentrations have been reported. Overexpression of MDM-2 mRNA occurs in up to 73% of patients with CLL, but no amplification of the MDM-2 gene has been demonstrated.34 35 Interestingly, MDM-2 overexpression is found not only in patients with advanced clinical disease, but 82% (14/17) of patients expressed wild type p53.35 Thus, it seems that overexpression of MDM-2 may play a role in the pathogenesis or progression of CLL. Unlike p53 mutations in CLL, however, MDM-2 overexpression does not seem to confer drug resistance.39

To date there have been no studies reported specifically looking at MDM-2 expression in cells from patients with CLL and trisomy 12. Caution should perhaps also be exercised in interpreting MDM-2 overexpression, as most studies have compared expression of CLL cells with peripheral blood, lymph node or splenic B lymphocytes rather than the normal counterpart (that is, CD19+/CD5+) of the B-CLL lymphocytes.

**CHROMOSOME 13**

Abnormalities of chromosome 13, although previously described in B-CLL, were brought to prominence by Fitchett in 1987, who described 14 patients with deletions (9) or translocations (5) involving 13q14.46 It is now known that karyotypic 13q14 abnormalities are the most frequent structural lesions found in typical CLL, occurring up to 30% of patients.11 40 Other deletions of 13q—for example, 13q11, 13q12, also occur, but in the vast majority of these 13q14 is also deleted.2 11 40 As with other chromosomal abnormalities in CLL 13q deletions/translocations may occur as subclones or as part of a more complex karyotype.40 45 Indeed, in only about 50% of patients is the 13q aberration the only abnormality.3

Interestingly, patients with single 13q abnormalities have the same prognosis as those with a normal karyotype.2 Karyotypic evolution of 13q deletions can occur and other unrelated clones may also appear.34 41 44

Fitchett herself emphasised in her original paper the proximity of the Rb-1 gene and sev-
eral studies using FISH, Southern blotting, PCR, single strand conformation polymorphism (SSCP), and comparative genomic hybridisation have now been reported. Monoallelic loss of Rb-1 is common in patients with del (13q14) and occurs in up to 26% of patients with a normal karyotype. Reduced levels of mRNA have also been reported in up to 57% of patients with del (13q14) using immunohistochemistry, northern blotting or RT-PCR. Loss of the Rb-1 gene occurs early in CLL but is probably not the initiating event; some authors have even suggested it is an indifferent bystander phenomenon and may have no relevance to the pathogenesis of CLL, although this is disputed. Evidence that the Rb-1 gene and its product do not play a role in the initiation/progression of CLL come from the observations that:

- the Rb-1 gene is believed to act as a recessive oncogene but homozygous deletions are extremely rare in CLL;
- patients with del (13q14) not involving the Rb-1 gene have been described;
- a recently described gene telomeric to the Rb-1 gene has been shown to be deleted more frequently.

The D13S25 gene is deleted in up to 90% of patients with del (13q14) and up to 50% of those with a normal karyotype. The D13S25 locus is also not critically involved in CLL. In a recent study Liu et al. demonstrated deletions of Rb-1, D13S25 and D13S319 in 21%, 39% and 44% of patients with CLL, respectively.

Furthermore, homozygous deletions of the D13S319 locus were present in 13% of patients compared with 5% for the D13S25 locus in this series. Although the homozygous deletion of D13S25 was lower than suggested by earlier reports, this study indicates that a previously unknown tumour suppressor gene, important in the pathogenesis of CLL, is present in the region of D13S319.

It is interesting to note that many patients with translocations involving 13q14 also have monoallelic loss of a region including the Rb-1 gene. This suggests that it is genetic loss rather than the formation of a novel fusion gene (as seen in some other cases of leukaemia—for example, chronic myeloid leukaemia; CML) that is important.

**CHROMOSOME 14**

Abnormalities of chromosome 14 occur in up to 9% of patients with CLL and may be associated with a poor prognosis. The commonest abnormality is t(11;14), but as indicated above many of these cases are atypical or represent leukemic phase non-Hodgkin’s lymphoma. Translocations may also occur with other chromosomes, notably 2 and 19, and they often form part of a more complex karyotype, especially in patients with advanced disease. In nearly all cases the immunoglobulin heavy chain (lgH), situated at 14q32, is involved. The t(14;18) (q32;q21), characteristically seen in follicular non-Hodgkin’s lymphoma, is extremely rare in CLL. If non-Hodgkin’s lymphoma, the IgH gene is rearranged and juxtaposed to bcl-2. It seems that IgH/bcl-2 rearrangements do not occur in the absence of a karyotypically detectable t(14;18).

### CHROMOSOME 17

Karyotypic abnormalities of chromosome 17 are rare in CLL, occurring in about 4% of all patients. The commonest abnormalities are monosomy 17, del (17p), iso (17q), and translocations involving both 17p and 17q (more common). Interest in chromosome 17 arose from the identification of the p53 tumour suppressor gene on 17p13, which plays an important role in cell growth, differentiation and ultimately apoptosis. It was shown to act as a tumour suppressor gene by preventing the onset of tumorgenesis and is inherited as an autosomal recessive trait. Inactivation of p53 can occur through gene deletions, mutation or single base insertion.

**Mutations of p53** are often associated with karyotypic abnormalities of chromosome 17 (usually monosomy 17 or del (17p) and loss of

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**Table 1** p53 mutations in CLL.
heterozygosity of the normal allele).  

Four studies have shown recently that p53 mutations are associated with drug resistance, which is independent of MDR1/MDR3 status, and results in a significantly worse prognosis.  

Indeed, in multivariate analysis it is one of the most important prognostic factors. Patients are resistant to a wide range of single chemotherapy agents—for example, chlorambucil, prednisolone, fludarabine, and deoxycoformycin, as well as the multidrug regime, CHOP (cyclophosphamide, adriamycin, vincristine, prednisolone). As the mode of action of many chemotherapy agents is to induce apoptosis, it is thought that p53 mutations prevent initiation of the apoptotic pathway.

The exact role of p53 mutations in the pathogenesis of CLL is unclear as it has not been studied extensively. It is known that p53 mutations are more frequent in Richter syndrome, but analysis of paired samples (that is, CLL and Richter sample) have not given consistent results.

Matolcsy et al. studied six patients but found p53 mutations in only two. One patient had a p53 mutation in the Richter clone but the immunoglobulin rearrangement was different to the preceding CLL clone, indicating the emergence of a totally unrelated clone. In the other patient an identical p53 mutation was present in the CLL and Richter clones, suggesting that the p53 mutation was not responsible for the histological transformation. A recent patient with atypical CLL (t(11;14)) reported by Cuneo et al. developed a p53 mutation (A to G at codon 234) during Richter transformation which was not present during the CLL phase; this suggested a pathogenic role for this p53 mutation. The identical p53 mutation, however, has been described in a patient with stage I typical CLL, a long doubling time and loss of 17p13 heterozygosity (that is, early non-progressive disease), indicating that this particular mutation alone is insufficient to lead to disease progression or transformation.

CHROMOSOME 18

Abnormalities of chromosome 18 are rare (about 3% of patients) in CLL.  

Monosomy, trisomy and translocations have been described. The commonest translocations usually involve 18q21, the bcl-2 gene, with 2p11 (κ light chain locus), 1q42 (IgH locus) and 25q11 (λ light chain locus). In all of these patients bcl-2 is juxtaposed to the above genes with rearrangement of bcl-2.

Various break-points have been described within the minor and major break-point regions and the variant cluster regions.

Bcl-2 rearrangements are not found in CLL cells lacking karyotypic abnormalities of 18q21.

Although rearrangements of bcl-2 are rare in CLL, all cells have increased expression of bcl-2 mRNA, with up to 70% exhibiting increased protein expression compared with normal CD5+ B lymphocytes. In CLL most malignant cells are in G0 of the cell cycle but when cultured undergo apoptosis. High concentrations of bcl-2 protect against apoptosis and it is therefore postulated that bcl-2 plays an important role in the pathogenesis of CLL, although the mechanism of overexpression is not understood.

Discussion

In recent years it has become increasingly recognised that it can be difficult to distinguish CLL from other lymphoproliferative disorders and that many early cytogenetic and molecular studies included many non-CLL patients. Unfortunately, there is no single diagnostic test for CLL and a combination of morphology and immunophenotyping is necessary. The morphology of CLL is diverse, but the term typical CLL should be reserved for small lymphocytes with a high N:C ratio, regular nuclear and cytoplasmic membranes and < 10% atypical cells or prolymphocytes. Atypical CLL cells are often large with a low N:C ratio, irregular nuclear or cytoplasmic membranes, cleat nuclei with small or inconspicuous nucleoli, and < 10% prolymphocytes. Mixed CLL/PLL refers to the presence of prolymphocytes (10–55%). Immunophenotyping can also be variable and Matutes et al. recently proposed a scoring system based on the reactivity of five commonly used antibodies (table 2). Widespread use of strict morphological criteria and the Matutes scoring system would allow comparisons to be made between patients from different studies. Indeed, the fact that some studies detected bcl-1 and bcl-2 rearrangements, whereas others did not, emphasises the need for strict diagnostic criteria.

It is now clear that typical CLL is associated with del (13q) and del (11q) whereas trisomy 12 is usually associated with atypical CLL. The argument for distinguishing between typical and atypical CLL is supported by the paucity of patients who possess both trisomy 12 and del (13q) or del (11q). This suggests strongly a different pathogenesis for these two disorders and hence they should probably be regarded as different, although related, diseases. There is now little doubt that a novel tumour suppressor gene, important in the pathogenesis of CLL, resides on 13q. The fact that single abnormalities of 13q have no prognostic significance suggests that although it has a role in the initiation of CLL it is only one step in what is probably a multistep, pathogenic
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process (for example, myelodysplasia (MDS) and CML). Indeed, del (13q), as part of a more complex karyotype, does have an adverse prognostic significance.

The observation that clones, subclones and unrelated clones often coexist and that karyotypic evolution occurs also argues for a multistep process.14 47 Recent studies indicating that there is probably a new tumour suppressor gene on 11q are extremely exciting. Many oncogenes relevant to the development of B cell leukaemia are already known to reside on 11q and studies are presently being undertaken to identify the relevant gene(s).117

We feel that all cases of t(11;14) are probably not CLL at all, as they share genetic, phenotypic and prognostic similarities with mantle cell lymphoma and probably represent an unusual presentation of this disorder or PLL.110

Karyotypic studies in CLL have been hampered by the failure to obtain adequate metaphases for analysis and the observation that many abnormal clones exist as subclones. Combined studies using FISH and immunophenotyping have shown that up to 60% of malignant cells have a normal karyotype.48 The origin and role of these karyotypically normal malignant cells is unclear and further studies are necessary. FISH can also be used to detect abnormal clones—for example, trisomy 12, in patients with a normal karyotype but the significance of subclones which exist at very low levels—for example, < 5% of cells, is unknown. Sequential FISH studies are needed to assess whether these clones eventually become dominant and have an impact on prognosis.

To date, no oncogenes have been identified which are critical to the development and progression of CLL. Bcl-1 and bcl-2 rearrangements are extremely rare or probably do not occur at all in true CLL. The mechanism of bcl-2 protein overexpression and its clinical importance certainly warrants further investigation. The recent observation that several cytokines—for example, interleukin-4, α or γ interferon, can increase bcl-2 concentrations and inhibit apoptosis is interesting and perhaps suggests that autocrine production of cytokines may be pathogenetically important.112-116

Similarly, the development and importance of p53 mutations, especially in exons other than 5-8, needs further evaluation as studies to date have given inconsistent results. Most studies have focused on CLL and Richter syndrome, but Richter transformation is not a common cause of death in CLL and patients usually die from complications of marrow failure. Does p53 play any role in CLL progression? Only sequential studies are likely to answer this question. The only study to look at p53 mutations and karyotype suggested that p53 mutations nearly always occur when there are karyotypic abnormalities of 17p.92 This observation requires confirmation. MDM-2 studies have shown overexpression in some patients with CLL but a clear role has yet to be established. Expression of MDM-2 in cells with trisomy 12 would be of interest, as would further studies looking at p53 status and MDM-2 expression.

In conclusion, although we are much closer to identifying genes relevant to CLL there is still a lot to be done. When dealing with relatively "benign" disorders, the urgency for the absolutely correct diagnosis is not so great but if we wish to increase our understanding, the distinction between typical, atypical and mixed CLL must be more fully appreciated and specific karyotypic and molecular studies devised for these different disorders. We are moving closer to a new classification of CLL based upon morphological, immunophenotypic, karyotypic, and perhaps genetic abnormalities, but CLL epitomises the well known fact that it is more difficult to study a common, heterogeneous disorder with a relatively good prognosis than a rare lethal disease such as acute myeloid leukaemia.


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