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POSSIBLE INVOLVEMENT OF TRANSFORMING GROWTH FACTOR-β IN THE INHIBITION OF RAT PITUITARY TUMOR GROWTH BY ESTRADIOL

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Summary—We have shown that growth of F_4Z_2 cells and F_4Z_2 tumors was stimulated by estradiol, that of $MtTF_4$ and F_4P tumors was inhibited and that of F_4P cells remained insensitive. In the present work we explore the possible role of transforming growth factor-β (TGF-β) as a mediator of estradiol action in these pituitary tumors and cell lines. *In vivo*, estradiol treatment increased the concentration of TGF-β_1 mRNAs in tumors whose growth was inhibited by estradiol ($MtTF_4$ and F_4P) but not in tumors whose growth was stimulated (F_4Z_2). F_4Z_2 and F_4P cell lines also contained TGF-β_1 transcripts. These cells and tumors differed by two points: the level of TGF-β_1 transcript was higher in F_4Z_2 than in F_4P cells while the opposite situation was observed *in vivo* and the concentration of TGF-β_1 mRNA in cultured cells was insensitive to estradiol (1 or 100×10^{-9} M). Moreover, the secretion of TGF-β like activity assayed by two different methods was estradiol insensitive and the growth of both cell lines was dose-dependently inhibited by TGF-β_1 (ED_{50}:2×10^{-11} M). Since estradiol increases TGF-β_1 mRNA in the tumors $MtTF_4$ and F_4P whose growth is inhibited by estradiol and that TGF-β_1 inhibits the proliferation of F_4P cells it is proposed as a working hypothesis that TGF-β_1 is one of the mediators of the inhibitory effect of estradiol in pituitary tumors. No data favor the hypothesis that estradiol stimulates pituitary tumor proliferation by decreasing TGF-β production.

INTRODUCTION

Members of the transforming growth factor-β (TGF-β) family control the proliferation of various types of cells. TGF-β_1 is the member of the family the most widely studied [1–3]. Both the *in vivo* and *in vitro* biologic effects of TGF-β are remarkably diverse. Depending on cell type and cell environment TGF-β_1 may stimulate or inhibit proliferation. However, TGF-β_1 is one of the most potent polypeptide growth inhibitors known for a variety of cell types including selected types of mesenchymal and the large majority of epithelial cells [4].

Estradiol is involved in the control of the proliferation of some epithelial cells including tumor cells of epithelial origin such as the human breast cancer cell line MCF-7 [5]. The effect of estradiol and antiestrogens were shown to be linked to the control of the production and/or secretion of some enzymes and growth factors [6, 7] in agreement with the autocrine–paracrine mechanisms of growth regulation. These cells were growth-inhibited by TGF-β_1 [8] and the amount of TGF-β_1 in conditioned medium (CM) was markedly increased by two antiestrogens which have a strong negative growth modulatory activity [5]. In contrast, growth stimulatory concentrations of 17β-estradiol depressed slightly the concentration of TGF-β in CM [5]. Thus, it was suggested that antiestrogens and 17β-estradiol exert their action on cell growth through the enhancement or the decrease of TGF-β secretion and activation.

We decided to check whether such a hypothesis might be extended to an experimental model that we had developed from the $MtTF_4$ pituitary tumor whose growth presented the outstanding peculiarity of being slowed down by 17β-estradiol [9]. This model included, in addition to the $MtTF_4$ tumor, the F_4Z_2 and F_4P cell lines established from it and the F_4Z_2 and F_4P tumors which were developed by injecting cells into syngenic rats. The estradiol sensitivity and estradiol receptor content of the tumors and

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Table 1. Some features of the experimental model

	Response of growth to estradiol	Estradiol binding sites	Response of TGF-β_1 transcripts to estradiol
$MtTF_4$ tumors	Inhibition[a]	34 ± 11 fmol/mg cytosol protein	Stimulation
F_4Z_2 cells	Stimulation[b]	6700 ± 4000 binding sites/cell	Insensitivity
F_4Z_2 tumors	Stimulation[a]	74 ± 34 fmol/mg cytosol protein	Insensitivity
F_4P cells	Insensitivity[b,c]	ND	Insensitivity
F_4P tumors	Inhibition[a]	28–30 fmol/mg cytosol protein	Stimulation

[a]In animal studies; [b]in culture studies; and [c]F_4P cells after subculture No. 13. ND, not detected.

cells used are summarized in Table 1 [10, 11]. To determine whether TGF-β might be involved in the positive or the negative control of tumor growth, experiments were performed with tumors themselves and with cells from which the tumors grew. In tumors, we looked for estradiol-control of TGF-β_1 mRNA concentration. In cell culture: (i) we checked whether the effects observed *in vivo* on TGF-β_1 transcripts might be due to a direct action of estradiol on tumor cells, (ii) we looked for an estradiol-sensitive accumulation of TGF-β-like activity in CM and finally, (iii) we tested the TGF-β_1 sensitivity of cell growth.

MATERIALS AND METHODS

Estradiol and growth factors

17β-Estradiol was from Vegetadrog (Paris, France) and Sigma (St Louis, MO, U.S.A.). For studies in cell culture, stock solutions were prepared in ethanol and stored at −20°C. TGF-β_1 of porcine platelet origin was from R and D systems (Minneapolis, MN, U.S.A.). Stock solutions were prepared in 4 mM HCl, 0.1% bovine serum albumin (Albumin FAF, Boehringer Mannheim, Fed. Rep. Germany) and stored at 4°C. EGF of mouse submaxillary gland origin was prepared in the laboratory according to Savage and Cohen [12]. Stock solution was prepared in 50 mM acetic acid and stored at −20°C.

Animals and tumors

Fischer 344 female rats were from our colony. $MtTF_4$ tumor was maintained by serial injections [9]. Other tumors (F_4Z_2 and F_4P) were obtained by injection of $\simeq 2 \times 10^6$ cells harvested from culture flasks by trypsin–EDTA treatment [10]. When tumors were palpable, 10–15 days after injection, rats were implanted s.c. with a silastic capsule (1 cm long, Ref. 602–265, Dow-Corning Corp., Midland, MI, U.S.A.) filled with 17β-estradiol allowing a 17β-estradiol concentration in blood of $1–10 \times 10^{-9}$ M [9]. Tumors were collected 4–16 days later. For shorter treatments (6–48 h) rats received daily i.m. 10 μg estradiol in sesame oil. In all cases a group of control tumor-bearing rats did not receive any treatment and the tumors were collected on the same day as those of the test group.

Cell culture

F_4Z_2 cells were routinely maintained in RPMI 1640 medium (Gibco, Paisley, Scotland) containing 5% fetal-calf serum (FCS). F_4P cells were maintained in the same type of medium in which the FCS had been previously treated for 45 min at 56°C with 0.1% dextran and 1% charcoal Norit A (CT–FCS). All media contained phenol red (8 mg/ml). Neither basal growth nor estradiol-sensitivity of the growth of these cells were modified by this dye. To study the effects on TGF-β_1 mRNA concentration, cells were seeded in RPMI 1640 medium supplemented with 5% CT–FCS and cultured for 5 days without estradiol or with 1×10^{-9} M (F_4Z_2 cells) or 100×10^{-9} M (F_4P cells) estradiol. To study the effects on TGF-β activity, cells were seeded as above. Three days later the medium was replaced by a serum-free RPMI 1640 medium supplemented with 2 mg/l transferrin (Sigma) and 2×10^{-4} M Hepes buffer pH 7.4 (Gibco) and the same concentrations of estradiol as those used at seeding time. Cells were cultured for 24 h, fed again with the same medium and CMs were collected 48 h later. Cells were counted with a Coulter counter. To analyze the effects of TGF-β_1 on cell yield, cells were seeded in RPMI 1640 medium containing 5% CT–FCS. TGF-β_1 was added every day and medium was changed one time. Cells were counted 7 days later with a Coulter counter.

RNA

Total RNAs were extracted from tumors and uterus immediately after collection and from cells immediately after harvesting with PBS containing 1 mM EDTA according to Chromczynski and Sacchi [13]. The A 260/A 280 ratios were $\geqslant 1.8$. RNA (20 μg) was denatured

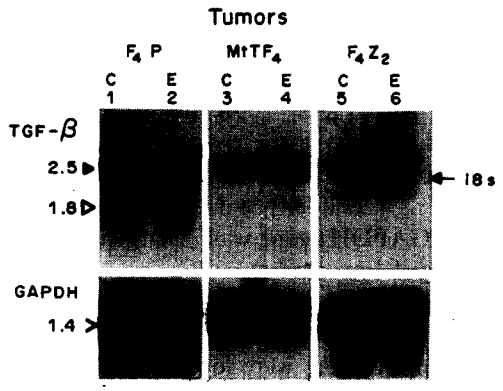

Fig. 1. Northern blots of TGF-β_1 (upper part) and glyceraldehyde-3-phosphate-dehydrogenase mRNAs (lower part) in tumors. Total RNAs were extracted from various tumors carried by control rats (C) and by rats treated for 8 days with an estradiol-implant (E). These RNAs (20 μg) were separated by electrophoresis, transferred onto a nylon membrane and were hybridized first to a single strand ^{32}P-labeled human TGF-β_1 probe (upper part), then to a ^{32}P-labeled glyceraldehyde-3-phosphate-dehydrogenase c-DNA probe (lower part). Arrows point to the position of 18 S rRNA. Similar results were obtained in series of 2–3 tumors grown from 3 different cell preparations for $MtTF_4$ and F_4P tumors and from 2 different cell preparations for F_4Z_2 tumors.

by heating (65°C for 5 min) just before electrophoresis on 1% agarose gel containing 10% formaldehyde. Thereafter, RNAs were transferred onto a nylon membrane (Hybond ™-M Amersham). Prehybridization and hybridization were carried out according to Church and Gilbert [14] and Van Obberghen Schilling *et al.* [15]. Briefly, prehybridization was performed for 1 h at 65°C in a 0.5 M disodium phosphate buffer pH 7.4 containing 1% crystallized bovine serum albumin (Sigma A-7638), 7% sodium dodecyl-sulfate and 1 mM EDTA. Hybridization was carried out in the same buffer containing labeled probe (5×10^4 cpm/cm2) for 20 h at 65°C. The single strand ^{32}P-labeled probe was prepared from the recombinant M 13 phage containing a 243 bp Pvu II fragment of the 3′ end of TGF-β_1 human cDNA non-coding strand. A specific 17-Mer oligonucleotide within the insert was used as a primer. Autoradiographs were obtained after 11–14 days exposure at −70°C to Kodak X-AR-K films with intensifying screens. Thereafter, filters were washed in water at 90°C and rehybridized with a rat glyceraldehyde-3-phosphate-dehydrogenase 1300 bp cDNA probe ^{32}P-labeled by random priming [16].

Processing of CMs

Immediately after their collection, CMs were combined with 1×10^{-3} M phenyl methyl sulfonyl fluoride (Sigma), cleared by centrifugation at 4°C (600 *g* × 4 min) and concentrated 10-fold with an Amicon ultracentrifugation cell (YM2 Diaflo ultrafiltration membranes, M_W cut-off: 1000). Transient acidification with HCl (pH 2) was performed on one half of each sample according to the method of Lawrence *et al.* [17] slightly modified. The samples, treated or not with acid, were dialyzed three times for 8 h at 4°C against 50 vol of ultrapure water (MilliQ, Millipore) using tubings of M_W cut-off 3500 (Spectrapor R, PolyLabo, Strasbourg, France), freeze dried and stored at −20°C. It was checked that pH values at the end of the dialysis were in the same range (pH 7–8) in all samples. Non-CM was processed simultaneously for each experiment. Just before TGF-β activity assay, PBS was added to achieve a 100-fold concentration.

Anchorage-independent growth assay

In 24-well tissue culture plates (Falcon), 3 layers were successively added: (1) 0.5 ml DMEM containing 0.8% agar (Difco, Detroit, MI, U.S.A.) and 10% FCS; (2) 0.25 ml DMEM containing 0.5% agar, 10% FCS and the indicator cells (2000 NRK-49F cells); and (3) 0.2 ml DMEM containing 10% FCS, 4 ng/ml EGF and one of the following: buffer, increasing amounts of TGF-β_1, increasing volumes of 100 × concentrated CM or increasing volumes of 100 × concentrated non-CM. Plates were incubated for 11–13 days in a 5% CO_2 atmosphere at 37°C. Colonies were observed by phase contrast microscopy, their number and their size were determined with a Quantimet 900 image analyzer (Cambridge Instruments Ltd, Monsey, NY, U.S.A.). The range of the areas of colonies scored was 9×10^{-4}–180×10^{-4} mm^2.

Cortisol production assays

Ovine adrenocortical cells were isolated from adrenal cortex by a sequential treatment with 0.25% trypsin in Dulbecco's modified Eagle's medium (DMEM–Ham's F-12 medium, v/v [18]. The cells were washed and cultured for 24 h in the same medium supplemented with 1% horse serum. The next day, medium was replaced by DMEM–Ham's F-12 without serum. Treatments with TGF-β_1 or CM were initiated the next day in serum-free medium for 24 h. Finally, following stimulation of cells for 1 h with ACTH (1×10^{-9} M), medium content of cortisol was measured by a specific radioimmunoassay [18].

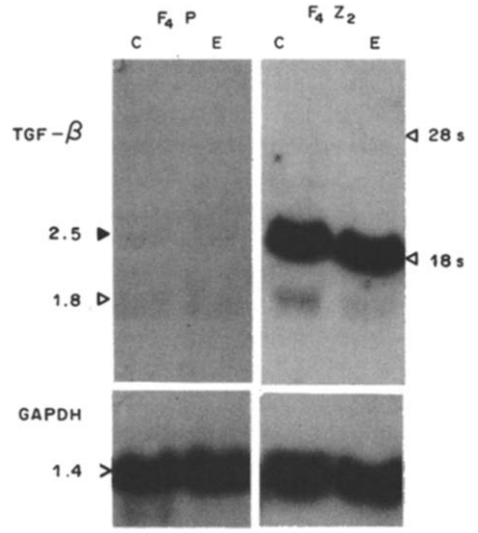

Fig. 2. Northern blots of TGF-β_1 (upper part) and glyceraldehyde-3-phosphate-dehydrogenase mRNAs in cells. Total RNAs were extracted from various control cells (C) and from cells treated with estradiol (E) for 5 days. F_4Z_2 cells were treated with 1×10^{-9} M and F_4P with 100×10^{-9} M estradiol. RNAs (20 μg) were analyzed as described in Fig. 1. Arrows point to the positions of 28 and 18S rRNAs. Similar results were found in 6 experiments with F_4Z_2 cells (passages 37 to 44) and 5 experiments with F_4P cells (passages 34 to 39).

RESULTS

TGF-β_1 mRNAs in tumors

The three types of tumors analyzed, those whose growth was inhibited by estradiol (F_4P and $MtTF_4$) and those stimulated by estradiol (F_4Z_2) contained the classical 2.5 kb TGF-β_1 mRNA (Fig. 1). In addition, a less abundant 1.8 kb transcript was observed. After estradiol treatment for 8 days (Fig. 1) or 2, 4 or 16 days (not shown) the concentrations of 2.5 and 1.8 kb transcripts increased in tumors whose growth was inhibited by estradiol (compare lanes 1 and 2, 3 and 4) and were unchanged in tumors whose growth was stimulated by estradiol (compare lanes 5 and 6). The increase, estimated from the absorbance ratio of the 2.5 kb bands varied between 1.5 to 3-fold. No modification was observed in any type of tumor for shorter treatments (6–24 h).

A technical point has to be mentioned. TGF-β_1 transcripts were revealed by hybridization with a ^{32}P-labeled single-strand probe complementary to the coding strand of a 243 bp Pvu II fragment of the 3′ end of TGF-β_1 human cDNA. Such a probe was chosen, although it was time consuming to prepare it because we found an unexpected cross hybridization in using a probe labeled by random priming. This probe prepared from the 1050 bp TGF-β_1 cDNA fragment of human λ-β C1 clone [19] revealed 18 and 28 S RNAs in addition to the TGF-β_1 transcripts. High stringency did not allow us to remove the cross hybridization.

The concentration of transcripts of the house keeping glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) gene was insensitive to estradiol (Fig. 1 lower part).

TGF-β_1 mRNAs in cultured cells

TGF-β_1 mRNAs were looked for in cultured cells from which tumors grew. In basal conditions both F_4P and F_4Z_2 cells contained the same transcripts as tumors (Fig. 2). However, the concentration of the classical 2.5 kb transcript was higher (1.5- to 10-fold according to the experiment) in F_4Z_2 than in the F_4P cells. In addition, the ratios of 2.5 to 1.8 kb transcripts determined from absorbance measurements were also higher (2- to 24-fold) in F_4Z_2 than in F_4P cells. Faint but constant signals for 2.5 and 1.8 kb transcripts were observed in all the experiments realized with F_4P cells. A common feature to both cell lines was that the concentrations of their TGF-β_1 related transcripts were insensitive to estradiol treatment for 5 days (Fig. 2) or 2 days (not shown). Estradiol concentrations used were those known to be optimal for stimulating the growth of F_4Z_2 cells (1×10^{-9} M) and inhibiting the growth of F_4P cells (1×10^{-7} M) before their growth became estradiol-insensitive [10]. We found 2.5 kb, but no 1.8 kb transcripts in MCF-7 and MB MDA 231 cell lines (not shown) suggesting that the 1.8 kb transcripts of other cells and tumors processed simultaneously might not be experimental artifacts.

TGF-β activity in CM

Due to the high background we observed in TGF-β assays using receptors carried by NRK-49 F cells or prostate cellular membranes we estimated TGF-β by two different bioassays. First, the stimulation of the anchorage-independent colony formation by NRK cells was used (Fig. 3). The transiently acidified CMs were used in order to measure the totality of TGF-β activity: the spontaneously active and the latent forms. CM of F_4P [Fig. 3(E)] and of F_4Z_2 cells [Fig. 3(F)] increased the number and the areas of colonies but that of F_4Z_2 was much more active (note, in the legend of Fig. 3, the volume of the samples and the number of cells which conditioned the medium). These effects were

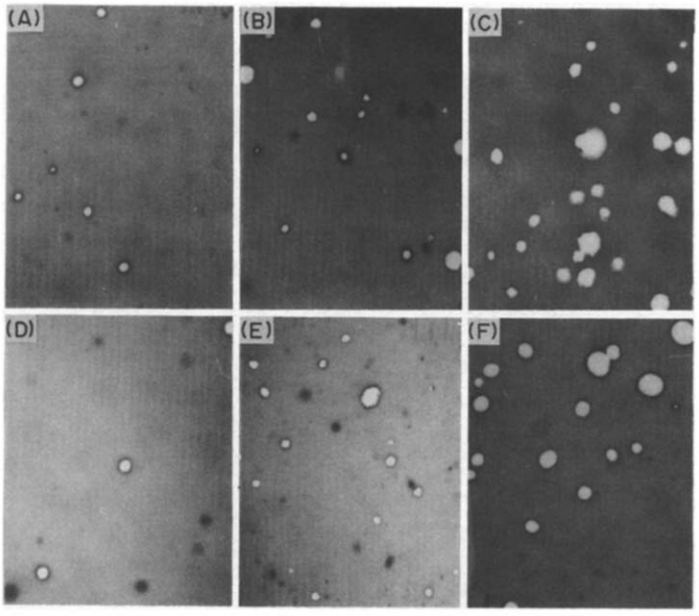

Fig. 3. Evidence for TGF-β like activity in CMs of F_4Z_2 and F_4P cells (stimulation of an anchorage-independent growth). NRK-49 F cells were cultured for 11 days in the presence of EGF (4 ng/ml) and with or without test products. Colonies were examined by phase contrast microscopy. (A) No test product. (B) TGF-β_1 1 ng. (C) TGF-β_1 2 ng. (D) 50 μl of 100-fold-concentrated medium not incubated with cells. (E) 20 μl of 100-fold-concentrated CM of 41 d F_4P cells (1 ml of medium was conditioned for 48 h by 16×10^5 cells counted at the harvesting time). (F) 5 μl of 100-fold-concentrated CM of 43 d F_4Z_2 cells (1 ml of medium was conditioned for 48 h by 7×10^5 cells counted at the harvesting time).

much more marked than with non-CM [Fig. 3(D)] and were in the range of those of TGF-β_1 [1 and 2 ng, Fig. 3 (B and C)]. Quantification by this method was found to be hazardous because the parallelism of the data from CM and the calibration curve (0.025–2 ng) was poorly documented. Rough estimations from two experiments were in the ranges 0.3–0.4 and 1.5–14 ng/10^6 cells/48 h for F_4P and F_4Z_2 cells, respectively confirming that F_4Z_2 cells were better producers than F_4P cells. Due to the large variations observed after addition of CM in soft agar we were unable to specify whether estradiol-treatment with 1×10^{-9} M for 5 days actually modified the TGF-β activity in F_4Z_2 CM (not shown). Thus, to look for possible limited estradiol effects which could not be seen in this essay and also to seek estradiol effects on F_4P cells we turned to another bioassay. This assay was supposed to be more discriminant because the end point was the result of a limited number of modifications including the inhibition of one enzyme activity involved in the control of cortisol production [18] and not the consequence of a cascade of numerous events leading to cell division. TGF-β_1 decreased in a dose-dependent manner the cortisol production by ovine adrenocortical cells [Fig. 4(A)]. TGF-β_1 activity was estimated in CMs which were either not acidified or transiently acidified [Fig. 4(B)]. Without acidification, the TGF-β like activity was in the range of 2–5 ng/10^6 cells/48 h in F_4Z_2 CM and at the limit of detection, 0.001–0.004 ng/10^6 cells/48 h, in F_4P CM. After acidification, the activity was found in the CMs of both cell lines but the F_4Z_2 cells were still higher producers than F_4P cells (2–7 vs 0.1–0.2 ng/10^6 cells/48 h). Whatever the conditions of assay, 5-day treatments with estradiol (1×10^{-9} M for F_4Z_2 or 100×10^{-9} M for F_4P cells) did not significantly modify the TGF-β activity in CM, transiently acidified or not [Fig. 5(B and D)].

Effects of TGF-β_1 on F_4Z_2 and F_4P cell proliferation

When F_4Z_2 and F_4P cells were cultured in the presence of 5% CT–FCS and increasing concentrations of TGF-β_1 the cell yield 7 days after seeding decreased in a dose-dependent manner (Fig. 6) The ED_{50} was approx. 2×10^{-11} M both for F_4Z_2 and F_4P cells.

DISCUSSION

It is not surprising that the tissues and cell lines examined in this work contained TGF-β_1 transcripts nor that the two cell lines examined

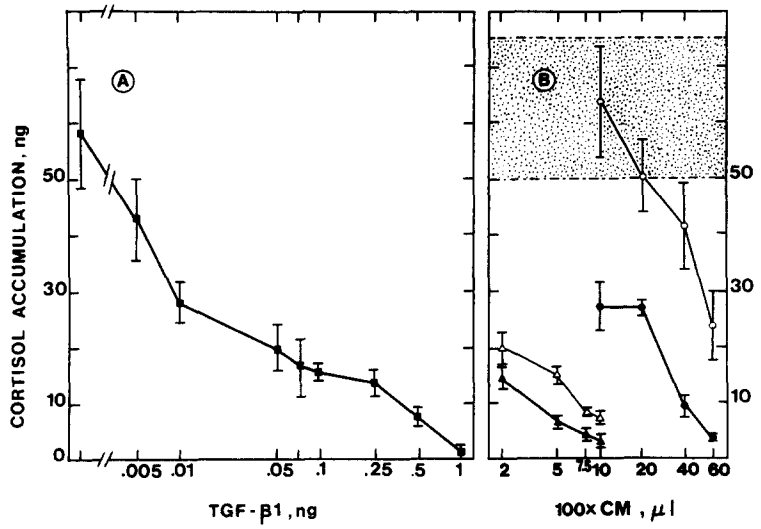

Fig. 4. Comparison of basal TGF-β activities in CMs of F_4Z_2 and F_4P cells (inhibition of cortisol production). Ovine adrenocortical cells were incubated in the presence of increasing concentrations of TGF-β_1 (A) or increasing volumes of 100-fold-concentrated CMs of 35 d F_4Z_2 cells (△, ▲) or 17 d F_4P cells (○, ●) (B). The cortisol accumulated in the medium after ACTH stimulation was measured in triplicate in 3 different wells. Results are means ± SD of a typical experiment of 4 carried out with F_4Z_2 cells and 2 with F_4P cells. The CMs were (●, ▲) or were not (○, △) transiently acidified before they were concentrated. When CMs were collected, 8×10^5 F_4Z_2 cells and 15×10^5 F_4P cells were recovered per ml of medium. The values (means ± SD) obtained with the culture medium not incubated with cells are located in the punctated area.

produced TGF-β like activity and had their growth inhibited by TGF-β_1. Indeed, the large majority of normal or tumor cells examined today contain, secrete or are sensitive to TGF-β_1 [19]. Before discussing the possible role of TGF-β in estradiol control of cell and tumor growth several features of our experimental model have to be mentioned.

F_4Z_2 and F_4P cells which were established from the same tumor display several other dissimilarities other than the difference of growth-sensitivity to estradiol. F_4Z_2 cells are better TGF-β producers and contain more TGF-β_1 transcripts than F_4P cells (this paper). F_4Z_2 cells secrete immunoreactive IGF-1 (50–200 ng/10^6 cells/5 days) while F_4P cells do not (unpublished). F_4P cells secrete PRL while F_4Z_2 do not [10] and only F_4P cells contained PRL transcripts (submitted for publication). In addition to the classical 2.5 kb TGF-β_1 transcript, a 1.8 kb transcript was revealed in all cells and tissues analyzed with a single-strand TGF-β_1 probe. Such mRNAs have already been found by others in Burkitt lymphoma B lymphoblasts Radji, in stimulated normal peripheral lymphocytes and hepatoma Hep G_2 cell line [1], in liver during regeneration after hepatectomy [20], in rat and bovine normal tissues including calvaria, diaphysis, ameloblast, bone cells [21], in the rat intestinal cell lines IEC-6 [22] and chicken embryo-chondrocytes [23]. The significance of this short transcript is not clear: cross hybridization with other members of the TGF-β family, e.g. TGF-β_4 as shown in chick embryo-chondrocytes [23], or products of alternative splicing, or products of a limited degradation of TGF-β_1 transcript. The fact that the concentrations of 1.8 kb transcript varied in parallel to 2.5 kb transcript after *in vivo* estradiol treatment (this paper) and after partial hepatectomy [20] do not favor the cross hybridization hypothesis.

The estradiol control of TGF-β_1 transcripts depended on the nature of cells and/or cell environment (Table 1). Indeed, *in vivo*, estradiol increased their concentrations in the tumors $MtTF_4$ and F_4P whose growth was inhibited by estradiol but not in the tumors F_4Z_2 whose growth was stimulated by estradiol. In cell culture, estradiol did not control TGF-β_1 transcripts neither in the F_4Z_2 cells nor in F_4P cells from which tumors grew. Two hypotheses may explain why estradiol enhanced TGF-β_1 transcripts in F_4P tumors but not in F_4P cells in culture. (1) Estradiol could increase TGF-β_1 transcripts in normal cells originating from surrounding tissues when tumors invaded them. However, a direct action of estradiol on these cells is unlikely since the same cells should be found in F_4Z_2 tumors whose TGF-β_1 transcripts

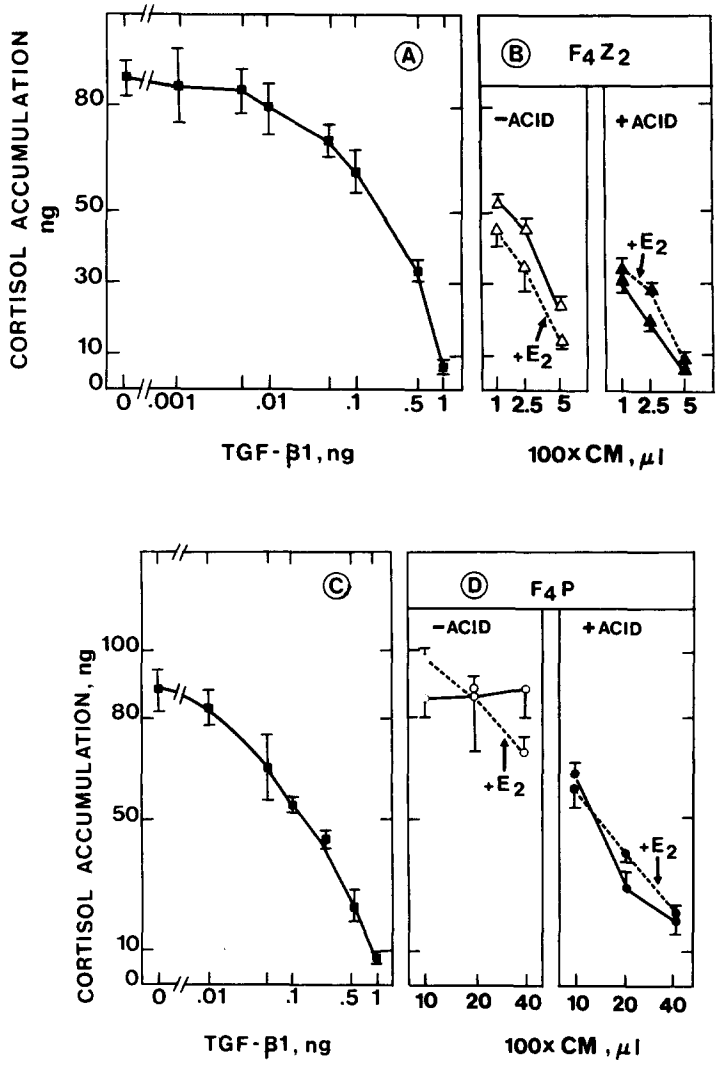

Fig. 5. TGF-β activity in CMs of cells treated or not with estradiol (inhibition of cortisol production). Ovine adrenocortical cells were incubated in the presence of increasing concentrations of TGF-β_1 (A, C) or increasing volumes of 100-fold-concentrated CM of 29 d F_4Z_2 (B) and 24 d F_4P (D) cells. CMs were acidified (+ACID) or not (−ACID) before they were concentrated. A-B and C-D show the results obtained with two different preparations of ovine adrenocortical cells. The cortisol accumulated in the medium after ACTH stimulation was measured in triplicate in 3 different wells. Results are means ± SD of a typical experiment. (B) F_4Z_2 cells were cultured in the absence (—) or in the presence (----) of estradiol (1×10^{-9} M). When CMs were collected, 6.4×10^5 cells were recovered per ml of medium both in the control and estradiol-treated flasks. (D) F_4P cells were cultured in the absence (—) or in the presence (----) of estradiol (1×10^{-7} M). When CMs were collected, 11×10^5 cells per ml of medium were recovered both in the control and estradiol-treated flasks. Similar results were obtained in three other experiments with F_4Z_2 cells and one other with F_4P cells.

were estradiol-insentitive. The role of cell population changes in the variation of TGF-β mRNA concentration in a tissue has already been reported. The TGF-β_1 mRNAs increase during liver generation has been attributed mainly to an increase of the non-parenchymal endothelial cells [20]. This hypothesis appears to us improbable in the F_4P and $MTtF_4$ tumors because their stroma is less developed [24] than in liver where it might account for 30–35% of the total liver cell population. (2) Thus, we favor the hypothesis that estradiol enhances TGF-β_1 mRNAs in tumor cells. We cannot specify whether this action is direct or indirect via endocrine or paracrine factors. The fact that the estradiol-insensitive F_4P cell line, from which the estradiol-inhibited F_4P tumor was obtained, did not contain estrogen receptor ([10] and unpublished results) does not rule out the direct hypothesis. Indeed, F_4P tumors contained estrogen receptors [10]. It is conceivable that F_4P cells recovered estrogen receptors when they

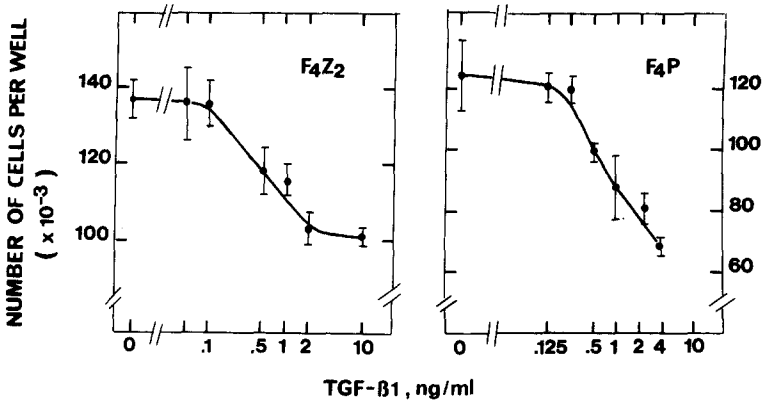

Fig. 6. Inhibition of F_4Z_2 and F_4P cell proliferation by TGF-β_1. Cells (10^4/cm^2) were seeded in RPMI 1640 medium supplemented with 5% CT–FCS in the presence of increasing concentrations of TGF-β_1. Cells were counted in triplicate 7 days later and results are expressed as means ± SD of a typical experiment on 3 realized with F_4Z_2 or F_4P cells.

grew *in vivo*. As far as the estradiol-regulation of the concentration of TGF-β_1 transcripts is concerned there is no discrepancy between F_4Z_2 cells and F_4Z_2 tumors. Indeed, both cells and tumors contained estrogen receptors [10] and their TGF-β_1 transcripts were insensitive to estradiol. The reason for such an insensitivity in cells whose growth was estradiol-responsive was not explored. This observation, taken with those of others, does not point to any simple correlation between the hormonal control of TGF-β_1 gene expression and cell growth. Indeed, the TGF-β_1 transcripts were reported: (i) to be insensitive both to the growth stimulator estradiol and to the growth inhibitory antiestrogens in MCF-7 cells [5]; (ii) to be slightly decreased by the growth stimulator dihydrotestosterone in the normal human osteoblastic cell line [25]; and (iii) to be markedly reduced by the growth inhibitor medroxy-progesterone acetate in the human breast tumor cells, T47D [26]. However, in human osteoblast-like osteosarcoma cells HOS TE 85, the estradiol responsiveness of which was not specified, estradiol increased the 2.5 kb transcript [27].

The accumulation of TGF-β like activity, including possibly TGF-β_1, TGF-β_2 and TGF-β_3, in CMs of F_4Z_2 and F_4P cells was not significantly modified by estradiol treatment. Such a result was expected for estrogen-receptor negative F_4P cells. That obtained with F_4Z_2 cells differs from those of Knabbe *et al.* [5] in MCF-7 cells whose growth was stimulated by estradiol and inhibited by the two antiestrogens 4-OH-tamoxifen and LY 117 018:estradiol was reported to decrease and antiestrogens to increase the production of TGF-β_1 at the post-transcriptional level in MCF-7 cells. It may be argued that the bioassays we used were not sensitive enough to detect small variations of TGF-β activity in F_4Z_2 CM. However, a 3–4-fold variation, like that reported in MCF-7 cells, should have been detected with our assays. Indeed, an increase of 20% TGF-β_1 activity was found after acid activation of F_4Z_2 CM. Thus, if estradiol has any effect on TGF-β production in F_4Z_2 it should be limited. Another difference has to be pointed out between TGF-β secretion by F_4Z_2 and MCF-7 cells: the latent TGF-β activity represents the large majority ($\simeq$95%) of the total TGF-β activity in MCF-7 cell secretions [5] as in numerous cell lines [17, 28, 29] while it accounts for $\simeq$20% only of the F_4Z_2 cell secretion. The proportion of the TGF-β activity in a latent form in F_4P cell secretion is similar to that of MCF-7 cells. Thus, there is no evident correlation between the growth sensitivity of cells to estradiol and the ratio of the spontaneous to the latent form of TGF-β activity.

The sensitivity of the anchorage-dependent growth of F_4Z_2 and F_4P cells to TGF-β_1 (ED_{50}: 2×10^{-11} M) is slightly lower than that of the anchorage-independent growth of NRK cells [$2–4 \times 10^{-12}$ M, 8] and is in line with the K_d of TGF-β_1 for their receptors, e.g. on GH_3 cells [30]. Thus, F_4Z_2 and F_4P cells are potential targets for TGF-β_1 *in vivo*.

In conclusion, we have shown that estradiol controls the level of TGF-β_1 transcripts in pituitary tumors whose growth is inhibited by estradiol but not in tumors whose growth is stimulated nor in cell lines from which these tumors grew. In this model, it is not obvious that TGF-β is one of the autocrine–paracrine factors involved in the estradiol control of tumor or cell growth. However, since estradiol

increases TGF-β_1 transcripts in tumors whose growth is inhibited by estradiol and that TGF-β_1 inhibits the cell proliferation, it is reasonable to propose, as a working hypothesis, that estradiol inhibits tumor growth by increasing the local concentration of TGF-β. No data favor the hypothesis that estradiol stimulates pituitary tumor growth by decreasing TGF-β production. The fact that the basal level of TGF-β_1 transcript and its control by estradiol differs in tumors and cells suggests that factors other than estradiol are involved. In the present experimental set up these factors were not investigated.

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