Increased frequency of the S allele of the L-\textit{myc} oncogene in non-Hodgkin's lymphoma

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Summary We studied 100 patients with non-Hodgkin's lymphoma, 44 patients with Hodgkin's disease and 100 controls for the prevalence of the EcoRI restriction fragment polymorphism of the L-\textit{myc} oncogene. No difference in the frequency of the three genotypes (LL, LS, SS) was found between the patient and control groups. However, the S allele was found to occur more frequently in the non-Hodgkin's lymphoma patients ($\chi^2 = 4.57$, $P = 0.032$). These data confirm an earlier report and suggest that the presence of the S allele is associated with susceptibility to non-Hodgkin's lymphoma.


dna isolation and Southern blot analysis

DNA was isolated from peripheral blood samples by the method of Ciulla \textit{et al.} (1988). Briefly, 5 ml of peripheral blood was lysed with 45 ml of cold lysis buffer (0.32 M sucrose, 10 mM Tris--HCl pH 7.5, 5 mM magnesium chloride and 1% Triton X-100) and then centrifuged for 10 min at 1,000 $g$. The pellet was resuspended in 5 ml of 4.0 M guanidine isothiocyanate, 25 mM sodium acetate and 0.84% $\beta$-mercaptoethanol and rocked gently for 20 min. The DNA was precipitated by the addition of an equal volume of isopropanol and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). A 3 $\mu$g aliquot of DNA was digested with EcoRI, electrophoresed in a 0.8% agarose gel, transferred to Hybond N$^+$ nylon membrane by alkaline transfer (Chomczynski & Sacaba, 1984) and hybridised with a $^{32}P$-labelled probe prepared by the random priming method of Feinberg and Vogelstein (1983). A 1.8 kb \textit{Smal}--EcoRI L-\textit{myc} fragment, pJB327 (Nau \textit{et al.}, 1985), excised from low gelling temperature agarose, was used as the probe. Washes were carried out at a final stringency of 0.3 x SSC and the autoradiographs exposed for 1--3 days on Kodak XAR-5 film at $-80^\circ$C.

Statistics

The frequency of the three genotypes and the two alleles in the patient and control groups were compared using the $\chi^2$ test.

Results

EcoRI-digested DNA probed with the L-\textit{myc} probe results in two fragments of 10 kb (L) and 6.6 kb (S) which are due to an EcoRI restriction site polymorphism (Nau \textit{et al.}, 1985). The distribution of the three genotypes (LL, LS, SS) in the control and the patient groups is shown in Table I. Although there is an increased number of patients with an SS genotype in the NHL group, $\chi^2$ analysis showed that there was no difference in the distribution of the three genotypes between either of the two patient groups and the controls, and all are in accord with Hardy--Weinberg equilibrium. However, there was a significant difference in the allele frequency between the controls and the NHL patients. The S allele occurred more frequently in the NHL patients than the normal control ($\chi^2 = 4.57$, 1 d.f., $P = 0.032$).

Discussion

Our finding of an increased frequency of the S allele in the NHL patients confirms the results of Chenevix-Trench \textit{et al.} (1989), who reported that the S allele was more common in a
combined group of acute lymphoblastic leukaemia (ALL) and NHL patients and suggested that it may be a factor which confers susceptibility to these haematopoietic cancers. Although we found an increased frequency of the S allele in the NHL patients, our data are not strictly comparable with those of Chenevix-Trench et al. (1989) because they compared a combined patient group of NHL and ALL patients, whereas in our study separate patient groups of NHL and HD were studied. However, if the ALL patients in Chenevix-Trench et al.’s study are removed the S allele still occurs more frequently in their NHL patients ($\chi^2 = 6.08, 1\,\text{d.f.}, \, P = 0.013$).

The results are, however, complicated because Chenevix-Trench et al. (1989) used two sets of normals, geriatric (mean age 77 years) and laboratory workers (age unknown, but presumably younger). The increased frequency of the S allele in the combined NHL and ALL patients was only found when they were compared with the unselected (laboratory workers) controls. In addition, they found a significant difference in the genotype frequency between the geriatric population and the unselected controls and suggested that the LH homozygotes are less likely to reach old age. Neither our patient nor control group falls into the geriatric category described by Chenevix-Trench et al. (1989) so we do not know if there is a reduced incidence of LH homozygotes in the New Zealand elderly population.

The frequency of the L allele in our control population as well as the unselected controls of Chenevix-Trench et al. (1989) is considerably higher than in the other reported studies of normals (Table II). The reason for this variation is unclear but may reflect differences in the ethnic composition of the various control groups. Table II shows that Norwegians, Japanese, Indians, American Whites, English Caucasians and geriatric Australians of European descent have similar allele frequencies, whereas American Blacks have an increased frequency of the S allele. In contrast, our normal controls, as well as the unselected controls of Chenevix-Trench et al. (1989), both of whom are of European descent, have an increased frequency of the L allele. Differing allele frequencies related to ethnicity are unlikely to be a factor in the increased frequency of the S allele in the NHL patients in our study because both our control and patient groups were Caucasians of European descent. Nevertheless, Table II does show the importance of ensuring that the ethnic composition of the control and patient groups is similar.

How the presence of a polymorphic EcoRI site is related not only to a susceptibility to NHL but also to an increased tendency to metastasis in other forms of cancer is not clear. The nucleotide sequence of the S allele has been determined (Kawashima et al., 1992) and, as expected, differs by 1 bp in the EcoRI site. In addition, there was a deletion of 8 bp in intron 2 and it was suggested that these differences may influence the transcription or splicing of the S allele. An alternative explanation is that the L-myc gene is not involved, but is in linkage disequilibrium with a gene or genes that are important in NHL as well as other forms of cancer. Further studies of well-characterised large patient groups of defined ethnicity are needed to ascertain the role of the S allele in carcinogenesis.

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Table I Distribution of L-myc genotypes and allele frequencies in patients and normal controls

| Genotypes | Controls | NHL | HD |
|-----------|----------|-----|----|
| LL        | 43       | 31  | 18 |
| LS        | 43       | 46  | 18 |
| SS        | 14       | 23  | 8  |
| Total     | 100      | 100 | 44 |

| Allele frequency | $\chi^2$ (P) |
|------------------|--------------|
| L                | 0.65 (0.35)  |
| S                | 0.54 (0.46)  |

Table II Frequency of L-myc alleles in control populations

| Reference               | Allele frequency | No. of individuals | Country  |
|-------------------------|------------------|--------------------|----------|
| Tefre et al. (1990)     | 0.5              | 129                | Norway   |
| Kato et al. (1990)      | 0.485            | 98                 | Japan    |
| Kakehi and Yoshida (1989)| 0.54            | 143                | Japan    |
| Saranath et al. (1990)  | 0.54             | 101                | India    |
| Kawashima et al. (1988) | 0.415            | 20                 | Japan    |
| Ishizaki et al. (1990)  | 0.49             | 100                | Japan    |
| Tamai et al. (1990)     | 0.57             | 16                 | USA      |
| White                   | 0.35             |                    |          |
| Black                   | 0.43             |                    |          |
| Farnon and Simmons (1987)| 0.43          | 45                 | England  |
| Chenexvix-Trench et al. (1989) | 0.65  | 100                | New Zealand |
| Geriatric               | 0.544            | 112                | Australia|
| Unselected              | 0.367            | 49                 |          |
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