Pergamon

European Journal of Cancer, Vol. 33, No. 13, pp. 2246–2251, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

PII: S0959-8049(97)00240-2

Original Paper

Expression of Macrophage Colony-stimulating Factor (M-CSF), Interleukin-6 (IL-6), Interleukin-1β (IL-1β), Interleukin-11 (IL-11) and Tumour Necrosis Factor-α (TNF-α) in p53-characterised Human Ovarian Carcinomas

J.G.W. Asschert,[1,2] E. Vellenga,[2] H. Hollema,[3] A.G.J. van der Zee[4] and E.G.E. de Vries[1]

[1]Division of Medical Oncology; [2]Division of Haematology, Department of Internal Medicine; [3]Department of Pathology; and [4]Division of Gynaecologic Oncology, Department of Gynaecology, University Hospital Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands

Ovarian carcinoma is often associated with overexpression of cytokines that may exert autocrine and paracrine growth effects, as well as genetic alterations in (proto)oncogenes and tumour suppressor genes, such as *p53*. The p53 protein is not only involved in the regulation of cell cycle and apoptosis, it is also involved in the *in vitro* regulation of *IL-6* gene expression. In this study, 30 tumours of patients with a primary diagnosis of human ovarian carcinoma were characterised for p53 expression with immunohistochemistry and analysed for the expression of M-CSF, IL-6, IL-1β, IL-11 and TNF-α with Northern blotting. Nuclear and cytoplasmic p53 staining was observed in 27% (8/30), cytoplasmic staining in 30% (9/30), and no p53 staining in 43% (13/30) of the tumours. In 70% (21/30) of the tumours, M-CSF mRNA was expressed, in 40% (12/30) TNF-α, and in 30% (9/30) IL-6. None of the tumours expressed IL-1β or IL-11. The expression of TNF-α occurred more frequently in M-CSF positive tumours compared to M-CSF negative tumours (52% (11/21) versus 11% (1/9), $P < 0.05$). TNF-α expression was also associated with better responses to chemotherapy ($P < 0.02$). M-CSF expression was associated with nuclear p53 staining ($P < 0.05$). The p53 positive tumours more frequently expressed one or more cytokines (88%) compared with p53 negative tumours (54%, $P < 0.05$). This study suggests that mutations in the *p53* gene might be associated with cytokine overexpression, especially M-CSF. © **1997 Elsevier Science Ltd.**

Key words: p53, cytokine expression, human ovarian carcinomas
Eur J Cancer, Vol. 33, No. 13, pp. 2246–2251, 1997

INTRODUCTION

DEVELOPMENT OF ovarian carcinomas is associated with multiple and sequential genetic changes which may involve oncogene activation, inactivation of tumour suppressor genes and inappropriate expression of growth factors [1, 2]. The induction of autocrine or paracrine loops may result in a growth advantage for malignant cells compared with benign cells [2]. In ovarian carcinomas and cell lines a variety of growth factors and their receptors have been found to be expressed, such as macrophage colony-stimulating factor (M-CSF) [3, 4], interleukin-1 (IL-1) [3, 5], interleukin-6 (IL-6) [3, 4, 6] and tumour necrosis factor-α (TNF-α) [7, 8]. These growth factors are often elevated in ascites and sera from patients with ovarian carcinoma [3, 9–12], while IL-6 and M-CSF have also been found to be elevated in cystic fluids from malignant tumours [13, 14]. In addition, normal cells can produce several cytokines by paracrine loops. For instance, IL-1 and M-CSF can trigger macrophages to produce IL-1, IL-6 and TNF-α [5, 7, 15]. In return, TNF-α stimulates ovarian tumour cells to produce TNF-α, which induces autocrine proliferation. Moreover, the stromal derived cytokine interleukin-11 (IL-11) exhibits IL-6-like bioactivity [16, 17] and is a ligand for the IL-6 gp130 signal transducing receptor which is present on cells of ovarian tumour cell lines [6].

Correspondence to E.G.E. de Vries.
Received 16 Sep. 1996; revised 15 Apr. 1997; accepted 21 Apr. 1997.

The mechanisms resulting in cytokine overexpression are largely unknown but may be related to an aberrant expression of (proto)-oncogenes or tumour suppressor genes. In ovarian carcinomas, mutation of the *p53* tumour suppressor gene resulting in overexpression of a non-functional protein frequently occurs and can be regarded as a negative prognostic factor [18–20]. Mutations not only abrogate the function of p53 in cell cycle control and apoptosis, but may also affect cytokine expression [21]. In the cervical carcinoma cell line (HeLa) wild-type p53 is able to repress the IL-6 promoter activity by binding to the transcription factor nuclear factor IL-6 (NF-IL-6) [22]. This repressive ability is lost in the presence of specific mutations in the *p53* gene, which results in an increased IL-6 expression. This phenomenon might enhance the malignant potential of the cell.

Until now, limited data are available concerning the relationship between p53 and cytokine expression in human ovarian carcinomas. The present study was performed to examine the potential relationship between p53 overexpression and expression of the cytokine messenger RNAs (mRNAs) M-CSF, IL-1β, IL-6, IL-11 and TNF-α in human ovarian carcinomas.

Our results show that the expression of M-CSF is associated with overexpression of p53. Furthermore, it is demonstrated that the expression of TNF-α is associated with M-CSF expression.

MATERIALS AND METHODS

Tissues

Tumour specimens were obtained from patients with a primary diagnosis of ovarian carcinoma operated on at cooperating hospitals in the northern part of The Netherlands in 1992. Tumour collection was supervised by a pathologist. The tumour samples were divided and either frozen in liquid nitrogen immediately after removal from the patient and stored at −80°C for p53 immunohistochemistry, and at −180°C for RNA isolation, or embedded in paraffin for routine histological analysis. All patients were staged according to the International Federation of Obstetrics and Gynaecology (FIGO) classification. Tumours were histologically classified according to the World Health Organization (WHO) classification using paraffin-embedded tissue sections [23]. Carcinomas were graded as well-, moderately and poorly differentiated adenocarcinomas [24]. Acetone fixed 4 μm tumour sections from the frozen material were stained with haematoxylin and eosin (H&E) to determine the volume percentage of tumour cells in the samples.

Immunohistochemical staining

Cryostat (4 μm) sections were air-flow dried for 20 min, fixed in acetone for 10 min at room temperature and thereafter air-dried again for 30 min before staining. After rinsing three times for 5 min in phosphate-buffered saline (PBS; 0.14 M NaCl, 2.7 M KCl 6.4 M $Na_2HPO_4.2H_20$, 1.5 M KH_2PO_4 pH 7.4), the samples were incubated with the first antibody, diluted 1:100 in PBS containing 1% bovine serum albumin (BSA, CLB, Amsterdam, The Netherlands) and 1% AB-serum for 60 min. The first antibody, the monoclonal antibody PAb1801 (Ab-2; Oncogene Science, Manhasset, New York, U.S.A.), recognises a denaturation-resistant epitope between amino acid residues 32 and 79 of the p53 protein and is considered to react with both wt- and m-p53 proteins [25]. The sections were rinsed three times for 5 min with PBS followed by incubation with the second antibody, biotinylated rabbit anti-mouse IgG (E354, DAKOPATTS, Glostrup, Denmark) diluted 1:300 in PBS, 1% BSA, 1% AB-serum, for 30 min. After rinsing the sections three times with PBS, the slides were incubated for 30 min with streptavidin-peroxidase (P397, DAKOPATTS) in PBS, 1% BSA, 1% AB-serum. The slides were washed with PBS and treated with 0.1% 3-amino-9-ethylcarbazole in 0.05 M Tris–HCl buffer, pH 7.6, containing 0.5% hydrogen peroxide. Thereafter, the slides were rinsed in tap water, counterstained with 5% haematoxylin, dehydrated and covered with permanent mounting medium. Positive controls were two p53-positive colorectal carcinomas. Negative controls were obtained by replacement of the first antibody by buffer. Tumour sections were evaluated for nuclear and cytoplasmatic localisation of p53 staining. The intensity of the staining was expressed as +, weak–moderate; ++, strong and +++, for very strong staining comparable to staining in p53 positive colorectal carcinomas. Only tumour cells were evaluated.

Beside p53 staining, tumour samples were also evaluated for the percentage of tumour cells in the slides. Tumour volume index (TVI) is the percentage of malignant epithelial tissue in a specimen. The TVI of the sections was estimated by two researchers independently. Also haematopoietic infiltration was studied in the slides.

Isolation of total RNA and Northern blot analysis

Frozen tumour samples were pulverised using a microdismembranator, and RNA was isolated with the guanidium/isothiocyanate caesium chloride method [26]. RNA (20 μg) was loaded on to a 1.2% agarose gel with 2.2 M formaldehyde. After size fractionation, RNA was blotted on to nylon membranes (Hybond N^+, Amersham, Frankfurt, Germany) [27] and cross-linked with 0.05 M sodium hydroxide. Membranes were prehybridised for at least 1 h at 65°C in hybridisation buffer, containing 0.5 M Na_2HPO_4, 1 mM EDTA, 7% sodium dodecyl sulphate (SDS, Sigma, St. Louis, Missouri, U.S.A.). Subsequently, the membranes were probed overnight at 65°C with 100 ng complementary DNAs (cDNAs). Membranes were washed once in 2x standard saline-citrate (2xSSC: NaCl, 17.53 g/l: sodium citrate, 8.82 g/l, pH 7.2), 0.1% SDS, once in 1xSSC, 0.1% SDS and finally in 0.3xSSC, 0.1% SDS for 30 min at 65°C. The probes were labelled for 1 h with [α^{32}P]-dCTP (11 Tbq/mmol, Amersham, Buckinghamshire, U.K.) using the hexanucleotide primer technique [28]. The specific activity was approximately 500×10^6 cpm/100 ng of DNA. For rehybridisation, membranes were first soaked in 0.01% SDS at 85°C. cDNAs corresponding to the *IL-1β* (gift from Dr S. Gillis, Immunex, Seattle, Washington, U.S.A.), *IL-6* (gift from Dr L.A. Aarden, CLB, Amsterdam, The Netherlands) [29], *IL-11* (gift from Dr S.C. Clark, Genetics Institute, Cambridge, Massachusetts, U.S.A.), *M-CSF* (gift from Dr S.C. Clark) and *TNF-α* (gift from Dr M.A. Brach, MDC, Berlin, Germany) genes were used. Total RNA from monocytes, stimulated with lipopolysaccharide (LPS, L9143, Sigma) was included in the assay to assure appropriate reaction of the *IL-6*, *M-CSF*, *IL-1β* and *TNF-α* probes and to standardise between different membranes. For IL-11 mRNA, fibroblasts were stimulated with 50 ng phorbol myristate acetate (PMA, Sigma)/ml for 6 h. To verify uniform loading and transfer of the RNA samples, membranes were reprobed with a cDNA fragment of human 28S. The membranes were exposed to X-ray films

(Kodak X-Omat XAR, Rochester, New York, U.S.A.) for one to several days at −80°C, using an intensifying screen. No densitometric analysis was performed. mRNA expression was designated + whenever specific hybridisation had occurred.

Statistics

Association between the clinicopathological parameters, p53- and cytokine mRNA expression were determined using chi-squared analysis with correction according to Yates if required. Differences in the progression-free survival (PFS) by stage, age, response and cytokine expression were analysed using log-rank statistics. Only P values < 0.05 were considered significant.

RESULTS

Table 1 summarises tumour and clinical characteristics of the ovarian carcinoma patients. 18 tumours had a tumour volume index (TVI) ≥ 50. There were 24 stage III–IV and 6 stage I–II tumours. Only one of the slides contained infiltrated haematopoietic cells, of which most were granulocytes.

Expression of p53 protein

Strong nuclear staining with varying intensity of cytoplasmic staining of the tumour cells was found in 8 (27%) tumours (Table 2). 9 (30%) tumours showed cytoplasmic staining with varying intensity, from weak to strong without nuclear staining. 13 tumours showed weak staining visible as small granular spots in the cytoplasm, regarded as negative, since wt-p53 containing control slides demonstrated similar patterns. Stromal cells showed no staining reaction with the p53 monoclonal antibody.

Table 1. Tumour and clinical characteristics of the ovarian carcinoma patients

Sample	TVI	Stage	Grade	Type of adenocarcinoma	Ascites
1	95	III	II	Serous	+
2	95	II	II	Serous	+
3	90	III	III	Serous	+
4	85	IV	II	Serous	+
5	85	III	II	Mucinous	+
6	80	Ib	II	Endometroid	−
7	80	IIc	II	Serous	−
8	80	IV	III	Serous	−
9	80	IV	III	Endometroid	−
10	75	III	III	NOS	−
11	70	III	II	Serous	nd
12	70	IV	II	NOS	+
13	65	III	III	Serous	−
14	60	III	III	Serous	nd
15	60	III	III	Serous	+
16	50	IV	II	Serous	+
17	50	III	I	Serous	−
18	50	III	III	Serous	−
19	45	III	I	Mucinous	−
20	45	III	III	Serous	+
21	40	III	I	Serous	+
22	30	IV	II	Serous	+
23	30	III	III	Serous	+
24	25	III	II	Serous	+
25	20	III	II	Serous	nd
26	10	Ia	I	Mucinous	−
27	5	III	I	Serous	+
28	5	Ic	I	Serous	−
29	5	IIc	nd	Clear cell	−
30	5	III	III	Serous	−

TVI, tumour volume index. Ascites is + when > 11. nd, not done; NOS, not otherwise specified.

Cytokine expression at mRNA level

Size fractionation of the RNA samples revealed intact mRNA in all samples as was visible by intact 28S and 18S bands. M-CSF was the most frequently expressed cytokine (21/30 tumours, 70%) (Table 3). TNF-α mRNA was detected in 12 tumours, IL-6 in 9. None of the tumours expressed IL-1 or IL-11 mRNA. At least one or more cytokines were expressed in 22/30 (73%) tumours. Tumours expressing M-CSF also expressed TNF-α (11/21, 52%) significantly more frequently than M-CSF negative tumours (1/9, 11%; $P < 0.05$). 4 of the M-CSF and TNF-α expressing tumours also expressed IL-6 mRNA. An example of the specific mRNA expression for 5 carcinomas plus controls is demonstrated in Figure 1.

Combination of p53 staining and cytokine expression

One or more cytokines were expressed more frequently in p53-positive tumours (88%) compared to the p53-negative tumours (54%, $P < 0.05$). In 15 out of 17 (88%) p53 positive tumours, and in 6 out of 13 (46%) p53 negative tumours, M-CSF expression was observed (Table 3). Detectable M-CSF was found in all 8 nuclear p53-stained tumours, which was more frequent than in p53-negative tumours (6/13, 46%; $P < 0.05$). There were no significant differences in IL-6 and TNF-α expression between p53 positive and negative tumours.

To evaluate the potential dilution of the tumour samples by normal cells, the results were also analysed for the 18 tumour samples with a TVI ≥ 50. Of these 18 tumours, all nuclear p53-stained tumours expressed one or more cytokines. 13 of 14 (93%) p53 positive tumours and 1 of 3 (25%) p53 negative tumours expressed M-CSF mRNA ($P < 0.05$). The expression of IL-6 occurred in p53 positive and negative tumours (4/14, 29% and 2/4, 50%, respectively). TNF-α was also expressed in both p53 positive and negative tumours (7/14, 50% and 1/4, 25%, respectively). In this group of 18 tumours, the 78% (14/18) p53 positive tumours more often expressed one or more cytokines than negative tumours (13/14, 93% versus 2/4, 50%, $P < 0.05$).

No significant difference in p53 staining was observed between stage III/IV (13/24, 58%) and stage I/II (4/6, 67%) tumours. M-CSF was preferentially expressed in stage III/IV tumours, 79% (19/24) compared with 33% (2/6; $P < 0.05$). No significant difference was observed for IL-6 expression (8/24, 33% in stage III/IV versus 1/6, 17% in stage I/II

Table 2. p53 expression in ovarian carcinoma samples

Samples ($n = 30$) n	(%)	Nuclear p53 staining*	Cytoplasmic p53 staining†
8	(27)	+/++	+/++
9	(30)	−	+/++
13	(43)	−	−

*++, strong nuclear staining; +, moderate nuclear staining; −, no nuclear staining. †++, strong cytoplasmic staining; +, moderate granular staining; −, weak granular cytoplasmic staining.

Table 3. p53 staining and cytokine mRNA expression in 30 ovarian carcinoma samples

mRNA	p53 positive tumour			p53 negative tumour	All tumours
	N+C ($n=8$) no. (%)	C ($n=9$) no. (%)	Total ($n=17$) no. (%)	($n=13$) no. (%)	($n=30$) no. (%)
M-CSF	8 (100)	7 (78)	15 (88)	6 (46)	21 (70)
IL-6	4 (50)	3 (33)	7 (41)	2 (15)	9 (30)
TNF-α	4 (50)	5 (56)	9 (53)	3 (23)	12 (40)
*	0 (0)	2 (22)	2 (12)	6 (46)	8 (27)

N+C, nuclear and cytoplasmic immunostaining; C, cytoplasmic staining; *no M-CSF, IL-6 or TNF-α mRNA. Percentages are calculated as percentage of number of tumours in the first row.

tumours). TNF-α was equally expressed in high and low stage tumours, namely 42% (10/24) and 33% (2/6).

Survival analysis and response to chemotherapy

In order to obtain insight in the consequences of all evaluated parameters, namely p53 and cytokine expression, on the survival of the patients, log-rank analysis of the parameters on progression free survival (PFS) was performed. First the established prognostic factors, such as stage of disease, age and response to chemotherapy, were evaluated to see whether this small group of patients was representative. Despite the relative small size of the population, age > 58 years ($P<0.0001$), stage III/IV ($P<0.02$) and no response to or stable disease during chemotherapy ($P<0.01$) were prognostic factors for a shorter PFS in this group of patients. Thereafter, p53 expression and cytokine expression were analysed. A borderline significant prognostic factor in the expression of TNF-α for a prolonged PFS ($P=0.0549$) was observed. This trend was supported by the observed association of TNF-α mRNA expression and a complete or partial response to chemotherapy ($P<0.02$). For the other parameters evaluated, no prognostic value could be ascribed nor were they found to be associated with the outcome of chemotherapy. The Kaplan–Meier curves for PFS for patients with and without TNF-α mRNA expression are shown in Figure 2.

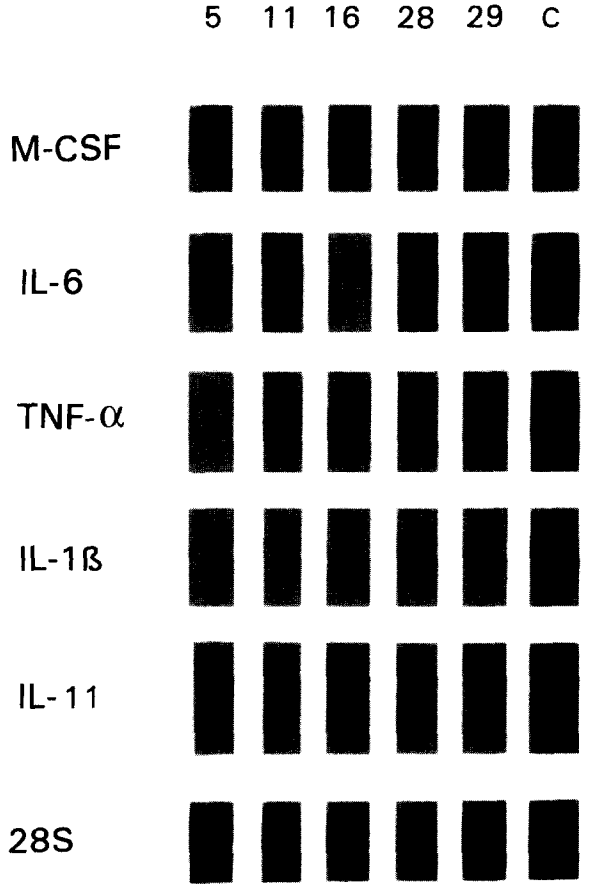

Figure 1. Northern blot analysis of M-CSF, IL-6, TNF-α, IL-1 and IL-11 mRNA in ovarian carcinoma samples 5, 11, 16, 28 and 29. Stimulated monocytes expressing IL-1β, IL-6, M-CSF, TNF-α mRNA, and stimulated fibroblasts expressing IL-11 mRNA were used as positive controls (C). Rehybridisation with 28S cDNA was performed for the degree of RNA loading.

DISCUSSION

In ascitic fluid and sera from patients with ovarian carcinoma, elevated levels of growth factors such as M-CSF [9–11, 14], IL-6 [3, 12] and TNF-α are often found [3]. M-CSF and IL-6 seem to be produced constitutively by ovarian carcinoma cell lines [4, 9, 10, 30], whereas IL-1α and β protein levels do not appear to be elevated in malignant ascites of ovarian carcinoma patients [3]. However, Li and associates reported that carcinoma cells, isolated from ascites of patients with ovarian carcinoma and cultured *in vitro*, expressed IL-1α and β transcripts [5]. In addition, it has been described that TNF-α can be produced by primary ovarian carcinomas, especially in a more advanced stage [8]. Some, but not all ovarian tumour cell lines seem to be susceptible to medium conditioned by lipopolysaccharide (LPS)-stimulated monocytes, indicating that growth stimulation may be induced by IL-1, IL-6 or TNF-α [7], which can also be produced by tumour cells.

In the present study, 73% (22/30) of the human ovarian tumours expressed one or more cytokine mRNAs. Most tumours were found to express M-CSF mRNA (70%), 40% (12/30) expressed TNF-α and 30% (9/30) IL-6 mRNA. Interestingly, M-CSF mRNA positive carcinomas expressed TNF-α more frequently compared with M-CSF mRNA negative tumours ($P<0.05$). This may indicate that either one of these growth factors has a stimulating effect on the expression of the other. M-CSF expressing tumours also expressed IL-6 more frequently than M-CSF mRNA negative, but this phenomenon did not prove to be significant. None of the tumour samples showed IL-11 or IL-1β mRNA expression, indicating that the observed cytokine expression is not due to contaminating macrophages since IL-1β is more abundantly expressed in the activated macrophage lineage [31]. This observation is supported by the histological analysis of infiltrating non-tumour cells. Furthermore, comparison of the complete group with samples having a TVI > 50 relatively increased the cytokine mRNA expression in the tumours. This supports the idea that M-CSF is indeed expressed by tumour cells instead of tumour-associated cells.

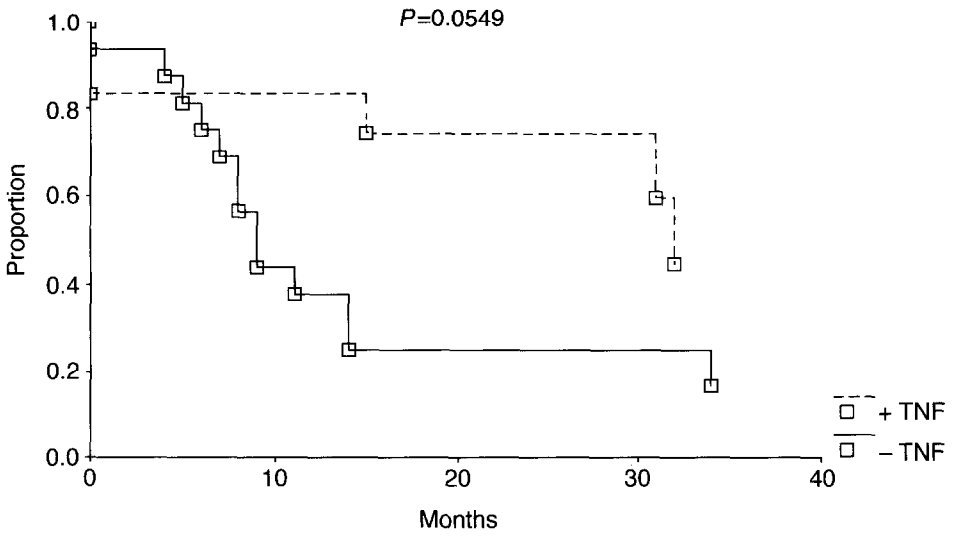

Figure 2. Progression free survival for all stages by TNF-α mRNA expression.

A frequent phenomenon in ovarian carcinoma is overexpression and mutation of the tumour-suppressor gene p53, which codes for a 53-kDa nuclear phosphoprotein [32, 33], hardly detectable with immunohistochemistry in normal cells. The mutant form of p53 is less vulnerable to degradation due to conformational modification and accumulates either in the nucleus or in the cytoplasm, where it can be detected with immunohistochemistry.

In the present study, 30 tumours were evaluated with regard to p53 expression. A total of 57% (17/30) of the carcinomas showed overexpression of the p53 protein, 27% with nuclear and cytoplasmic localised p53, and 30% with cytoplasmic p53 staining only. As reported in the literature [18–20], 35–56% of ovarian carcinomas overexpress p53, mostly in the nucleus, whereas additional studies also report cytoplasmic p53 staining [20, 34]. Overexpression of mutant p53 is frequently observed, especially in more advanced stages of the disease [18, 19]. However, in this study no clear association was found between tumour stage and p53 expression.

Previous studies have demonstrated a distinct link between aberrant p53 expression and the constitutive expression of IL-6. Especially in the HeLa cell line, it has been demonstrated that wt-p53 can repress IL-6 promoter activity by binding to NF-IL-6 [22]. Furthermore, Watson and associates found that some ovarian tumour cell lines produced high levels of IL-6 mRNA, which was associated with expression of a dysfunctional p53 [30, 35].

However, in the present study, no relationship was found between spontaneous expression of IL-6 mRNA and p53 overexpression. The discrepancy may be ascribed to the fact that mutated p53 protein can result from several gene mutations resulting in different conformations and functions of the protein. The conformation or activity of the mutant protein used in the HeLa study may differ from the mutant p53 proteins detected in this study. Furthermore, it must be taken into account that immunohistochemistry, although ideal for protein detection, is not the most optimal assay to detect specific mutations or truncated proteins. In contrast to IL-6 mRNA, M-CSF mRNA expression was correlated with p53 expression ($P < 0.05$). Of the tumours expressing M-CSF mRNA, 71% (15/21) also stained positive for p53. This could indicate that expression of the M-CSF gene is somehow stimulated in the presence of mutant p53. In addition, M-CSF was expressed more frequently in stage III/IV tumours compared with stage I/II. This could indicate a possible role for M-CSF in tumour development.

Studies with M-CSF promoter constructs have not demonstrated the presence of a NF-IL-6 binding site, which was the link between m-p53 and IL-6 promoter activation. Nuclear factor-κB (NF-κB), however, seems to be a prominent transcription factor involved in IL-6, M-CSF and TNF-α transcriptional activity. Of interest is the fact that the p50 subunit of the NF-κB transcription factor can associate with NF-IL-6 [36], suggesting an interactive process between both components. To what extent p53 can interfere in this process or creates conditions under which M-CSF overexpression can occur requires further study. In addition, TNF-α, which was found to be preferentially expressed in M-CSF producing carcinomas, can induce NF-κB, resulting in M-CSF promoter activation [37].

In this study, log-rank analysis of established adverse prognostic values were all found significant for shorter PFS. This indicates that, despite its size, this study evaluated a representative population of ovarian carcinoma patients. Statistical analysis of p53, IL-6 and M-CSF expression could reveal no prognostic value of these factors for PFS. In contrast, patients expressing TNF-α showed a trend, although not significant, for prolonged PFS compared to patients without TNF-α expression (Figure 2). The association of TNF-α expression with a better response to chemotherapy suggests a possible role for autocrine production of TNF-α in the sensitivity of human ovarian carcinoma to chemotherapy. This adds to earlier findings that cisplatin sensitivity of ovarian cancer cell lines can be modulated by the addition of exogenous TNF-α [38, 39].

In summary, this study demonstrates that human ovarian carcinomas frequently overexpress p53, localised in the nucleus and the cytoplasm, which is predominantly associated with the expression of M-CSF mRNA.

1. Berchuck A, Kohler MF, Boente MP, Rodrigues GC, Whitaker BS, Bast RC. Growth regulation and transformation of ovarian epithelium. *Cancer* 1993, 71 (Suppl.), 545–551.

2. Boente MP, Hurteau J, Rodrigues GC, Bast RC, Berchuck A. The biology of ovarian cancer. *Curr Opin Oncol* 1993, **5**, 900–907.
3. Moradi MM, Carson FL, Weinberg JB, Haney AF, Twiggs LB, Ramakrishnan S. Serum and ascitic fluid levels of interleukin-1, interleukin-6, and tumour necrosis factor-alpha in patients with ovarian epithelial cancer. *Cancer* 1993, **72**, 2433–2440.
4. Lidor YJ, Xu FJ, Martínez-Maza O, *et al.* Constitutive production of macrophage colony-stimulating factor and interleukin-6 by human ovarian surface epithelial cells. *Exp Cell Res* 1993, **207**, 332–339.
5. Li B-Y, Mohanray D, Olson MC, *et al.* Human ovarian epithelial cancer cells cultured *in vitro* express both interleukin-1α and β genes. *Cancer Res* 1992, **52**, 2248–2252.
6. Guillaume T, Sekhavat M, Rubinstein DB, Hamdan O, Symann ML. Transcription of genes encoding granulocyte-macrophage colony-stimulating factor, interleukin 3, and interleukin 6 receptors and lack proliferative response to exogenous cytokines in nonhematopoietic human malignant cell lines. *Cancer Res* 1993, **53**, 3139–3144.
7. Wu S, Boyer CM, Whitaker RS, *et al.* Tumour necrosis factor α as an autocrine and paracrine growth factor for ovarian cancer: Monokine induction of tumour cell proliferation and tumour necrosis factor α expression. *Cancer Res* 1993, **53**, 1939–1944.
8. Naylor MS, Stamp GW, Foulkes WD, Eccles D, Balkwill FR. Tumour necrosis factor and its receptors in human ovarian cancer: Potential role in disease progression. *J Clin Invest* 1993, **91**, 2194–2206.
9. Kacinsky BM, Bloodgood RS, Schwartz PE, Carter DC, Stanley ER. The macrophage colony stimulating factor CSF-1 is produced by human ovarian and endometrial adenocarcinoma-derived cell lines and is present at abnormally high levels in the plasma of ovarian carcinoma patients with active disease. *Cold Spring Harbor Symp Quart Biol* 1989, 7, 333–337.
10. Xu FJ, Ramakrishnan S, Daly L, *et al.* Increased serum levels of macrophage colony stimulating factor in ovarian cancer. *Am J Obstet Gynecol* 1991, **165**, 1356–1362.
11. Elg SA, Yu Y-H, Carson LF, *et al.* Serum levels of macrophage colony-stimulating factor in ovarian cancer patients undergoing second look laparotomy. *Am J Obstet Gynecol* 1992, **166**, 134–137.
12. Berek JS, Chung C, Kaldi K, Watson JM, Knox RM, Martínez-Maza O. Serum interleukin-6 levels correlate with disease status in patients with epithelial ovarian cancer. *Am J Obstet Gynecol* 1991, **164**, 1038–1043.
13. Van der Zee AGJ, De Cuyper EMJ, Limburg PC, *et al.* Higher levels of interleukin-6 in cystic fluids from patients with malignant versus benign ovarian tumours correlate with decreased hemoglobin levels and increased platelet counts. *Cancer* 1995, **75**, 1004–1009.
14. Price FV, Chambers SK, Chambers JT, *et al.* Colony-stimulating factor-1 in primary ascites of ovarian cancer is a significant predictor of survival. *Am J Obstet Gynecol* 1993, **168**, 520–527.
15. Wu S, Rodabaugh K, Martínez-Maza O, *et al.* Stimulation of ovarian tumour cell proliferation with monocyte products including interleukin-1, interleukin-6, and tumour necrosis factor-α. *Am J Obstet Gynecol* 1992, **166**, 997–1007.
16. Yin T, Taga T, Tsang ML-S, Yasukawa K, Kishimoto T, Yang Y-C. Involvement of IL-6 transducer gp 130 in IL-11-mediated signal transduction. *J Immunol* 1993, **151**, 2555–2561.
17. Du XX, Williams DA. Interleukin-11: a multifunctional growth factor derived from the hematopoietic microenvironment. *Blood* 1994, **83**, 2023–2030.
18. Marks JR, Davidoff AM, Kerns BJ, *et al.* Overexpression and mutation of p53 in epithelial ovarian cancer. *Cancer Res* 1991, **51**, 2979–2984.
19. Kohler MF, Kerns B-JM, Humphrey PA, Marks JR, Bast RC, Berchuck A. Mutation and overexpression of p53 in early-stage epithelial ovarian cancer. *Obstet Gynecol* 1993, **81**, 643–650.
20. Van der Zee AGJ, Hollema H, Suurmeijer AJH, *et al.* The value of P-glycoprotein, glutathione S-transferase pi, c-erbB-2 and p53 as prognostic factors in ovarian carcinomas. *J Clin Oncol* 1995, **13**, 70–78.
21. Santhanam U, Ray A, Seghal PB. Repression of the IL-6 gene promoter by p53 and the RB susceptible gene product. *Proc Natl Acad Sci USA* 1991, **88**, 7605–7609.
22. Margulies L, Seghal PB. Modulation of the human interleukin-6 promoter (IL-6) and transcription factor C/EBPβ (NF-IL-6) activity by p53 species. *J Biol Chem* 1993, **268**, 15096–15100.
23. Serov SF, Scully RE, Sobin LH. *Histological Typing of Ovarian Carcinomas.* Geneva, World Health Organization, 1973, 17–18.
24. Sobre B, Frankendal B, Veress B. Importance of histological grade in the prognosis of epithelial ovarian carcinomas. *Obstet Gynecol* 1982, **59**, 567–573.
25. Banks L, Matlashewski G, Crawford L. Isolation of human-p53-specific monoclonal antibodies and their use in the studies of human p53 expression. *Eur J Biochem* 1986, **159**, 529–534.
26. Vellenga E, Rambaldi A, Ernst TJ, Ostapovicz D, Griffin JD. Independent regulation of the M-CSF and G-CSF gene expression in human monocytes. *Blood* 1988, **71**, 1529–1532.
27. Maniatis T, Fritsch EF, Sambrook J. *Molecular Cloning: A Laboratory Manual.* New York, Spring Harbor Laboratory, 1982, 7.49–7.50.
28. Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments of high specific activity. *Anal Biochem* 1983, **132**, 6–13.
29. Brakenhoff JPJ, Groot ER, Pannekoek H, Aarden LA. Molecular cloning and expression of hybridoma growth factor in *E. coli*. *J Immunol* 1987, **139**, 4116–4121.
30. Watson JM, Sensintaffar JL, Berek JS, Martínez-Maza O. Constitutive production of interleukin 6 by ovarian cancer cell lines and by ovarian tumour cultures. *Cancer Res* 1990, **50**, 6959–6965.
31. Dokter WHA, Dijkstra AJ, Koopmans SB, *et al.* G(Anh)MTetra, a natural bacterial cell wall breakdown product, induces interleukin-1β and interleukin-6 expression in human monocytes. *J Biol Chem* 1994, **269**, 4201–4206.
32. Rogel A, Popliker M, Webb CG, Oren M. p53 cellular tumour antigen: analysis of mRNA levels in normal adult tissues, embryos, and tumours. *Mol Cell Biol* 1985, **5**, 2851–2855.
33. Levine AJ, Nomand J, Finlay CA. The p53 tumour suppressor gene. *Nature* 1991, **342**, 705–708.
34. Midgley CA, Fisher CJ, Bártek J, Vojtèsek B, Lane D, Barnes DB. Analysis of p53 expression in human tumours: an antibody raised against human p53 expressed in *Escherichia coli*. *J Cell Sci* 1992, **101**, 183–189.
35. Yaginuma Y, Weatphal H. Abnormal structure and expression of the p53 gene in human ovarian carcinoma cell lines. *Cancer Res* 1992, **52**, 4196–4199.
36. Le Clair KP, Blanar MA, Sharp PA. The p50 subunit of NF-κB associates with the NF-IL6 transcription factor. *Proc Natl Acad Sci USA* 1992, **89**, 8145–8149.
37. Yamada H, Iwase S, Mohri M, Kufe D. Involvement of a nuclear factor-κB-like protein in induction of the macrophage colony-stimulating factor gene by tumour necrosis factor. *Blood* 1991, **78**, 1988–1995.
38. Mizutani Y, Bonavida B. Overcoming *cis*-diamminedichloroplatinum (II) resistance of human ovarian tumor cells by combination treatment with *cis*-diamminedichloroplatinum (II) and tumor necrosis factor-alpha. *Cancer* 1993, 72, 809–818.
39. Uslu R, Bonavida B. Involvement of the mitochondrion respiratory chain in the synergy achieved by treatment of human ovarian carcinoma cell lines with both tumor necrosis factor-α and *cis*-diamminedichloroplatinum. *Cancer* 1996, 77, 725–732.

Acknowledgements—We wish to thank Drs L.A. Aarden, M.A. Brach, S.C. Clark and S. Gillis for providing the cytokine cDNAs.