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Original Paper

Transforming Growth Factor-β Isoform Expression in Human Ovarian Tumours

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The expression patterns of members of the transforming growth factor-β (TGF-β) family were analysed in 96 primary ovarian tumours by RNAse protection assay. mRNA for the three mammalian isoforms, TGF-β1, TGF-β2 and TGF-β3, was detected in 46, 66 and 66% of 74 malignant tumours, respectively, with the predominant patterns of expression being either dual or triple co-expression. TGF-βII receptor expression, detected by reverse-transcription PCR, was present in 92% malignant tumours. Expression patterns were similar between malignant, borderline and benign tumours, although TGF-β1 incidence was reduced in benign tumours. In malignant tumours, the incidence of TGF-β1 expression was less than that of either TGF-β2 ($P = 0.02$) or TGF-β3 ($P = 0.0014$), while in both malignant and borderline tumours, TGF-β2 and TGF-β3 tended to be co-expressed. Aneuploid tumours were more likely than diploid tumours to express multiple rather than single forms of TGF-β ($P = 0.018$). The incidence of TGF-β1 expression was reduced in PR-moderate/rich (PR > 20 fmol/mg protein) relative to PR-negative/poor tumours ($P = 0.048$), while TGF-β3 expression was increased in ER-moderate/rich (ER > 20 fmol/mg protein) tumours compared to ER-negative/poor tumours ($P = 0.0012$). Expression of TGF-β3, but not TGF-β1 or TGF-β2, was associated with advanced stage disease ($P = 0.014$) and, in the malignant group, reduced survival ($P = 0.02$) with a hazard ratio of 2.6. These data suggest a possible role for TGF-β3 in the progression of ovarian cancer. © **1997 Elsevier Science Ltd.**

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INTRODUCTION

THE TRANSFORMING growth factor-β family of polypeptide growth factors is involved in cell growth regulation, tissue remodelling and immune suppression [1]. Three forms of TGF-β have been identified in human systems, namely TGF-β1, TGF-β2 and TGF-β3, and these exist as homodimeric chains of between 111 and 113 amino acids, with molecular weights of 25 kDa. These growth factors interact with cell surface serine–threonine kinase linked receptors which mediate their tissue regulatory effects [2, 3]. The TGF-β isoforms bind directly to the TGF-βII receptor, whereupon the type I receptor is recruited into the complex, becomes phosphorylated and in turn propagates the signal to downstream substrates [2].

Originally identified from the culture media of transformed cells, evidence has accumulated to suggest that TGF-β may have a central role in many aspects of transformed cell function [1]. The immune suppressive and tissue modelling activities of these growth factors suggest a role in tumour metastasis, while angiogenic effects may influence tumour growth [1]. However, in most epithelial systems, TGF-βs have been shown to inhibit tumour cell proliferation [1]. Clearly, therefore, in any *in vivo* system, a complex interaction between stromal and tumoral effects of these factors will exist.

The TGF-β family has been shown to inhibit growth in ovarian carcinoma cell lines *in vitro* [4–9] and has been

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implicated in the regulation of normal ovarian function and granulosa cell proliferation [10]. TGF-β 1 has been shown to inhibit the growth of both normal and malignant ovarian epithelial cells grown in monolayer culture [11] and to induce apoptosis in malignant cultures [12].

However, the expression patterns of TGF-β isoforms in primary ovarian cancer and their relationships with clinical or pathological features have not previously been reported. With interest focusing on the role of TGF-β in tumour behaviour and its signalling pathways, this study sought to investigate the patterns of TGF-β isoform expression in primary ovarian tumours using RNAse protection and reverse transcription–polymerase chain reaction (RT–PCR) assays and their relationships with tumour behaviour.

MATERIALS AND METHODS

Patients

Ovarian tumour material was collected from patients undergoing surgery at the Eastern General Hospital, Edinburgh, the Royal Infirmary of Edinburgh and the University Hospital, Turin. Tumour samples were collected at the time of primary surgery, frozen in liquid nitrogen and stored at −180°C until used. Tumour histology and grade were assessed on paraffin-embedded sections and classified according to WHO criteria [13]. 96 patients with histologically confirmed primary ovarian tumours were included in this study and their tumours were classified into the following histologies: 74 malignant, 7 borderline and 15 benign. The malignant group consisted of 32 serous, 23 endometrioid, 4 mucinous, 5 clear cell carcinomas, 3 malignant mixed mesodermal tumours and 7 others (including a steroid cell tumour, a teratoma, a Leiomyosarcoma, a granulosa cell tumour, 2 undifferentiated tumours and 1 of mixed histology). The 7 borderline tumours consisted of 2 of serous and 5 of mucinous histology; the 15 benign included 7 of epithelial origin (5 mucinous, 1 serous and 1 Brenner tumours), 4 fibromas, 1 thecoma, 1 dermoid cyst, 1 granulosa cell tumour and 1 teratoma. Cell pellets from 8 patients with ovarian ascites were also analysed. All 8 of these were derived from serous primary tumours and 2 of these were obtained from individuals whose primary tumours were also investigated. Patients with ovarian cancer were staged according to the International Federation of Gynecology and Obstetrics (FIGO) criteria [14].

Survival data were available for 69 of the patients with malignant tumours (of whom 60 were epithelial). Of these 69 patients, 41 patients received cisplatin-containing regimens, 8 received chlorambucil monotherapy and one was treated with ^{32}P intraperitoneal radiotherapy. 19 received no further treatment after surgery (mainly stage Ia, grade 1 disease). No patient received therapy before surgery.

mRNA extraction

Total cellular RNA was extracted from frozen tissue using the lithium chloride/urea method [4]. Prior to RNA extraction, 400 mg of tumour tissue was homogenised using a tissue dismembrator (Braun, Germany) at −20°C.

Synthesis of riboprobes

Labelled RNA was prepared from linearised template DNA using a Gemini II system (Promega Ltd, Southampton, U.K.). Template DNA was incubated in the presence of an RNAse inhibitor (human placental RNAsin; Amersham plc, Amersham, U.K.), cold ribonucleosides, dithiothreitol and ^{32}P-rCTP with the appropriate RNA polymerase (T3, T7 or SP6) for 1 h at 37°C. The DNA template was then removed by incubation with RQ1 DNAse (Promega) for 15 min at 37°C. Labelled RNA was precipitated in the presence of added tRNA (Sigma) as carrier and full-length transcripts were isolated by polyacrylamide gel electrophoresis. Following identification of full-length transcripts by autoradiography, the bands were excised, and labelled RNA was eluted from the gel, precipitated under ethanol and resuspended in hybridisation buffer prior to use in RNAse protection assays.

RNAse protection assays

mRNA for the individual isoforms of TGF-β was evaluated using RNAse protection assays. This assay is more sensitive and specific (100% homology is required) than Northern blot analysis and assays were performed as described previously [4, 15]. Briefly, test RNA (20 μg) was precipitated under ethanol, dried and resuspended in 30 μl hybridisation buffer (80% formamide, 40 mM Pipes (pH 6.7), 400 mM NaCl, 1 mM EDTA); tRNA was prepared in a similar manner as a negative control. Test probe (10^6 c.p.m.) plus γ-actin probe (10^6 c.p.m.) were added to each sample. Samples were incubated at 85°C for 20 min, transferred to a water bath and left to hybridise overnight at 51°C. After hybridisation, single-stranded RNA (both labelled and cold) was removed by incubating with single-stranded specific RNAses A and T1 (Boehringer Mannheim, Lewes, U.K.) at 37°C for 30 min, followed by incubation with proteinase K in sodium dodecyl sulphate (SDS) at 37°C for 15 min. Protein was extracted by using phenol/chloroform-isoamyl alcohol. Double-stranded probe: test RNA was precipitated with carrier tRNA (5 μg) and separated by gel electrophoresis. Tumour samples were scored positive for TGF-β mRNA expression when a protected fragment RNA of the correct size (see Figure 1) was identified by autoradiography in the presence of a positive signal for γ-actin.

Reverse transcription–polymerase chain reaction (RT–PCR)

RT–PCR for Type II TGF-β receptor and γ-actin were carried out using a Techne PHC-3 thermocycler. For the reverse transcription assay, 20 μg aliquots of total cellular RNA were reverse transcribed by incubation with 300 ng of a random hexamer oligonucleotide with 2 mM each of dATP, dTTP, dCTP and dGTP (Pharmacia, U.K.), 200 units of Superscript reverse transcriptase (Life Technologies, Paisley, U.K.) for 1 h at 42°C in a total volume of 20 μl. Reverse transcribed RNA was stored at −20°C prior to analysis by PCR.

For all PCRs, 0.2–1 μl of reverse transcribed RNA were added to 100 ng of each primer in a volume of 50 μl. Prior to PCR, this reaction was heated to 94°C for 10 min and then cooled rapidly to 4°C. PCR reactions were performed in a final volume of 100 μl containing the following: 0.5 units of TAQ polymerase (Promega), 1.25 μM dATP, dTTP, dCTP and dGTP (Pharmacia, U.K.), 100 ng of each primer, 50 mM KCl, 10 mM Tris–HCl, 0.1% Triton-X and 2.5 mM $MgCl_2$. Reactions were overlaid with 100 μl paraffin oil.

The amplification reaction was carried out over 40 cycles with the following parameters: Step 1, 94°C for 38 s; Step 2, 50 C for 53 s; Step 3, 72°C for 68 s. For the final cycle, the 72°C step was extended to 7 min to ensure all transcripts were full length. The following primers were used in these reactions:

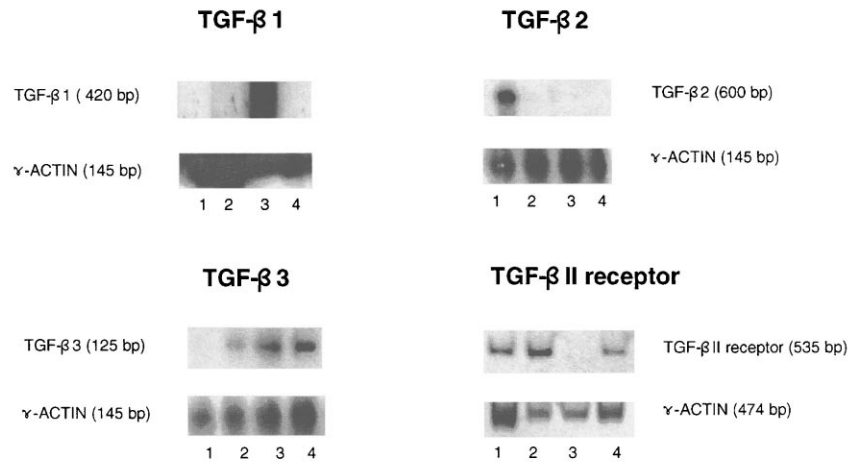

Figure 1. TGF-β mRNA expression in human ovarian tumours. The RNAse protection assay was used to detect TGF-β1, TGF-β2 and TGF-β3 mRNAs and RT–PCR was used to identify TGF-βII receptor mRNA. Representative data are shown. For TGF-β1, only lane 3 contains a positive transcript, while for TGF-β2 lane 1 contains a positive transcript. For TGF-β3, lanes 3 and 4 contain strong positive signals with a weak signal in lane 2. TGF-βII receptor mRNA was present in lanes 1, 2 and 4. All samples shown demonstrate signals for γ-actin confirming the integrity of RNA samples. Transcript sizes are indicated.

TGF-β II receptor primers (accession number M85079 in GenBank database):

Sense 5′-ACTGTGCCATCATCCTGG-3′ (positions 979–996)
Antisense 5′-GCAGGTTAGGTCGTTCTTCACG-3′ (positions 1514–1535)

Expected transcript size = 535 base pairs.

γ-Actin primers (accession number M16247 in GenEMBL database):

Sense 5′-ACGAGACCACCTTCAACTCC-3′ (positions 849-868)
Antisense 5′-CAAGTTCTACAATCCAGTGC-3′ (positions 395–414)

Expected transcript size = 474 base pairs.

PCR products were visualised after electrophoresis on polyacrylamide gels and staining with ethidium bromide. Tumours were scored as positive for TGF-βII receptor when a PCR product of the expected molecular size was amplified and identified following electrophoresis. Samples were sized using a 100 bp ladder (Gibco, U.K.). As an additional control, PCR of a known housekeeping gene γ-*actin* was performed to establish the integrity of transcribed RNA. Use of these mRNA samples in other studies wherein the target mRNA was not observed indicated that genomic DNA contamination could be ruled out.

Measurement of oestrogen receptor (ER) and progesterone receptor (PR)

Tissue fragments (50–200 mg) were weighed, pulverised in liquid nitrogen and resuspended in buffer (10 mM Tris, 0.25 M sucrose, 1 mM ethylenediaminetetracetate, pH 8.0 at 22°C, plus 1% monothioglycerol and 10% v/v glycerol). After centrifugation at 105 000 *g*, the supernatant cytosol was assayed by enzyme immunoassay using ER–EIA and PR–EIA kits (Abbot Ltd, Basingstoke, U.K.) according to the manufacturer's instructions. The protein content of the cytosol was determined by the Bradford method [16].

Ploidy analysis

Tumours were treated with trypsin/detergent and DNA was stained with propidium iodide [4]. Samples were then analysed on a FACScan flow cytometer (Becton Dickinson) equipped for doublet discrimination using Cellfit software. Those samples showing a single G0/G1 peak were classified as DNA diploid, whereas those demonstrating additional G0/G1 peaks were classified as aneuploid. Ploidy data were obtained for 66 of the 74 malignant tumours.

Statistics

Differences between subgroups in contingency tables were analysed by Fisher's exact test. Differences of survival used the graphical Kaplan–Meier method and groups were compared using the log-rank test. Cox regression analysis was used with stage, residual disease, grade and histology included among the variables in the initial step of a backward stepwise selection procedure to assess if the TGF-β mRNA variables were independently prognostic for survival. The results are given as hazard ratios. A hazard ratio of 2 for a given marker indicates that the risk of death at any time for a patient with that marker is twice that of a patient without the marker, all other prognostic variables being the same [17].

RESULTS

Expression of mRNA for TGF-β1, TGF-β2, TGF-β3 isoforms and TGF-βII receptor in ovarian tumours

TGF-β mRNA isoform expression was investigated by RNAse protection assay in all 74 malignant primary tumours and typical examples of transcript signals are shown in Figure 1; 34 (46%) expressed TGF-β1 mRNA, 49 (66%) expressed TGF-β2 mRNA and 49 (66%) expressed TGF-β3 mRNA (Table 1 and Figure 2). The incidence of TGF-β1 expression was significantly less than that of either TGF-β2 ($P = 0.02$) or TGF-β3 ($P = 0.0014$). TGF-βII receptor mRNA expression was detected by PCR in 92% of tumours examined (60 of 65).

Of the 7 borderline tumours, 5 (71%) expressed TGF-β1, 4 (57%) expressed TGF-β2 and 4 (57%) expressed TGF-β3

Table 1. *TGF-β isoform and TGF-β II receptor mRNA expression in primary ovarian tumours*

Tumour type	Group	TGF-β1	TGF-β2	TGF-β3	TGF-βII receptor
Malignant ($n=74$)	All	34/74 (46%)	49/74 (66%)	49/74 (66%)	60/65 (92%)
Epithelial ($n=64$)	Serous	16/32*	20/32	22/32	28/31
	Endometrioid	11/23	15/23	13/23	18/20
	Mucinous	1/4	3/4	3/4	4/4
	Clear cell	3/5	4/5	4/5	3/3
	Stages I and II	11/22	17/22	11/22	18/21
	Stages III and IV	21/39	24/39	31/39	37/41
	Well differentiated	2/7	6/7	4/7	5/7
	Moderately differentiated	7/17	12/17	13/17	13/15
	Poorly differentiated	22/36	22/36	23/36	32/33
Non-epithelial ($n=10$)	All pathologies	3/10 (30%)	7/10 (70%)	7/10 (70%)	7/7 (100%)
	Stage I and II	0/3	2/3	1/3	3/3
	Stages III and IV	1/5	4/5	4/5	2/2
	Well differentiated	0/1	0/1	0/1	—
	Moderately differentiated	0/1	1/1	1/1	1/1
	Poorly differentiated	1/3	2/3	3/3	1/1
Malignant ascites ($n=8$)	Epithelial	5/8 (63%)	1/8 (13%)	5/8 (63%)	7/8 (88%)
Borderline ($n=7$)	Epithelial	5/7 (71%)	4/7 (57%)	4/7 (57%)	6/7 (86%)
Benign ($n=15$)	All	3/15 (20%)	8/15 (53%)	9/15 (60%)	9/11 (82%)
	Epithelial	0/7	3/7	3/7	3/5
	Non-epithelial	3/8	5/8	6/8	6/6

*Ratios shown are number of tumours positive/number assayed.

(Table 1 and Figure 2). TGF-βII receptor mRNA was detected in 6 (86%) tumours.

Of the 15 benign tumours studied, TGF-β1 expression was found in 3 (20%), TGF-β2 in 8 (53%) and TGF-β3 in 9 (60%) tumours (Table 1 and Figure 2). Comparison of the total benign group (which consisted of 7 epithelial and 8 non-epithelial tumours) with malignant or borderline groups revealed no significant differences in the patterns of expression, but comparison of the epithelial tumours alone (64 malignant tumours, 7 borderline tumours and 7 benign tumours) indicated that benign epithelial tumours were significantly less likely to express TGF-β1 (0 of 7 positive) than their malignant (31 of 64 positive; $P=0.016$) or borderline (5 of 7 positive; $P=0.021$) counterparts. TGF-βII receptor was expressed in 82% (9 of 11) benign tumours.

TGF-β expression was also investigated in samples of malignant ascites cells from 8 patients. Of these, 5 (63%) were positive for TGF-β1, 1 (13%) was positive for TGF-β2, 5 (63%) were positive for TGF-β3 and 7 (88%) were positive for TGF-βII receptor. The incidence rates of TGF-β1 and TGF-β3 were similar to those found in malignant primary tumours but that for TGF-β2 was significantly lower (13% versus 66%; $P=0.005$). For two of these ascites, the primary tumours were also investigated; in 1 patient TGF-β2 expression in the primary was positive, but was then negative in a subsequent ascites sample, in the other patient, both primary and subsequent ascitic samples were negative.

Co-expression of TGF-β isoforms

The co-expression patterns of the different isoforms in this tumour series are illustrated in Figure 3. The predominant

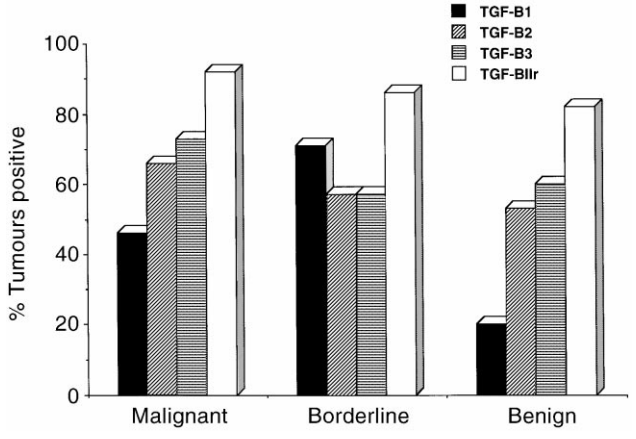

Figure 2. TGF-β isoforms and TGF-βII receptor mRNA expression in primary ovarian tumours. 74 malignant, 7 borderline and 15 benign tumours were examined. TGF-β1, -β2 and -β3 mRNA expression were assayed by RNAse protection assay, TGF-βII receptor was detected by RT-PCR.

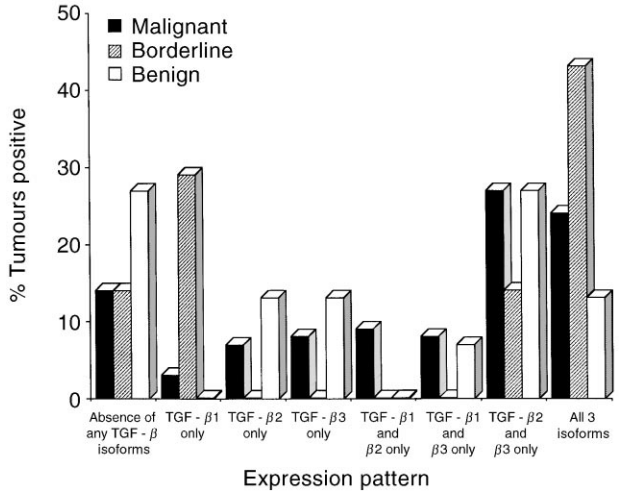

Figure 3. Co-expression of TGF-β isoform mRNA in primary ovarian tumours.

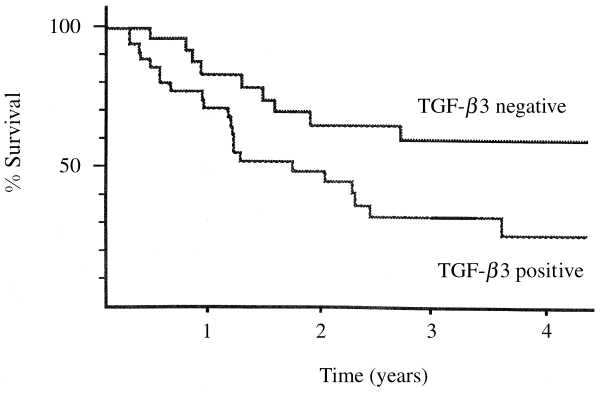

Figure 4. Relationship of TGF-β3 expression to survival in epithelial ovarian cancer. Data for 60 patients are shown, of whom 35 were TGF-β3 positive and 25 were TGF-β3 negative. The difference between the two curves was significant; $P = 0.032$, log-rank test.

pattern found was expression of multiple isoforms of TGF-β (in 64% of malignant tumours) and only rarely was TGF-β expression not detected (in 11% of tumours) although γ-actin RNA was demonstrated in all specimens. A significant association was found between the expression of TGF-β2 and expression of TGF-β3 in both malignant ($P = 0.017$) and borderline tumours ($P = 0.029$). No significant association was found between expression of TGF-β1 and either TGF-β2 or TGF-β3 in any of these groups.

Relationship of TGF-β with clinicopathological factors

Analysis of the different TGF-β isoforms in the various histological subtypes of epithelial ovarian cancer indicated no major differences in expression between different histologies or grades of differentiation (Table 1). However, advanced stage tumours (stages III/IV) were more likely to be positive for TGF-β3 mRNA expression than early stage (I/II) tumours (80% versus 48% positive, respectively; $P = 0.014$).

Analysis of the ploidy of this series of malignant tumours indicated that aneuploid tumours were more likely to express multiple as opposed to single isoforms of TGF-β than diploid tumours (37 of 46 aneuploid tumours contained multiple expression versus 10 of 20 diploid tumours; $P = 0.018$).

Relationship with oestrogen receptor and progesterone receptor

Oestrogen and progesterone receptor concentrations were available for 64 of the 74 malignant tumours. The incidence of TGF-β3 expression was increased in ER-moderate/rich (> 20 fmol/mg protein) tumours compared to ER-negative/poor (< 20 fmol/mg protein) tumours (74% versus 44%, respectively; $P = 0.0012$). There was no association between ER status and either TGF-β1 ($P = 0.46$) or TGF-β2 ($P = 0.61$). In contrast, a weak association between PR-negative/poor (< 20 fmol/mg protein) and TGF-β1 expression was present with 57% (27 of 47) of PR-negative poor tumours expressing TGF-β1 compared with 29% (5 of 17) of PR-moderate/rich tumours ($P = 0.048$).

Relationship of TGF-β isoforms with survival

Analysis of the survival of this group of patients by univariate analysis indicated that the patients whose tumours were positive for TGF-β3 had reduced survival compared to those patients whose tumours were negative ($P = 0.032$ for epithelial cancers, $n = 60$, Figure 4; $P = 0.045$ for all malignant tumours, $n = 69$; log-rank test). In contrast, the presence or absence of TGF-β1 ($P = 0.31$), TGF-β2 ($P = 0.25$) or TGF-βIIr ($P = 0.84$) was not significantly related to survival for either the epithelial cancers alone or the complete malignant group (P values shown are for epithelial cancers). Multivariate analysis of TGF-β3 along with other prognostic variables (including debulking, stage, histology and grade) disclosed debulking and TGF-β3 as independently prognostic variables with hazard ratios of 4.9 for non-debulked versus debulked disease (2.3–10.5, 95% confidence interval; $P \leq 0.001$) and 2.6 for TGF-β3 positive versus negative disease (1.2–5.6, 95% confidence interval; $P = 0.02$).

DISCUSSION

The data presented here represent the first investigation of the expression of TGF-β isoforms in primary ovarian cancer and ascites. In total, 104 samples were examined representing the major subtypes of ovarian tumours, and comparisons with clinicopathological and oestrogen and progesterone receptor content were made. Overall, expression rates of individual isoforms of TGF-β were variable ranging from 46 (for TGF-β1) to 66% (for TGF-β2 and TGF-β3) for the expression of the individual isoforms in malignant tumours; absence of expression for all three isoforms was found in only 11% of tumours. The predominant patterns of expression consisted of either dual expression of TGF-β2 and -β3 or triple expression of TGF-β1, -β2 and -β3 and this is compatible with data obtained from our previous study of isoform expression in ovarian cancer cell lines [4]. The incidence of expression for these isoforms was comparable for malignant, borderline and benign tumours, although TGF-β1 expression was significantly lower in the malignant and benign groups relative to that of TGF-β2 and TGF-β3 and these latter two isoforms were frequently co-expressed. The lower rate of expression of TGF-β1 relative to the other forms contrasts with our findings in breast cancer, where all three forms were equally expressed [15]. The expression of TGF-β receptors was observed in almost all tumours, even when limited to mRNA for a single class of receptor. Tumours expressing one or more TGF-β isoforms together with receptor mRNA comprised over 80% of this tumour population, indicating the potential for TGF-β regulation in most tumours. The actions of TGF-βs are known to be mediated by complex interactions between their receptor/binding proteins [2]. Despite this diversity of signals, the TGF-βII receptor appears central to cellular signalling for the TGF-β receptor complex [2, 18]. The downstream consequences of TGF-β receptor activation are not yet fully known, but evidence supports a central role for TGF-β in the control of cellular apoptosis and cell cycle control [18]. Evidence from many studies, including our own [4], support a role of TGF-β in blocking the cell cycle late in G1, and the pathways by which this activity may be controlled has recently been identified [18]. The ability of TGF-β1 to induce apoptosis, possibly via modification of the Bcl2/Bax ratio, within cells has also been established [12, 19]. This duality of function would be consistent with a role for TGF-β in the control of cellular proliferation by shifting cells from proliferative to apoptotic pathways.

TGF-β expression is regulated by oestrogen in a variety of normal and malignant tissues and to investigate the possibility that expression *in vivo* was under oestrogen control, the relationship between ER and PR expression and TGF-β was

examined in this series of tumours. A significant association was observed between ER expression and the presence of TGF-β3, but not with the other two isoforms, suggesting the possibility that TGF-β3 expression is regulated by oestrogen. Takahashi and associates have recently reported that all three isoforms of TGF-β are regulated by oestrogen in reproductive tract tissues of the mouse and, of these three forms, TGF-β3 is the first to be upregulated upon exposure to oestrogen [20]. Experimental studies of oestrogens and anti-oestrogens in breast cancer cell lines also indicate that TGF-β2 and TGF-β3 are more likely than TGF-β1 to be under oestrogen control [21, 22]. This possible association between oestrogen receptor and TGF-β3 requires further investigation in ovarian cancer cell lines. The progesterone receptor was found to be inversely associated with TGF-β1 expression and this is also consistent with the observation of TGF-β1 regulation by progestins in breast cancer cells [23].

Comparison of primary malignant tumours with a small series of malignant ascites indicated a reduced rate of expression of TGF-β2 in ascites relative to the primary tumour group, suggesting that reduction of this isoform might be associated with tumour spread; alternatively the expression of TGF-β2 in the primary tumour may be associated more with stromal and endothelial components than malignant epithelial cells and these will not be seen in the ascitic samples. Of the three isoforms, however, TGF-β3 appeared to be most strongly associated with disease progression. Analysis of the relationship between TGF-β expression and clinicopathologic parameters revealed that TGF-β3 was significantly more likely to be expressed in advanced stage than in early stage tumours. Furthermore, the survival of patients with tumours positive for TGF-β3 was significantly reduced compared to those whose tumours were negative. These data support a positive role for TGF-β3 in ovarian tumour progression *in vivo* and this is in line with studies in breast cancers where associations between TGF-β isoforms (not necessarily TGF-β3) and disease progression have been observed. For example, an immunohistochemical study of biopsies from a series of breast cancer patients has shown that increased expression of TGF-β1 was associated with a greater likelihood of progression [24]. Therefore, while these factors can clearly act as growth inhibitors on ovarian carcinoma cells when cultured *in vitro* [4–9, 11, 12], their positive effects on processes such as angiogenesis, immunosuppression and the extracellular matrix may enhance the progression of established ovarian tumours *in vivo* [24–26]. In a recent study, investigating the expression and distribution of TGF-β mRNA isoforms in breast cancers by *in situ* hybridisation, TGF-β3 expression, although seen in malignant epithelial cells, was most strongly associated with blood vessels, compatible with a role in mesenchymal tissues [27]. Since all three isoforms have marked sequence homology and are likely to have very similar effects on growth regulation, it is perhaps the differences in distribution within tumours (and TGF-β3's possible localisation in the stroma) that produces differential effects on tumour progression. To determine whether this is the explanation will require immunohistochemical or *in situ* testing on ovarian cancer sections. Although these isoforms share a 64–85% amino acid homology [28], the differential expression in many tissue and tumour types suggests not only differential regulation of these genes, but also different functions of the gene products. Differential biological effects have been demonstrated in, for example, wound repair wherein TGF-β1 and TGF-β2 induce cutaneous scarring while TGF-β3 inhibited this effect [29]. Differences in potency have also been observed, for example, TGF-β3 was more potent than TGF-β1 or TGF-β2 at inhibiting DNA synthesis in keratinocytes [30]. The data reported here suggest that TGF-β3 may have a more important role in ovarian cancer progression than the other isoforms and that its expression may be regulated by oestrogen.

In this study, mRNA expression has been investigated by the sensitive and highly specific techniques of RNAse protection assay and RT–PCR as this has allowed identification of individual isoforms. As interesting associations have been identified with clinical and pathological parameters with only a qualitative assessment of expression data, it seems likely that improved quantification of levels of expression of each isoform might yield more detailed information. It will also be valuable to explore expression at the protein level but this will require antibodies which are totally specific for each isoform.

In conclusion, this is the first report of the isoform expression of TGF-β in ovarian cancer. The majority of ovarian tumours express multiple isoforms of TGF-β, most commonly TGF-β2 and TGF-β3. The expression of TGF-β3 related to advanced stage, while univariate and multivariate analysis indicated that patients with tumours positive for TGF-β3 were likely to have reduced survival with a 2.6-fold increased risk of death. While previous studies of ovarian cancer have demonstrated a direct growth inhibitory effect of TGF-β on ovarian cancer cells grown in culture, the present study suggests that the situation in clinical tumours may be more complex and the pleiotropic effects of TGF-β on other cell types may enhance the progression of this disease. The nature of these effects remains to be determined.

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