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Original Paper

Detection of the *TP53* Tumour Suppressor Gene Product and p53 Auto-antibodies in the Ascites of Women with Ovarian Cancer

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Antibodies against the *P53* tumour suppressor gene product are present in the serum of many cancer patients, but with varying frequencies ranging from 0 to 30%. Approximately 15–20% of patients with ovarian carcinoma develop auto-antibodies against p53 that circulate in the serum. Since many ovarian cancer patients develop ascites during their disease, we speculated that p53 antigen and/or p53 auto-antibodies may be present in this biological fluid. Ninety-six ascites fluids from women with primary ovarian cancer and one from a patient with cancer of the breast, which metastasised to the ovaries, were analysed for p53 auto-antibodies. Seventeen ascites fluids (18%) contained auto-antibodies. For 30 of these patients, serum was also available. Auto-antibodies were present in both serum and ascites in 6/30 patients; 22 patients were negative in both ascites and serum; and 2 patients had auto-antibodies only in their serum. All 97 ascites fluids were also analysed for p53 antigen and 7 (7%) were positive. None of the 17 p53 auto-antibodies-positive ascites fluids were positive for p53 antigen suggesting that p53 auto-antibodies may interfere with p53 antigen detection by capturing the antigen. In total, 24 patients (25%) had either p53 auto-antibodies or p53 protein in their ascites fluid. These data demonstrate that p53 auto-antibodies and/or p53 protein are present in ascites and may have some value for tumour diagnosis, prognosis or monitoring.

Key words: ovarian cancer, tumour suppressor genes, p53, auto-antibodies, tumour markers, ascites fluid

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INTRODUCTION

THE *P53* TUMOUR suppressor gene product appears to be one of the most important molecules involved in the maintenance of normal cell growth and differentiation and in the prevention of cancer development. *P53* accomplishes these tasks by blocking the division of cells that have sustained DNA damage, and in those cases that the damage is irreparable, by triggering cell death via apoptosis [1]. For these reasons, *P53* has been characterised as a "molecular policeman which guards" the integrity of the genome [2]. These observations indicate that the inactivation of *P53* allows for an accumulation of genetic rearrangements that eventually lead to tumorigenesis.

In most cases, *P53* function is inactivated by genetic mutations. Mutations of the *P53* gene have been identified in a wide range of tumours including those of the colon, breast, lung, brain and the haematopoietic system [3, 4]. Currently, *P53* is considered to be the most frequently mutated gene in human cancers [4].

The p53 protein is present at very low levels in normal tissues and cannot be detected by conventional immunohistochemical methods. Mutant p53 appears to have an increased life span, accumulating in the tumour cells at relatively high levels, and, therefore, it can be detected by

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immunohistochemical, immunological or immunoblotting techniques. The tumour-derived p53 has not as yet been identified in human serum or in any other biological fluid, either due to the lack of highly sensitive measurement techniques or because it undergoes degradation by enzymes or complexes with other proteins in the circulation. Alternatively, p53, a nuclear protein, may not reach the general circulation, or may be released intermittently only during tumour necrosis.

The overexpression of mutant p53 is believed to lead to intolerance and the subsequent development of antibodies against this protein by a proportion of cancer patients. These antibodies are released into the circulation and can be readily detected in the serum. The immune response against p53 was originally observed by Crawford and associates, who detected p53 antibodies in 9% of patients with breast cancer [5]. This work was later extended by Caron de Fromentel and associates, who identified such auto-antibodies in the sera of children bearing a wide variety of tumours [6]. Recently, the immune response against p53 has attracted much attention, but the underlying molecular mechanisms of this phenomenon still remain poorly understood. It has been suggested that the development of p53 antibodies is dependent upon complexing of the mutant protein with heat shock protein 70 (HSP70) [7]. Others have found that the immune response is associated with the type of the mutation, and that only missense mutations give rise to an immunogenic protein [8]. More recently, Soussi's group has shown that p53 antibodies are directed towards immunodominant epitopes localised in the amino and carboxy terminal end of the p53 protein [9–11]. From the wide variety of tumours studied so far, the most immunogenic seem to be those of the breast (9–26%) [5, 7, 9, 12], lung (13–24%) [8, 11], colon (23%) [13, 14] and ovary (15%) [13].

Ovarian cancer is the most lethal gynaecological malignancy [15]. Although the molecular mechanisms of ovarian cancer development are not yet fully understood, it has been suggested that allelic losses and mutations of the *P53* gene are critical genetic alterations for the development of this cancer [16]. It has been found that 50% of epithelial ovarian carcinomas overexpress the p53 protein [17, 18]. Our group has recently shown that 30% of these patients with p53 overexpression develop antibodies against this protein [13]. In this study, we investigated p53 auto-antibodies and the p53 gene product in the ascites of patients with ovarian carcinoma.

MATERIALS AND METHODS

Ascites fluid and serum samples

Ascites from 96 patients with primary ovarian cancer and 1 patient with breast cancer that had metastasised to the ovaries were either collected during surgery or withdrawn at paracentesis, and kept at −20°C until analysis. Paired serum samples from 30 of these patients were obtained either at the time of surgery or after surgery, during therapeutic monitoring of the patients, and stored at −20°C until analysis. All samples were collected over a 4-year period.

Cell line

The cell line COLO 320 HSR(+), obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.), was used as a source of p53 protein. This is a colorectal carcinoma cell line that has been shown to express high levels of p53 as a result of a mutation at codon 248 [19, 20]. Cells were grown in suspension in RPMI culture medium containing 10% foetal calf serum and antibiotics.

Antibodies

The mouse monoclonal anti-p53 antibodies PAb240 and DO-1, which recognise epitopes on mutant p53 protein between amino acids 213–217, and 37–45, respectively, were used to detect the p53 antigen. The antibodies were produced in our laboratory from hybridoma cells lines donated by Dr D. Lane, University of Dundee, Scotland. The mouse monoclonal anti-lutropin antibody was from Medix Biochemica, Helsinki, Finland. The human anti-p53 antibodies in serum or ascites were detected either with a goat anti-human IgG antibody conjugated to alkaline phosphatase (ALP) (for the immunoassay procedure), or with a sheep anti-human IgG antibody conjugated to horseradish peroxidase (HRP) (for the Western blot analysis). The two secondary antibodies were purchased from Jackson Immunoresearch, West Grove, Pennsylvania, U.S.A., and from Amersham Life Sciences, Oakville, Ontario, Canada, respectively. The rabbit polyclonal anti-p53 antibody CM-1 (Dimension Labs, Mississauga, Ontario, Canada) was used for detection of the p53 protein. In the immunoassay, CM-1 was recognised by a goat anti-rabbit antibody conjugated to ALP (also obtained from Jackson) and on the immunoblot, by a donkey anti-rabbit antibody conjugated to HRP (Amersham). The mouse monoclonal anti-p53 antibody DO-7 (Dimension Labs) was used for immunohistochemistry. This antibody recognises an epitope that resides between amino acids 35–45 of the p53 protein and reacts with both wild-type and mutant forms of p53 [21]. The biotinylated goat anti-mouse secondary antibody was purchased from Vector Laboratories, Inc., Burlington, California, U.S.A. Biotinylation of the DO-1 and anti-lutropin antibodies was carried out as previously described [22].

Immunoassays

For the quantitative analysis of p53 antibodies in ascites and the serum samples, we used a time-resolved immunofluorometric technique described in detail elsewhere [23, 24]. Two other immunofluorometric procedures were used for the detection of the p53 protein in the ascites fluid [20, 25]. The only difference between these two assays is the specific antibody used to capture the p53 protein on the microtitre wells (either PAb 240 or DO-1). The method is based on the measurement of alkaline phosphatase activity with a detection system previously described by our group, which involves the alkaline phosphatase substrate 5-fluorosalicylphosphate, the metal-ion Tb^{3+}, EDTA and time-resolved fluorimetry [26, 27]. This method is extremely sensitive and can quantify analytes at attomole levels.

Quantification

The ratio between the fluorescence in the presence of p53 and in the absence of p53 (blank) during the p53 antibody assay was 1.7 or higher, as previously described [24].

During validation of the p53 antibody assay, we analysed 230 sera from healthy individuals and found that 228 of them (>99%) had ratios <1.7. Because of the lack of a suitable standard preparation, we devised an arbitrary system to calibrate our methods [24]. One of the p53 antibody-positive sera, possessed from our previous work, was selected as standard and its relative titre was arbitrarily defined to be 20000 Units/l (U/L). This serum sample was used in dilutions to construct calibration curves from which the titres of all other positive sera were calculated.

For the p53 antigen assay, samples were considered positive if the ratios of the signal obtained with the p53-specific antibody (pAB240 or DO-1) versus an irrelevant antibody (antilutropin) or no coating antibody at all (blank) was higher than 2.

Western blot

Lysates from COLO 320 HSR(+) cells were mixed with an equal volume of Tris-glycine-sodium dodecyl sulphate (SDS) buffer containing 2-mercaptoethanol, denatured by heating at 90°C for 5 min and loaded on to 8–16% polyacrylamide mini gels (Novex, San Diego, California, U.S.A.). After electrophoresis (125 V, 90 min), the proteins were transferred to a nitrocellulose membrane (Hybond™-ECL, Amersham) by electroblotting at 30 V for 2 h. The membrane was then treated overnight in a blocking solution (5% non-fat dried milk in wash solution; Tris-buffered saline, pH 7.6, 0.1% Tween 20) and subsequently cut into strips, which were probed for 1 h at room temperature with the human sera or ascites fluids [diluted 1000-fold in a 6% (w/v) bovine serum albumin (BSA) solution] or the rabbit polyclonal CM-1 anti-p53 antibody (diluted 1000-fold in a 6% BSA solution). After washing the blot was incubated for 1 h with a sheep anti-human IgG conjugated to HRP in the case of the human sera and ascites, and with a donkey anti-rabbit IgG conjugated to HRP in the case of the CM-1. After a final washing, antibody binding was visualised by chemiluminescence, captured on X-ray film, using the ECL–Western blot detection kit from Amersham.

Protein A affinity chromatography

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

Immunoprecipitation

Two different tubes containing 100 µl of each ascites sample were spiked with either 30 µl biotinylated DO-1 antibody or 30 µl biotinylated anti-lutropin antibody (control). 100 µl of the same sample were spiked with 30 µl of a 6% bovine serum albumin (6% BSA) diluent, pH 7.8. All three tubes were incubated for 2 h at room temperature (RT) with mechanical shaking. Subsequently, 20 µl of streptavidin-coated magnetic beads (Dynal A. S., Oslo, Norway) were added in each sample, and incubated for 30 min at RT shaking. The magnetic beads were then precipitated and the supernatants were analysed with the immunofluorometric procedure for the presence of p53.

RESULTS

p53 Antibodies in the ascites fluid of patients with ovarian cancer

For 30 patients with ovarian cancer, both serum samples and ascites were available. These were analysed for the presence of p53 antibodies with the highly sensitive immunofluorometric technique [23, 24]. Results are presented in Table 1. Antibodies against the p53 protein were detected in the ascites of 6 of the 30 patients. The corresponding sera of these 6 patients were also positive for p53 antibodies. For 2 patients (patients 13 and 27), p53 antibodies were present in the serum sample, but not in the corresponding ascites. No such antibodies were identified in either the ascites or serum of the 22 patients.

Another 66 ascites from patients with primary ovarian cancer and 1 from a patient with primary breast cancer, which had metastasised to the ovaries (patient 97), were also tested for the presence of p53 antibodies (Table 2). The results revealed that 11 patients developed an immune response against p53, detectable in the ascites.

In summary, from a total of 97 patients analysed, 17 (17.5%) produced antibodies which were detected in the ascites surrounding the ovarian tumour. 8 of the 30 cancer patients tested had p53 antibodies in their serum.

Quantification of p53 antibodies in ascites and serum samples

The titres of the p53 antibodies were measured in all positive ascites and sera. The results are shown in Tables 1 and 2. For 6 of the 8 patients with paired ascites–serum samples, more than one serum sample was analysed. These samples were obtained at different times during the course of the disease. Patients 13 and 27 had no detectable antibodies in their ascites and low levels of these antibodies in their sera. For patient 24, the antibody levels in the ascites was approximately 10-fold higher than that in the three corresponding sera, whereas for patient 30 the p53 antibody titre in the ascites was more than 10-fold higher than the antibody titre in the serum taken on the same day (day of surgery). The second serum sample was drawn two months later and the p53 antibody titre had decreased significantly, most probably due to the surgical debulking and chemotherapy. For patient 14, more detailed clinical data were available and the p53 antibody titres measured in the ascites and each of the 11 consecutive sera are shown in Figure 1. The ascites was drawn on the day of surgery (time 0 in Figure 1) and the antibody titre was higher than in any of the sera. The antibody titres in the serum fluctuated for 9 months post-surgery, presumably reflecting the response of the tumour to chemotherapy. Changes in CA-125 during the same period followed a similar pattern.

p53 auto-antibody titres were also measured in the ascites of patients for whom no serum was available (Table 2) and they were found to be extremely high in some of the patients (e.g. patients 33, 58, 68 and 76).

p53 Antibodies in the ascites specifically react with the p53 protein

The specific reactivity of the antibodies detected in the ascites was demonstrated with immunoblotting. The colorectal carcinoma cell line COLO 320 HSR(+), which is known to overexpress mutant p53, was used as the source of the p53 gene product. These ascites and serum samples

Table 1. p53 gene product and antibodies in the ascites and serum of ovarian cancer patients

Patient	Ascites*		Serum†	Sampling time
	p53 Antibodies (U/L)	*P53* gene product	p53 Antibodies (U/L)	
4‡	—	+	—	Serum and ascites taken on same day
6	+ (34 600)	—	+ (53 542)	Serum and ascites taken on same day
10‡	—	+	—	Serum and ascites taken on same day
12	—	+	—	Serum and ascites taken on same day 1 year after surgery
13	—	—	+ (1933)	Ascites taken 1 year after surgery, serum taken a year later
14	+ (1 628 000)	—	+ (Figure 1)	Ascites taken 2 months before 1st serum sample—see Figure 1
22‡	+ (3856)	—	+ (1423; 6390)	Ascites taken 1.5 years before the first serum specimen; second serum specimen taken 4 months later
23	+ (522)	—	+ (1387; 3195)	First serum specimen taken 15 months after ascites; second serum specimen taken 19 months after ascites
24	+ (500 290)	—	+ (61 472; 29 323; 54 831)	Serum samples taken within one month of each other. Sampling of ascites unknown
27	—	—	+ (618; 1295)	First serum specimen taken 2 months after surgery; second serum specimen taken 4 months after surgery; ascites taken 18 months after second serum specimen
30	+ (637 775)	—	+ (59 330; 4475)	Ascites and 1st serum taken on same day; 2nd serum taken 2 months later

* Each of the ascites shown was accompanied by one or more serum samples; individual titres in serum are shown in parentheses. Patient samples with numbers between 1 and 30 (not shown) were negative for p53 antibodies in ascites and serum and for *P53* gene product in ascites.
† For 6 of the 8 positive serum samples, more than one serum sample was analysed, obtained at different times during the course of the disease—see sampling time.
‡ Date of surgery unknown.

from patients 6, 14 and 24 specifically recognised a 53 kD protein that comigrated with the protein detected by the specific anti-p53 polyclonal antibody CM-1 (positive control) (Figure 2a). Figure 2b is a representative blot, showing that while positive ascites exhibited immunoreactivity with the 53 kD protein, three ascites, which had been negative for p53 antibodies by the immunofluorometric method, lacked such reactivity (Figure 2b). These data clearly demonstrate that the antibodies detected in the ascites and sera are specific for the p53 protein (a further ten p53 antibody-negative ascites were also tested by immunoblotting, and did not recognise p53).

Table 2. p53 antigen and antibodies in the ascites fluid of ovarian cancer patients

Patient*	Ascites	
	p53 Antibodies (U/L)	p53 Antigen
32	+(1858)	—
33	+(143 717)	—
56	+(658)	—
58	+(1 697 200)	—
62	—	+
64	+(4886)	—
65	+(1183)	—
68	+(623 600)	—
69	—	+
71	—	+
76	+(256 445)	—
78	+(945)	—
84	+(3346)	—
89	—	+
97	+	—

* Ascites samples with numbers between 31 and 96 (not shown) were negative for p53 antibodies and p53 antigen.

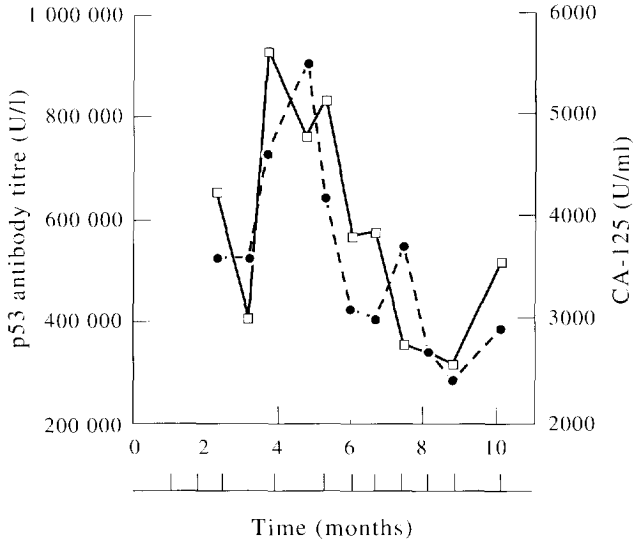

Figure 1. Serum CA-125 (broken lines) and serum p53 antibody titres (solid lines) in patient 14. The concentration of p53 antibodies in the ascites was measured in a sample drawn at the time of surgery and it was found to be 1 628 000 U/l. Vertical lines below the *X*-axis indicate the time of chemotherapy administration. The patient underwent surgery at time 0.

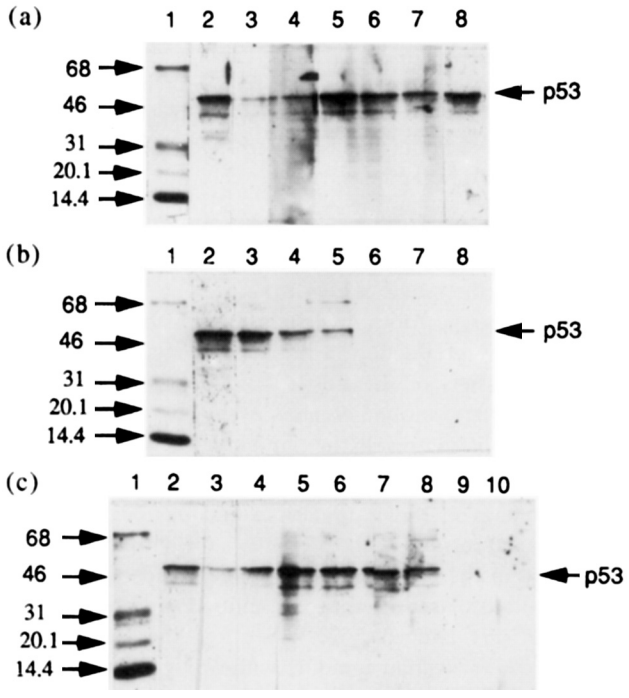

Figure 2. Western blot analysis serum samples and ascites for p53 antibodies. (a) Lane 1: molecular weight markers with molecular weights in kD shown; Lane 2: CM-1 antibody (positive control); Lanes 3 and 4: ascites and serum sample, respectively, from patient 6; Lanes 5 and 6: ascites and serum sample, respectively, from patient 14; Lanes 7 and 8: ascites and serum sample, respectively, from patient 24. (b) Lanes 1 and 2: as per (a); Lanes 3–5: ascites from patients 58, 68 and 30, respectively (tested p53 antibody-positive with the immunoassay); Lanes 6–8: ascites from patients 34, 35 and 36, respectively (negative). (c) Lanes 1 and 2: as per (a); Lanes 3–10: protein A purified ascites from patients 6 (Lane 3), 14 (Lane 4), 24 (Lane 5), 58 (Lane 6), 68 (Lane 7), 30 (Lane 8), 34 (Lane 9) and 35 (Lane 10).

To confirm that the moieties detected in the ascites of the ovarian cancer patients were human immunoglobulins, 6 positive ascites (presented in Figure 2) and two of the negative ones were purified by protein A affinity chromatography. The protein A column is known to bind only immunoglobulin molecules. After elution of the moieties bound to the column, the collected eluates were again used to probe the p53 protein immobilized on nitrocellulose membranes. As shown in Figure 2c, the purified ascites retained their p53-specific immunoreactivity.

p53 Protein detected in the ascites of patients with ovarian cancer

The 97 ascites were also analysed for the presence of p53 protein, using two different immunological assays [20, 25] that differ in the captive antibody. The capture antibodies used were PAb240 and DO-1. To control for any non-specific binding, each sample was analysed in parallel with either of the two anti-p53-specific antibodies and with an irrelevant antibody (monoclonal anti-lutropin antibody) as well as with no antibody at all. Of the 97 ascites, 7 were considered positive for p53 protein (Tables 1 and 2). Representative results are illustrated in Figure 3. For 6 of the positive samples (patients 4, 10, 12, 62, 69 and 71), the ratio varied between 2 and 5 while for patient 89, the ratio was approximately 10 (Figure 3b).

To further verify these findings, the 7 p53-positive ascites, two negative ones, and in addition, extracts from the COLO 320 HSR(+) cell line (as positive control) were incubated with biotinylated DO-1 antibody, biotinylated anti-lutropin antibody or antibody diluent. We then treated all samples with magnetic streptavidin beads to separate the biotinylated antibodies. The supernatants were analysed for the presence of p53. The 7 positive samples, when incubated with biotinylated DO-1 antibody, exhibited a 20–50% decrease in signal compared to the signal obtained in the presence of the lutropin antibody or the diluent (data not

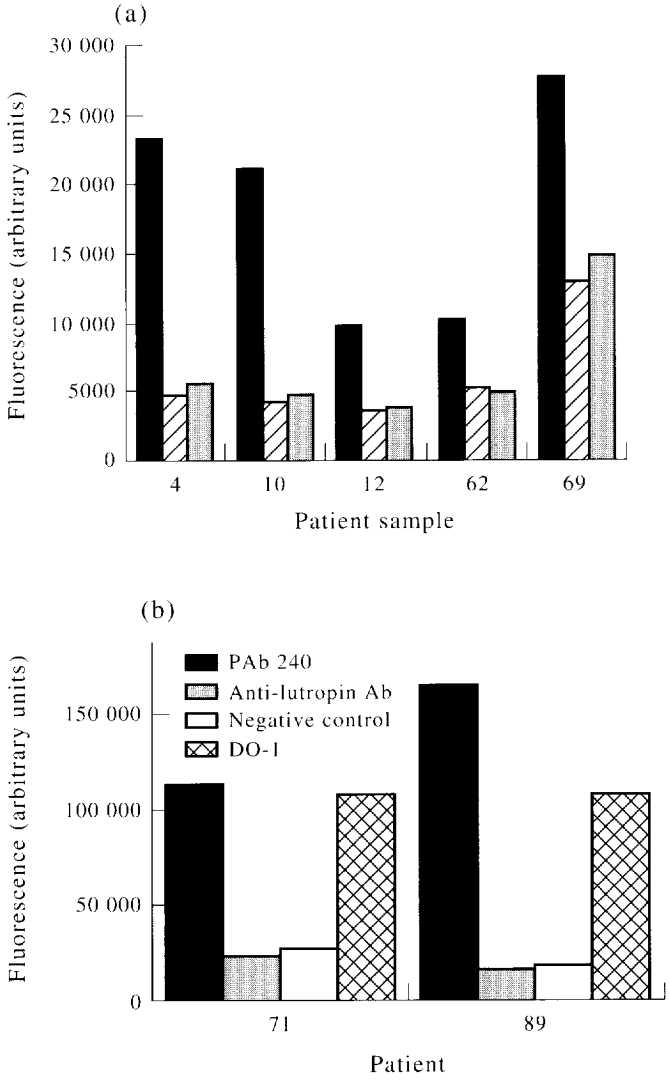

Figure 3. p53 antigen-positive ascites samples from patients with ovarian cancer. The results are expressed in arbitrary fluorescence units, obtained after analysis of the samples with the time-resolved immunofluorimetric assay. (a) Patients 4, 10, 12, 62 and 69, solid bars: PAb240 antibody; hatched bars: anti-lutropin antibody; stippled bar: negative control (see Materials and Methods). (b) Patients 71 and 89.

shown). No decrease was observed in the case of the 2 negative ascites, while the positive control also exhibited a 50% decrease. These data further support the proposal that p53 protein was indeed present in the p53 protein-positive ascites. Remarkably, none of the p53 protein-positive ascites was positive for anti-p53 antibodies.

DISCUSSION

The realisation that *P53* gene alterations are the most frequent molecular events in human cancer has led to intensive work on the identification of this molecule in a wide variety of cancers. The expression of the protein by the tumour has been extensively investigated by immunohistochemical, immunoblotting and immunoassay techniques. The mutational status of the gene has been thoroughly examined by molecular methods including polymerase chain reaction (PCR), single-stranded conformational polymorphism (SSCP) and sequencing. More than 4000 mutations have already been identified [28]. Clinical and epidemiological studies describing the usefulness of p53 as a marker of patient prognosis have also been performed. However, serological analysis of p53 has not as yet become feasible since the protein appears to be absent from serum. Another approach for serological evaluation of p53 status in cancer patients is the analysis of serum for the presence of p53-specific antibodies.

Recently, many tumour types have been studied for their ability to elicit anti-p53 antibodies. The most immunogenic tumours are those of the breast, lung, colon, and ovary [5, 7–9, 11–14]. p53 antibodies, detected in the serum of cancer patients, have some potential for diagnosis or monitoring of patients during therapy [13, 14]. They also seem to have prognostic value in breast cancer patients [9, 12], but not in colon cancer patients [14].

Recently, our group has studied the immune response against p53 in ovarian cancer [13]. Anti-p53 antibodies were detected in the serum of 15% of these patients. This finding led us to speculate that p53 antibodies might also be present in ascites that frequently accompany these tumours. The ascites, which surround the tumour, is rich in B-lymphocytes that may be stimulated by mutant proteins expressed by the tumours. The analysis of 97 ascites from ovarian cancer patients revealed that our hypothesis was correct (Tables 1 and 2).

Quantification of the p53 antibody titres in all positive ascites and sera was also performed. Some patients exhibited very high levels of antibodies ($>10^5$ U/L), while for two the levels exceeded 10^6 U/L. We do not yet know if antibodies are first developed in serum or ascites and then diffuse from one fluid to the other, or if they are produced by different populations of plasma cells.

The p53 protein has not yet been identified in the serum of cancer patients. However, during tumour development or necrosis, p53 protein or malignant cells could be released into the ascites. Analysis of the ascites revealed that p53 protein was present in 7.2% (7/97) of the patients. A rough comparison of the ascites p53 antigen levels with those of ovarian tumour cytosolic extracts [18] revealed that samples 4, 10, 12, 62 and 69 had borderline positivity while samples 71 and 89 had levels comparable to the 50–75 percentile of the ovarian tumour extracts. To our knowledge, this is the first time that p53 protein has been detected in a biological fluid.

In this study, we show that all 17 ascites that contained p53 antibodies were negative for p53 protein and all 7 ascites that contained p53 protein were negative for p53 antibodies. These findings allow us to speculate that during ovarian tumour development, tumour cells are shed into the peritoneal cavity where they lyse and release proteins, including mutant p53. Host immune cells are triggered by the presence of such mutant proteins and secrete antibodies against them. Our data suggest that not all p53 mutants appear to be immunogenic since in 7 patients, p53 antigen was present in the ascites without the presence of p53 antibodies. However, we do not exclude the possibility that the lack of antibody production in these 7 patients may be due to the suppression of the host's immune system. It also appears that once antibodies are developed, they bind to p53 and this binding results in elimination of any immunoreactive p53 in ascites. This would explain why no p53 protein was detected in any of the 17 p53-antibody-positive ascites.

It has been shown that approximately 40–50% of ovarian tumours contain *P53* gene mutations and demonstrate p53 protein overexpression [18]. We have shown that approximately 25% of these tumours can be identified by measuring ascites fluid or serum anti-p53 antibodies (17–20%) or ascites fluid p53 antigen (~7%). We hypothesise that the other 15–25% of ovarian tumours with *P53* gene mutations either do not shed tumour cells into the peritoneal cavity or the immune response is such that the predominant species in ascites are immune complexes between p53 antibodies and p53 antigen. Such complexes, if they exist, are not measurable by our methods, which detect either free antibodies or free antigen. We are currently investigating if alternative methods could be developed that can measure immune complexes containing p53 antigen.

Our data could also offer a reasonable explanation for why p53 protein has not been detected in serum of any cancer patients despite its high levels in many tumours. As we have shown here, approximately 20% of ovarian cancer patients have circulating antibodies against p53 which would mask any free p53 and render it unmeasurable even by highly sensitive techniques.

In summary, we have demonstrated for the first time that high levels of specific anti-p53 antibodies are present in ascites of 18% of ovarian cancer patients. Furthermore, the p53 antigen is also present in the ascites of about 7% of these patients, but the presence of one of the moieties is exclusive for the presence of the other. In total, 25% of the cases have either p53 protein or antibodies in their ascites fluid. This novel finding suggests that p53 autoantibodies and/or antigen may have some value as analytes for monitoring patients during therapy. Our data shed more light into the immune response of the host against mutant, tumour-specific proteins. As we have already proposed elsewhere [13], we believe that the immune response of the host against tumour-specific antigens is not restricted to p53, and that the study of specific populations of antibodies into the ascites may ultimately lead to the discovery of novel immunogenic molecules that are present in tumour cells.

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