Expression of p53 in Human Leukemia and Lymphoma

By M. Prokocimer, M. Shaklai, H. Ben Bassat, D. Wolf, N. Goldfinger, and V. Rotter

Analysis of fresh human tumors have indicated that patients with B type lymphoproliferative diseases and the majority of patients with acute lymphoblastic leukemia (ALL) express elevated levels of p53. It is suggested that in these human malignancies, p53 may provide a novel tool for monitoring cancer activity. Conversely, p53 is not expressed in acute myeloid leukemias, myeloproliferative diseases, or myeloid leukemic cell lines. Analysis of the p53 gene structure indicated the existence of similar patterns of p53 restriction fragments in producer and nonproducer cells, which suggests that the p53 gene is not altered in the latter. However, in one case of acute promyelocytic leukemia (APL), we have observed a rearrangement in the p53 gene. Karyotype analysis has indicated that these APL cells do not contain the typical 15:17 translocation. In other APL patients who exhibit a 15:17 translocation, we found no genomic changes of the p53, suggesting that the p53 gene, which was recently mapped to the short arm of chromosome 17 in the human, is not structurally related to the typical chromosomal break point found in the long arm of chromosome 17 of APL patients.

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The Cellular encoded protein p53 is overproduced in cancer cells. Accentuated concentrations of this protein have been detected in a large number of transformed cell lines and in primary tumors in mice. It was found in a variety of tissue types, including sarcoma and leukemia cells of several species.

A direct role for p53 in the process of carcinogenesis was suggested by experiments that showed that expression of the p53 gene can immortalize cells and that the p53 gene can replace the myc oncogene in a myc-ras immortalization/transformation assay. In addition, we found that the introduction of a functional p53 gene into p53 nonproducer L12 Ab-MuLV–transformed cells that have a rearranged p53 gene changed their phenotype from cells that induce regres- sor tumors into cells that induce the appearance of lethal tumors. These and other experiments suggest a correlation between expression of p53 in tumor cells and the capacity of the latter to exhibit a fully transformed phenotype.

Recently we and others became interested in evaluating the possible role of the p53 oncogene in human neoplastic growth and differentiation. We were especially interested in studying the expression and gene structure of p53 in human leukemia-lymphoma cells in comparison with analogous normal tissues. A study of the hematologic malignant diseases was attractive because we have recently mapped the p53 gene to the short arm of chromosome 17. Gain or loss of the whole chromosome 17 or parts of it has been a frequent finding in these malignancies.

We were in particular interested in studying patients with acute promyelocytic leukemia (APL), since a reciprocal translocation involving chromosomes 15 and 17 at bands q22 and q21, respectively, is a characteristic abnormality in APL. The study group consisted of patients with (1) acute leukemias, acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL); (2) myeloproliferative diseases, chronic myeloid leukemia (CML) with Philadelphia chromosome–positive cells (ph+), myeloblastosis (MF), myeloid metaplasia (MM), and polycythemia vera (PV); (3) chronic lymphocytic leukemia (CLL); and (4) lymphoma in leukemic phase. We extended our study to include a large number of established human lymphoma-lymphoma cell lines. The availability of a cloned human p53 cDNA and specific anti-p53 monoclonal antibodies permitted us to survey p53 expression and its gene structure in human lymphoma-lymphoma cells.

Materials and Methods

Cell lines. The following human leukemia-lymphoma cell lines were tested: ALL cell lines including Reh, Km-3, Peer, Amsalem, Molt-4, Nalm-6, Nalm-1; Burkitt’s lymphoma cell lines including Ramos, Bjab, Bjab-B958, Ra-B958, Raji; and the Dekabuto myeloid cell line (Ben Bassat, unpublished observations). In addition we tested SV80, a human transformed fibroblastic cell line expressing the SV40 DNA tumor virus, and a normal human permanent B cell line established from peripheral blood lymphocytes infected by Epstein-Barr virus (EBV) (B958) (Ben-Bassat, unpublished observations).

All cell lines were grown in RPMI 1640 enriched with 10% heat-inactivated fetal calf serum (Biolab, Jerusalem, Israel) and 2 x 10^{-5} mol/L β-mercaptoethanol. Hybridoma cell lines PAb421, PAb122, and RA3-2C2 were grown in RPMI 1640 medium enriched with 20% heat-inactivated fetal calf serum supplemented with 20 mmol/L L-glutamine and 20 mmol/L sodium pyruvate.

Patients. Samples of heparinized venous blood and bone marrow cells were obtained from patients and normal healthy volunteers who gave informed consent. The samples were collected at the time of diagnosis or at relapse from patients with ALL, AML, CML ph+ or MF, lymphoma in leukemic phase, and CLL. Diagnoses were made by using standard clinical, morphologic, cytochemical, and immunologic criteria. Eighty percent or more of the cells in each of these cases was malignant by the aforementioned criteria.

Antibodies. Monoclonal anti-p53 antibodies were obtained from the established hybridoma cell lines PAb421, PAb122, and RA3-2C2. Monoclonal antibodies were obtained from either supernatants of the hybridoma cell lines or ascitic fluid of syngeneic mice.

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injected intraperitoneally (IP) with the hybridoma cell lines. Antibodies were purified and concentrated by binding to Sepharose-protein A columns (Sigma Chemical Co, St Louis).

Synthesis of p53 protein. A quantity of 1 × 10⁵ cells of each individual cell line, sample of bone marrow, peripheral blood mononuclear cells, or one plate of fibroblasts at the logarithmic stage of growth were washed several times in phosphate-buffered saline (PBS), resuspended in 1.5 mL Dulbecco’s modified Eagle’s medium without methionine, and enriched with 10% dialyzed heat-inactivated fetal calf serum and 250 μCi ³⁵S-methionine (purchased from Amersham Corp, Buckinghamshire, England). Cells were incubated for one hour at 37 °C. washed in PBS, and extracted into 2 mL of lysis buffer (10 mmol/L Na₂HPO₄-NaH₂PO₄, pH 7.5, 100 mmol/L NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecylsulfate) at 4 °C. Labeled cell lysates were precleared by repeated absorption on *Staphylococcus aureus* and nonimmune serum. Equal amounts of radioactive protein were immunoprecipitated with specific antibodies. Antigen-antibody complexes were collected by *S aureus*. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli.²⁹

Southern blot analysis. Genomic DNA was prepared from individual cell lines and fresh leukemia-lymphoma cells. Cells were washed twice with PBS, resuspended in lysis buffer (0.5% SDS, 50 mmol/L Tris-HCl, 5 mmol/L EDTA, pH 7.5) and 0.2 mg/mL proteinase K (Bohringer Mannheim, FRG), and incubated for 14 hours at 37 °C. The solution was extracted twice with an equal volume of phenol and twice with equal volumes of chloroform/isoamylalcohol 24/1. The DNA solution was adjusted to a final concentration of 0.3 mmol/L Na-acetate, and 2 vol of cold ethanol was added. The DNA precipitate formed at room temperature was washed twice with 80% ethanol and resuspended in 10 mmol/L Tris-HCl and 1 mmol/L EDTA (pH 7.5). Aliquots of 10 μg DNA digested with *EcoRI* and *HindIII* were electrophoresed on 0.8% agarose gel, blotted onto nitrocellulose filter,³⁵ and hybridized to nick-translated p53 DNA probes.³⁴

RESULTS

Expression of p53 protein in established human cell lines. Using the human specific anti-p53 monoclonal antibodies PAb421 and PAb122, we screened a large number of human lymphoma-leukemia cell lines for expression of p53. Figure 1 illustrates an example of several human cell lysates immunoprecipitated with PAb421. As shown in this figure, human cell lines express more than one p53 protein species. Some lines, like SV-80-transformed fibroblasts and the Reh-ALL cell lines, synthesize two physically distinct proteins, whereas others express either the lower or the upper p53 protein species (Molt-4 and Nalm-6, respectively). Table 1, which summarizes results with all the human lines we have studied, indicates that all T and B leukemia cell lines as well as a normal human permanent B cell line infected by EBV B958 expressed the p53 protein evaluated by immunoprecipitation with specific anti-p53 monoclonal antibodies. These findings are in agreement with the detection of the p53 protein in human cell lines previously documented.³⁵ p53 synthesis, however, was not detected in the following human myeloid leukemic cell lines: HL-60,²¹ K-562 (Fig 1), and Dekabuto (not shown).

Analysis of the p53 gene in the p53 producer and unproducer cell lines tested by Southern blotting using a human specific cDNA probe, p53-H-13,¹⁵ indicated a similar pattern of restriction fragments digested with *EcoRI* or double digested with *EcoRI* and *HindIII* in all cell lines. The HL-60 cells are the only exception; they were found to contain a major deletion in the p53 gene (Fig 2).²⁷

Expression of p53 in fresh leukemia-lymphoma cells. Fresh human leukemia-lymphoma cells were tested for expression of the p53 protein using human-specific anti-p53 monoclonal antibodies, PAb421,¹⁶ and a control of

| Table 1. Expression of p53 in Established Human Lymphoma-Leukemia Cell Lines |
|--------------------------|-----------------|-----------------|--------------------------|
| Cell Line               | Origin          | p53 Expression  | Properties of p53 Antigens |
| Reh                     | N-ALL           | +               | D                         |
| Km-3                    | N-ALL           | +               | D                         |
| Peer                    | T-ALL           | +               | S                         |
| Molt-4                  | T-ALL           | +               | S                         |
| Amsalem                 | T-ALL           | +               | S                         |
| Nalm-6                  | pre-B-ALL       | +               | D                         |
| Daudi                   | BL              | +               | S                         |
| Raji                    | BL              | +               | D                         |
| Ramos                   | BL              | +               | S                         |
| Re-B958                 | BL              | +               | S                         |
| BJAB                    | BL              | +               | S                         |
| BJAB-B958               | BL              | +               | S                         |
| K-562                   | CML-BC          | —               | —                         |
| Nalm-1                  | CML-BC          | +               | S                         |
| HL-60                   | APL             | —               | —                         |

Abbreviations: BL, Burkitt’s lymphoma; CML-BC, CML in blast crisis; S, single p53 protein; D, doublet of p53 protein; +, p53 detected in ³⁵S-methionine-labeled cell extracts by immunoprecipitation with human specific anti-p53 monoclonal antibodies PAb421 and PAb122; —, p53 undetected by immunoprecipitation.
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9.4Kb 6.5 Kb 4.3Kb 2.3Kb 2.0Kb
SV-80 HL-60 K562 Nalm-6

Fig 2. Southern blot analysis of p53 producer and nonproducer human cell lines. Equal amounts of DNA digested with EcoRI (a) or double digested with EcoRI and HindIII (b) were separated by electrophoresis and hybridized with a human-specific cDNA probe, p53-H-13.15

RA3-2C2, mouse-specific anti-p53 (Fig 3).18 p53 expression was detected in all of the following patients: (1) B-CLL, (2) B lymphoma in leukemic phase, and (3) in five out of six patients with ALL (Table 2). Overall, ALL and lymphoma samples displayed more intense signals than did the CLL samples. In the latter, prolonged exposures of 3 to 4 weeks' duration were usually required for visualization of the signals. As in human cell lines, fresh tumors express more than one p53 species. Indeed, we observed several tumors that synthesize a single p53 protein, whereas in others, two and occasionally three physically distinct proteins were detected.

Table 2. Expression of p53 in Fresh Human Lymphoma-Leukemia Cells

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. Studied</th>
<th>No. Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-CLL</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>B lymphoma in leukemic phase</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>ALL</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>AML*</td>
<td>17</td>
<td>—</td>
</tr>
<tr>
<td>CML-ph1+</td>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>MF</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>PV</td>
<td>4</td>
<td>—</td>
</tr>
</tbody>
</table>

*The study group includes M1-M4 morphologic subtypes (French-American-British classification, FABs).

Evaluation of p53 expression in nontransformed human cells indicated the absence of detectable levels of p53 in thymocytes, in peripheral blood lymphocytes, and in bone marrow progenitor cells. p53 protein synthesis, however, was detected in fetal cord blood obtained immediately after delivery.

p53 synthesis was not detected in patients with AML, CML (ph1+), MF, or PV (Table 2). Prolonged exposures of autoradiograms for longer than four weeks, did not yield a p53 signal in these samples.

Southern blot analysis of genomic DNA isolated from these samples was performed using the p53 human-specific cDNA probe (p53-H-13).15 The results illustrated in Fig 4 showed a similar pattern of restriction fragments in producer and nonproducer cells, suggesting that the p53 gene analyzed

Fig 3. Expression of p53 protein in human fresh leukemia-lymphoma cells. Patients with the following diseases were studied: lymphoma (A), CLL (B) ALL (C), and AML (D). Equal amounts of [35S]-methionine-labeled cell lysates were immunoprecipitated with either nonimmune serum (a), RA3-2C2 mouse-specific anti-p53 (b), or PAb421 human-specific anti-p53 (c). The dried gel was autoradiographed for 4 weeks.

Fig 4. Southern blot analysis of the p53 gene in fresh leukemia and lymphoma samples. DNA analyzed was obtained from patients with the following diseases: A, B, lymphoma; C, D, CML; E and F, AML. Equal amounts of DNA (10 μg), digested with EcoRI (a) or double digested with EcoRI and HindIII (b) were separated by electrophoresis and hybridized with the human-specific cDNA probe p53-H-13.15
under these resolution conditions is intact in p53 nonproducer fresh tumor cells.

Analysis of the p53 gene in leukemic cells of patients with APL. Previously we found that most of the p53 gene in the HL-60 promyelocytic cell line was deleted.27 In these cells only one chromosome 17 was detected.29 The fact that the human p53 gene was shown to map to chromosome 17 as well as the observation of Larson et al showing a consistent 15q22 to 17q21.1 chromosomal translocation in patients with APL prompted us to extend our survey and analyze p53 expression in APL patients. To that end five patients with APL were analyzed. The criteria for the diagnosis of APL were as described previously.31 All patients either had disseminated intravascular coagulation on presentation, or the syndrome developed after initiation of chemotherapy. Cytogenetic evaluation of bone marrow was performed in all the patients. Four patients had the classic translocation 15;17, and one had a normal karyotype. Results shown in Fig 5 indicated that four patients who had the classic translocation seem to present an intact p53 gene as evaluated by Southern blotting. A typical pattern of restriction fragments was obtained with these patients (Fig 5, lanes a, b, c, and e). However, in the single patient diagnosed as having APL exhibiting a normal chromosomal karyotype with no 15;17 translocation, a rearrangement of the p53 was observed (Fig 5, lane d).

**DISCUSSION**

Recently we and others became interested in studying p53 expression in human cancers. These studies have been directed toward the question of whether the p53 gene is overexpressed in human malignancies.

Our study has focused on evaluation of the p53 protein expression and its gene structure in the fresh human lymphoma-leukemia cells and in established lymphoma-leukemia cell lines. Using the human-specific anti-p53 monoclonal antibodies PAB421, PAb122, and a cloned human p53 cDNA as a probe, we screened a variety of human primary tumors. We have observed that most of the malignant tissues studied expressed an elevated level of p53 synthesis compared with analogous normal tissues. All Burkitt's lymphoma and ALL (T and B) human cell lines as well as all patients with B-CLL, B lymphoma in leukemic phase, and a majority of patients with ALL expressed elevated levels of p53 protein synthesis. This suggests that p53 overexpression is an authentic phenomenon rather than a tissue culture artifact.

We found that fresh ALL, B lymphomas generally displayed an increase in signal intensity compared with the majority of CLL cells, suggesting that the rate of synthesis of p53 in leukemic cells derived from patients with ALL lymphomas is increased over that in CLL cells. Since p53 is a nuclear-associated protein that may be involved in the control of DNA replication, it is possible that p53 is more frequently synthesized in the more rapidly proliferating acute leukemia or lymphoma cells than in the less-proliferating CLL cells. Analysis of the p53 protein in fresh tumors has indicated the presence of several p53 protein species varying in their molecular size. As in human cell lines, some synthesize a single p53 protein, whereas others synthesize two or even three physically distinct proteins. This differential expression of several p53 species by the single human p53 gene could be controlled by an alternative splicing mechanism (manuscript in preparation).

Previously we observed that the p53 gene of HL-60 cells, a promyelocytic leukemic cell line, was significantly altered. Most of the p53 gene was deleted in these cells and therefore failed to produce the p53 protein.27 In this study we further observed the lack of a detectable level of p53 protein in other myeloid cell lines such as the K-562 (erythroleukemic cell line derived from the pleural effusion of a patient with CML in blastic crisis) and Dekabuto cell lines. These findings are in agreement with the results reported by Miller et al.34 It should be noted, however, that p53 is expressed in the Nalm-1 cell line.35 This line was established from the peripheral blood of a patient who was in blastic crisis of ph1 CML. It was derived from the lymphoid type of CML blasts. Lack of p53 expression was also evident in fresh samples obtained from patients with AML and myeloproliferative diseases such as CML, MF, and PV. These observations suggest that this gene may play a role in early hematopoietic differentiation. The p53 gene may be completely shut off through gene inactivation in myeloid differentiation. Alternatively, lack of the p53 protein in myeloid leukemias might suggest the existence of a different transformation mechanism in these malignancies.

Southern blot analysis of p53 producer and most of the nonproducer cell lines or fresh tumors studied here indicate that the p53 gene seems intact. Under the present resolution conditions, we have observed similar patterns of p53 restriction fragments in p53 producer and nonproducer cells.

In the group of APL patients, we observed one of the five in which the p53 gene was rearranged. In that particular case no 15;17 translocation typical for APL was evident. In the other four cases of APL in which a typical translocation of the long arm of chromosome 17 to chromosome 15 was found, no modifications in the p53 gene were observed. This
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strongly suggests that the 15;17 translocation break point and the p53 gene are not structurally related. This is further emphasized by the fact that the p53 gene was mapped to the short arm of chromosome 17, which is usually not altered by the typical 15;17 q22-21.1 translocation.12 Taken together, it seems that the assumption that p53 has a critical involvement in the transformation of normal myeloid cells into leukemic cells put forth by LeBeau et al10 seems to be unlikely. These findings are not consistent with our previous observation of a major deletion of the p53 gene in the HL-60 promyelocytic cell line.27 We would like to speculate that the rearrangement of the p53 gene in HL-60 cells was caused by a change in the karyotype involving the loss of chromosome 17 during the continuous passage of these cells in culture. Alternatively, it is possible that APL patients exhibit at a low frequency an additional rearrangement occurring in the short arm of chromosome 17. Therefore, the rearrangement in one APL patient as well as the deletion in the p53 gene in HL-60 cells may be an authentic observation.

In summary, the p53 protein is expressed in B-type lymphoproliferative diseases and in the majority of ALL patients as well as in lymphoid cell lines of both T and B origin, including Nalm-1 cells. In these malignancies, p53 may provide a novel tool for monitoring the activity of malignancies. Conversely, p53 is not expressed in AML, myeloproliferative diseases, and myeloid leukemic cell lines. Understanding of these variations in p53 expression in the different tumor cells may provide some insight into the biologic roles of p53 in normal and malignant cells.

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