PREVALENCE OF SERUM ANTIBODIES AGAINST THE p53 TUMOR SUPPRESSOR GENE PROTEIN IN VARIOUS CANCERS

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We have developed 2 new quantitative methods for measuring anti-p53 antibodies in human serum. Using these methods we analyzed 1,392 sera from patients with various malignancies and 230 sera from individuals without malignancy. Highest prevalence of anti-p53 antibodies was associated with ovarian and colon cancers (15%), followed by lung (8%) and breast (5%) cancers. Prevalence in other malignancies was lower (<4%). In hospitalized patients and apparently healthy individuals, prevalence was very low (<2% and 1% respectively). Extremely high antibody concentrations (>10⁴ U/L) were found in 5 ovarian, 2 breast, 1 lung and 1 colon cancer. Sequential analysis of 6 positive samples has shown that the p53 antibody test may have potential for patient monitoring. The p53 antibody–positive sera from breast cancer patients were associated with tumors that were steroid hormone receptor negative (p < 0.001). We propose that the measurement of p53 antibodies is a relatively specific serological test for cancer, which can be performed with easily automatable and quantitative methodologies and may be further exploited for patient monitoring, prognosis, diagnosis and probably screening for selected cancers.

Cancer diagnosis is not possible using currently available serological tumor markers because these serum proteins are not sensitive and/or specific for cancer. It has been proposed that oncogenes and tumor suppressor genes and their products may form new biochemical tests for cancer (Diamandis, 1992). This proposal has merit because it is currently believed that the changes in oncogenes and tumor suppressor genes are not merely late sequelae or epiphenomena of cancer, as is the case with most known tumor markers, but initiating and pathogenetic events (Fearon and Vogelstein, 1990). Thus monitoring of such events may provide novel ways for the early detection and monitoring of cancer.

In most cancers, genetic changes are somatic and are present exclusively in the tumor. Thus to study such changes one must obtain tumor cells. This is possible in selected cancers which can release tumor cells in easily obtainable specimens, e.g., stool (colon cancer), urine (bladder cancer) and sputum (lung cancer). In these cases, extremely sensitive techniques may provide a means of identifying the genetic changes in a minute number of tumor cells, which can be used for diagnosis (Sidransky et al., 1991, 1992).

The release of measurable mutant proteins from the tumor into the bloodstream offers another possibility for cancer diagnosis. However, many mutant proteins are either cytoplasmic or nuclear components and are released into the circulation in either minute amounts or only during tumor cell necrosis. In the circulation, these proteins may appear for only short periods of time because of degradation or clearance. Other mutant proteins are membrane components and are not usually released into the circulation. It is thus not surprising that some mutant proteins, e.g., the p53 tumor suppressor gene protein, have not as yet been identified in serum, even when the tumor is very rich in mutant p53 protein levels. Probably, mutant p53 is released into the bloodstream discontinuously or at minute, unmeasurable amounts or it is bound to other proteins, degraded or cleared quickly.

We here examine another possible way of diagnosing and monitoring cancer based on mutant proteins by taking advantage of 3 distinct features of the immunological system: (i) identification of non-self antigens or loss of tolerance to self antigens, (ii) immunological amplification and (iii) stability of antibodies in the circulation. The concept, diagrammatically presented in Figure 1, is similar to the one used to identify pathogens and has not been systematically studied for cancer diagnosis and monitoring. Antibody generation against a few oncoprotein and tumor suppressor gene products, i.e., myb (Sorokine et al., 1991), myc (Ben-Mabrec et al., 1990) and p53 (Crawford et al., 1982; Caron de Fromentel et al., 1987; Winter et al., 1992; Davidoff et al., 1992; Schlichtholz et al., 1992), has already been described. We present an extensive study of antibody generation against the p53 protein in various, largely unstudied cancer types and in groups of healthy and non-cancer hospitalized patients.

MATERIAL AND METHODS

We used 2 time-resolved immunofluorometric techniques, described in detail elsewhere (Hassapoglou and Diamandis, 1992; Angelopoulos and Diamandis, 1993), to measure anti-p53 antibodies in serum. Both methods are quantitative, non-isotopic and easily automatable. Briefly, the methods are based on the following principles. Method A (Hassapoglou and Diamandis, 1992): white, opaque polystyrene microtiter wells were coated with goat anti-mouse immunoglobulin. We added mutant p53 antigen (produced in COLO 320 HSR⁺ cells) and mouse monoclonal antibody (MAb) PAB240 anti-p53 (mutant-specific), incubated for 3 hr and washed 6 times, followed by patient serum (diluted 10-fold or more, as necessary) incubated for 1 hr and washed as above. We then added alkaline phosphatase-labeled goat anti-human immunoglobulin, incubated for 1 hr and washed as above. The alkaline phosphatase activity was measured with a new detection method previously described by our group (Christopoulos and Diamandis, 1992; Papasstasiou-Diamandis et al., 1992), involving the alkaline phosphatase substrate 5-fluorosaliciliphosphate, Tb³⁺ and EDTA. This method is extremely sensitive and can quantify analytes at attomole quantities. Method B (Angelopoulos and Diamandis, 1993): microtiter wells were coated as in Method A. Patient serum (undiluted or diluted, as necessary, for samples with high antibody titers) was then incubated in tubes with a standard amount of p53 antigen (from COLO 320 HSR⁺ cells). The mixture was then added to the microtiter wells along with PAB240. After incubation for 3 hr and washing, we added the CM-1 anti-p53 rabbit antibody (mutant and wild-type-specific), followed by 1

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**Figure 1** - Concept for the serological diagnosis of cancer. Early during cancer development, alterations in oncogenes and/or tumor suppressor genes may lead to the production of mutant forms or abnormal levels of proteins within tumor cells or on tumor cell membrane (black sections). Host’s immunological system detects such altered or abnormally abundant proteins and produces antibodies against them. Antibodies are produced in amounts vastly higher than the immunogen (immunological amplification), circulate in the serum for long periods and could be used to spot the cancer initiation event. The immune response can be initiated even if the offending immunogen is only transiently released from the tumor.

hr incubation and washing. We then added an alkaline phosphatase-labeled goat anti-rabbit immunoglobulin, incubated for 1 hr and washed 6 times. The alkaline phosphatase activity was measured as in Method A.

Method A is a non-competitive procedure based on the reaction of serum anti-p53 antibodies with mutant p53 antigen immobilized on the solid-phase through the PAb240 antibody. Method B is essentially a p53 antigen assay (Hassapoglou et al., 1993), but during the incubation of serum with exogenously added p53 antigen, any p53 antibodies present in the serum react with p53 antigen and render it unmeasurable by the p53 assay. This is a “competitive” assay because the exogenously added p53 binds either to the serum antibodies or to the coating PAb240 antibody.

High performance liquid chromatography (HPLC) was performed with a Shimadzu (Kyoto, Japan) system with an absorbance monitor at 280 nm. The mobile phase was 0.1 mol/L Na₂SO₄-0.1 mol/L NaH₂PO₄ solution, pH 6.8. The flow rate was 0.5 mL/min and the HPLC was run isocratically. The gel-filtration column was a Bio-Sil SEC-250 column, 600 x 7.5 mm (Bio-Rad, Richmond, CA). The column was calibrated with a m.w. standard solution from Bio-Rad, containing thyroglobulin (670 kDa), IgG (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and cytochrome b (1.4 kDa). HPLC fractions (0.5 mL) were collected with a fraction collector (model FRAC-100; Pharmacia, Upptala, Sweden).

Protein A affinity chromatography was performed manually using the kit system MAPS, purchased from Bio-Rad. The instructions of the manufacturer were followed throughout.

The specificity of detection of serum anti-p53 antibodies by Methods A and B was checked in some positive sera by Western blot analysis as follows: lysates from COLO 320 HSRS cells were mixed with an equal volume of Tris-glycine-SDS buffer containing 2-mercaptoethanol, denatured by heating at 90°C for 5 min and loaded onto a 4–20% polyacrylamide gels. After electrophoresis (125 V, 90 min), proteins were transferred to a nitrocellulose membrane (Hybond-ECL, Amersham, Arlington Heights, IL) by electroblotting at 30 V for 2 hr. The membrane was then treated overnight in a blocking solution (5% non-fat dried milk in wash solution, consisting of Tris-buffered saline, pH 7.6, 0.1% Tween-20). The membrane was then cut into strips, which were probed for 1 hr at room temperature with the human sera or the polyclonal anti-p53 antibody CM-1, diluted 1,000-fold in a 5% BSA solution. After washing with solution the blot was incubated for 1 hr with a goat anti-human IgG conjugated to horseradish peroxidase (HRP), in the case of the human sera, and with a goat anti-rabbit IgG conjugated to HRP, in the case of the CM-1. After a final washing, antibody binding was visualized by chemiluminescence and captured on X-ray film, using the ECL-Western blot detection kit from Amersham.

**Quantitation**

Because of the lack of a suitable standard solution, we devised an arbitrary system to calibrate Methods A and B. Among the highly p53 antibody-positive sera we selected one and arbitrarily defined its concentration to be 10,000 Units/L (U/L). This serum sample was then used at various dilutions to construct calibration curves for assays A and B from which the concentration of the other samples was calculated (Angelopoulou and Diamandis, 1993).

**Assays for tumor markers**

For the analysis of CA-125 and CEA we used commercially available kits, i.e., the TRU-QUANT OV RIA (Biomira, Edmonton, Canada) and the Amerlite CEA-60 assay (Kodak, Rochester, NY). Estrogen and progesterone receptors were measured with enzyme immunoassay kits from Abbott, North Chicago, IL.

**Statistical analysis**

The chi-square ($\chi^2$) test was used to determine the statistical significance of differences in distributions and all $\chi^2$ values and corresponding $p$ values were calculated by the statistical software SAS (Cary, NC).

**RESULTS**

We collected sera from various groups of patients over a 3-year period and stored them at −70°C until analysis. Most sera were collected within 6 months from diagnosis. No effort was made to subclassify the patients in categories according to disease stage, mode of treatment, duration of disease, sex, age or any other clinical parameter. Our testing strategy was as follows: all specimens were analyzed only by Method A, and positive samples were identified based on a cut-off fluorescence ratio of 1.7, between fluorescence in the presence or absence of p53 antigen, as previously described (Hassapoglou and Diamandis, 1992). All samples positive by Method A were then analyzed by Method B for confirmation. The results are summarized in Table I and Figure 2.

The highest positivity rate was observed with colon and ovarian cancer sera (15–16%). These groups of patients have not been previously systematically studied for p53 antibody response. Relatively high positivity rates were also obtained with lung (8%) and breast (5%) cancer sera in accordance with previous reports (Crawford et al., 1982; Winter et al., 1992; Davidoff et al., 1992; Schlichthoz et al., 1992). Lower positivity rates (3–4%) were seen in patients with pancreatic and prostate cancer and in patients with multiple myeloma and lymphoma. Positivity rates similar to those obtained for blood
donors or hospitalized patients (<2%) were obtained in patients with hepatoma, melanoma, leukemia, Kaposi's sarcoma and testicular carcinoma.

<table>
<thead>
<tr>
<th>Patient diagnosis</th>
<th>Samples tested</th>
<th>Positive samples¹</th>
<th>Method A</th>
<th>Methods A and B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td>73</td>
<td>6(8.3)</td>
<td>6(8.3)</td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>290</td>
<td>14(4.8)</td>
<td>10(3.4)</td>
<td></td>
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<tr>
<td>Ovarian cancer</td>
<td>86</td>
<td>13(15.1)</td>
<td>11(12.8)</td>
<td></td>
</tr>
<tr>
<td>Colon cancer</td>
<td>82</td>
<td>13(15.8)</td>
<td>15(15.8)</td>
<td></td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>144</td>
<td>0(0)</td>
<td></td>
<td></td>
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<tr>
<td>Prostate cancer</td>
<td>65</td>
<td>2(3.1)</td>
<td>2(3.1)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>46</td>
<td>2(4.3)</td>
<td>2(4.3)</td>
<td></td>
</tr>
<tr>
<td>Hepatoma</td>
<td>150</td>
<td>1(0.7)</td>
<td>1(0.7)</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>58</td>
<td>1(1.7)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>165</td>
<td>5(3.0)</td>
<td>5(3.0)</td>
<td></td>
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<tr>
<td>Leukemia</td>
<td>107</td>
<td>1(0.9)</td>
<td>1(0.9)</td>
<td></td>
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<tr>
<td>Lymphoma</td>
<td>115</td>
<td>3(2.6)</td>
<td>3(2.6)</td>
<td></td>
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<tr>
<td>Kaposi's sarcoma</td>
<td>11</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycythemia rubra vera</td>
<td>14</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>10</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood donors and healthy volunteers</td>
<td>150</td>
<td>1(0.7)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Hospitalized patients</td>
<td>56</td>
<td>1(1.8)</td>
<td>1(1.8)</td>
<td></td>
</tr>
</tbody>
</table>

¹Samples found positive by Method A were also tested by Method B. Percentages of positive samples are given in parentheses.

Our methods for measuring p53 antibodies are quantitative. Serum p53 antibody concentrations were calculated for all positive samples, and the results are presented in Figure 3. Concentrations ranging from 10² U/L to almost 10⁷ U/L were obtained. The highest titers (>10⁷ U/L) were seen in the sera of 5 ovarian, 1 lung, 2 breast and 1 colon cancer patients. We were interested in the changes of p53 antibody titers with time and their relation to disease progression and regression or to therapeutic manipulations. The results for 6 patients from whom we had serial samples are shown in Figure 4.

Patient A (70 years old) was diagnosed with an ovarian papillary invasive serous adenocarcinoma, grade 3 and stage III, and was treated with bilateral salpingooophorectomy (BSO) and omentectomy, but residual tumor remained. After surgery, she was treated with cisplatinum plus cyclophosphamide. The same chemotherapy was given another 5 times over a 5-month period, as shown in Figure 4a. CA-125 levels and p53 antibody levels were monitored in 8 consecutive samples. Patient B (62 years old) was diagnosed with ovarian papillary serous invasive cystadenocarcinoma and was treated with BSO and omentectomy/hysterectomy, but residual tumor remained. After surgery, she was treated with cisplatinum plus cyclophosphamide. The same chemotherapy was repeated over a 10-month period, as shown in Figure 4b. CA-125 levels and p53 levels were monitored in 10 consecutive samples. Patient C (74 years old) was diagnosed with serous ovarian adenocarcinoma, grade 2, stage III, with widespread metastasis to liver and colon and presence of ascites fluid. She was operated and treated with chemotherapy as per patient B. The

![Figure 2](image-url) - Positivity of the p53-antibody test (Method A) in the serum of patients with various malignancies, blood donors and patients with non-malignant diseases. The number of patients tested per group is given in Table I.
Figure 4 – Monitoring 5 ovarian cancer patients (a–e) and a breast cancer patient (f) with CA-125 (a–e) or CEA (f) (solid lines) and with the p53-antibody test (broken lines). Surgery was performed at time 0 in all cases; laparoscopy was performed for patient e. Adjuvant chemotherapy was administered repeatedly, as shown by vertical lines below the X-axis. R = radiation therapy. For more details on the patients see text.
frequency of chemotherapy is shown in Figure 4e. Twenty-nine consecutive samples were available for CA-125 analysis over 30 months, and from these, 13 were available for p53 antibody levels. Patient D (71 years old) was diagnosed with carcinoma of the fallopian tube and treated with surgery and radiation. After a notable elevation of CA-125, she was further treated repeatedly with carboplatin, as shown in Figure 4d. We had available 12 samples for CA-125 analysis, 7 of which were also available for p53 antibody levels. Patient E (63 years old) was diagnosed with grade 3, stage III ovarian carcinoma and laparotomy was considered non-operable. She was treated with chemotherapy (4 courses over 3 months with carboplatin and cyclophosphamide). The changes in 7 and 5 sera for CA-125 and p53 antibody levels, respectively, are given in Figure 4e. Patient F (73 years old) was diagnosed with unilateral infiltrating intraductal breast carcinoma and was treated with radical mastectomy plus radiation. The tumor was estrogen- and progesterone receptor-negative and a non-CEA producer. No axillary node infiltration was found. The patient is clinically relapse-free, but p53 antibody titers in 3 consecutive samples tend to increase with time (Fig. 4f).

It is evident from Figure 4 that the temporal patterns of changes between the serological marker CA-125 and the p53-antibody concentration are similar but that the latter lags behind the CA-125 changes by approximately 1–3 months. This delay would be expected if the relatively long serum half-lives of antibodies and the time required for the immune system to respond to an immunological stimulus were considered. The p53-antibody concentration in the serum of cancer patients appears to be dependent on tumor volume because it increases in relapse. Our data indicate that patient immunization represents a continuous event driven by the tumor and is not a temporally isolated process. These preliminary observations suggest that p53-antibody levels could be used to monitor therapy in patients who are positive for p53 antibodies.

Among 300 cancer patient sera tested by both methods, 2 were positive by Method B and negative by Method A and 6 exhibited titers which were 2–4 times higher with Method B. We speculated that these sera may contain antibodies of the IgA or IgM immunoglobulin classes or other non-immunoglobulin p53 binders. To examine this possibility, we re-analyzed 12 samples with Method A but substituted the goat anti-human immunoglobulin G antibodies with goat anti-human immunoglobulin A or M. From the 6 samples that showed differences in p53-antibody concentrations between Methods A and B, we found 5 that were positive for IgA and 2 that were positive for IgM. However, in all 6 samples, the fluorescence signals with goat anti-human immunoglobulin G were much higher. These data suggest that, although IgA and IgM antibodies against p53 exist in some patient sera, their concentrations or affinities are much lower in comparison to the co-existing IgG antibodies. From the 6 samples with good agreement in p53-antibody concentrations between Methods A and B, 2 were positive for IgA and 1 was positive for IgM. HPLC with a molecular sieve column of one sample that gave about 4-fold higher p53-antibody concentration with Method B was performed and fractions analyzed by both Methods A and B. This experiment failed to identify any p53-antigen binders, which could be detected only by Method B. Taken together, these data suggest that the differences between titers observed between Methods A and B in these few samples are likely due to lower-affinity anti-p53 IgG, IgA or IgM antibodies that are detected only by Method B. This notion is suggested because in Method B, low-affinity antibodies could effectively block p53 antigen captured by the coating antibody. In Method A, low-affinity
antibodies could escape from the p53 antigen and pass undetected during the 2 washing steps of the assay.

The specificity of the methodologies used was examined in 3 different experiments. (i) Some sera positive for p53 antibodies were separated on an HPLC system using a gel filtration column and fractions were analyzed for anti-p53 antibodies by Methods A and B. The anti-p53 antibody-positive HPLC fractions corresponded to a m.w. consistent with human immunoglobulins (160–180 kDa, data not shown). (ii) Some sera were passed through a protein A column, which is known to bind only immunoglobulins. After elution, we tested the eluate with Methods A and B and detected anti-p53 antibodies, further confirming that the measured moieties are human immunoglobulins. (iii) We performed Western blot analysis using a COLO 320 HSR cell lysate as a source of mutant p53 protein (Hassapoglou et al., 1993). These data (Fig. 5) clearly show that sera positive for p53 antibodies but not sera negative for p53 antibodies react with a 53-kDa protein, which is also visualized with a specific polyclonal anti-p53 antiserum (CM-1 antibody). These data taken together strongly suggest that Methods A and B identify human immunoglobulins reacting specifically with the p53 protein. Previous studies have established that the serum anti-p53 antibodies react with both wild-type and mutant forms of p53 (Winter et al., 1992; Davidoff et al., 1992; Schlichtholz et al., 1992).

From the 290 breast cancer patient sera analyzed, we had estrogen and progesterone receptor data for 178. When we classified the sera in groups, as shown in Table II, we found that the p53 antibody–positive sera were strongly associated with estrogen and/or progesterone receptor–negative tumors (p < 0.002). These findings are in agreement with the data of Schlichtholz et al. (1992) and strongly suggest that tumors eliciting antibody responses define a subgroup with poor prognosis.

**DISCUSSION**

The prevalence of p53 antibodies in cancer patient sera has only been occasionally studied, the cancer types examined being few and the results qualitative (Crawford et al., 1982; Caron de Fromentel et al., 1987; Winter et al., 1992; Davidoff et al., 1992; Schlichtholz et al., 1992). We here report serum p53 antibody prevalence in a group of 1,392 cancer patients and in 230 sera from patients without malignancy or normal volunteers. Our studies were conducted using 2 new quantitative methodologies based on different principles. Both methods employ a detection methodology that uses alkaline phosphatase as label and time-resolved fluorometry with terbium chelates. This detection method is among the most sensitive reported and is suitable for measuring analytes at attomole levels (Christopoulos and Diamandis, 1992; Papanastasiou-Diamandis et al., 1992). Highest antibody prevalence was obtained in sera from ovarian and colon cancer patients (15–16%), 2 tumors that were not previously systematically studied for p53-antibody generation. One report described the presence of anti-p53 antibodies in 1 ovarian cancer patient (Labrecque et al., 1993). Antibody prevalence was 5–8% in patients with lung and breast tumors, in fair accordance with previous reports (Crawford et al., 1982; Winter et al., 1992; Davidoff et al., 1992; Schlichtholz et al., 1992). We found relatively low prevalence of anti-p53 antibodies (3–4%) in patients with pancreatic and prostate cancer and in patients with multiple myeloma or lymphoma. In patients with other malignancies (hepatoma, melanoma, leukemia, Kaposi’s sarcoma and testicular carcinoma), p53-antibody prevalence was similar to that of non-cancer patients (<2%).

Among the sera from the blood donor group (n = 150), we found 1 sample that was positive by Method A only (Table I). We could not obtain any clinical information for this patient. Among the 56 hospitalized patient sera, we found 1 sample which was positive by both Methods A and B, with a p53-antibody concentration of 4,000 U/L. This serum belonged to a non-insulin-dependent diabetic who had undergone colectomy and colectomy for complications of an abscess 3 years before the serum sampling. No malignancy has as yet been diagnosed in this patient.

In some patient sera, p53-antibody concentration was astro

**Table II**

<table>
<thead>
<tr>
<th>Receptor status</th>
<th>p53 Antibody status</th>
<th>p value</th>
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<tbody>
<tr>
<td>(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER (+)</td>
<td>2 (20%)</td>
<td>128 (76%)</td>
</tr>
<tr>
<td>ER (-)</td>
<td>8 (80%)</td>
<td>40 (24%)</td>
</tr>
<tr>
<td>PR (+)</td>
<td>2 (20%)</td>
<td>113 (67%)</td>
</tr>
<tr>
<td>PR (-)</td>
<td>8 (80%)</td>
<td>55 (33%)</td>
</tr>
<tr>
<td>ER (+), PR (+)</td>
<td>1 (10%)</td>
<td>105 (62%)</td>
</tr>
<tr>
<td>ER (+), PR (-)</td>
<td>1 (10%)</td>
<td>23 (14%)</td>
</tr>
<tr>
<td>ER (-), PR (+)</td>
<td>1 (10%)</td>
<td>8 (5%)</td>
</tr>
<tr>
<td>ER (-), PR (-)</td>
<td>7 (70%)</td>
<td>32 (19%)</td>
</tr>
</tbody>
</table>

1ER, PR, estrogen and progesterone receptors. Cut-off values were < 10 fmol/ml of total protein (Hassapoglou et al., 1993).
reports, are as follows: lung, 30–70% (Ozturk et al., 1992); breast, 20–46% (Hassapoglou et al., 1993; Ozturk et al., 1992); ovarian, 36–80% (Ozturk et al., 1992); colon, 20–69% (Ozturk et al., 1992); prostate, 10–79% (Ozturk et al., 1992; Van Veldhuizen et al., 1993); pancreatic, 40% (Ruggeri et al., 1992); melanoma, 47–85% (Stretch et al., 1991; Yamamoto and Takahashi, 1993); multiple myeloma, 13% (Neri et al., 1993); leukemia, 3% (Ozturk et al., 1992); lymphoma, 15–50% (Ozturk et al., 1992). Tumors with high frequency of p53 gene mutations appear to be associated with high prevalence of serum p53 antibodies, but this does not appear to be the sole contributing factor. Many tumors bearing p53 gene mutations are not immunogenic (Winter et al., 1992; Davidoff et al., 1992; Schlichtholz et al., 1992). Davidoff et al. (1992) have shown that tumors which elicit an antibody response contain complexes between heat shock protein 70 (HSP 70) and mutant p53. Winter et al. (1992) have shown that only tumors with p53 gene missense mutations are able to induce antibodies. No antibody generation was observed in tumors bearing stop, splice/stop, splice or frameshift mutations. Generally, it has been suggested that tumors bearing p53 gene mutations in exons 7–8 are not immunogenic, whereas those with mutations in exons 5–6 are (Winter et al., 1992; Davidoff et al., 1992). Although in our study we did not examine the tumor p53 gene mutations in patients with serum p53 antibodies, it is tempting to speculate that the high incidence of p53 antibodies in patients with ovarian cancer may be due to the frequent clustering of the p53 gene mutations in exon 6, as has been suggested by Teneriello et al. (1993).

Schlichtholz et al. (1992) have shown that the p53-antibody response may be a clinically useful indicator associated with poor prognosis. Our finding that the p53 antibody–positive breast tumors are associated with estrogen and progesterone receptor-negative tumors supports this proposal (Table II).

Because the assays used were quantitative, we were able to monitor levels of p53 antibodies during the course of the disease or during therapeutic manipulations in some patients from whom we had serial samples. In these patients we have shown that the temporal changes of p53 antibodies correlate with disease progression or regression. This finding suggests that the p53-antibody test may have some value for monitoring, especially in cases where other tumor markers are normal.

It has been suggested that the appearance of anti-p53 antibodies in serum of cancer patients is a very early event, independent of disease progression (Schlichtholz et al., 1992). Combined with previous reports on antibody generation against other oncogene products (Sorokine et al., 1991; Ben-Mabree et al., 1990) and the development of fast, quantitative and economical methodologies for antibody measurements in serum, we propose the further exploitation of such testing for cancer diagnosis in high-risk groups and for screening for selected cancers. As we and others have shown, the diagnostic specificity of the p53-antibody test is very high, approaching 100%. While the diagnostic sensitivity for cancer diagnosis is only around 15% for ovarian and colon cancers, the test may still be a viable alternative since the prevalence of these cancers is relatively high and the proposed test is non-invasive.

Following the paradigm of p53, we are currently investigating whether panel testing for antibodies against other oncogene products will improve the diagnostic sensitivity without compromising specificity.

In conclusion, we here report prevalence of anti-p53 antibodies in over 1,600 patient sera and establish relatively high positivity rates in ovarian and colon cancers. We also report quantitative antibody concentration data and demonstrate significant variability of antibody titers in various cancers and association of very high antibody titers with ovarian cancer. Furthermore, we show that antibody titers change with disease progression or regression and that the test may have some value in patient monitoring. We also confirm a previous report of an association between the presence of anti-p53 antibodies and other unfavorable prognostic indicators in breast cancer.

ACKNOWLEDGEMENTS

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