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Urinary Excretion of Growth Factors in Patients with Ovarian Cancer

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The levels of epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) were analysed in 24-h urine samples from patients with ovarian malignancies, benign ovarian tumours, and healthy controls by specific radioimmunoassays. No significant difference in total urinary immunoreactive EGF excretion between the groups was detected. However, 79% (23/29) of the patients with ovarian carcinomas excreted TGF-α (median 12.6 pmol/24 h), whereas only 17% (2/12) of the patients with benign ovarian tumours and 23% (3/13) of the controls did so. The difference between cancer patients and controls was highly significant ($P < 0.001$). Analyses of the urine samples separated by gel filtration revealed a greater molecular heterogeneity of EGF and TGF-α in cancer patients than in controls. High and low molecular weight forms of EGF were able to bind to the EGF receptor and to induce anchorage-independent growth. After surgical reduction of the tumour, a distinct decrease of urinary high molecular weight forms was observed. Thus, some macromolecular growth factors seem to be associated with epithelial ovarian carcinomas.

Key words: ovarian carcinoma, urinary, transforming growth factor-α, epidermal growth factor, high molecular weight form

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INTRODUCTION

OVARIAN CANCER has the highest mortality of the gynaecological cancers [1], with an overall 5-year survival rate of only 20–30%. The lack of symptoms, difficulties in early diagnosis, insufficient tumour markers [2] and lack of information on ovarian tumour biology contribute to the poor prognosis of ovarian cancer patients.

Ovarian cancer cells, like many other malignant cells, can produce and secrete growth factors. Bauknecht and associates [3] have shown enhanced epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) levels in human ovarian carcinoma tissue compared to non-malignant tissue. Moreover, a TGF-α-mediated autocrine loop was identified in ovarian cancer tissue [4, 5], supporting the concept that ovarian tumour development and growth may be related to inappropriate growth factor regulation. Although the secretion of growth factors has been investigated in detail *in vitro*, the clinical significance of the secretion of growth factors *in vivo* is not well understood.

The increased excretion of growth factors (EGF and TGF-α) into effusions, plasma and urine by tumour patients was described by several authors [6–12]. The elevated excretion of TGF-α into effusions of ovarian cancer patients was described by Arteaga and colleagues [13]. Sherwin and associates [14] reported increased urinary levels of TGF-α in a group of tumour patients, including 1 patient with ovarian carcinoma.

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The aim of this study was to characterise growth factors in 24-h urines of ovarian cancer patients, to quantify the renal elimination of growth factors and to analyse their immunological and biological properties. It was of additional interest to compare the ovarian cancer patients with patients with benign ovarian tumours and healthy women. We report the experimental strategy used to characterise the growth factors, TGF-α and EGF, after renal excretion, with respect to their molecular distribution and biological activity.

MATERIALS AND METHODS

Cell lines

49F, a clone of fibroblastic cells from the normal rat kidney line NRK, and epidermoid cancer cells (A431) were routinely maintained in humidified 5% CO_2/95% air at 37°C. Standard growth medium (GM) was based on Eagle's minimal essential medium (Biochrom, Berlin, Germany) and was enriched with 2.5 mg transferrin/l (Sigma, München, Germany), 67 mg gentamicin sulphate/l (Biochrom), 40 U insulin/l (Hoechst, Frankfurt, Germany) and 10% fetal calf serum (FCS; Boehringer-Mannheim, Germany). The serum-free medium used for testing receptor binding contained standard growth medium with 0.1% bovine serum albumin (Sigma), 4 mM L-glutamine (Merck, Darmstadt, Germany), 1 mM sodium pyruvate, 0.25 μM glycyl-L-histidyl-L-lysine acetate (Serva, Heidelberg, Germany), 10 nM 3,3′, 5-triiodo-DL-thyronine (Sigma) and 5 mg fetuin/l (Sigma).

Growth factors

Recombinant human EGF (rhEGF) as well as ^{125}I-labelled hEGF (specific activity approximately 1300 Ci/mmol) were obtained from Amersham/Buchler (Braunschweig, Germany).

TGF-β, purified from human platelets, platelet-derived growth factor AB (PDGF AB) (Sigma), rhTGF-α and insulin-like growth factor-I (IGF-I) (Bissendorf, Hannover, Germany), fibroblast growth factor (FGF) (Paesel and Lorei, Frankfurt, Germany) and bovine insulin (Hoechst) were used.

Collection and storage of urine samples

Individual urine samples were collected for a period of 24 h from patients with ovarian tumours as well as healthy, age-matched, female volunteers. The collection of 24-h urine samples started 1 day before removal of the tumour, and was repeated once within 10–20 days after operation. The stage of each tumour was determined by pre-operative diagnostic procedures, intra-operative inspection and histopathological assessment following surgery. The urine was centrifuged at 200 *g* for 10 min to remove cellular debris and stored at −80°C. These original urine samples were adjusted to 1 M acetic acid and stored at 4°C overnight to precipitate acid insoluble proteins. The samples were centrifuged at 1500 *g* for 10 min and concentrated 10–20-fold in an Amicon concentrator (Amicon, Witten, Germany) equipped with a 1000 cut-off cartridge. Known amounts of EGF and TGF-α were treated in the same manner as the urine samples. As a result, approximately 80% of the growth factors were found after concentration and purification.

Gel chromatography

Approximately 6 ml of concentrated urine were applied to a 2.6 × 100 cm column containing Sephadex G-100 SF (Pharmacia, Uppsala, Sweden), and eluted with 0.1 M acetic acid. Fractions of 5.7 ml were collected. The column was calibrated in separate runs with dextran blue, serum albumin, carbonic anhydrase, cytochrome c and aprotinin (Sigma), and with ^{125}I-labelled hEGF and hTGF-α. For further analyses, fractionated samples were lyophilised and resuspended in 0.5 ml phosphate buffer pH 7.3 containing 0.1% BSA.

Radioimmunoassays

The radioimmunoassay (RIA) kits for human EGF from Amersham and the TGF-α RIA-kits from Peninsula (Merseyside, U.K.) were used according to the manufacturers instructions. Assays were routinely performed in duplicate on multiple dilutions of the same sample. The EGF antiserum showed no crossreactivity with human TGF-α. The TGF-α antiserum did not detect 100-fold concentrations of either EGF or the EGF receptor. The TGF-α antibody detected human TGF-α as well as rat TGF-α, confirming the 100% crossreactivity stated by the manufacturer.

No inhibitory substances were present in urines as evaluated by measuring the urine samples alone and in combination with known amounts of EGF and TGF-α. The urinary EGF and TGF-α levels in the crude urines were compared to the concentration after acid treatment, dialysis and lyophilisation, and a median of 67% of the growth factors was detected in the concentrated urines compared to the crude urines.

Radioreceptor assay

The radioreceptor assay was performed as decribed by DeLarco and Todaro [15]. Briefly, A431 cells were seeded in multiple four-well cluster dishes (Nunc, Roskilde, Denmark) at a density of 3 × 10^4 cells/well in standard growth medium. After a 1-day attachment phase, the medium was removed and serum-free medium was added. On the next day, cells were washed once with serum-free medium containing 25 mM HEPES (binding buffer) at 4°C. Binding buffer (250 μl), containing 7.5 fmol [^{125}I]EGF and various concentrations of the samples or the EGF standard, were added. After a 3-h incubation period at 4°C in 5% CO_2/95% air, the cells were washed twice with binding buffer, detached with 0.5 ml trypsin/EDTA solution (2000 U/l trypsin) and transferred to counting vials. Non-specific binding was determined with a 1000-fold excess of unlabelled EGF. The EGF radioreceptor assay responds to all members of the EGF family including EGF and TGF-α. K_d values of 1.23 and 2.84 nM were calculated for rh-EGF and hTGF-α, respectively. Assays were performed in duplicate. In the radioreceptor assay, serial dilutions of the samples (crude urines, concentrated urines, urine fractions) inhibited the binding of [^{125}I]EGF to the EGF receptor in a similar way as authentic EGF.

Assay for anchorage-independent growth

Normal rat kidney fibroblasts (20 000 cells) were suspended in 1 ml of GM containing 7.5% FCS and 0.3% agarose (FMC Bio Product, Rockland, U.S.A.) and serial dilutions of test samples. This 1-ml top layer was added to a 1-ml bottom layer of GM containing 7.5% FCS and 0.5% agarose in 35-mm plastic Petri dishes (Greiner, Nürtingen, Germany) as reported by Rizzino [16]. TGF-α/EGF activities were determined alone or in the presence of 250 pg/ml TGF-β. Plates were incubated for 10–14 days in a 5% CO_2/95% air atmosphere at 37°C. Colonies larger than 60 μm in diameter were counted by an Omnicon Image analysis system (BioSys GmbH, Karben, Germany), programmed to count a 5-cm^2 area of a 3.5-cm diameter plate.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Concentrated urine samples were dialysed twice over 2 days against 25 mM Tris-HCl buffer pH 7.5 before electrophoresis. Samples were solubilised in 25 mM Tris-HCl buffer pH 6.8 containing 12% SDS and 10% glycine by heating at 100°C for 10 min and analysed on 15% polyacrylamide gels containing 0.1% SDS with a discontinuous buffer system [17]. Protein standards (rainbow marker) were obtained from Amersham. Gels were cut into 6×6-mm slices after electrophoresis, and incubated with radioimmunoassay (RIA) buffer for 20 h. Growth factors were determined by EGF and TGF-α RIAs.

CA 12.5 determinations

Serum CA 12.5 levels were analysed routinely by RIA kits from Centocor.

Statistical analysis

Comparison between more than two groups were made using non-parametric statistics (Kruskal–Wallis test). For evaluation of differences between two subgroups, the Mann–Whitney test was used. Differences between pre- and postoperative levels were analysed with the Wilcoxon matched-pairs signed-ranks test. All tests were performed at the 5% significance level.

RESULTS

Growth factor concentrations in urine of patients and of controls

Figures 1 and 2 show scattergrams of the amount of immunoreactive EGF (irEGF) and TGF-α (irTGF-α) in 24-h urine samples of female controls and patients with malignant or benign ovarian tumours.

Immunoreactive EGF was found in all urines tested. The median level of EGF was 2.34, 1.75 or 1.81 nmol/24 h in the urine of controls (n = 13), patients with epithelial ovarian cancer (n = 29) and women with benign ovarian tumours (n = 12),

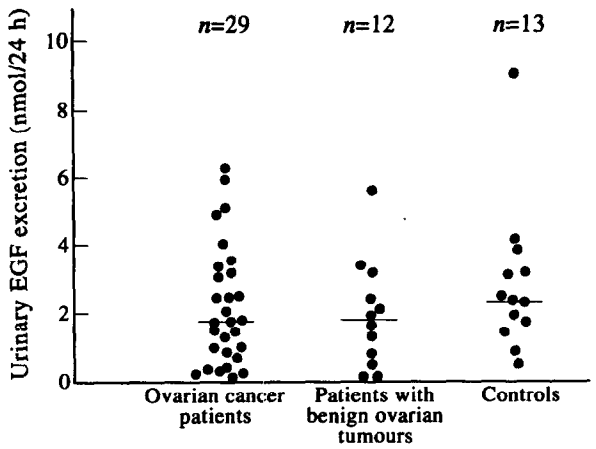

Figure 1. Scattergram representation of irEGF in crude urines of cancer patients, patients with benign ovarian tumours and age matched healthy controls. After acidic precipitation and ultrafiltration of the urines, irEGF concentrations were determined using a commercial EGF-RIA kit. n, number of samples. Lines indicate median values.

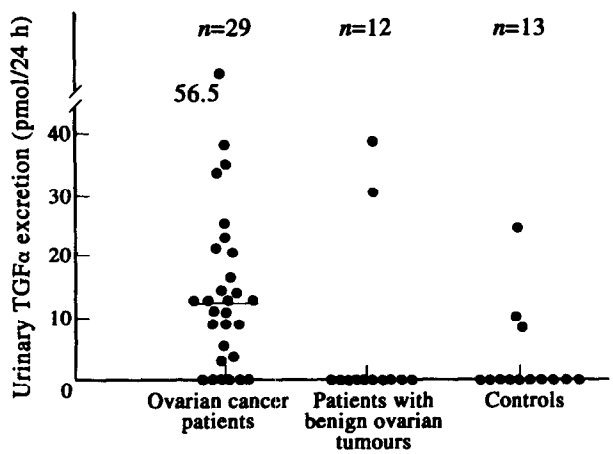

Figure 2. Scattergram representation of irTGF-α in crude urines. For details see legend to Figure 1.

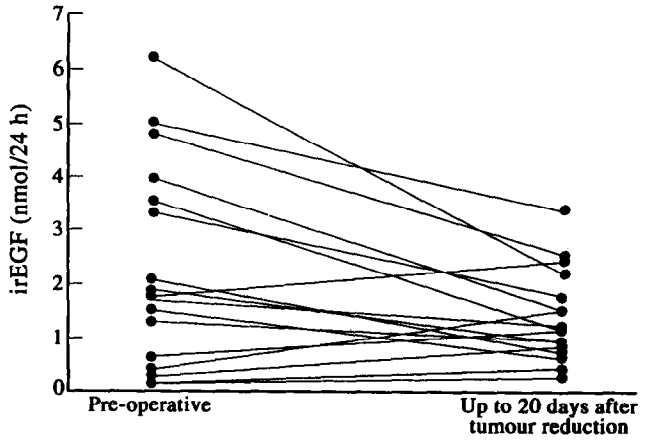

Figure 3. Level of irEGF in the urine of ovarian cancer patients before and up to 20 days after surgical tumour reduction.

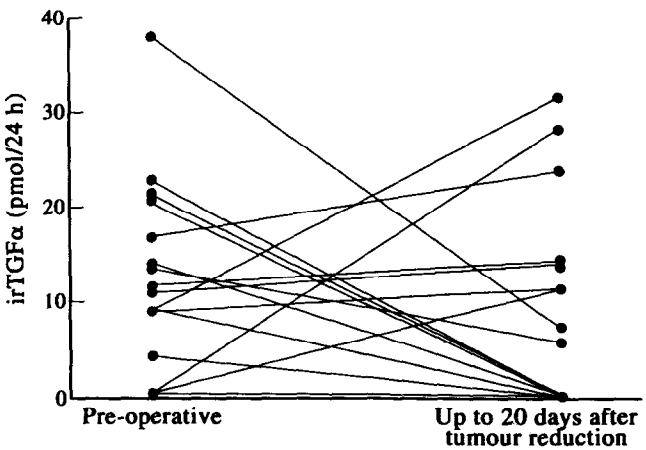

Figure 4. Level of irTGF-α in the urine of ovarian cancer patients before and up to 20 days after surgical tumour reduction.

respectively. There was a wide variability within each group and considerable overlap (Figure 1). Therefore, no statistically significant differences between these three groups were observed. The level of urinary EGF declined significantly ($P < 0.01$) within 20 days after tumour reduction (Figure 3).

Figure 2 shows that immunoreactive TGF-α was detected in the urine of 23 of 29 (79%) ovarian carcinoma patients. Excretion rates ranged from 0 to 56.5 pmol/24 h with a median of 12.6 pmol/24 h. The limit of detection of the TGF-α RIA was 0.73 ± 0.52 fmol/tube. In contrast, TGF-α was renally eliminated by only 2 of 12 (17%) patients with benign tumours and 3 of 13 controls (23%). The differences between the ovarian cancer patients and the other groups were highly significant ($P < 0.001$, Kruskal–Wallis test). The sensitivity was 79% and the specificity 77% for ovarian carcinomas. TGF-α levels decreased after tumour reduction in 53% ($n = 17$), increased in 35% and were unchanged in 2 cases (Figure 4).

Urinary irTGF-α was found in all tumours with stage I ($n = 1$), II ($n = 4$) and IV ($n = 4$) and in 70% ($n = 20$) of women with stage III tumours, and it was detected in well and poorly differentiated tumours. No correlation between the amount of TGF-α and stage, morphological grading or with the serum concentration of the tumour marker CA 125 was found.

High and low molecular weight forms of urinary EGF and TGF-α

Urine samples from tumour patients and healthy controls were chromatographed on a Sephadex G-100 SF column to investigate the molecular weight distribution of renally excreted EGF and TGF-α. The eluted fractions were subjected to rhEGF- and TGF-α RIAs. In the control urine (Figure 5a), large amounts of 6–8 kDa EGF were excreted, together with small amounts of high molecular weight forms (30–40 kDa) of EGF (HMW-EGF). Similar molecular weight forms were also found in cancer patients (Figure 5b). However, the urine samples of ovarian cancer patients were characterised by a higher molecular weight heterogeneity of excreted EGF forms. High molecular weight EGF forms with approximate molecular weights of > 60 kDa and/or 20 kDa and/or 12 kDa (Figure 5b) were additionally found in 79% of the urine samples of the ovarian cancer patients ($n = 14$). None of the control urine samples ($n = 7$) contained these special EGF forms. The fractions were grouped into two molecular weight categories of > 8 kDa (HMW-EGF) and ≤ 8 kDa (LMW-EGF), because authentic rhEGF was eluted in the later fractions. As shown in Table 1, HMW-EGF comprised 17.2% (median) of total EGF in the urine of carcinoma patients

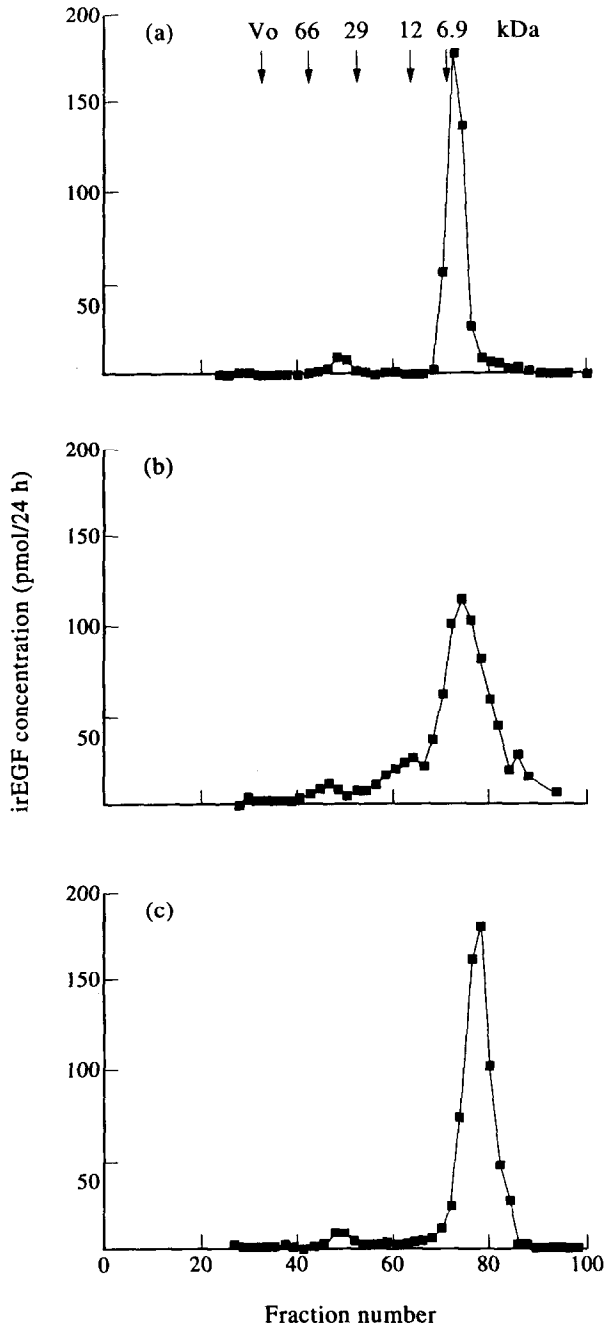

Figure 5. IrEGF in urine samples fractionated by gel permeation. Urines from a healthy donor (a) and from an ovarian cancer patient before (b) and 10 days after resection of approximately 95% of the malignant tumour (c) were chromatographed on Sephadex G-100 SF. Fractions were lyophilised and resuspended. Aliquots were tested for irEGF using an EGF RIA kit. [^{125}I]EGF was eluted in fraction number 70–74.

and 7.1% in control urines ($P < 0.01$, Mann–Whitney test). After reductive surgery, only the 6–8 kDa and the 30–40 kDa forms of EGF were detected, and the concentration of higher molecular weight EGF forms was reduced to basal levels in all samples tested ($P < 0.05$, Wilcoxon matched pairs signed-ranks test). Thus, elevated levels of these growth factor forms appear to be dependent on tumour burden.

As mentioned previously, TGF-α was found in only three of 13 control urines. The urine samples were concentrated before gel filtration was performed. TGF-α that was not detected in crude urine samples was detectable after concentration in some cases. After gel filtration, low amounts of irTGF-α were found in the urine of three controls, whose crude urine samples did not contain detectable amounts of TGF-α. In the irTGF-α positive control urines, TGF-α immunoreactivity corresponded to authentic TGF-α and/or to TGF-α fragments with a molecular weight < 6 kDa (Figures 6a and 7a).

Table 1. Amounts of high and low molecular immunoreactive EGF (irEGF) in 24-h urines

	n	HWM irEGF (pmol/24 h)	LMW irEGF (pmol/24 h)	HMW EGF/ Σ EGF (%)
Patients without tumours	7	97 (5–151)	1352 (79–1859)	7.1† (0.5–11.8)
Patients with ovarian carcinomas				
Pre-operative	14	131* (12–639)	452 (63–3468)	17.2†‡ (8.9–40.1)
Postoperative	9	94* (14–152)	663 (118–1549)	10.6‡ (5.2–24.5)

Median values and minimum and maximum amounts are indicated. *n*, number of analysed urines. For statistical analysis, all irEGF-proteins with a molecular weight > 8 kDa (HMW EGF) and ≤ 8 kDa (LMW EGF) were evaluated. Statistical significant results are indicated: *$P < 0.05$ (Wilcoxon matched-pairs signed-ranks test), † $P < 0.01$ (Mann–Whitney test), ‡ $P < 0.05$ (Wilcoxon matched pairs signed-ranks test).

The elution pattern of TGF-α of a woman with ovarian cancer is shown in Figure 6b. IrTGF-α was detected in fractions corresponding to a molecular weight range of less than 6–30 kDa. High molecular weight forms of TGF-α (MW > 10 kDa) were detected in 36% of the samples ($n = 14$) tested. The majority of patients with disseminated cancer excreted higher amounts of TGF-α than the controls. Moreover, the molecular weight distribution was more heterogeneous in one third of these patients (Figure 7b).

After surgical tumour reduction, only 1 of 8 patients excreted HMW TGF-α (MW > 10 kDa, Figure 7c).

Concentrated urine samples were also separated by SDS-PAGE. After electrophoresis, immunoreactive forms of EGF and TGF-α were determined by RIA in extracts of gel slices. SDS-PAGE confirmed the presence of HMW-forms of EGF and TGF-α (results not shown).

Radioreceptor assay

The ability of the different molecular weight forms of EGF and TGF-α to bind to EGF receptors was determined by a radioreceptor assay using A431 cells. As both EGF and TGF-α bind to the same receptor, these two growth factors cannot be distinguished in this assay. Urinary immunoreactive TGF-α values were very low, if compared to the amount of urinary immunoreactive EGF (see Figures 1 and 2). Moreover, the urinary TGF-α concentration is in the concentration range of the detection limit of the radioreceptor assay and has, therefore, almost no effect on EGF receptor binding. The profiles of EGF receptor binding after gel chromatography (Figure 8) are similar to those of the EGF concentrations detected by RIA (Figure 5). These results demonstrate that all molecular weight forms of irEGF bind to the EGF receptor.

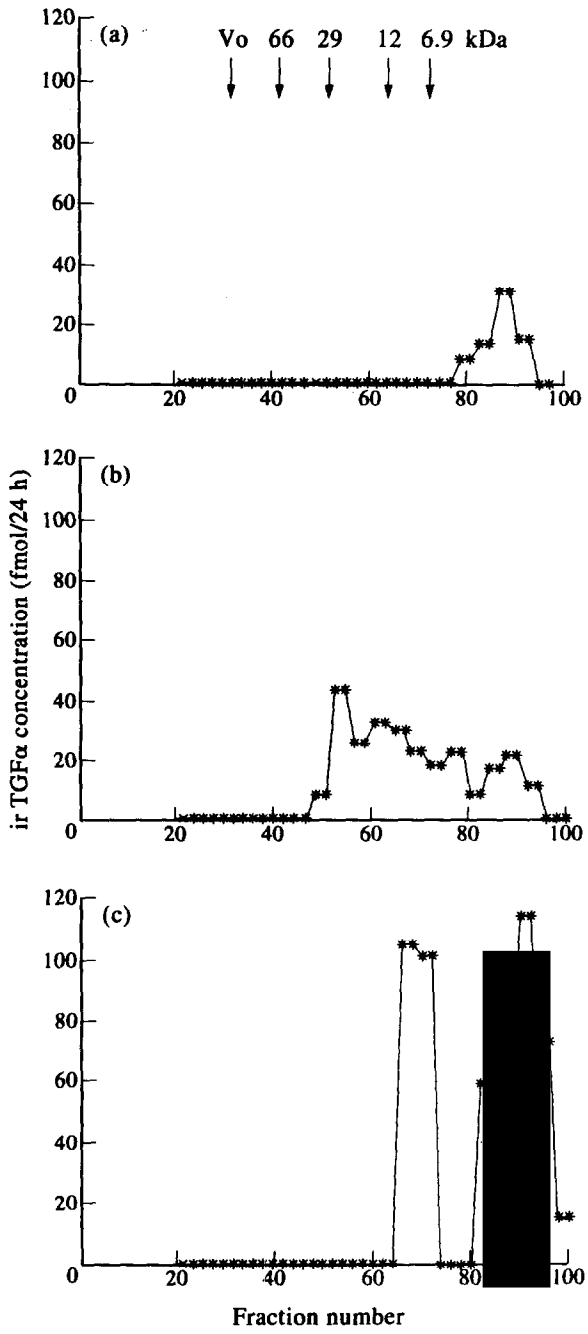

Figure 6. IrTGF-α in urine samples fractionated by gel permeation. For details see Figure 5. [^{125}I]TGF-α was eluted in fraction number 66–70.

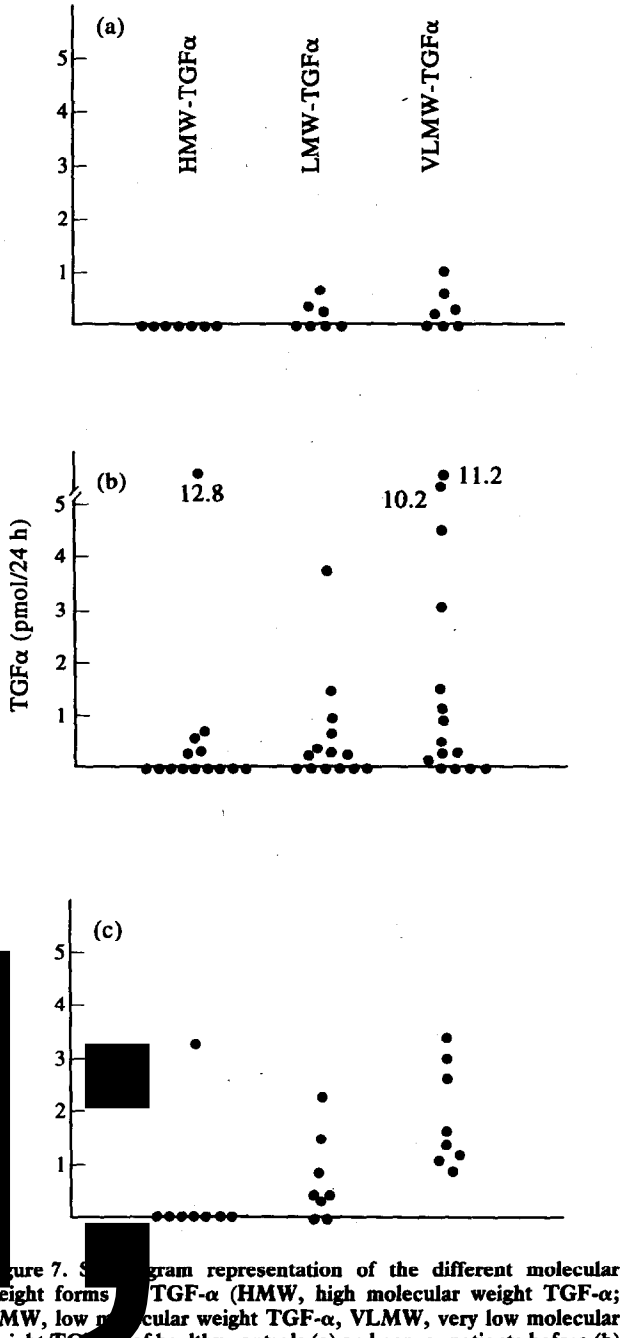

Figure 7. S[illegible]gram representation of the different molecular weight forms [illegible]TGF-α (HMW, high molecular weight TGF-α; LMW, low m[illegible]cular weight TGF-α, VLMW, very low molecular weight TG[illegible] of healthy controls (a) and cancer patients before (b) and after surgical tumour reduction (c) in gel permeation fractionated urines.

These analyses of the fractionated urines of ovarian cancer patients revealed that 16.7% (median) of the EGF-equivalents were HMW-EGF forms (corresponding to a median absolute amount of 359 pmol HWM-EGF/24 h). In contrast, only 9.0% of the EGF equivalents of the controls were HMW-EGF forms (absolute amount 146 pmol/24 h HMW-EGF).

Surgical reduction of the tumour masses caused a decrease or complete loss of these HMW-EGF equivalents (193 pmol/24 h). The differences in pre- and postoperative HMW levels were significant ($P < 0.05$).

Fractions containing peak amounts of LMW-EGF were quantified in parallel by EGF RIAs and by EGF radioreceptor assays. EGF concentrations measured using the radioreceptor assay were generally higher than the EGF concentration of the same samples analysed by RIA. Interestingly, the difference between RIA and radioreceptor assay values was 6-fold (median) for low molecular weight fractions of ovarian cancer patients ($n = 7$), but only 2-fold for samples of healthy controls ($n = 6$). This might indicate a modified affinity of EGF from ovarian cancer patients and controls to the RIA-antibody and/or to the EGF-receptor of A431 cells. A similar discrepancy between RIA and radioreceptor assay data has been described by Nexo and colleagues [18].

Assay for anchorage-independent growth

Normal rat kidney (NRK) cells provide a non-tumorigenic mesodermal cell line sensitive to TGF-α and members of the

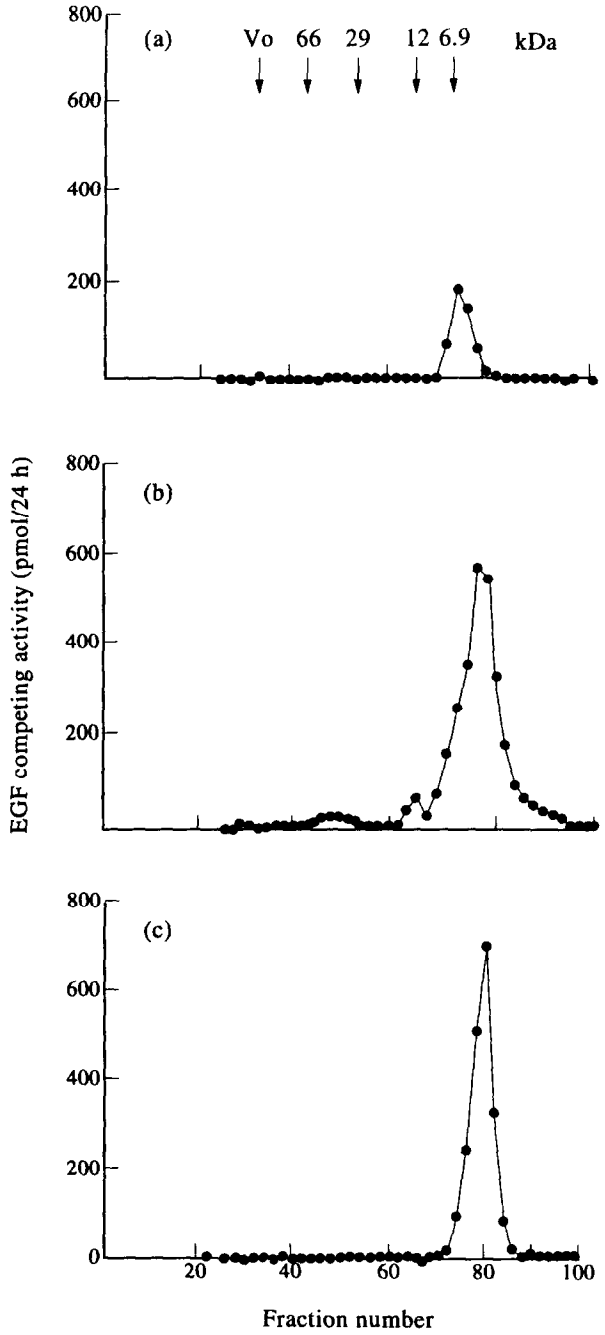

Figure 8. EGF receptor binding of fractionated urine samples determined by radioreceptor assay (RRA). Urines from the same control donor (a) and the same ovarian cancer patient before (b) and after (c) tumour reduction as described in Figure 5 were chromatographed on Sephadex G-100 SF. Collected fractions were lyophilised and resuspended. Aliquots were tested for their ability to compete with [^{125}I]EGF for EGF receptor binding sites of A431 cells. EGF equivalents were determined using a standard curve. [^{125}I]EGF was eluted in fraction number 70–74.

EGF family. 0.3 ng of EGF or TGF-α were sufficient to induce colony formation in serum-supplemented medium. Ten nanograms PDGF-AB, 77 ng FGF, 10 ng IGF-I, 100 ng insulin and 250 pg TGF-β were not able to induce colony formation of NRK cells in soft agar. The low levels of immunoreactive TGF-α levels in the fractionated urines as measured by TGF-α RIA were insufficient to induce colony formation.

The result of a typical experiment is presented in Figure 9. Fractions containing RIA-detectable amounts of EGF stimulated anchorage-independent growth of NRK cells. All control urines showed colony formation with peak maxima at 6–8 kDa and 30–40 kDa. Eighty-five per cent of the ovarian carcinomas (n = 13) contained additional activity of > 60 kDa and/or 15–20 kDa. These results indicate that urinary LMW, as well as HMW, forms of EGF-induced colony formation in all samples tested so far. In postoperative urine samples, the clonogenic potential of the HMW-EGF levels was reduced in 78% of the

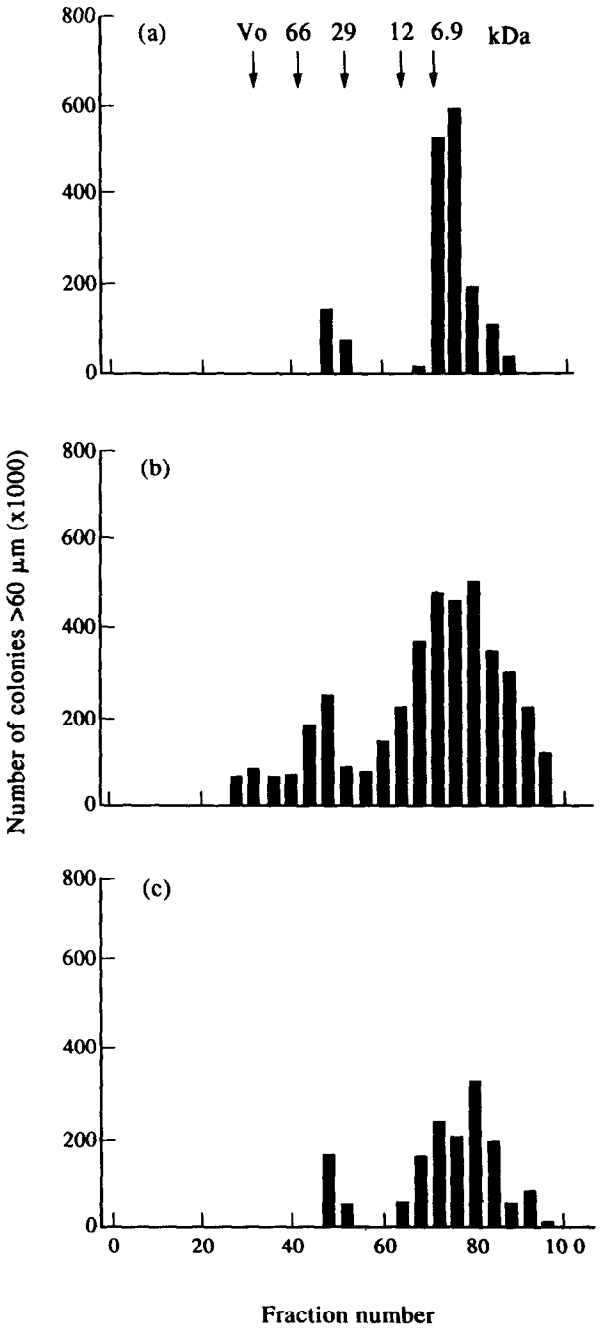

Figure 9. Induction of anchorage-independent growth of NRK 49F cells. Urines from the same control donor (a) and the same ovarian cancer patient before (b) and after (c) tumour reduction as described in Figure 5 were chromatographed on Sephadex G-100 SF. NRK 49F cells (2 × 10^4) were incubated with 25 µl aliquots of concentrated fractions. The experiment was carried out in triplicate. Number of colonies was calculated for the 24-h urines.

patients (n = 9), and the pattern of anchorage-independent growth was similar to that of controls.

DISCUSSION

In addition to therapeutic advances in human ovarian cancer, early diagnosis seems to be the best hope for significantly improving the prognosis. Currently, no single test is of value as a screening test for early stage ovarian cancer. The present investigation was initiated to evaluate the clinical relevance of urinary growth factors as diagnostic and prognostic markers for ovarian cancer.

Urinary growth factor levels

The excretion of urinary irEGF did not differ significantly between patients with ovarian carcinoma, patients with benign ovarian tumours and healthy controls. Therefore, the measurement of urinary EGF has no clinical application. Our results are consistent with other studies reporting unchanged levels of urinary irEGF derived from patients with breast [19], colon [19] and ovarian carcinoma [20], when compared to normal subjects. In contrast to these findings, Ushiashi and colleagues [21] demonstrated elevated levels of urinary EGF in patients with malignant diseases, suggesting a possible clinical application as a tumour marker.

Normal human urine contains EGF, most of it being derived from the kidneys [22]. Tumour derived EGF is also excreted, via the blood, into the urine but this, according to Sweetenham and associates [19], would be unlikely to produce a significant increase in the urinary EGF level. If elevated EGF levels are measured, they may represent an indirect effect of a tumour product on renal EGF production [20].

In contrast to EGF excretion, we found that urinary TGF-α levels were significantly increased in ovarian carcinoma patients. Seventy-nine per cent of the patients with ovarian carcinomas excreted TGF-α, whereas only 23% of the controls did so. Human tumour cells produce mainly TGF-α rather than EGF [23–25], and all components necessary for a TGF-α-mediated autocrine loop were detected in ovarian cancer cell lines [4, 5, 26]. Bauknecht and coworkers [3, 27] reported enhanced TGF-α concentrations in 66% of ovarian tumour material when compared to normal tissue by immunohistochemical methods. Whether the TGF-α in tumour patients is tumour-derived, or represents a host reaction to the presence of malignancy needs to be elucidated by further investigations.

Our report of increased TGF-α levels in the urine of patients with disseminated ovarian cancer suggests that this may be a clinically useful tumour marker. However, urinary TGF-α levels were also high in 35% of ovarian cancer patients after surgical reduction of the tumour. Todaro and colleagues [28] pointed out that TGF-α may be bound to receptors on the cancer cell membrane. Receptor-bound TGF-α may be released from cell membrane receptors during surgery, leading to elevated levels of TGF-α soon after surgical tumour reduction. Moreover, TGF-α is involved in the process of wound healing [6], which could also explain the elevated postoperative TGF-α levels. Therefore, it may be necessary to investigate urinary TGF-α levels over a longer period of time after surgical tumour reduction. The urinary TGF-α activity appears to be tumour associated, but not tumour specific. Increased urinary TGF-α levels in patients with bronchogenic carcinoma, melanoma, hepatocellular carcinoma, colon carcinoma and one woman with an ovarian tumour have been reported [3, 11, 13]. The usefulness of TGF-α as an early diagnostic marker may be limited, because it could be detected only in the urine of 79% of the patients with ovarian carcinoma. However, TGF-α was detected in all stage I and II tumours (n = 5), whereas the tumour marker CA 125 exceeded the cut off level (> 35 U/ml) in only 2 of these cases. TGF-α was detected in only 17% (n = 12) of patients with benign ovarian tumours, whereas 42% presented with enhanced CA 125 serum concentrations. Therefore, the measurement of urinary TGF-α levels may give additional diagnostic and/or prognostic information, and a combination of the measurement of TGF-α in urine and CA 125 in serum may augment the value of either parameter. It must be further investigated whether the quantitative expression of TGF-α in urine is related to the extent of tumour burden, and if its measurement is useful in following the patients' response to treatment. Kohler and associates [27] and Stromberg and colleagues [5] observed poor prognosis for ovarian carcinoma patients with high levels of EGF-like factors in tumour extracts.

High molecular weight forms of EGF and TGF-α

TGF-α and EGF were detected in a wide molecular weight range in urine from ovarian cancer patients. High molecular weight EGF forms of > 60, 20 and 12 kDa and high molecular weight TGF-α forms of > 6 kDa seem to be tumour specific. Urinary EGF has been found in various forms of molecular weight ranging from 6 kDa to more than 30 kDa [29, 30]. A 43-kDa EGF form has been detected in the urine of breast cancer patients [9]. HMW TGF-α (30 kDa) has been detected in urine from patients with melanoma, and could be distinguished from TGF-α found in normal controls using HPLC [31]. Sherwin and associates [14] reported an EGF-related HWM TGF activity (30–35 kDa) and a LMW TGF activity (6–8 kDa) in the urine of patients with solid disseminated tumours. Hudgins and colleagues [32] investigated the contribution of human tumours in rat hosts to urinary EGF-related peptide growth factors. They reported that all EGF-receptor binding activity appears to be derived from the rat and not from the xenografted tumour. Therefore, the observed increased TGF-α levels, as well as the HMW-EGF forms, may represent a host reaction to the presence of malignancy. However, we have investigated the conditioned medium of ovarian carcinoma cell lines and identified immunoreactive HMW-EGF and HMW-TGF-α forms in the supernatant of the ovarian cancer cell lines EFO-21 [33] and EFO-27nu [34], respectively (data not shown).

The smaller fragments found in urine could be due to degradation during the collection, storage and processing of the urine. The soluble bioactive higher molecular weight TGF-α species may correspond to the glycosylated extracellular forms released from the transmembrane precursor [35, 36].

Induction of a biological response

All high and low molecular weight EGF forms were able to bind to the EGF-receptor and to induce a biological effect, as analysed by the induction of anchorage-independent growth. Interestingly, there seems to be a modified affinity of urinary EGF from cancer patients to the EGF receptor or to the RIA antibody. Further studies wil help to reveal if this depends on EGF modifications or if other urinary substances reacting with the EGF receptor are present.

This study provides further evidence for the clinical relevance of renally excreted TGF-α, as well as of high molecular weight forms of EGF and TGF-α. These growth factors may be secreted by the tumour, transported in the blood and excreted by the

kidneys, or they may be synthesised in increased amounts by the renal distal tubules in response to some stimulus stemming from the tumour. The results demonstrate that the urinary excretion of some growth factors is elevated in patients wth ovarian cancer, suggesting that tumour growth may be stimulated by auto- and paracrine effects of these growth factors *in vivo*. It supports the possibility that these activities may serve as biological tumour markers for certain types of cancer including ovarian cancer. Even though we observed a lack of complete tumour specificity, measurements of these growth factors may play an important role in evaluating prognosis and control of therapy.

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