Rearrangements of c-myc and c-abl genes in tumour cells in Burkitt’s lymphoma

S Casares, J M Rodriguez, A Martin, A Parrado

Abstract
Rearrangements of oncogenes c-myc and c-abl were detected by non-radioactive hybridisation in a case of Burkitt’s lymphoma/leukaemia. The surface phenotype of Burkitt’s cells were positive for CD19, CD20, HLA-DR, CD14, CD33 and surface immunoglobulin markers. Although cytogenetic analysis was not performed, the c-myc and heavy immunoglobulin genes had the same 14-2 kilobase EcoRI molecular size fragment, suggesting a possible t(8;14) translocation which is a common marker of this malignancy. The c-abl oncogene was also rearranged in DNA digested BamHI and EcoRI. The physiopathological implications of the rearranged c-abl gene are unknown, this being the first case, as far as is known, of Burkitt’s lymphoma/leukaemia with a rearranged c-abl gene.

Some haematological malignancies represent the best human tumour models of well defined mutations in the development of carcinoma. The first example was the finding of the Philadelphia chromosome in chronic myelogenous leukaemias (CML) due to a t(9;22) translocation in which the c-abl oncogene (9q34) is juxtaposed into the bcr region (22q11).

In Burkitt’s lymphoma the c-myc oncogene (8q24) is rearranged into the immunoglobulin heavy (14q32), k light (2p13), or l light (22q11) chain genes by t(8;14), t(2;8), or t(8;22) translocations, respectively.

Case report
A 10 year old boy was admitted for multiple lymphadenopathy, leukocytosis (21 x 10^9/L)—with 14% blast cells—anaemia, and thrombocytopenia. A bone marrow aspirate showed 96% infiltration of small non-cleaved cells (Burkitt’s type). Cytotechnical examination showed that the blast cells were negative for myeloperoxidase, Sudan black B, periodic acid Schiff, acid phosphatase, a- naphthyl acetate esterase and toluidine blue. Blast cell vacuoles were often intensely positive when using the oil red staining technique. The phenotype of the bone marrow blast cells was obtained using the alkaline phosphatase antialkaline phosphatase (APAAP) technique and the following percentages for positivity obtained: HLA-DR 94%; CD19 75%; CD20 97%; CD10 4%; CD33 69%; CD14 94%. The cells were negative for Tdt, CD7, CD3, CD13, and CD61 markers. The surface phenotype was also studied in peripheral blood smears by flow cytometric analysis on a FACScan (Becton Dickinson, San José, California), revealing 14% Burkitt’s cells (CD19, CD20, HLA-DR, CD14, CD33) and surface total immunoglobulin (sIg) positivity, using the former antibodies in a double labelling technique. Cytogenetic analysis was not possible. These findings justified a diagnosis of Burkitt’s lymphoma/leukaemia (FAB L3 subtype) despite CD14 and CD33 positivity. The patient rapidly became comatose and died before effective treatment could be started.

NON-RADIOACTIVE SOUTHERN BLOTTING
High molecular weight DNA extracted from peripheral mononuclear cells was digested with restriction enzymes, fractioned in 0.8% agarose gels, and transferred to nylon membranes. DNA size standards used were fragments from phage λ digested with HindIII, SmaI, BstEIII, or KpnI; in some cases digests with different enzymes were mixed to obtain a more complex standard. Purified probes and phage λ were labelled by random primer in 0.1 mmol/L dATP, 0.1 mmol/L dGTP, 0.1 mmol/L dCTP, 0.065 mmol/L dTTP, 0.035 mmol/l dUTP-digoxigenin (Boehringer Mannheim, Tutzing, Germany) for 24 hours at 37°C, and precipitated in 0.8M LiCl and 2.5 volumes ethanol. Hybridisation was performed in 50% formamide, 5% dextran sulphate, 0.1% Denhart’s solution, 1% sodium dodecyl sulphate and 150 μg/ml salmon sperm in 3 x SSC sodium citrate and sodium chloride at 42°C for 40 hours. Development by enzyme immunoassay was carried out following the manufacturer’s instructions (DIG Nucleid Acid Detection Kit; Boehringer Mannheim).

Restriction fragment sizes were calculated interpolating to a third grade function obtained from the migration distance of DNA size standards.

PROBES
The c-myc probe was a 1.5 kilobase EcoRI-HindIII human DNA fragment containing a second myc exon (Amersham International, Buckinghamshire, England). The c-abl probe was a 0.45 kilobase KpnI-BamHI human DNA fragment containing v-abl homologous sequences (Amersham International). The heavy chain joining gene probe (H-Ig) was a 6 kilobase BamHI-HindIII DNA fragment (from Dr P W Tucker, Health Science Center, Dallas, Texas, USA).
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Figure 1 Nonradioactive hybridisation analysis in Burkitt's lymphoma/leukaemia (BL) of the c-myc and immunoglobulin heavy chain (H-Ig) genes in EcoRI digested DNA. G; DNA from normal cells; P; molecular size standards expressed in kilobases. Arrows indicate rearranged bands.

Figure 2 Rearrangement of the c-abl gene in Burkitt's lymphoma/leukaemia (BL), P; molecular size standards expressed in kilobases; B; BamHI; E; EcoRI. Arrows show the rearranged bands.

Results
The Burkitt's lymphoma cells with a mixed B lymphoid-myeloid phenotype showed rearrangements of the c-myc and c-abl oncogenes. The probes detected the germline fragments generated by the restriction enzymes acting on normal DNA and additional rearranged fragments. The rearranged bands were weaker than the germline bands, as expected from the observation that malignant cells comprised only 14% of the cells studied. The c-myc probe detected rearranged fragments of 14·2 kilobase EcoRI and 6·2 kilobase PsiI; the immunoglobulin heavy chain gene probe revealed two additional fragments of 14·2 and 9·5 kilobases (fig 1). The similar fragment size (14·2 kilobases) suggests that due to the rearrangement the parts of the c-myc and H-Ig genes recognised by the probes might lie together in the same EcoRI fragment. The rearranged c-abl gene had 3·4 kilobase BamHI and 7·9 kilobase EcoRI molecular fragment sizes (fig 2).

Discussion
We found c-myc and c-abl rearranged genes in a CD14 and CD33 positive Burkitt's lymphoma. The presence of more than one mutant oncogene in malignant cells is considered a sequential process, representing progression from low to high grade malignancy.

Some oncogenes are rearranged in relatively common tumours such as a follicular lymphoma, with bcl-2 rearranged in a t(14;18), or such as chronic myelocytic leukaemia with c-abl rearranged in t(9;22), both considered to be low grade malignancies. The expansion of a malignant clone increases the probability of new mutations which would exacerbate the aggressiveness of the tumour. This fact has already been reported for some follicular lymphomas with a bcl-2 rearrangement which evolve into high grade lymphomas8 and for those CML progressions in chronic phase which evolve into an acute phase as a result of an amplification or rearrangement of the c-myc oncogene.

We detected a rearrangement of the c-myc gene in a restricted fragment of 14·2 kilobase EcoRI in Burkitt's lymphoma. It coincides with that observed in the rearrangement of the H-Ig gene, suggesting a possible t(8;14) translocation, a common marker of this malignancy. The physiopathological implications of a rearranged c-abl gene in this aggressive Burkitt's lymphoma/leukaemia and its possible role in the pathogenesis of this malignancy are unknown, although the results obtained by the authors suggest that this Burkitt's lymphoma/leukaemia evolved from a low grade malignancy. The highly aggressive nature of this malignancy which killed the patient, might be determined by the association of these two mutated oncogenes, or by the expression of a mixed lymphoid/myeloid phenotype, as generally speaking, the haematological malignancies of a mixed phenotype indicate a worse prognosis and poorer survival.10

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