The Pattern of p53 Mutations in Burkitt’s Lymphoma Differs from That of Solid Tumors

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Abstract

Available evidence suggests that, among hematomal malignancies, p53 is most often mutated in Burkitt’s lymphoma (BL). However, much of the published data is based on cell lines. We have, therefore, analyzed BL biopsies to determine more accurately the frequency and pattern of p53 mutations in primary tumors and to determine whether there are differences among the various subtypes of BL. Among 27 BL biopsies from South America, we have observed mutations in the p53 gene (exons 5 through 8) in 37% of tumors. The higher frequency of mutations in cell lines (70%) suggests that mutation of p53 may be associated with tumor progression. Summarizing available data we conclude that the presence of mutated p53 in BL is independent of the geographic origin of the tumor, the 8:14 chromosomal breakpoint locations and Epstein-Barr virus association. We also find that the mutational spectrum of p53 in BL differs from that observed in nonlymphoid tumors. More than 50% of mutations in BL are clustered in a small stretch of 33 amino acids (codons 213 to 248). Interestingly, codon 213 appears to be as frequently mutated as codon 248. Conversely, codon 273, often mutated in solid tumors, is rarely involved in BL.

Introduction

Mutation of the p53 gene appears to be the most common genetic lesion in many forms of inherited and acquired human neoplasia. These mutations have some characteristic features: (a) mutations usually cluster in four highly conserved regions of the protein (amino acid residues 132–145; 171–179; 239–248; and 272–286) (1); (b) most of these mutations are missense and the mutated protein appears to gain the ability to accumulate normally cryptic in the p53 protein, which is recognized by the antibody PAb 240 (3). Whether p53 acts as a true tumor suppressor gene, i.e., mutation results in a loss of its function, or acts as a dominant oncogene, i.e., mutation results in a gain of function, is at present a matter of debate (4) and, indeed, both may be correct. Nevertheless, several lines of evidence suggest that p53 mutation may be relevant either to the development or to the progression of tumors (5).

Malignant transformation of a cell results from the accumulation of multiple genetic lesions. Thus the presence of p53 mutations in a wide variety of tumors suggests that p53 may act as an additional, if not the primary, genetic abnormality in these neoplasias. For example, the analysis of p53 mutations in colorectal tissues suggests that p53 plays a role in the progression of adenomas to adenocarcinomas (6). Recent data have implicated mutant p53 in a category of lymphoid tumors carrying a genetic lesion resulting in deregulated c-myc (Burkitt’s lymphoma and acute lymphocytic leukemia-L3) (7). Furthermore, observed regulatory interactions between p53 and c-myc (8) provide a basis for the potential involvement of p53 in lymphomagenesis. However, much of the published information on p53 mutations in Burkitt’s lymphoma is based on tumor-derived cell lines (7, 9, 10) and, although unlikely, the p53 mutations could have occurred in vitro rather than being selected in vivo during the development of the neoplasm. We report here the frequency and pattern (mutational spectrum) of p53 mutations in primary Burkitt’s lymphoma biopsies.

Materials and Methods

The presence of mutated p53 (exons 5 through 8) was determined by SSCP3 analysis (11) in 27 BL tumors from South America (Argentina, Chile, and Brazil). The PCR primers were derived from intronic sequences immediately flanking the respective exons (7). Two hundred ng of genomic DNAs were amplified with 20 pmol of each primer in 20-μl reactions containing 5 μM concentrations of each dNTP, 2 μCi [32P]-dCTP, 1 unit Taq polymerase, in a buffer of 10 mM Tris (pH 8.3), 50 mM KCl, and 1 mM MgCl2. Thirty cycles of denaturation (58°C for exons 5 and 8; 62°C for exons 6 and 7) and extension (72°C) were done. Aliquots of the PCR reactions were loaded onto a 6% polyacrylamide (acylamide: bisacrylamide, 19:1) nondenaturing gel and run for 14–16 h at room temperature.

DNAs from tumors showing aberrantly migrating bands were further characterized by direct DNA sequencing of PCR-purified products using the Sequenase Version 2.0 Kit (United States Biochemical Corp.) and 35S-dATP.

Results and Discussion

SSCP analysis revealed altered electrophoretic mobility in 19 tumors, 7 in exon 5, 4 in exon 6, 5 in exon 7, and 3 in exon 8 (Fig. 1). Sequence analysis (Fig. 2) of these polymorphic bands confirmed the SSCP results in only 13 tumors and demonstrated that mutations in these tumors resulted from single base changes in the coding region of the respective exons (Table 1). The remaining 6 tumors showed an identical aberrant band in exon 5 (Fig. 1) which on sequencing was shown to be identical in each case, but not to originate from p53. Repeat sequencing of these 6 tumors using different exon 5 primers revealed no mutations (data not shown).

The most frequent base changes were transitions (six), but three transversions, one deletion, and one insertion were also observed. Among the four mutations in exon 6, three occurred in codon 213 but only one resulted in an amino acid substitution. We have since demonstrated this silent A-G transition (third base in codon 213) to be due to a polymorphism in the South American population (12).

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Mutational Spectrum of p53 in Burkitt's Lymphoma

Fig. 1. SSCP analysis of p53 mutations in Burkitt's lymphoma from South America. PCR-amplified genomic DNA fragments generated from exons 5, 6, 7, and 8 of human p53 were electrophoresed on 6% polyacrylamide gels at room temperature. Nondenatured (double-stranded) fragments for each exon are shown on the left. ●, positive controls with mutated p53, specific for each exon. Selection of these controls was based on previously published data. +, wild type p53 (non-tumor DNA). Tumors with aberrant migration patterns are individually numbered. Samples 2 to 7 show an identical-sized aberrant band (faster migrating). Sequence analysis revealed that they do not correspond to the p53 gene. Multiple exposures of the autoradiograph were used to score mutations.

Mutations of p53 exons 7 and 8 are often present in inherited tumors (13, 14). Changes in codons where germ line mutations have been found (245 and 248) were present in 4 of 27 South American BLs. However, as in most of the tumors observed in this panel with mutated p53, the wild type allele was also present in each of these four tumors. Since the starting material was a tumor biopsy, we cannot exclude the possibility that the presence of the normal sequence represents DNA from normal cells in the sample. Alternatively, the heterozygosity in the case of these "heritable" lesions, may represent a stage in progression to homozygosity and abnormal p53 function. The presence of heterozygosity in the case of nonheritable p53 mutations in
MUTATIONAL SPECTRUM OF p53 IN BURKITT'S LYMPHOMA

Fig. 2. Sequence analysis of mutations in the p53 gene observed by SSCP in South American Burkitt's lymphomas. An asterisk (*) is placed above the band representing the mutation.

Table 1 p53 mutations in South American Burkitt's lymphomas

<table>
<thead>
<tr>
<th>Patient</th>
<th>Origin</th>
<th>Exon</th>
<th>Mutated codon</th>
<th>Amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. G. O</td>
<td>Brazil</td>
<td>5</td>
<td>141 TGC—TAC</td>
<td>Cys—Tyr</td>
</tr>
<tr>
<td>V. A.</td>
<td>Argentina</td>
<td>6</td>
<td>213 CGA—CGG</td>
<td>Silent</td>
</tr>
<tr>
<td>S. G.</td>
<td>Argentina</td>
<td>6</td>
<td>213 CGA—CGG</td>
<td>Silent</td>
</tr>
<tr>
<td>B. D.</td>
<td>Argentina</td>
<td>6</td>
<td>213 CGA—TGA</td>
<td>Arg—Stop</td>
</tr>
<tr>
<td>J. R.</td>
<td>Argentina</td>
<td>6</td>
<td>220 TAT—CAT</td>
<td>Tyr—His</td>
</tr>
<tr>
<td>B. D.</td>
<td>Argentina</td>
<td>7</td>
<td>236 ATG—A G</td>
<td>Frameshift</td>
</tr>
<tr>
<td>C. V.</td>
<td>Argentina</td>
<td>7</td>
<td>245 GGC—GAC</td>
<td>Gly—Asp</td>
</tr>
<tr>
<td>S. R. C.</td>
<td>Brazil</td>
<td>7</td>
<td>245 GGC—AGC</td>
<td>Gly—Ser</td>
</tr>
<tr>
<td>O. J. I.</td>
<td>Argentina</td>
<td>7</td>
<td>248 CGG—CTG</td>
<td>Arg—Leu</td>
</tr>
<tr>
<td>I. N.</td>
<td>Brazil</td>
<td>7</td>
<td>248 CGG—CAG</td>
<td>Arg—Gin</td>
</tr>
<tr>
<td>B. E.</td>
<td>Brazil</td>
<td>7</td>
<td>272 GTG—GAG</td>
<td>Val—Glu</td>
</tr>
<tr>
<td>P. M.</td>
<td>Chile</td>
<td>8</td>
<td>278 CCT—CAT</td>
<td>Pro—His</td>
</tr>
<tr>
<td>A. G.</td>
<td>Argentina</td>
<td>8</td>
<td>282 CGG—TCGG</td>
<td>Frameshift</td>
</tr>
</tbody>
</table>

In conclusion, from a total of 13 single base changes in this panel, 2 are identical to a recognized germ line polymorphism (12), 8 are missense, 1 results in a stop codon, and 2 cause a frameshift (Table 1). The overall frequency of South American BL with mutant p53 is 37% (10 of 27 tumors; B. D. carries 2 mutations).

Since we have previously demonstrated molecular differences in the subtypes of BL, each represented by various combinations of breakpoint locations on chromosomes 8 and 14 (15), we analyzed all available data on p53 mutations, in both tumor biopsies and cell lines (over 70 tumors), with respect to breakpoint locations on chromosomes 8 and 14 and Epstein-Barr virus association. The presence or absence of mutated p53 in BL in this and previous studies (7, 9) appears to be independent of the geographic origin, the breakpoint locations, or the presence of Epstein-Barr virus.

To determine the pattern of p53 mutations observed in BL, we have pooled data from this and earlier studies, including data from primary BL from North America and Africa (7, 9), and plotted the mutations to reflect the frequency of codon involvement (Fig. 3). Fifty-three percent of the mutations are
clustered in a small stretch of 33 amino acids (codons 213 to 248). Very few mutations have been observed in this region in breast and lung cancer, and such mutations represent a small fraction of mutations in colon cancer (16). In colon cancer, the majority of mutations are in codons 175, 248, and 273. Interestingly, in BL codon 213 appears to be as frequently mutated as codon 248. Codon 213 lies within the region of p53 involved in binding to heat shock proteins (17). In an earlier summary of 280 mutations in p53 (16) only one 213 mutation was reported in a nonlymphoid tumor, significantly different from 5 of 47 in BL. Since mutations at codon 213, which represents a CpG dinucleotide, have often been transitions, it is possible that the high frequency of 213 mutations in hematological disorders reflects tissue-specific differences in CpG methylation or different levels of spontaneous deamination in lymphoid cells. Codon 273, on the other hand, is rarely involved in BL. Thus, the pattern of p53 mutations in BL differs in some respects from that observed in colorectal, breast, and lung tumors.

This pattern of mutation suggests the existence of a targeted region specific to BL which may define a functional region of p53 which is relevant to the process of lymphomagenesis. In this respect, it is of interest that among lymphoid tumors, p53 mutations have more frequently been observed in neoplasms bearing c-myc/immunoglobulin translocations. However, the presence of mutated p53 seems to be independent of deregulated c-myc since mutations in p53 are rarely found in murine plasmacytomas (18), a lymphoid tumor with a similar molecular pathogenetic pathway as BL. However, the reported involvement of p53 in blocking the differentiation of pre-B-cells (19, 20) suggests that the presence of p53 mutations in BL and their absence in plasmacytomas might be due to the differentiation stage of the target cells. The reason for the low frequency of p53 mutations in other immature lymphoid neoplasias, e.g., acute lymphocytic leukemia (21), remains unexplained. It is also possible that mutations in the p53 gene are prognostic of tumor progression, particularly in BL. Two observations support this possibility: (a) the frequency of p53 mutations in primary biopsies (33–37%) is lower than that found in cell lines (70%), and (b) whereas cell lines can be derived from only a small fraction (<20%) of primary tumors, they are more readily (80%) derived from relapsed tumors.

Whether or not the presence of a p53 mutation in BL at the time of presentation is of prognostic significance remains to be determined.

References

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