SHORT COMMUNICATION

Somatic changes in B-lymphoproliferative disorders (B-LPD) detected by DNA-fingerprinting

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Specific genomic alterations may be involved in the transformation of a normal cell to a malignant one (Nowell, 1986). Characteristic translocations, often related to cellular oncogenes, are frequent in B-non-Hodgkin's lymphoma (B-NHL). In Burkitt's lymphoma, a translocation t(8;14) activates the c-myc oncogene and in 85% of follicular lymphomas a translocation t(14;18) is detected, involving the putative oncogene bcl-2 (Dalla Favera et al., 1982; Tsujimoto et al., 1985). The clonal evolution of B-NHL is marked by successive chromosomal changes (Yunis et al., 1987), some of which correlate with histologic signs of progression and a more malignant course of the disease (Koduru et al., 1987).

Karyotype analysis, however, is technically difficult in solid tumours and impossible in cases where only frozen tissue is available. By detecting a large amount of chromosomal loci dispersed throughout the genome, minisatellite probes may serve as probes for chromosomal changes (Jeffreys et al., 1985). Thein et al. used this approach to screen the DNA of 35 patients with a variety of cancers and found differences between the constitutional DNA and tumour DNA in ten cases (Thein et al., 1987). The changes were interpreted as either unequal sister-chromatid exchanges producing new fragments, or variations in fragment intensity and hence chromosomal copy number. Using minisatellite probe 33.15, we studied two EBV-negative cell lines, HH2 and HH3, derived from pleural effusion cells of a single B-immunoblastic NHL. In parallel, karyotype analysis was performed to relate the changes seen in DNA-fingerprint directly to cytogenetic differences. Cell line HH2 and the original malignant pleural effusion cells showed 48XY +7 +del12(q24) t(14;18)(q32;q21). Cell line HH3 showed the same karyotype except that no partial trisomy 12 was present. On hybridizing to the minisatellite probe 33.15, the fingerprints of cell lines HH2 and HH3 and of the original tumour were indistinguishable, but for 5.1 and 2.7 kb fragments present in HH2 and in the original tumour and absent in HH3 (Figure 1). This finding suggests the location of these fragments on the partially deleted extra chromosome 12. Moreover, since this chromosome must be derived from one of the two normal chromosomes 12 neither of which contains a 5.1 and 2.7 kb band, the deletion may have created the altered minisatellite fragments, underscoring that chromosomal aberrations may be detected by DNA-fingerprinting.

DNA-fingerprints of successive lymph node biopsies of 19 patients with B-LPD were analysed for somatic changes with time. DNA was extracted according to standard methods from 35 lymph node biopsy samples from 17 patients with B-NHL and 7 peripheral blood samples from two patients with CLL and digested with Hinfl or HaeIII. Blots on Genescreen plus® (New England Nuclear) were made (Jeffreys et al., 1985) and hybridized to minisatellite probe 33.15. 32P-labelled by primer extension. The filters were washed in 2x SSC/0.1% SDS at 65°C and autoradiographed at −70°C for 12 to 36 h.

Out of 19 patients, 8 showed histologic signs of progression. Loss of follicular pattern or predominance of blasts

Figure 1 Minisatellite patterns of cell lines HH2 and HH3 and of the original immunoblastic non-Hodgkin's lymphoma from which they were derived. Two fragments, which are absent in HH3, but present in HH2 and in the original tumour are marked by an arrowhead.

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Figure 2 Minisatellite patterns of successive biopsies of cases 5, 1, 11 and 14. The initial biopsy is marked A, the second biopsy B. Changes in minisatellite pattern are marked by arrowheads. Size markers are given in kilobase pairs. Case 5 shows no changes. In case 1, two fragments are lost in the second biopsy. One fragment shows an increase in relative hybridization intensity. In case 11, one fragment is lost and a new one appears, while two bands show diminished relative intensities in the second biopsy. Case 14 shows loss of one fragment.
were seen in 5 cases with follicular centroblastic/centrocytic lymphoma and diffuse centroblastic/centrocytic lymphoma. A more pleomorphic cell population and/or predominance of large blast cells was seen in 2 cases of immunocytoma and one of diffuse centrocytic lymphoma (Table 1). Alterations in DNA-fingerprints arose with time in 7 cases (Table 1, Figure 2). Extensive rearrangement of chromosomal material was indicated by loss and appearance of bands and changes in relative intensities. Since changes in relative intensity were accompanied by loss as well as acquisition of fragments, this observation is more consistent with numerical chromosomal differences between the first and second biopsy than with the inclusion of different amounts of tumour material, as may happen in biopsies. A similar relative contribution of tumour cells and admixed normal cells in both biopsies was independently confirmed by study of the tumour specific immunoglobulin gene rearrangements in the same samples as were used for minisatellite analysis (de Jong et al., submitted). The correlation between histological progression and alterations in minisatellite pattern was significant as tested by a $\chi^2$-test ($P<0.02$).

In a study on a variety of human malignancies Thein et al. (1987) have detected considerably less differences in DNA-fingerprints of constitutional DNA and tumour DNA than we report here for consecutive samples of B-NHL. Since we were not able to obtain constitutional DNA of the patients due to the retrospective nature of our study, the DNA-fingerprint differences between constitutional DNA and tumour DNA in B-NHL have probably even been underestimated. The discrepancy between these studies may be explained as follows: Both in B-NHL and in solid tumours numerous chromosomal aberrations occur with time (Nicolson, 1987). Solid tumours, however, show grossly aberrant karyotypes, polyploidy and random loss of chromosomes (Cram et al., 1983) and consist of large numbers of subclones with heterogeneous genomic variations. Individually, each of these clones contributes too little, quantitatively, so that most variations are lost in the background signal. In strong contrast, B-NHL show less bizarre karyotypes. The predominance of one or few subclones with distinct chromosomal variations, as in non-Hodgkin's lymphoma (Diamond et al., 1980), creates a far more favourable situation for their detection by DNA-fingerprinting. For future studies of genomic heterogeneity in malignancy, therefore the use of a panel of chromosome specific minisatellite probes (Wong et al., 1987) would be of great interest to identify specific chromosomes and chromosomal regions involved in tumour progression.

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