Transforming Growth Factor–β Modulates the High-affinity Receptors for Epidermal Growth Factor and Transforming Growth Factor–α

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ABSTRACT The epidermal growth factor (EGF) receptor mediates the induction of a transformed phenotype in normal rat kidney (NRK) cells by transforming growth factors (TGFs). The ability of EGF and its analogue TGF–α to induce the transformed phenotype in NRK cells is greatly potentiated by TGF–β, a polypeptide that does not interact directly with binding sites for EGF or TGF–α. Our evidence indicates that TGF–β purified from retrovirally transformed rat embryo cells and human platelets induces a rapid (t1/2 = 0.3 h) decrease in the binding of EGF and TGF–α to high-affinity cell surface receptors in NRK cells. No change due to TGF–β was observed in the binding of EGF or TGF–α to lower affinity sites also present in NRK cells. The effect of TGF–β on EGF/TGF–α receptors was observed at concentrations (0.5–20 pM) similar to those at which TGF–β is active in promoting proliferation of NRK cells in monolayer culture and semisolid medium. Affinity labeling of NRK cells and membranes by cross-linking with receptor-bound 125I-TGF–α and 125I-EGF indicated that both factors interact with a common 170-kD receptor structure. Treatment of cells with TGF–β decreased the intensity of affinity-labeling of this receptor structure. These data suggest that the 170 kD high-affinity receptors for EGF and TGF–α in NRK cells are a target for rapid modulation by TGF–β.

Transforming growth factors (TGFs) are hormonally active polypeptides that induce a transformed phenotype when added to normal cells (1). The transformed phenotype in normal rat kidney (NRK) cells, a known target cell line for TGFs (1), is induced by the synergistic interaction of at least two types of TGFs (2, 3). The first type, or TGF–α, is an analogue of epidermal growth factor (EGF) and is released by various neoplastic cell lines (1, 3, 4, 5). EGF and TGF–α are 6-kD polypeptides that exhibit significant amino acid sequence homology (3, 5, 6), and similar affinity and mode of interaction with a common receptor type (6, 7). The second type of TGF, or TGF–β, is a 23–25 kD molecule which consists of two disulfide-linked 11–12 kD polypeptide chains (8–11). TGF–β binds to specific receptors that do not recognize other growth factors (12–14). TGF–β has no transforming action on NRK cells when it acts alone but strongly potentiates the transforming action of TGF–α and EGF (8–11). TGF–β is found in normal tissues including human platelets, human placenta and bovine kidney (8–10), and in retrovirally transformed fibroblasts that also produce TGF–α (2, 3).

The biochemical events elicited by the interaction of TGF–β with its receptor are unknown at present. However, it has been shown that the EGF receptor is a target for regulation by agents that complement the proliferative action mediated by this receptor type. These include platelet-derived growth factor (15–18) and phorbol diester tumor promoters (19–23). The modulation of EGF receptors appears to be mediated by receptors specific for each of these agents. These receptor-receptor interactions are of interest because they can help identify primary biochemical events involved in the cellular actions mediated by growth factor receptors. Thus, direct phosphorylation of certain serine and threonine residues in
the EGF receptor by protein kinase C, the putative cellular receptor for tumor-promoting phorbol esters, has been recently implied as the biochemical basis for the modulatory action of phorbol esters on EGF receptors (22, 24). Because of the biological interaction between TGF-β and EGF analogues, we examined whether the action of TGF-β on target cells also involves modulation of EGF/TGF-α receptors. The results indicate that TGF-β from Snyder-Theilen feline sarcoma virus-transformed rat embryo (FeSV-Fre) cells and human platelets induces a rapid decrease in the binding of EGF and TGF-α to high-affinity receptors in NRK cells.

MATERIALS AND METHODS

**Growth Factors:** TGF-α and TGF-β from serum-free media conditioned by FeSV-Fre cells were purified to homogeneity as described previously (3, 11). TGF-β from human platelets (9) was purified to homogeneity by a modification (14) of a previously described method (11). No differences have been found in molecular structure or biological properties between TGF-β and FeSV-Fre cells and human platelets. When indicated, preparations of TGF-β from FeSV-Fre cells purified 2,500-fold through the C18 Bondapak reverse phase chromatography step (C18 TGF-β) (11) were used instead of homogeneous preparations of TGF-β. The purity of C18 TGF-β preparations estimated by the two bioassays described below ranged between 0.7 and 4%. Mouse submaxillary gland EGF was purified as described (25, 26). TGF-α and EGF were labeled with ¹²⁵I by the lactoperoxidase-glucose oxidase method as described previously (7) to a specific activity of 60–70 Ci/g and 80–100 Ci/g, respectively.

**Cells and Membrane Preparations:** NRK clone 49F cells (Dr. J. E. DeLarco, National Cancer Institute, and American Type Culture Collection, CRL 1570) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 5% serum plus nonessential amino acids (Gibco Laboratories, Grand Island, NY) or with 10% calf serum alone, respectively. Membranes from NRK and A431 cells were isolated as described (7).

**Treatment of Cells with TGF-β:** NRK cells (6 x 10⁴ cells/well) or A431 cells (2 x 10⁴ cells/well) were plated on 35-mm wells and incubated for 2 d at 37°C with 2.0 ml of Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum. The sparse cell monolayers were then washed with Waymouth’s medium that contained 0.2% calf serum, and were incubated for 16–24 h at 37°C in 1.0 ml of this medium before additions of TGF-β were made. TGF-β-labeled ligands measured in the presence of 5–10 μg of bovine serum albumin (BSA) and reconstituted in 50 μl of Waymouth’s medium was added to the wells to reach the desired final concentrations. Incubations continued for the indicated time at 37°C and were arrested as described below for binding measurements. In a control experiment described below, incubation of cells with TGF-β at 4–5°C was done in the presence of binding buffer instead of culture medium.

**Binding Assays:** At the end of the treatment with TGF-β, the medium was replaced with ice cold binding buffer (125 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 1.2 mM CaCl₂, 2 mg/ml of BSA, and 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonate, pH 7.4). Cell monolayers were then incubated at 0–4°C with 1.5 ml of binding buffer that contained 125I-TGF-α or 125I-EGF at 0.3 nM final concentration unless otherwise indicated. Incubations proceeded for 3.5 h on a platform oscillating at 2 cycles/s. The medium was then aspirated, and the monolayers were washed four times with ice cold binding medium. The cell-associated radioactivity was determined after solubilization of cells. Radioactivity of the supernatant was used to determine the amount of ligand bound to the cells. The cell-associated radioactivity was determined after solubilization of cells and was subtracted in all cases. Nonspecific binding was not changed by treatment of cells with TGF-β. Total binding was in all cases no more than 0.5% (NRK cells) on 7% (A431 cells) of the input radioactivity not changed by treatment of cells with TGF-β at 0–4°C was done in the presence of binding buffer instead of culture medium. After 60 min, samples were placed on ice, diluted with 4.0 ml of ice cold binding medium, and filtered under negative pressure through 0.2 μm cellulose ester microporous filters (Amicon Corp., Danvers, MA). Filters were washed twice with 4.0 ml portions of cold medium and counted in a gamma counter.

Receptor down-regulation experiments were done with A431 cells plated in 16-mm wells (8 x 10⁴ cells/well). 48 h after seeding, the spent medium was removed, and 1.0 ml of fresh medium with the indicated concentrations of TGF-β, TGF-α, or EGF was added. Incubations continued at 37°C for 16 h and were terminated by aspiration of the media. Cells were prepared for determination of ¹²⁵I-TGF-α binding as described in detail previously (7). Degradation of TGF-α and EGF during incubation for 16 h at 37°C with A431 cells in receptor down-regulation experiments was significant (8–15% of hormone released) only at the highest (0.1–0.5 nM) concentrations of TGF-α and EGF tested in these experiments, as determined by a standard radioreceptor assay (7). The concentration values in the experiment shown in Fig. 3 have been corrected to account for this limited decrease in the concentrations of active hormone during experimentation.

**Receptor Affinity-labeling:** Sparse (2.5 x 10⁴ cells/dish) NRK cell monolayers in 150-mm cell culture dishes were incubated in the cold with 15 ml of binding medium that contained 0.33 nM ¹²⁵I-TGF-α or 0.33 nM ¹²⁵I-EGF and unlabeled ligands as indicated. After 3.5 h, this medium was aspirated and cells were washed four times with ice cold binding medium. 25 ml of binding medium plus 320 μl of 27 mM disuccinimidyl suberate (Pierce Chemical Co., Rockford, IL) in dimethyl sulfoxide (28) were immediately added to the cells, and incubation proceeded for 10 min at 0–4°C. Cells were then washed twice with 0.25 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.0, detached from the plate by gentle scraping in the presence of this buffer, and centrifuged at 1,000 x g for 5 min. The cell pellet was resuspended with 10 mM Tris, 1 mM EDTA, pH 7.0, and solubilized by heating for 3 min at 100°C with 100 μl of twice-concentrated electrophoresis sample buffer (29). Insoluble material was removed by centrifugation at 12,000 x g for 10 min. Samples were electrophoresed on dodecyl sulfate-polyacrylamide slab gels as described (29). Electrophoresis gels were fixed, stained for protein, dried, and subjected to autoradiography as described (30). Molecular weight protein standards were carbonic anhydrase (30 kD), ovalbumin (45 kD), BSA (68 kD), phosphorylase b (94 kD), β-galactosidase (116 kD), and myosin (200 kD).

Affinity labeling of receptors in isolated membrane preparations was done essentially as described before (31) using 0.20 mM disuccinimidyl suberate. The efficiency of affinity labeling of cells and membranes using this methodology is ~7 and 8%, respectively, with ¹²⁵I-TGF-α and ~6 and 4%, respectively, with ¹²⁵I-EGF (reference 7; and Massagué, J., unpublished results).

**Bioassays for TGF-β:** For the assay of [³H]thymidine incorporation into DNA, sparse NRK cell monolayers were treated with TGF-β as described above. After 16 h of incubation with TGF-β, 0.5 μCi of [³H]thymidine (New England Nuclear, Boston, MA) in 25 μl of Waymouth’s medium was added to the cells. 8 h later, wells were washed twice with PBS, and cells were solubilized with 0.5 ml of 10 mM Tris, 5 mM EDTA, 0.5% sodium dodecyl sulfate, pH 7.4. Lysates were transferred to tubes which contained 1.5 ml of ice cold 10% trichloroacetic acid. Acid-precipitable material was recovered by filtration on 0.2 μm microporous cellulose filters. Filters were washed twice with 10% trichloroacetic acid and counted for radioactivity in the presence of 4.0 ml Ecoscintfluor (New England Nuclear). Control experiments in this laboratory have demonstrated that under the conditions of our assay, a strictly linear relationship exists between [³H]thymidine incorporation into DNA and the index of nuclear labeling by [³H]thymidine as determined by autoradiography of fixed cell monolayers. Thus, basal incorporation of [³H]thymidine into growth-arrested NRK-49F monolayers is obtained under conditions in which 2.4% of the nuclei become labeled; maximal [³H]thymidine incorporation elicited by full serum supplementation is paralleled by > 95% nuclear labeling; and a fractional stimulation of [³H]thymidine incorporation by TGF-β or other mitogens is closely met by the corresponding increase in the percent of labeled nuclei.

The assays for induction of anchorage-independent growth of NRK cells was performed in the presence of 0.3 mM mouse submaxillary EGF, and read as previously described (2).

RESULTS

**TGF-β Decreases the Binding of TGF-α and EGF to Mitogenically Responsive NRK Cells**

Sparse monolayers of NRK cells incubated for 24 h in medium that contained 0.2% calf serum were used in this study because under these conditions, NRK cells respond mitogenically to TGF-β as determined by [³H]thymidine incorpo-
corporation into DNA, nuclear labeling determinations, and cell number measurements (reference 11; and Massagué, J., unpublished results). TGF-β from FeSV-Fre cells and human platelets markedly stimulated the incorporation of [3H]thymidine into acid-precipitable material at concentrations (0.5–20 pM) which were also effective in inducing anchorage-independent proliferation of NRK cells in the presence of EGF analogues (Fig. 1B and reference 11). We examined the ability of TGF-β-treated NRK cells to bind 125I-TGF-α and 125I-EGF. The binding of 125I-TGF-α and 125I-EGF to NRK cells was determined at 0–4°C to minimize the contribution of post-binding events, i.e., internalization, intracellular degradation and release, or retention of 125I-ligand, that rapidly occur at higher temperatures (27, 32, 33) and might lead to erroneous quantitations of cell surface receptors. Incubation at 37°C with TGF-β from FeSV-Fre cells or human platelets decreased the ability of NRK cells to bind 125I-TGF-α or 125I-EGF when radioligands were present in the binding assay at a subsaturating (0.3 nM) concentration (Fig. 1A). The effect of TGF-β on TGF-α and EGF binding to NRK cells was concentration-dependent and was half-maximal at ~0.5–1 pM TGF-β (Fig. 1A). This effect was also rapid and time-dependent, being half-maximal after ~20 min, and was completed after ~2 h of cell exposure to TGF-β (Fig. 2). It persisted for at least 6 h after addition of TGF-β (Fig. 2). The modulation of 125I-TGF-α and 125I-EGF binding to NRK cells by TGF-β was temperature-dependent. No effect on 125I-TGF-α or 125I-EGF binding was observed in NRK cells exposed to TGF-β at 0–4°C instead of 37°C (not shown).

TGF-β did not appear to interact directly with EGF/TGF-α binding sites. Thus, biologically saturating concentrations of TGF-β did not compete with 125I-TGF-α for binding to membrane preparations from NRK or A431 cells (Table I). No detectable decrease in the binding of 50 pM 125I-TGF-α or 50 pM 125I-EGF to A431 cells was observed when cells were exposed for 2 h at 37°C to concentrations of TGF-β that were active on NRK cells (not shown). Exposure of A431 cells to TGF-α or EGF decreases the number of cell surface binding sites for these two ligands in parallel (7). This receptor down-regulation phenomenon is a function of the persistent occupancy of binding sites by the corresponding ligand (33, 34).

![Figure 1](image1.png)

**Figure 1** Dose dependent inhibition of TGF-α and EGF binding to NRK cells by TGF-β. (A) Sparse NRK monolayers were incubated for 24 h at 37°C with medium that contained 0.2% calf serum. The indicated concentrations of TGF-β from FeSV-Fre cells (○) or human platelets (■) were then added to the cells, and incubations continued for 2 h at 37°C. The medium was then aspirated, and monolayers were incubated for 3.5 h at 0–4°C with binding buffer that contained 0.3 nM 125I-TGF-α (●) or 0.3 nM 125I-EGF (○, ▲). The specifically bound radioactivity was then determined after the unbound ligands were washed out. Data are the mean (±SD, bars) of triplicate determinations and are expressed as the percentage of the specific binding of 125I-TGF-α (810 cpm/well) or 125I-EGF (390 cpm/well, ○; 405 cpm/well, ▲) respectively, to cells not treated with TGF-β. The data corresponding to FeSV-Fre-cell-derived TGF-β and platelet-derived TGF-β were obtained in separate experiments. Five repeated experiments with TGF-β from either source yielded similar results. (B) Induction of [3H]thymidine uptake by various concentrations of TGF-β from FeSV-Fre cells (○) or human platelets (▲) added to NRK monolayers cultured as described above, and proportion of NRK cells that developed large colonies in semisolid medium in the presence of 0.3 nM EGF plus the indicated concentrations of TGF-β from FeSV-Fre cells (●) or human platelets (▲). Data are the mean of duplicate determinations. The incorporation of [3H]thymidine by NRK cell monolayers treated with 10% calf serum was 185–215 pmol/10⁶ cells.

![Figure 2](image2.png)

**Figure 2** Time-dependent inhibition of 125I-EGF binding to NRK cells by TGF-β. Sparse NRK monolayers were incubated for 30 h at 37°C in the presence of 0.2% calf serum–supplemented medium. At the indicated times before the end of this incubation, 100 pM TGF-β from human platelets was added to the cell cultures. After this incubation, the medium was aspirated, and monolayers were incubated for 3.5 h at 0–4°C in the presence of ice cold binding buffer that contained 0.3 nM 125I-EGF. The specifically bound radioactivity was then determined, and is plotted as the percentage of 125I-EGF binding (785 cpm/well) to monolayers not treated with TGF-β. Data are the mean (±SD, bars) of triplicate determinations. This experiment was repeated twice with similar results.

**Table 1**

| Membrane source | Competing ligand | 125I-TGF-α bound |
|-----------------|-----------------|-----------------|
| NRK cells       | None            | 1.2             |
|                 | 66 nM TGF-α     | 5.6             |
|                 | 66 nM EGF       | 5.6             |
|                 | 330 nM TGF-β    | 2130            |
|                 | 330 nM TGF-β    | 2170            |
| A431 cells      | None            | 1.6             |
|                 | 66 nM TGF-α     | 140             |
|                 | 66 nM EGF       | 140             |
|                 | 66 nM TGF-β     | 2130            |
|                 | 330 nM TGF-β    | 2170            |

Membranes were incubated for 60 min at 22°C in the presence of 0.25 nM 125I-TGF-α alone or with the indicated concentrations of TGF-α, EGF, or platelet-derived TGF-β. Total binding was then determined as described in Materials and Methods. Data are the mean of duplicate determinations.
34). However, biologically active concentrations of TGF-β did not cause down-regulation of EGF/TGF-α receptors in A431 cells under conditions in which TGF-α and EGF did (Fig. 3). These data suggest that the modulation of TGF-α and EGF binding to NRK cells by TGF-β involves an indirect mechanism that is operative in NRK cells but is apparently missing in A431 cells.

**TGF-β Decreases Binding of EGF and TGF-α to High-affinity Cell Surface Receptors**

To gain more information on the nature of the change in EGF/TGF-α receptors induced by TGF-β on NRK cells, we analyzed the binding of various concentrations of $^{125}$I-TGF-α to control and TGF-β-treated cells. Scatchard analysis of the binding of $^{125}$I-TGF-α to control NRK monolayers at 0–4°C yielded a curvilinear isotherm (Fig. 4). The curvilinearity of Scatchard plots in other hormone receptor systems has been explained by either negative cooperativity among binding sites whereby increasing occupancy of receptors by the ligand leads to a decreasing affinity, or the simultaneous presence of binding sites with different affinities for the ligand. However, it has been shown (21) that EGF receptors in rat fibroblasts are not subject to negative cooperativity. Therefore, the simplest interpretation for the complex binding of EGF and TGF-α to receptors in NRK cells is the presence of two classes of binding sites with high and low affinity for the ligand, respectively. According to this interpretation, control NRK cells exhibit on their surface a small (~6 x 10^3 sites/cell) population of binding sites with high (K_d = 0.2–0.3 nM) affinity for $^{125}$I-TGF-α, and a larger (~1.8 x 10^4 sites/cell) population of binding sites with low (K_d = 2.7–2.9 nM) affinity for this ligand, as estimated graphically from the slope and abcissa intercept of the two rectilinear components of the Scatchard plot (Fig. 4). Treatment of NRK cells for 2 h at 37°C with platelet-derived TGF-β induced a marked decrease in high-affinity binding of $^{125}$I-TGF-α to the cells, whereas little or no decrease was observed in binding to the low-affinity sites (Fig. 4). Similar results were obtained when the experiment was repeated with TGF-β from FeSV-Fre cells (not illustrated). Thus, TGF-β appears to affect selectively the high-affinity binding sites for $^{125}$I-TGF-α in NRK cells.

**A 170-kD Receptor Species is the Target for Modulation by TGF-β**

We sought to define structurally the EGF/TGF-α receptor species that was the target for modulation by TGF-β in NRK cells. Intact NRK cells and isolated NRK membranes were cross-linked with bound $^{125}$I-TGF-α and $^{125}$I-EGF using di-succinimidyl suberate, and were displayed by electrophoresis on dodecyl sulfate–polyacrylamide gels and autoradiography (Fig. 5). NRK membrane preparations affinity-labeled with $^{125}$I-TGF-α or $^{125}$I-EGF exhibited a 170-kD labeled component (Fig. 5, lanes a and d) whose labeling could be inhibited by an excess native TGF-α or EGF added during incubation of membranes with $^{125}$I-labeled ligands (Fig. 5, lanes b, c, e, and f). We identify this 170-kD membrane component as a common receptor species for TGF-α and EGF similar in molecular size and binding properties to EGF/TGF-α receptors in other cell types (7, 33–36). No other specifically labeled components could be identified in cross-linked NRK membranes even at the relatively high (10 nM) concentrations of $^{125}$I-TGF-α and $^{125}$I-EGF used in these experiments. At these ligand concentrations, various membrane components were labeled in addition to the 170-kD species, but none of them was sensitive to the presence of an excess of native TGF-α or EGF (Fig. 5, lanes a–f).

A 170-kD EGF/TGF-α receptor species could also be identified after electrophoresis and autoradiography of intact NRK cells affinity-labeled with 0.3 nM $^{125}$I-TGF-α or 0.3 nM $^{125}$I-EGF (Fig. 5, lanes g and i). In addition to this 170-kD species, NRK cell preparations also exhibited trace amounts of a 140-kD component specifically labeled with $^{125}$I-TGF-α and $^{125}$I-EGF (Fig. 5, lanes g and i; and Fig. 6). This lower Mr species may have originated by limited proteolysis of the affinity-labeled 170-kD receptor species during scraping and homogenization of the affinity-labeled cells, as has been previously documented (37, 38). No other components specifically labeled by $^{125}$I-TGF-α or $^{125}$I-EGF could be detected in intact NRK cells (Figs. 5 and 6).

When intact NRK cell monolayers were affinity-labeled...
FIGURE 5 Affinity-labeling of EGF/TGF-α receptor species in NRK membranes and intact cells. Aliquots (0.3 mg of membrane protein) of NRK membranes suspended in 0.3 ml of binding medium were incubated for 60 min at 23°C in the presence of 10 nM 125I-TGF-α (lanes a–c) or 10 nM 125I-EGF (lanes d–f). An excess (1 μM) of unlabeled TGF-α (lanes b and e) or EGF (lanes c and f) was also present in the indicated samples. Monolayers of NRK cells were incubated for 3.5 h at 0–4°C in the presence of 0.33 nM 125I-TGF-α (lanes g and h) or 0.33 nM 125I-EGF (lanes i and j), alone (lanes g and i) or with 100 nM EGF (lanes h and j). At the end of incubations, membranes and cells were washed free of unbound ligand and cross-linked at 0°C with bound ligands using disuccinimidyl suberate as described in Materials and Methods. Electrophoresis of the affinity-labeled samples was performed on a 6–10% polyacrylamide gradient gel (lanes a–f) or on a 5–9% polyacrylamide gel (lanes g–j). Shown are the autoradiograms of the fixed, dried gels. Numbers indicate the molecular weight in kilodaltons and the position of protein standards run on parallel tracks. Arrowheads point at the 170-kD and the 140-kD labeled species.

FIGURE 6 TGF-β induces a decrease in the labeling of the 140–170 kD EGF/TGF-α receptor in NRK cells. (A) Sparse NRK monolayers cultured for 24 h in medium that contained 0.2% calf serum received 0.2 μg/ml of C18 TGF-β (equivalent to 100 pM TGF-β) (lane b) or no additions (lanes a and c). After 2 h at 37°C, monolayers were washed with binding medium and incubated for 3.5 h at 0–4°C with binding medium that contained 0.3 nM 125I-TGF-α alone (lanes a and b) or in the presence of 0.2 μM native EGF (lane c). Monolayers were then washed, cross-linked to bound ligands, solubilized, and electrophoresed on a 6–10% polyacrylamide gradient gel. An autoradiogram from the fixed, dried gel is shown. (B) Densitometry of gel lanes a, b and c, between lane origin and the 68-kD position. Arrowheads point at specifically labeled bands.

with 0.3 nM 125I-TGF-α after preincubation with TGF-β, the intensity of labeling of the 140–170 kD receptor species was only about one half of that in control cells (Fig. 6). This decrease in labeling is consistent with a lower occupancy of the 170-kD receptor species in TGF-β–treated cells. These data suggest that the 170 kD EGF/TGF-α receptor species is the target for modulation of EGF and TGF-α binding to NRK cells by TGF-β.
DISCUSSION

The results reported here indicate that exposure of NRK cells to biologically active concentrations of TGF-β leads to a change in EGF/TGF-α cell surface receptors. In confirmation of previous results (39), we find that EGF/TGF-α receptors in NRK cells that are mitogenically responsive to TGF-β exhibit a complex ligand binding pattern (Fig. 4). Complex binding of 125I-EGF that yields curvilinear Scatchard plots has also been observed in other cell types (7, 16, 20, 21, 32). The simplest interpretation of these binding properties is the presence of a mixed cell surface receptor population minimally consisting of two classes of binding sites with high (Kd = 0.2–0.3 nM) affinity and low (Kd = 2.7–2.9 nM) affinity for the ligand, respectively. According to this model, our data suggest that TGF-β induces a rapid (t1/2 = 0.3 h) and persistent inhibition of ligand binding to high-affinity sites on the surface of NRK cells without significantly altering the binding to lower affinity sites. This effect of TGF-β is temperature- and concentration-dependent. The effect on 125I-TGF-α and 125I-EGF binding to NRK cells was half-maximal at 0.5–1.0 pM TGF-β, a concentration lower than the concentration (2–10 pM) of TGF-β required for half-maximal stimulation of [3H]thymidine incorporation into DNA or anchorage-independent proliferation of NRK cells. The apparent discrepancy in the range of TGF-β concentrations required for these two types of effects is likely due to substantial degradation of TGF-β during prolonged (1–10 d) incubation with cells in the mitogenic activity assays.

EGF and TGF-α share a common 170-kD receptor type in NRK cells as determined by receptor-affinity labeling methodology (Figs. 5 and 6). The sharing of a common receptor species by EGF and TGF-α has been documented in other cell types as well (1, 7, 39, 40). The decreased affinity labeling of this receptor species in TGF-β-treated NRK cells as compared with untreated cells is consistent with it being the target for TGF-β regulation of TGF-α and EGF binding.

No other receptor species for TGF-α or EGF could be identified in affinity-labeling experiments performed in this laboratory with five different NRK cell membrane preparations or with intact NRK cells obtained from two different sources. Similarly, only 140–170 kD receptor species common for EGF and TGF-α were detected by affinity labeling of membranes from other tissues and cell types including rat and human liver, rat and human placenta, rat adipocytes, A431 human epithelioid carcinoma, T24 human bladder carcinoma, T85 human osteosarcoma, WI38 human lung fibroblasts, FeSV-Fre cells, and Swiss 3T3 mouse fibroblasts (reference 7; and Massagué, J., manuscript submitted for publication). The results from ligand binding experiments (Table I and reference 7) are also inconsistent with the hypothesis of separate receptor types existing for EGF and TGF-α. These results are in contrast to the previously reported presence of a 60-kD membrane component in NRK cells that can interact with “sarcoma growth factor” (partially purified mouse TGF-α that also contains TGF-β, see reference 2) but not with mouse EGF (40). This difference could be due to the use in previous studies of crude preparations of “sarcoma growth factor” derivatized with [125I]iodo N-succinimidyl-3-(4-hydroxyphenyl) propionate and purified by binding and elution from A431 cells (40) instead of homogeneous preparations of chemically and biologically intact 125I-TGF-α (7) as used in the present studies. The 60-kD labeled component could be a minor TGF-β receptor species (14) affinity labeled by 125I-TGF-β present in the radiolabeled sarcoma growth factor preparations.

The modulation of EGF/TGF-α receptors by TGF-β does not appear to result from a direct interaction of this factor with EGF or TGF-α binding sites for various reasons. First, TGF-β does not compete with 125I-TGF-α (Table I) or 125I-EGF (not shown) for binding to receptors in isolated membrane preparations. Other studies (12–14) show that 125I-TGF-β binds with high affinity to membrane receptors in various cell lines including NRK cells, and binding is competed for by TGF-β but not by EGF or TGF-α. Furthermore, receptor affinity–labeling studies indicate that the receptor for 125I-TGF-β in rat fibroblasts is a glycosylated, disulfide-linked complex that contains a 280-kD ligand binding subunit, and is therefore distinct from EGF/TGF-α receptor structures (14, 41, 42). Secondly, active concentrations of TGF-β do not cause down-regulation of EGF/TGF-α receptors in A431 cells under conditions in which mitogenically equivalent concentrations of EGF or TGF-α do. Third, we did not observe any decrease in the binding of low concentrations of 125I-TGF-α or 125I-EGF to A431 cells treated with TGF-β under conditions in which TGF-β elicited a marked decrease in the binding of these ligands to NRK cells. These observations indicate that the effect of TGF-β on EGF/TGF-α receptors in NRK cells is mediated by a mechanism that may not be operative in other cell types (such as A431 cells), or under other cell culture conditions.

The results reported here are in contrast to the observation (43) of a small and transient decrease of EGF binding to NRK cells after addition of TGF-β, then a two-fold increase in binding at later (6 h) time points. The reason for this discrepancy is unclear, but it is interesting to note that the different effects of TGF-β on EGF binding observed in these two studies are correlated with differences in the ability of NRK cell cultures to respond mitogenically to TGF-β. Thus, whereas other studies (43) were carried out with confluent monolayers of NRK cells reported to be mitogenically unresponsive to TGF-β, the results reported here were performed with sparse, serum-deprived cultures of NRK cells that respond mitogenically to TGF-β. It is possible that TGF-β has a dual action on EGF/TGF-α receptors, and that the inhibitory effect of TGF-β on EGF/TGF-α binding predominates in situations in which cells evolve from a growth-arrested state to a mitogenically active one.

The effect of TGF-β on EGF/TGF-α receptors described here is similar, and may be related to the modulation of the affinity of this receptor type by platelet-derived growth factor (14–17), fibroblast-derived growth factor (18), and tumor promoters including phorbol diterpene esters, certain indole alkaloids, and polyacetates (20, 21, 23). These agents have been reported to potentiate or complement the cellular actions of EGF analogues, yet they acutely decrease the binding of 125I-EGF to high-affinity receptors in cultured cells. It is possible as proposed before (21) that potentiation of long-term actions of EGF by these agents is a consequence of a decreased degradation of EGF in the medium due to its decreased binding and internalization by cellular receptors. However, the exact significance of these paradoxical observations may not be clear until the role and cellular dynamics of EGF/TGF-α receptors with high and low apparent affinity for the ligand are established.

The observations reported here may help identify primary
molecular events involved in the action of TGF-β. Thus, the effect of TGF-β on EGF/EGF-α receptors may be accomplished by the induction of a molecular change in this receptor molecule that could convert it into a lower affinity state or target it for rapid removal from the cell surface. In an analogous situation, a molecular change of the EGF receptor, namely its phosphorylation on discrete sites by protein kinase C, has been proposed as the basis for modulation of EGF receptors by phorbol ester tumor promoters (22, 24, 44). Alternatively, TGF-β may induce a redistribution between the cell surface and an intracellular location(s) of preexisting EGF/EGF-α binding sites with different affinity for the ligands. Efforts to address the molecular basis for the modulation of EGF/EGF-α receptors by TGF-β are underway.

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