Eur J Cancer, Vol. 28A, No. 8/9, pp. 1432–1437, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Epidermal Growth Factor Receptor and Transforming Growth Factor Alpha Expression in Human Ovarian Carcinomas

Manuela Kohler, Thomas Bauknecht, Monika Grimm, Gerlinde Birmelin, Friedrich Kommoss and Edgar Wagner

The varying tumorbiological behaviour of ovarian carcinomas probably influences operability, response to chemotherapy, being one of the most relevant prognostic factors. Because it is believed that an activation of the epidermal growth factor/transforming growth factor alpha (EGF/TGFα) signal pathway could be involved, we analysed the expression of epidermal growth factor receptor (EGFR) and TGFα with molecular-chemical, biochemical and immunohistochemical methods in 42 ovarian carcinomas, 4 ovarian metastasis, 2 other malignant ovarian tumours, and in 25 nonmalignant tissues (ovary, myometrium). No major rearrangements or amplification of the EGFR or TGFα genes were found. In non-malignant tissues no strong EGFR or TGFα signals were detected. TGFα is mainly produced by the tumour cells as shown by immunohistochemistry. Four different high molecular weight forms (20–48 kD) were detected in malignant tissues by western blot analysis.
Eur J Cancer, Vol. 28A, No. 8/9, pp. 1432–1437, 1992.

INTRODUCTION

THE ABNORMAL expression and gene rearrangements of protooncogenes and growth factors (GF) have been implicated in the induction of malignant tumours [1]. The malignant phenotypes of distinct tumours and the biological differences within tumour groups are also believed to be caused by activated oncogenes or GF. One of the best characterised GF/protooncogene signal pathways is the epidermal growth factor/transforming growth factor alpha (EGF/TGFα) system [2] which takes part in control of the proliferation and differentiation of meso- and ectodermal tissues. TGFα was initially discovered in retrovirally transformed cells binding specifically to the epidermal growth factor receptor (EGFR) as an EGF-like factor, and it has been implicated in the malignant transformation process [3, 4]. Enhanced production of TGFα and EGF receptor (EGFR) is frequently detected in human tumour specimens and transformed cells of ectodermal origin [5, 6]. Furthermore, cells transfected with TGFα expression vectors become transformed. Later studies, however, showed TGFα to be present in benign normal tissues and cell types as well [7, 8] contributing to the normal development, possibly through autocrine or paracrine mechanisms. The EGF-like structure of TGFα is produced as part of a membrane-anchored precursor and can be released as a diffusible bioactive factor, defined by 50 aminoacids and six characteristically spaced cysteines [9, 10]. The membrane precursor consists of an extracellular domain that contains the mature TGFα followed by a membrane-spanning hydrophobic sequence and a cytoplasmic domain of unknown function. The processing of these TGFα precursors is incomplete in many cells including tumour cells which leads to an accumulation of pro-TGFα on plasma membranes [10]. The contact of pro-TGFα with the EGF/TGFα receptor of the adjacent cell may activate the receptor thereby mediating by cell–cell adhesion the promotion of cell proliferation. An early response in the cascade of the EGF/TGFα action is the stimulation of nuclear protooncogenes such as c-*fos*, c-*jun* and c-*myc* [12–14].

In view of the key role of the EGF/TGFα pathway for the development of the normal but also malignant epithelial tissues we analysed this pathway in ovarian carcinomas. These tumours encompass a broad spectrum of lesions from borderline malignant potential to highly aggressive tumours with a rapid growth pattern and distant spread [11]. In earlier reports we described the differences of EGFR/ligand expression in ovarian carcinomas and its correlation with the results of chemotherapy and patients prognosis [15–17]. An important question raised by these findings is whether ovarian carcinomas produce or process TGFα precursors with different biological activities and whether the EGF/TGFα pathway is intact or disrupted in certain tumours which could explain growth pattern and thus prognosis. Therefore, we examined the EGFR and TGFα genes, mRNA levels and the protein products in the ovarian carcinomas. The occurrence of TGFα preforms was analysed by western blottings and was compared with the results of TGFα immunohistochemistry.

MATERIALS AND METHODS

The surgical specimens were immediately frozen in liquid nitrogen and stored at −70°C for subsequent determination of EGFR, TGFα immunohistochemistry and RNA analysis. The tumour specimens were received from 42 cases with advanced ovarian carcinomas (stage III and IV), from 7 cases with other ovarian tumours and 25 non-malignant tissues. Routinely, all specimens were examined histologically and contributed to the histological subtype and grade of differentiation. Many of the investigated cases (about 80%) had a similar histological subtype, medium or low differentiated (G2 or G3) serous cyst-adeno carcinoma. An *Eco*RI (Böhringer) 1.8 kb fragment of EGFR cDNA corresponding with the extracellular domain of the

Correspondence to T. Bauknecht.
T. Bauknecht, M. Grimm, G. Birmelin and F. Kommoss are at the Universitäts-Frauenklinik, Hugstetterstr. 55, 7800 Freiburg, F.R.G.; and M. Kohler and E. Wagner are at the Institut für Biologie, Schänzlestr. 1, 7800 Freiburg, F.R.G.
Revised 31 Dec. 1991; accepted 19 Feb. 1992.

receptor protein, obtained from M. Waterfield (London) and a 1.4 kb fragment of human TGFα cDNA, obtained from R. Derynck (San Francisco) were labelled by random primer extension [8] to a specific activity of 5×10^8 dpm/mg. X-ray film XAR-5 was obtained from Kodak.

The TGFα antibodies (Tab 8, Tab 2, TBR9) were obtained from R. Walker (Triton), prepared as described previously [8]. Biotinylated horse anti-mouse IgG antibody, avidin (A) and biotinylated peroxidase (BC) were from Vector Laboratories. Biotinylated goat anti-rabbit and rabbit anti-mouse IgG antibody and streptavidin horseradish peroxidase were obtained from BRL/GIBCO.

EGFR binding assay (EGFR RRA)

The EGFR binding capacities were estimated by the use of a single point assay on a crude membrane preparation from the tumour specimens as described previously [15, 16, 18]. The relative amounts of binding capacities were calculated using the specimen with the highest number of binding sites as 100%, the specimens without detectable specific binding as 0% and the remaining samples between those.

DNA analysis

Tissues were ground in liquid nitrogen and digested with 100 μg/ml proteinase K (Boehringer) in detergent containing lysis buffer. DNA was purified by subsequent phenol and chloroform extractions and precipitated with ethanol. For Southern analysis, DNA samples (10 μg) were digested with *Eco*RI, *Hind*III and *Rsa*I, electrophoresed through 1% agarose gels, and transferred to nylon membranes (Zeta probe, BIO RAD) as described previously [19]. Hybridisation was conducted at 65°C in 0.5 mol/l sodium phosphate/7% (v/v) sodium dodecyl sulphate (SDS), pH 7.2. The membranes were washed twice under conditions of 40 mmol/l sodium phosphate/5% (v/v) SDS, pH 7.2 at 60°C and then exposed to X-ray films for 24 h.

RNA analysis

The amounts of EGFR or TGFα mRNA were analysed by Northern blotting with total cellular RNA isolated from frozen tissues by the guanidinum–isothiocynatate–caesium chloride method as described elsewhere [8, 17]. Briefly, 10 μg RNA was electrophoresed in a 1% agarose/2.2 mol/l formaldehyd gel, stained in ethidium bromide as to control quality and amount of RNA loaded onto the gel and transferred to nylon membranes. The membranes were hybridised for 24 h at 42°C in the presence of 50% formamide/10% dextran sulphate/1 mol/l NaCl/1% SDS and were afterwards washed in 2 × sodium saline citrate (SSC)/0.5% SDS for 30 min at room temperature and twice in 0.2 × SSC/0.1% SDS for 30 min at 65°C. The membranes were exposed to X-ray film for 3 days for EGFR and 10 days for TGFα. Quantitative evaluation of autoradiograms was performed by densitometric analysis using the specimen with the highest amount of mRNA as a 100% standard, the specimens without specific banding as 0% and the residual samples between those. The samples with >25% relative amounts of mRNA were contributed to the group with a high mRNA expression rate.

Immunohistochemistry

The TGFα immunohistochemical staining reaction and the semiquantitative analysis of the TGFα score on 6 μm cryostat sections were performed as has been previously described [8]. Negative controls consisted of sections incubated with mouse IgG instead of primary antibody. 500 tumour cells from representative microscopic fields were evaluated by two independent observers. Immunoreactive scores ranging from 0 to 9 were calculated by multiplying staining intensity (0: no staining, 1: weak staining, 3: strong staining) by percentage of positive epithelial cells (0–33%: factor 1, 34–66%: factor 2, and 67–100%: factor 3). Such semiquantitative scores have been shown to be of value in the assessment of immunohistochemical stainings [20]. The type of staining distribution was assessed as described [8], type-A tumours showing uniform staining of all epithelial tumour cells, while type-B carcinomas had both clearly negative and a second population of uniform strong positive cells. Finally, type-C tumours showed a wide spectrum of staining intensity from negative to weakly positive to intensely positive. The type-B and type-C staining patterns were shown to be reproducibly different by image analysis of breast carcinoma immunohistochemical staining of progesterone receptor [21].

Western blot analysis

TGFα extraction was performed as described previously [18]. SDS–PAGE (polyacrylamide gel electrophoresis) was performed in reducing conditions as described by Schägger and von Jagow [22]. Molecular weight standards (Sigma) were routinely added for protein size calibration. After electrophoresis, the gel was removed from the glass plates and rolled onto a sheet of submerged nitrocellulose paper (0.22 μm) avoiding air bubbles between the gel and the paper. The electroblot unit (Fröbel), containing the gel and nitrocellulose paper were assembled according to the manufacture's instructions. Electroblotting was performed in the presence of 25 mmol/l Tris/HCl, pH 9.4/20% methanol/40 mmol/l 6-aminocaprionic acid (cathode), 0.3 mol/l Tris/HCl, pH 10.4/10% methanol and 25 mmol/l Tris/HCl, pH 10.4/10% methanol (anode). The proteins were transferred for 1 h at 0.8 mA/100 mm^2. After the transfer the gels were routinely stained with Coomassie blue to proof the efficiency of the transfer. For immunodetection the nitrocellulose paper was submerged in block buffer (phosphate buffered saline/0.1% Triton X-100/3% BSA) for 2 h at room temperature and incubated with TBR9 for 24 h at 4°C. This was followed by incubation with biotinylated goat anti-rabbit IgG and streptavidin horseradish peroxidase conjugate. The peroxidase specific staining was carried out with 4-chloro-1-naphthol.

RESULTS

48 different ovarian tumours, including 42 ovarian carcinomas, 4 ovarian metastasis, 1 sarcoma and 1 teratoma of the ovary, were analysed. Placenta from 20 weeks of gestation as well as term placenta, which are known to express EGFR and TGFα, were taken as positive control tissues. Furthermore, 25 different non-malignant tissues (ovary and myometrium) were investigated.

Table 1 shows for the ovarian tumours the results of EGFR binding capacities, the amounts of EGFR and TGFα mRNA together with the TGFα immunostaining indices and the results of TGFα Western blot analysis. Examples of EGFR and TGFα northern blots of tumours with different amounts of mRNA's, of non-malignant tissue and of placenta are shown in Fig. 1. The ethidium bromide staining reaction demonstrates intact ribosomal RNA and the same quantities of total RNA. An EGFR transcript with a size of 10 kb and a TGFα transcript with a size of 4.4 kb was found in the tumour probes as well as in the non-malignant tissues. The TGFα western blots of TGFα producing tumours and non-malignant tissue are demonstrated in Fig. 2.

Table 1. Malignant ovarian tumours. Relative amounts of EGFR and TGFα mRNA (%) Results of TGFα western blot and immunohistochemistry

Nr	EGFR RRA fmol (%)		EGFR mRNA (%)	TGFα mRNA (%)	TGFα western	TGFα immunoh*
Ovarian carcinomas						
27	0		0	0	ND	ND
49	3	(7.5)	5	59.9	+	ND
67A	2	(5)	53	12.6	ND	ND
89	0		0	0	ND	0
138	1	(2.5)	0	0	+	ND
146	5	(12.5)	7	0	ND	ND
157	5	(12.5)	4	21	+	0
159	5	(12.5)	4	5	ND	0
166	6	(15)	0	0	ND	ND
183	3	(7.5)	4	0	ND	ND
186	2	(5)	0	0	ND	ND
207	0		12	17	+	0
209	4	(10)	9	0	ND	1/C
276	0		9	21	+	3/C
277	0		0	19	+	3/A
292	12	(30)	36	12	+	6/A
296	0		1	9	+	ND
301	1	(2.5)	1	0	+	6/B
301	4	(10)	0	0	ND	6/C
328	3	(7.5)	7	25	+	2/C
329	ND		9	29	ND	ND
359	46	(115)†	9	78	ND	6/A
363	5	(12.5)	2	4	+	6/B
387	38	(100)	100	0	0	0
445	7	(17.5)	7	8	+	3/C
448	ND		0	0	+	6/C
451	0		0	0	+	0
466	0		0	2.4	+	ND
480	0		0	13	+	3/A
483	7	(17.5)	3	0	+	6/C
484	27	(67.5)	25	0	+	6/C
487	0		0	0	+	3/C
488	5	(12.5)	44	9	+	6/C
491	2	(5)	22	7	ND	ND
502	0		4	7	+	6/C
505	0		7	57	+	6/A
506	2	(5)	6	67	+	1/A
513	0		7	100	+	12/A
564	2	(5)	3	52	ND	6/A
566	2	(5)	3	88	+	3/A
573	3	(7.5)	0	93	+	4/C
583	13	(32.5)	7	80	ND	6/A
Other malignant ovarian tumours						
123	0		0	0	0	0
258	0		58	88	+	1/C
286	0		20	48	+	6/A
378	ND		4	20	+	4/A
443	4	(10)	0	3	+	9/C
450	10	(25)	0	63	+	6/C

*TGFα Immunoh. = TGFα immunohistochemistry.
†EGFR gene arrangement (Southern blot).
The EGF binding capacities (fmol/mg), the mRNA amounts, the TGFα Western blot and immunohistochemistry results are presented from 42 ovarian carcinomas and 6 additional malignant ovarian tumours. The relative amounts of EGFR binding capacities (%) and of the mRNA bandings (values in per cent compared with the specimen with the highest expression rate) were calculated as described in Materials and Methods.

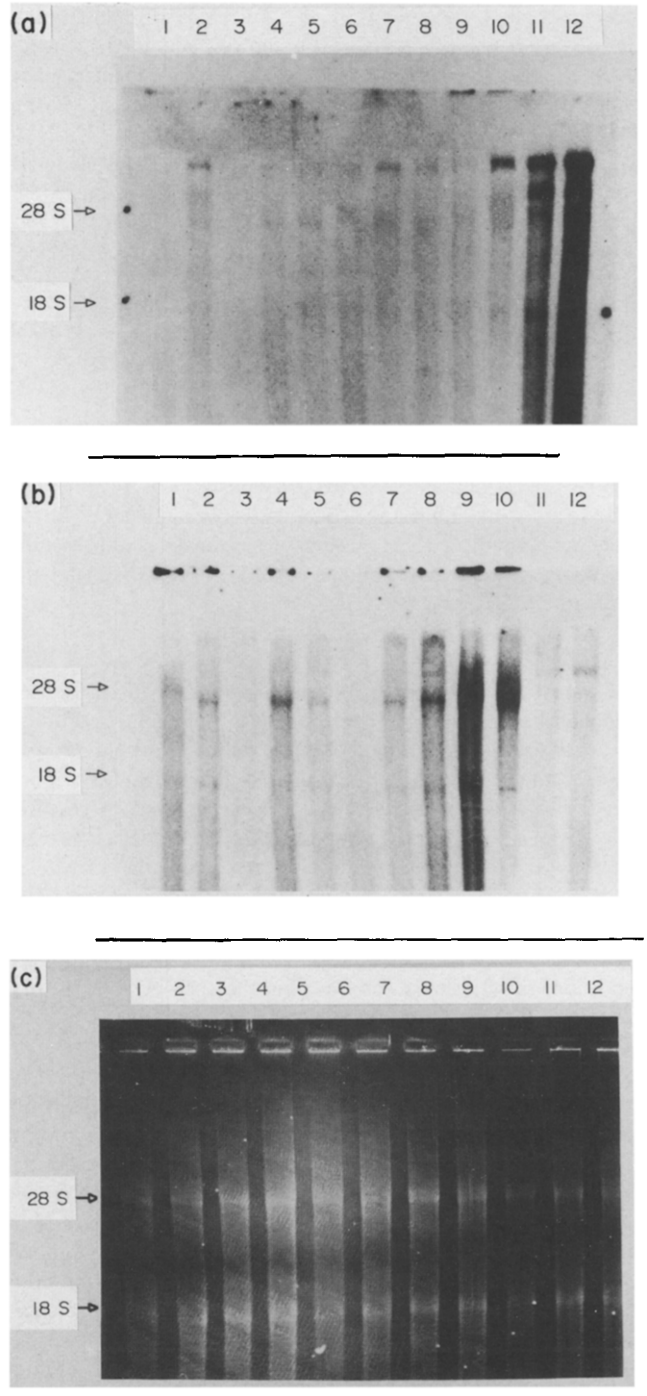
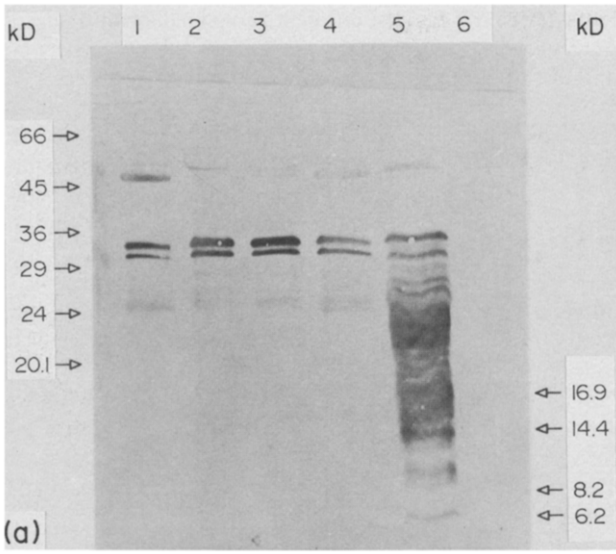


Fig. 1. Northern blotting. 10 μg total RNA isolated by guanidinium–isothiocynate–caesium chloride method from different ovarian tumours, non-malignant tissue and placenta were put on each lane. Lane 1: 123 (ovarian sarcoma), 2: 328, 3: 186, 4: 157, 5: 296, 6: 301, 7: nonmalignant tissue, 8: 378, 9: 513, 10: 286, 11, 12: placenta. (a) Hybridisation with a labelled 1.8 kb *Eco*RI EGFR fragment. (b) Hybridisation with a 1.4 kb *Eco*RI TGFα fragment. (c) The ethidium bromide staining shows the intact ribosomal RNA and that equal amounts were put onto the gel.

For the investigation of the EGFR and the TGFα gene, DNA isolated from the ovarian carcinomas and placenta was digested by *Eco*RI, *Hind*III, *Pst*I and *Rsa*I. Figure 3 shows an example of a Southern blot.

The biochemical EGF RRA detected specific EGF binding (n = 27) in about 68% of the analysed ovarian carcinomas



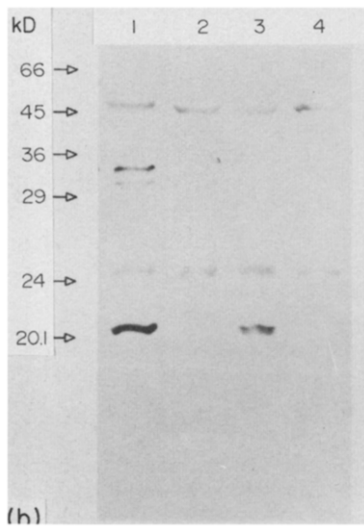


Fig. 2. Western blotting. Equal amounts of protein isolated from different ovarian tumours and non-malignant tissue as described above were resolved on an SDS-polyacrylamide gel and blotted onto a nitrocellulose filter. TAB2 antibody was diluted 1:1000 and biotinylated rabbit anti-mouse and streptavidin peroxidase conjugate were employed to visualise immunospecific bands. Molecular weight markers are indicated on the left side of the figure. (a) lane 1: 363, 2: 378, 3: 480, 4: 483, 5: 513, 6: non-malignant tissue. (b) lane 1: 573, 2: 506, 3: 138, 4:448.

(n = 40) with binding capacities from 1 to 46 fmol/mg, whereas the mRNA analysis (n = 42) revealed specific mRNA bandings in six additional biochemical EGF-R (−) cases (Table 1). In order to compare the biochemical and mRNA EGFR data, the tumours were categorised according to their binding capacities or hybridisation signals as described in Materials and Methods. About 10% of the analysed specimens expressed high amounts of EGFR mRNA which is similar to the number of cases with high EGF binding capacities. However, high binding capacities coincide in only a few cases (n = 3) with high expression rates.

Figure 4 shows the correlation (P = 0.423) between the biochemical and the mRNA hybridisation EGFR signals of all investigated specimens. In 11 out of 48 cases, the biochemical

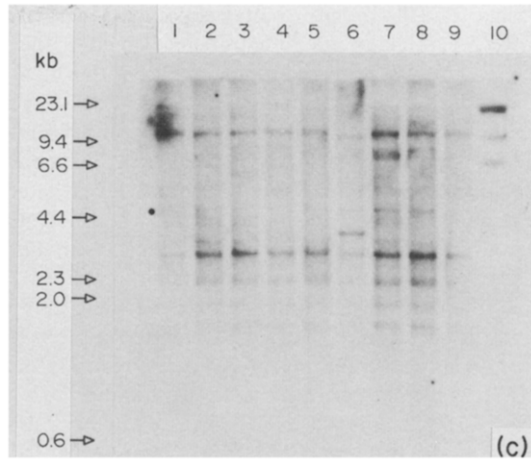


Fig. 3. Southern blotting. 10 μg DNA purified by phenol and chloroform extraction were digested with *Eco*RI restriction enzyme hybridised to the EGFR probe. Lane 1: 258, 2: placenta, 3: 513, 4: 483, 5: 480, 6: 207, 7: 566, 8: 286, 9: 583, 10: lambda, *Hind*III digested.

signals were lower and in 10 cases, they were higher as compared with the mRNA amounts.

The western analysis of the TGFα products revealed four different TGFα species with the molecular weights of about 48 kD, 30 kD, 25 kD and 20 kD. Among all 33 analysed specimens, we found the 48 kD form in 16 cases, the 30 kD form in 16 cases, in 22 cases the 25 kD and in 9 cases the 20 kD form were present. Furthermore, in a tumour (513) with an extraordinarily high level of TGFα mRNA we found additional TGFα specific bandings between 6 and 20 kD and in tumour 528/90 a 14 kD TGFα form was detected. In many cases a good relationship exists between the results of the northern blotting and those of the western blot analysis. However, discordant results were obtained in 6 out of 33 cases indicating a possible alteration of the TGFα preform processing. The results of TGFα immunohistochemistry indicate the tumour cells as the TGFα source with varying production rates, the stromal cells are TGFα negative. The investigation of the TGFα gene in ovarian tumours did not show rearrangements or amplifications (data not shown).

The case number of adnexal tumours other than common epithelial carcinomas (metastasis, teratoma, sarcoma) is too low to detect significant differences between both groups of ovarian

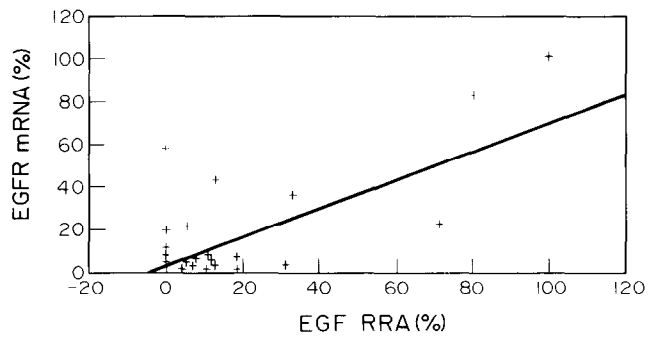


Fig. 4. The EGFR binding capacities were measured by EGF RRA and the EGFR mRNA expression by northern blotting and densitometry of the mRNA bandings. The relative quantities were evaluated taking into account the specimens with the highest value as 100%. Several biochemically EGFR negative tumours express low mRNA bandings as an indication of reduced sensitivity of the EGF RRA.

neoplasms. The discordant results between the EGFR binding and mRNA expression can perhaps be explained by the TGFα production rate. We observed some tumours with a high TGFα but a low EGFR or a high EGFR and a low TGFα expression level without a clear connection between these mRNA findings. The TGFα expression analysis of nonmalignant tissues revealed low amounts of TGFα mRNA in 44% of the investigated cases whereas no bandings were found in the western blots. The discrepancy could be explained by the findings of immunohistochemistry. TGFα specific immunostaining reactions were detected in epithelial cells only, and stromal cells were TGFα negative. The analysis of the EGFR gene by Southern blotting detected no amplification, but in two cases, rearrangements of the EGFR fragments were found. Case number 207 had an itensified 7.7 kb banding (*Eco*RI), and case number 359 had an additional 3.4 kb banding (*Pst*I). It is of interest that specimen 359 is characterised by a high EGF binding capacity (46 fmol/mg) but only moderate amounts of EGFR mRNA (9.4%). The investigation of the TGFα gene in ovarian tumours did not show rearrangements or amplifications of the enzyme digested TGFα fragments.

DISCUSSION

In the present study we have analysed the expression of EGFR together with the EGFR binding ligand TGFα in ovarian carcinomas. Comparing results of biochemical and mRNA EGFR detection methods, it can be seen that in about 10% of the biochemically EGFR negative specimens, a weak mRNA signal was found as an indication for a lower sensitivity of the EGFR RRA as compared with the northern blot. Considering the sensitivity limits of mRNA analysis, it may be assumed that probably all ovarian carcinomas express EGFR as an essential parameter for their viability. Further experiments with the polymerase chain reaction (PCR)-method should clarify this question. The discordant results of EGF binding assay and mRNA analysis need careful interpretation. Only in two cases EGFR gene rearrangements have been found. Modifications of the receptor molecule especially influence receptor recycling [23], and a modified ligand binding domain may influence EGF/TGFα binding properties [24]. The mitogenic properties of the tumour cells is not only dependent on the presence of the growth factor receptor but also on the local concentration and the biochemical structure of the ligand. In earlier reports we described the tissue levels of EGF and EGF like factors/TGFα in specimens of ovarian and other gynaecological cancers [18]. Immunohistochemistry demonstrated that the malignant cells of the ovarian carcinomas produce EGFR [17] and its ligand TGFα [8]. TGFα is probably produced by the cells in a glycosylated transmembrane-bound preform [25, 26]. The estimation of the TGFα gene products by western blotting revealed high molecular forms in 31 out ot 33 ovarian carcinomas. Two cases were factor negative, and one of the remaining two tumours had a 14 kD form and in the second tumour, TGFα peptides with molecular weights from 6–20 kD were found. Other groups describe similar results and the existence of biologically active TGFα preforms [25, 26]. Bringman *et al.* report an activation step to the smaller 6 kD peptide by the action of elastase-like enzymes as an important step for its action [8]. The detection of the low molecular weight 6 kD form in the tumour with the extremely high TGFα expression rate can be taken as an evidence that TGFα usually exists in its preforms and may be activated locally to the 6 kD form by the action of proteases or glycosidases. However, the concentration of the 6 kD form is usually too low to be detected if it is not purified. In nonmalignant tissues, we were unable to detect TGFα in western blots, whereas both immunohistochemistry and mRNA analysis revealed TGFα expression by epithelial cells. Dilution by stromal proteins is a possible explanation for this discrepancy. However, it may also be speculated that in nonmalignant tissues, the TGFα preforms are rapidly activated to the low molecular weight 6 kD forms which will be metabolised after receptor binding [24] and thus could explain the low tissue concentration of the 6 kD form. There is no information about the metabolic half-life of the different TGFα species. TGFα producing tumours may activate TGFα in such a manner which increases the local TGFα concentration and causes a constitutive TGFα signal for the tumour cells. Comparison of TGFα mRNA analysis with the western blots and the immunostaining scores exhibit a good correlation in some cases, but discordant results also exist. The reason for this is probably a post-translational TGFα modification as described by others [26].

Comparison between EGFR and TGFα expression showed reduced EGFR binding capacities in tumours with a high EGFR mRNA signal, if tumours had a high TGFα expression level. From these results we conclude that EGFR can be down regulated in tumours producing high amounts of TGFα.

We believe that the estimation of the EGFR and TGFα expression in ovarian cancer will be helpful for the clinical characterisation of such tumours. We currently prepare the publication of clinical data indicating that the results of chemotherapy are related to the expression rates of these parameters. These findings will add further to the body of evidence that tumour biology is one of the important factors to estimate the clinical course of malignant disease.

1. Cross M and Dexter TM. Growth factor in development, transformation and tumorigenesis. *Cell* 1991, **64**, 271–280.
2. Carpenter G, Cohen S. Epidermal growth factor. *Ann Rev Biochem* 1979, **48**, 193–216.
3. Todaro G, Fryling C, DeLarco G. Transforming growths factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptor. *Proc Natl Acad Sci USA* 1980, **77**, 5258–5262.
4. Derynck R, Goeddel D, Ullrich A, Gutterman R, Bringman T. Synthesis of RNA for transforming growth factor alpha and the EGF receptor by human tumors. *Cancer Res* 1987, **47**, 707–712.
5. Mydlo JH, Michaeli J, Cordon-Cardo C, Goldenberg AS, Heston WDW, Fair WR. Expression of transforming growth factor alpha and epidermal growth factor receptor messenger RNA in neoplastic and nonneoplastic human kidney tissue. *Cancer Res* 1989, **49**, 3407–3411.
6. Todd R, Donoff BR, Gertz R, *et al.* TGF-α and EGF-receptor mRNAs in human oral cancer. *Carcinogenesis* 1989, **10**, 1553–1556.
7. Coffey R, Derynck R, Wilcox J, *et al.* Production and auto-production of transforming growth factor-α in human keratinocytes. *Nature* 1987, **328**, 817–820.
8. Kommoss F, Wintzer HO, von Kleist S, *et al. In situ* distribution of transforming growth factor and in normal human tissues and in malignant tumours of the ovary. *J Pathol* 1990, **162**, 223–230.
9. Derynck R, Roberts AB, Winkler ME, Chen EY, Goeddel DV. Human transforming growth factor α: precursor structure and expression in *E. coli*. *Cell* 1984, **38**, 287–297.
10. Brinjman T, Lindquist P, Derynck R. Different transforming growth factor α species are derived from a glycosylated and palmitoylated transmembrane precursor. *Cell* 1987, **48**, 429–440.
11. Lyons RM, Moses HL. Transforming growth factors and the regulation of cell proliferation. *Eur J Biochem* 1990, **187**, 467–473.
12. Quantin B, Breathnach R. Epidermal growth factor stimulates transcription of c-*jun* protooncogene in rat fibroblasts. *Nature* 1988, **334**, 538–539.
13. Wilding G, Lippman M, Gelman E. Effects of steroid hormones

and peptide growth factors on protooncogene c-*fos* expression in human breast cancer cells. *Cancer Res* 1988, **48**, 802–805.
14. Ran W, Dean M, Levine R, Henkle C, Campisi J. Induction of c-*fos* and c-*myc* mRNA by EGF or calcium ionophore is cAMP dependent. *Proc Natl Acad Sci USA* 1986, **83**, 8216–8220.
15. Bauknecht T, Runge M, Schwall M, Pfleiderer A. Occurrence of epidermal growth factor receptor in human adnexal tumors and their prognostic value in advanced ovarian carcinomas. *Gynecol Oncol* 1988, **29**, 147–149.
16. Bauknecht T, Janz I. Kohler M, Pfleiderer A. Human ovarian carcinoma: correlation of malignancy and survival with the expression of epidermal growth factor receptor and EGF like factor. *Med Oncol Pharmacother* 1989, **6**, 121–127.
17. Kohler M, Janz I, Wintzer HO, Wagner E, Bauknecht T. The expression of epidermal growthfactor receptor, EGF like factors, and c-*myc* in ovarian and cervical carcinomas and their potential clinical significance. *Anticancer Res* 1990, **9**, 1537–1548.
18. Bauknecht T, Kohler M, Janz I, Pfleiderer A. The occurrence of epidermal growth factor receptor and the characterization of EGF like factors in human ovarian, endometrial, cervical and breast cancer. *J Cancer Res Clin Oncol* 1989, **115**, 193–199.
19. Southern E. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975, **98**, 503–517.
20. Remmele W, Stegner HE. Immunohistochemischer Nachweis von Östrogenrezeptoren (ER-ICA) in Mammakarzinomgewebe: Vorschlag zur einheitlichen Formulierung des Untersuchungsbefundes. *Deutsches Ärzteblatt* 1986, **83**, 3362.
21. Kommoss F, Bibbo M, Colley M, *et al.* Distribution patterns and quantitation of hormone receptors in breast carcinoma by immunocytochemistry and image analysis. Part I: progesterone receptors. Analyt Quant Cytol Histol 1989, **11**, 298–306.
22. Schägger H, von Jagow G. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kD. *Anal Biochem* 1987, **166**, 368.
23. Lax I, Burgess WH, Bellot F, Ullrich A. Schlessinger J. Givol D. Localization of a major receptor-binding domain for epidermal growth factor by affinity labeling. *Mol Cell Biol* 1988, **8**, 1831–1834.
24. Schlessinger J. The epidermal growth factor receptor as a multifunctional allosteric protein. *Biochemistry* 1988, **27**, 3119–3123.
25. Teixido J, Gilmore R, Lee DC, Massague J. Integral membrane glycoprotein properties of prohormone pro-transforming growth factor-α. *Nature* 1987, **326**, 883–885.
26. Teixido J, Massague J. Structural properties of a soluble bioactive precursor for transforming growth factor-α. *J Biol Chem* 1988, **263**, 3924–3929.

Acknowledgements—We gratefully acknowledge the technical assistance of Ms. Eva Mauch. This work was supported by the Deutsche Forschungsgemeinschaft (DFG).

Eur J Cancer, Vol. 28A, No. 8/9, pp. 1437–1441, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00
Pergamon Press Ltd

Comparison of Intra-arterial Versus Intravenous 5-Fluorouracil Administration on Epidermal Squamous Cell Carcinoma in Sheep

Garry J. Harker and Frederick O. Stephens

Clinical evidence that intra-arterial chemotherapy is more effective in regressing head and neck cancers than equivalent intravenous doses is lacking. Intra-arterial versus intravenous 5-fluorouracil infusion was compared in a naturally occurring, auricular epidermal squamous cell cancer in sheep. Of 18 lesions infused intra-arterially and of 18 infused intravenously with the same dose, 39 and 11%, respectively responded objectively (over 50% regression); mean (S.E.) tumour volume reduction was 37(23) and 18(22)%, respectively. There was a statistically significant difference in the mean tumour response and in numbers of tumours regressing by at least 40% of tumour volume (50% of intra-arterial treated tumours compared with 11% of intravenous treated lesions) after the 16 day total infusion time in favour of intra-arterial treatment. Technically, the intra-arterial route in this model was an improvement on previous small animal models. These findings lend support to the need for continuing clinical study of intra-arterial infusion.
Eur J Cancer, Vol. 28A, No. 8/9, pp. 1437–1441, 1992.

INTRODUCTION

THE STATE of the art concerning the theory, development, application and criticism regarding the intra-arterial chemotherapeutic technique in the treatment of cancer, has been reviewed comprehensively [1–4]. In spite of vast clinical experience, appropriate experimental and clinical trials have not been conducted in the management of head and neck carcinoma, in contrast with hepatic cancer [5–8], to confirm the principle that intra-arterial drug administration results in higher regional tumour drug concentrations and regression rates than are attainable by systemic drug delivery [1, 9]. This is primarily due to the fact that relatively small patient numbers are managed in diverse units where the required clinical and surgical expertise are available. On this basis, multi-institutional trials, enabling standardisation of therapeutic regimens, have been difficult to establish. Several investigators have sought further information from experimental small animal models, however, most series have had drawbacks for both biological and technical reasons [10–12]. This report documents the suitability of sheep epidermal squamous cell carcinoma in the head and neck region (Fig. 1) as a model for the study of intra-arterial 5-fluorouracil (5-FU) administration.

Correspondence to G.J. Harker.
The authors are at the Department of Surgery, The University of Sydney, NSW 2006, Australia.
Revised 27 Feb. 1992; accepted 28 Feb. 1992.