c-myc Hypermutation Is Ongoing in Endemic, But Not All Burkitt’s Lymphoma

By J. Martin Johnston, Monica T. Yu, and William L. Carroll

Deregulation of c-myc oncogene secondary to chromosomal translocation appears to play an essential role in the genesis of both endemic (African) Burkitt’s lymphoma (eBL) and sporadic Burkitt’s lymphoma (sBL). In most eBL, mutations in or near exon 1 disrupt normal c-myc regulatory sites. We examined c-myc sequences from a patient with eBL and two patients with eBL to determine (1) whether mutation is ongoing as the tumor clone expands, (2) the nature of mutations in the protein-coding exons 2 and 3, and (3) the extent of c-myc hypermutation in the two clinical forms of BL. Using the polymerase chain reaction (PCR), we amplified the c-myc from bulk tumor samples, cloned the products into plasmid vectors, and sequenced multiple subclones of each segment. The mutation frequencies in the control (remission bone marrow) and sBL tumor subclones were 0.65 × 10⁻⁴ and 3.0 × 10⁻⁴ (mutations/base), respectively (P > .25). Subclones from the two eBLs exhibited mutation frequencies of 20 × 10⁻⁴ and 16 × 10⁻⁴, respectively (P < .001 vs control). In addition to the consensus mutations seen in one eBL, a random pattern of unshared mutations was observed throughout c-myc in both samples, demonstrating that mutations may be introduced in a stepwise fashion. We noted a clear excess of transitions over transversions (30:9), which is qualitatively similar to the pattern observed in diverse examples of eukaryotic gene mutation. These data demonstrate that c-myc hypermutation is an ongoing process as the eBL tumor clone expands, is qualitatively different from immunoglobulin gene hypermutation, and is not a universal feature of BL, perhaps reflecting the nature of the translocation or the stage of tumor cell maturation.

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B URKITT’S LYMPHOMA (BL) or its more clinically advanced form, B-cell acute lymphoblastic leukemia (B-ALL), is the most common childhood tumor in Central Africa and also accounts for a major portion of non-Hodgkin’s lymphomas in industrialized countries. These tumors are characterized by distinct clinicopathologic features. Two clinical subtypes exist. Endemic (African) BL (eBL) typically arises in the mandible and is epidemiologically associated with the Epstein-Barr virus and holoendemic malaria. Sporadic BL (sBL) usually arises in the abdomen and has a high incidence of associated bone marrow involvement. Despite clinical dissimilarity, both tumors usually express surface immunoglobulin (Ig) and are characterized by cytogenetic translocation between the c-myc oncogene (8q24) and one of the Ig loci (most commonly, Ig heavy chain, 14q32; or κ light chain, 2p12; λ light chain, 22q11). Deregulation of c-myc, which occurs as a byproduct of the translocation, appears to be an essential step in tumorigenesis.

A number of studies indicate that c-myc deregulation is due to somatic alteration of essential sequences within or close to transcription regulatory sites in exon 1. A normal transcriptional block at the 3’ end of exon 1 is obliterated by the translocation breakpoint itself or by extensive mutation of this area. The degree of mutation observed is particularly striking and includes single base substitutions, deletions, and insertions. It is not known whether these mutations are introduced at a discrete step in tumorigenesis or represent an ongoing phenomenon as the tumor clone expands. In some cases, c-myc sequences from cell lines indicate that these mutations are clustered at the exon 1/intron 1 border and do not extend into the protein coding domains. However, clonal cell lines represent a single transformed cell; minor subpopulations that exhibit further alterations may be detected by examining multiple transcripts from bulk lymphoma samples. Similar mutations in the protein coding domains, for example, might produce a biologically altered product with greater transforming potential.

In order to determine (1) whether hypermutation is ongoing, (2) the degree and nature of mutations in protein coding exons 2 and 3, and (3) differences in mutation frequency in the two clinical forms of the disease, we used the polymerase chain reaction (PCR) to generate multiple c-myc clones from two patients with eBL and one with sBL. We noted no hypermutation in exons 2 and 3 among sBL subclones, but a substantial degree of intraclonal diversity in c-myc among eBL tumor subclones.

MATERIALS AND METHODS

Tumor cells. A 4-year-old white boy (J.S.) developed orbital swelling associated with low back and right knee pain. Biopsy of an orbital mass demonstrated undifferentiated lymphoma, Burkitt-type, and a bone marrow aspirate contained 40% L3 (French-American-British [FAB] classification) lymphoblasts, which were positive for surface Ig (κκ). Cytogenetic analysis showed duplication of 1q14 → q32 and a translocation, t(14;?) (q32;?). The patient was given aggressive multiagent chemotherapy and remains in remission 1 year after completing treatment. Tumor biopsies from two representative patients (Watra, K962) with endemic Burkitt’s lymphoma were kindly provided to us by Dr Ian Magrath (Pediatric Branch, National Cancer Institute, Bethesda, MD). Bone marrow cells obtained from J.S. during clinical remission served as a control; mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia LKB, Piscataway, NJ) centrifugation.

DNA and RNA isolation. High molecular weight DNA and RNA were simultaneously isolated from the African tumor samples from bulk tumor samples. Similar mutations in the protein coding domains, for example, might produce a biologically altered product with greater transforming potential.

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The c-myc oncogene was pursued in the sBL tumor. Southern blot analysis confirmed the presence of c-myc sequences. DNA from the sBL tumor served as a template for first-strand cDNA synthesis. Ten micrograms of total RNA from the K962 tumor served as a template for amplification.

**cDNA synthesis**

Four micrograms of total RNA from the K962 tumor served as a template for first-strand cDNA synthesis. Ten picomoles each of three c-myc antisense primers (5'GGCAAGGCT-TCGGTTACCAGAGTGC [intron 2, 16 bp 3' of the end of exon 2], 5'GGCAAGGCTTGGCGGTGGTGTATTA [exon 3, 100 bp 3' of the stop codon]), and 5'AAAAGCTTGAAACCAGGAG-CCA [exon 3, 619 bp 3' of the stop codon]) served to initiate synthesis by AMV reverse transcriptase (RT; Life Sciences, St Petersburg, FL). RNA and primers were heated to 65°C and cooled slowly to room temperature. Annealed primers were extended on the template using 30 U of RT in a 40-μL reaction containing 0.1 mol/L Tris HCl (pH 8.3), 0.14 mol/L KCl, 0.01 mol/L MgCl2, 1.0 mmol/L dithiothreitol (DTT), oligo-dT primer 0.02 μg/mL, RNAsin 500 μg/mL (Promega, Madison, WI), bovine serum albumin (BSA) 10 μg/mL, and 1.0 mmol/L of each dNTP at 42°C for 1 hour. One fourth (10 μL) of the cDNA reaction was directly diluted into PCR reactions as described below.

**PCR amplification.** Using the published genomic c-myc sequence,15 sense and antisense primers were constructed for amplification of portions of the c-myc gene. An exon 1 sense primer (5'AAAAGCTTCAATAGCGCAGGAATG) and an intron 1 antisense primer (5'AAAGGCTTACATAGCGCAAATG) yielded a 257-bp PCR product overlapping the exon 1/intron 1 border. An intron 1 sense primer (5'CGGGATCCCCCT-CAAGTTAGCTT) and the intron 2 antisense primer (see above) yielded a 763-bp product containing all of exon 2. An intron 2 sense primer (5'ATGGGATCTTGGCTAAAGGAGTGAT) and the first of the two exons 3 untranslated region antisense primers (see above) yielded a 1079-bp product encompassing the translated (5') portion of exon 3. Restriction enzyme cleavage sites (BamHI in the sense primers, HindIII in the antisense primers) were incorporated to facilitate directional cloning of PCR products.

One to two micrograms of genomic DNA served as a template for amplification. Standard reaction conditions were used (200 μmol/L each dNTP, 50 mmol/L KCl, 10 mmol/L Tris-HCl [pH 8.4], 1.5 mmol/L MgCl2, 100 μg/mL gelatin). Thirty to 40 cycles of denaturation (1 minute, 94°C), annealing (1 minute, 52°C to 57°C), and extension (1.5 minute, 72°C) were used to generate amplified segments using 2.5 U/100 μL Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). PCR products were digested with BamHI and HindIII, isolated from low-melting point agarose gels (SeaPlaque; FMC Bioproducts, Rockland, ME), and cloned into Bluescript phagemid vectors (Stratagene). Transformed bacterial colonies were screened by colony hybridization with the c-myc cDNA probe and positive subclones were then sequenced by the dideoxy method.16

**Statistical analysis.** The mutation frequency is defined as the number of base-pair substitutions and single-base deletions seen in a set of subclones, divided by the total number of base pairs sequenced. A chi-square statistic with 1 df is calculated to compare mutation frequencies between c-myc isolates.

**RESULTS**

Since karyotype analysis of tumor cells from sBL patient J.S. failed to identify the derivative chromosome involved in the t(14;?), EcoRI digested DNA was hybridized with a full-length c-myc cDNA probe (Fig 1). While both our patient’s normal (remission bone marrow) cells and a neuroblastoma cell line exhibit the expected 14-kb EcoRI fragment, the J.S. tumor is heterozygous for a smaller 8-kb fragment. This rearrangement demonstrates involvement of the c-myc locus in the translocation and the band size is consistent with the (8;14) translocation seen most commonly in sBL.10 Hybridization with an exon 1-specific probe showed a distinct band of 23 kb, confirming a translocation breakpoint in or near the first intron of c-myc (data not shown).

The genomic c-myc exon 2 and 3 subclones from patients J.S. (sBL) and Watra (eBL) are shown schematically in Fig 2. A total of 16 subclones from the J.S. remission bone marrow were isolated and sequenced. Only one base-pair substitution is noted, yielding a mutation frequency of 0.65 × 10^-4 (Table 1). Similarly, the mutation frequency among 24 subclones from the J.S. tumor is 3.0 × 10^-4, with five base-pair substitutions seen (P > .25). No subclone contains more than one substitution. Both mutation frequencies are somewhat less than the error rate reported for Tag polymerase.20 In contrast, 21 base-pair substitutions and three single-base deletions are seen among 17 subclones from the African tumor, for a mutation frequency of 20 × 10^-4 (P < .001). Most mutated subclones contain multiple substitutions. While half (six of 11) of the Watra exon 3 subclones contain at least one base-pair...
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Fig 2. Subclones of exons 2 and 3. The normal segments are shown schematically at the top of the figure. Numbers refer to codons. Open boxes are introns, triple lines are protein-coding domains, and the stippled area is the 3' untranslated region. Remission bone marrow subclones are indicated by N, J.S. tumor (sBL) subclones by T, and Watra tumor (eBL) subclones by W. Two T subclones are truncated (cloning artifact). Closed triangles indicate base substitutions and open triangles represent single base deletions.

substitution or deletion, all six of the exon 2 subclones contain at least one base change. However, the mutation frequencies are not significantly different between the two exons (Table 1). Since the translocation breakpoint in the J.S. tumor transects the exon 1/intron 1 region, only Watra exon 1/intron 1 subclones were examined, and these are shown schematically in Fig 3. While only 2 of 10 subclones contain substitutions, the mutation frequency (16 × 10^-4) is not significantly different from that for exons 2 or 3 (P > .75).

The Watra mutations are distributed uniformly across the regions of the c-myc gene examined. There is a suggestion of a cluster of mutations in the 5' end of the exon 3 untranslated region, including a specific mutation (C → T in the 26th base of the untranslated region) seen in two distinct subclones. Importantly, no mutation is shared by all tumor subclones. Of the base-pair substitutions seen among the Watra subclones, 21 (84%) are transitions and only four are transversions (Table 2). Fifteen of the substitutions occurring in coding regions of the gene result in amino acid substitutions; two are silent (Table 3). The three observed single base deletions (thymine in codon 19, guanine in codon 236, and adenine in codon 336) result in frame shifts within the coding regions of c-myc, each of which leads to premature termination of the polypeptide.

Seven c-myc cDNA subclones from the K962 tumor are shown schematically in Fig 4. All of these share six consensus mutations in a 104-bp region of exon 1, as well as a single base substitution in exon 2 (C → T in codon 44). While four of the subclones exhibit a C → G substitution at the exon 1/exon 2 splice site, three consecutive base pairs have been deleted at precisely the same location in the other three subclones. The consistent presence or absence of this 3-bp deletion in subclones which share other mutations (ie, K2 and K3, K6 and K7) suggests that it is a somatic alteration and is not due to defective splicing.

The degree of intraclonal diversity among the K962 subclones is somewhat less than that seen in Watra (Table 1), despite the fact that presumably only translocated alleles are represented. The K962 mutation frequency is nonetheless significantly higher than background due to Taq error (P < .001). If each shared mutation is counted only once, there are nine transitions, five transversions (Table 2), and two single base deletions. As compared with the Watra tumor, there are more silent mutations within the protein-coding regions (Table 3). One of the two deletions occurs in the untranslated portion of exon 3 and the other (guanine in codon 180) results in premature polypeptide termination.

DISCUSSION

Translocation involving the c-myc oncogene and one of the Ig loci is seen in nearly every sample of BL, indicating a pivotal role for this oncogene in tumorigenesis. Transcrip-

<table>
<thead>
<tr>
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<th>Exon 2</th>
<th>Exon 3</th>
<th>Exon 2 and 3</th>
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<td>2.2</td>
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<td>K962 tumor</td>
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</table>

Mutation frequencies as defined in Methods; a mutation seen in more than one subclone is counted only once.

*Versus Watra exons 2 and 3.
†Versus J.S. normal.
‡Versus J.S. normal exons 2 and 3.
tional regulation of c-myc is normally provided by sequences within exon 1, which interrupt mRNA elongation initiated from either of two promoters.21 Extensive studies have demonstrated that this attenuation site is lost in most BL cells by one of two mechanisms. The translocation breakpoint may occur within the region itself, thereby “decapitating” the regulatory sequences from protein-coding exons 2 and 3.6 Alternatively, the breakpoint may lie 5' or 3' of c-myc, often at a considerable distance.6 In these cases, somatic mutations (base substitutions, small deletions or insertions) are observed within the regulatory region.20 The byproduct of these events is transcriptional deregulation of the c-myc gene and/or disruption of a second c-myc protein originating from a non-AUG codon in exon 1.15 Increased mRNA levels from the translocated allele may silence the normal c-myc allele. However, increased c-myc transcripts are not a universal feature, since many tumors express lower c-myc mRNA levels despite the alterations described above.22

Although specific exon 1 mutations have been characterized previously, it is not known whether c-myc mutation is an ongoing event as the tumor expands or a phenomenon that is ablated on further transformation of the tumor clone. Additional mutations in the regulatory sequences could further compromise normal regulation of c-myc expression, whereas mutations in exon 2 or 3 could affect the biologic function of c-myc protein (dimerization, substrate specificity) or mRNA half-life. Mutations that result in greater transforming potential may be positively selected and would be expected to be shared by multiple tumor subclones. Random mutations without any selective advantage may only be seen in an individual clone. The presence or absence of ongoing mutation would have implications not only for the biology of the particular tumor, but also for our understanding of the mechanisms underlying the extensive mutation observed in translocated c-myc alleles.

Our data indicate that somatic mutation of the translocated c-myc gene is not a consistent feature of BL. Hypermutation is not seen in the c-myc gene from a sample of sBL. The number of base-pair substitutions seen among the normal and sBL subclones is lower than expected, given the 2.1 \times 10^{-4} error rate (mutations/base pair/generation) previously demonstrated for Taq polymerase.26 Even though the breakpoint is much closer to c-myc exons 2 and 3 in sBL than in eBL, mutation is apparently not a universal byproduct of the translocation itself.

In contrast, the substitutions and deletions among the Watra (eBL) subclones are much more numerous than the background attributable to Taq. Moreover, they occur in a pattern that is distinct from that described with eBL amplification, where A \rightarrow G transitions predominate.27 The most common substitution seen in the Watra subclones is G \rightarrow T.

### Table 2. Base Substitutions in c-myc Subclones

<table>
<thead>
<tr>
<th>Codon</th>
<th>Wild-type</th>
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### Table 3. Base Substitutions in c-myc Protein Coding Regions

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<tr>
<td>319</td>
<td>TAT</td>
<td>TAC</td>
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*Includes consensus and intraclonal mutations.
†Seen in two subclones.
C → T (11 of 25). The ratio of transitions to transversions is 5:1, while the number of single base deletions approximates the number of transversions. The mutations are distributed fairly evenly among the three portions of c-myc analyzed, suggesting that this is a consistent characteristic across the c-myc locus in these cells and, most importantly, they occur at a significantly higher frequency than is seen in either the normal or the J.S. tumor subclones, which were generated in precisely the same fashion.

Because our initial data were obtained from genomic DNA, it is impossible to determine whether a particular subclone represents sequence from the translocated c-myc allele. There would appear to be no bias in favor of selective Taq amplification of either allele, in which case approximately 50% of subclones should represent each allele. Increased mutation has been shown previously to occur only in the translocated c-myc allele, suggesting that alterations in the c-myc subclones in excess of that expected from Taq error would represent the translocated allele. The Watra exon 3 clones are obviously consistent with such a schema, suggesting that the five unmutated subclones all derive from the untranslocated allele, while the six mutated subclones represent the translocated c-myc gene. If this is the case, then the actual mutation frequency in the translocated c-myc locus would be proportionally higher. All six subclones of Watra exon 2 contain at least one mutation. However, the sole "mutation" seen in one subclone is precisely the same base-pair substitution seen in a "normal" exon 2 subclone (G → A in codon 68), suggesting that it represents a Taq amplification error and that this Watra subclone is, in fact, not mutated. Clustering of Taq errors has been reported previously. While only two of 10 Watra exon 1/intron 1 subclones are mutated, these are shorter segments of DNA; thus, the mutation frequency is similar to that for exons 2 and 3. In the Watra tumor, no mutation was shared by all tumor subclones. Failure to identify a consensus mutation may indicate that such a lesion lies outside of the regions sequenced to date, perhaps in the proposed intron 1 protein binding site shown to be a target for mutation by Zajac-Kaye et al.

To directly assess mutation in the translocated allele, we examined expressed sequences in eBL K962. Since the normal allele may be transcriptionally silent, mRNA is more likely to represent products of the translocated allele. The consensus mutations in these seven subclones confirm that all are derived from the translocated allele. Six base changes in exon 1 are shared by all tumor subclones, as is a C → T base change that results in an amino acid substitution in exon 2. Whether this latter change represents a polymorphism or is the result of selection is uncertain. Intraclonal diversity in the K962 cDNA subclones is less extensive than in Watra, but nonetheless greater than can be attributed to Taq amplification error. The error rate of AMV reverse transcriptase is an order of magnitude lower than that associated with Taq polymerase and thus can be expected to have a limited impact on the introduction of artifactual base substitutions and/or deletions in these cDNA subclones. Furthermore, the presence of shared substitutions within two or more subclones suggests that these are authentic mutations. Two "families" of subclones are distinguished by the presence of a C → G change or a 3-bp deletion, respectively, at the exon 1/exon 2 splice site. Two pairs of subclones (K2, K3; K6, K7) share distinct additional somatic alterations, suggesting an ongoing stepwise accumulation of mutations in eBL. A preference for transitions is again displayed in this tumor, as in Watra.
Although six of the seven subclones contain mutations resulting in amino acid changes, we did not see the bias for such replacement changes that was suggested in the Watra tumor.

What is the molecular mechanism underlying the increased c-myc mutations in these samples of eBL? As noted above, mutation is often an apparent consequence of translocation, since the untranslocated allele is faithfully maintained. However, the data from the sBL tumor J.S. demonstrate that translocation alone is not sufficient for mutations to arise; no significant increase in c-myc mutation is seen, even though exons 2 and 3 are 10 to 100 times closer to the translocation breakpoint than in the African tumors. Mutations in c-myc may result from “spillover” of the somatic hypermutation that has been described to occur in the IgH locus of lymphoid cells as a normal part of the evolution of the antibody response. Hypermutation within the IgH locus occurs during a very specific stage of B-cell development. It has been shown to occur in human B-cell follicular lymphomas that do not express the CD5 antigen, but studies have failed to demonstrate IgH hypermutation in pre-B cell acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia, and sBL, suggesting that the latter tumors are not representative of the normal B-cell developmental stage where the process takes place in vivo. Previous studies have demonstrated that this “focused” Ig hypermutation may affect non-Ig DNA, which has been juxtaposed as a result of chromosomal translocation, as occurs in BL. The different mutation frequencies in sBL and eBL could reflect differences in their breakpoints within the Ig locus. Although we have not yet examined the IgH locus or the expressed Ig molecules in the African tumors for hypermutation, such an effect would have to operate over a large distance, since the translocation breakpoint in eBL may lie up to 50 kb 5′ of c-myc exon 1. In contrast, Klopp et al noted mutations only within a few hundred bp of a t(2;8) breakpoint involving the light chain locus.

Importantly, the pattern of mutations observed in these c-myc isolates is distinct from that reported previously for Ig genes. Ig variable region mutations that result in greater antigen affinity are selected in the late primary immune response making them difficult to compare directly with c-myc mutations, where a different selective pressure may take place. However, both et al analyzed somatic mutations in the untranslated portions of IgH where the absence of antigenic selection should allow the hypermutation mechanism to demonstrate its inherent error specificity. They observed 38 transitions, 38 transversions, and four single base deletions. While this suggests a preference for transitions over transversions (when compared with the expected random pattern of 1:2), our results indicate a much greater number of transitions (5:1). However, the relative numbers of transitions, transversions, and deletions seen in the eBL tumors are quite consistent with the pattern cited by Sommer in a review of somatic mutations in the factor IX, p53, and Rb genes of patients with hemophilia B, retinoblastoma, brain tumors, and breast or colon carcinoma. Sommer suggested that such a distribution of mutations is a hallmark of changes resulting from “endogenous processes” and contrasts with the predominance of transversions seen in lung cancer, presumably reflecting exposure to extrinsic mutagens.

Mutated Watra subclones display an excess of mutations that result in amino acid replacement over silent substitutions. In addition, three deletions result in premature polypeptide termination. Similarly, only one of the seven K962 subclones codes for a structurally normal c-myc protein. The generation of such structurally abnormal c-myc proteins is somewhat inconsistent with a persistent role for c-myc as a dominantly acting oncogene in eBL. Although c-myc is presumed to function as a transcriptional activator, experimental evidence for this function is largely indirect. c-myc contains a leucine zipper motif allowing dimerization with itself or other proteins. Dimerization appears to be essential for DNA binding. Recently, Smith et al demonstrated that mutation of all four leucine residues diminished but did not completely obliterate the ability of c-myc to block Friend murine erythroleukemic differentiation. Thus, c-myc may be more tolerant of mutations than other DNA binding oncogene proteins such as jun and fos family members. Alternatively, c-myc overexpression may be only a temporary step in tumorigenesis: transgenic mice expressing c-myc linked to the Ig enhancer show a stochastic pattern of tumor formation, indicating that additional events are necessary for neoplastic transformation.

In summary, we have demonstrated that c-myc hypermutation is not a universal feature of BL. In eBL, mutation appears to be an ongoing event in tumorigenesis and random mutations may extend into the protein coding domains. However, the lack of consensus mutations in these regions suggests that protein coding changes are unlikely to result in greater transforming potential. The nature of the mutations indicates that their occurrence may be related to background mutation in the human genome and not due to unique aspects of the c-myc locus or associated Ig genes.

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REFERENCES

4. Dalla-Favera R, Bregni M, Erickson J, Patterson D, Gallo R, Croce CM: Human c-myc oncogene is located on the region of
c-myc HYPERMUTATION IN BURKITT'S LYMPHOMA

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chromosome 8 that is translocated in Burkitt lymphoma cells. Proc Natl Acad Sci USA 79:7824, 1982


c-myc hypermutation is ongoing in endemic, but not all Burkitt’s lymphoma

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