0013-7227/98/$03.00/0
Endocrinology

Vol. 139, No. 3
Printed in U.S.A.

Developmental and Hormonal Regulation of Transforming Growth Factor-α and Epidermal Growth Factor Receptor Gene Expression in Isolated Prostatic Epithelial and Stromal Cells*

NAOKI ITOH, URVASHI PATEL, AND MICHAEL K. SKINNER

Center for Reproductive Biology, Department of Genetics and Cell Biology, Washington State University, Pullman, Washington 99164-4231

ABSTRACT

Androgen has an important role in development of the prostate, and the actions of androgen are mediated, in part, by locally produced growth factors. These growth factors are postulated to mediate stromal-epithelial interaction in the prostate to maintain normal tissue physiology. Transforming growth factor-α (TGF-α) is one of the growth factors that can stimulate prostatic growth. The expression of TGF-α is thought to be regulated by androgen. The expression of epidermal growth factor receptor (EGFR), which is the receptor of TGF-α and EGF, also may be regulated by androgen. The hormonal and developmental regulation of TGF-α and EGFR messenger RNA (mRNA) levels in isolated epithelial and stromal cells from rat ventral prostate was investigated. The expression of mRNA for TGF-α and EGFR was analyzed by a quantitative RT-PCR (QRT-PCR) procedure developed. Observations from this assay demonstrated that both epithelial and stromal cells expressed the mRNA for TGF-α and EGFR. TGF-α mRNA expression was constant during postnatal, pubertal, and adult development of the prostate. EGFR mRNA expression was elevated at the midpubertal period and decreased with age. After castration of 60-day-old adult rats, both TGF-α and EGFR mRNA were significantly enhanced. TGF-α mRNA expression was stimulated by EGF in stromal cells (4.5-fold increase) but was not changed by any treatment in epithelial cells. EGFR mRNA levels were stimulated by EGF and keratinocyte growth factor treatment and inhibited by testosterone treatment in epithelial cells. Stromal cell EGFR mRNA levels were not affected by any treatment. Both testosterone and EGF stimulated incorporation of ^{3}H-thymidine into prostatic stromal and epithelial cells. Anti-TGF-α antibody significantly inhibited testosterone-stimulated ^{3}H-thymidine incorporation into stromal cells and epithelial cells. Immunocytochemical localization of TGF-α and EGFR demonstrated expression on the luminal surface of epithelial cells within prostatic ducts, and minimal expression was observed in stromal cells.

Results indicate that testosterone does not directly regulate TGF-α mRNA levels but does inhibit EGFR mRNA levels. Interestingly, anti TGF-α antibody suppressed the effect of testosterone on ^{3}H-thymidine incorporation into prostatic stromal and epithelial cells. This finding suggests that testosterone may act indirectly on prostatic cells to influence TGF-α actions. TGF-α mRNA levels were influenced by EGF in stromal cells only, and EGFR mRNA levels were influenced by testosterone, EGF, and keratinocyte growth factor in epithelial cells. These observations suggest that regulation of TGF-α and EGFR is distinct between the cell types. In conclusion, a network of hormonally controlled growth factor-mediated stromal-epithelial interactions is needed to maintain prostate development and function. (*Endocrinology* **139:** 1369–1377, 1998)

ANDROGEN and growth factors orchestrate proliferation, differentiation, and function of prostatic cells (1–3). These factors are required to maintain the homeostasis of prostate growth and function through stromal-epithelial interactions (4). Several growth factors have been shown to mediate stromal and epithelial cell interactions in the prostate (5–7). Transforming growth factor-α (TGF-α), epidermal growth factor (EGF), basic fibroblast growth factor, keratinocyte growth factor (KGF), and insulin-like growth factors stimulate growth of prostatic epithelial cells (6, 8, 9). Transforming growth factor-β (TGF-β) has an inhibitory effect on prostatic cell growth (10–12). Because androgen is essential to maintain growth, development, and function of the prostate, the regulation of these growth factors by androgen has been studied (7, 10, 13, 14).

TGF-α and EGF are mitogenic polypeptides that consist of 50 and 53 amino acids, respectively, and act on cells by binding the EGF receptor (EGFR) (15). TGF-α is structurally and biologically similar to EGF. High concentrations of EGF were determined in rat prostate (16, 17). EGFR expression was observed in rat epithelial cells (18). After castration, both EGF and EGFR expression increase, and expression is decreased with subsequent androgen treatment (13, 19, 20), although some investigators have found that EGF expression may fall after castration (for review, see Ref. 8). These observations suggest that EGF/TGFα may have a crucial role in the prostate and that EGF and EGFR seem to be negatively regulated by androgen. TGF-α expression has been demonstrated in normal rat ventral prostate (18). TGF-α is thought to have a role also in prostate cancer. TGF-α expression has been observed in prostatic carcinoma and prostatic carcinoma cell lines such as LNCaP, PC-3, and DU-145 (21–23). It has not been reported whether TGF-α expression is directly regulated by androgen. Regulation of growth factor expression is complex and influenced not only by androgens but also by a network of locally produced factors within the prostate. We have recently reported that TGF-β expression is

Received August 18, 1997.
Address all correspondence and requests for reprints to: Michael K. Skinner, Ph.D., Center for Reproductive Biology, Department of Genetics and Cell Biology, Washington State University, Pullman, Washington 99164-4231. E-mail: skinner@mail.wsu.edu.
* This study was supported by NIH Grants DK-45889 and CA-59831.

regulated by EGF and KGF but not testosterone (24), whereas TGF-β1 is negatively regulated by androgen *in vivo*.

The aim of the current study was to investigate the hormonal and developmental regulation of TGF-α and EGFR messenger RNA (mRNA) expression in rat ventral prostate. A quantitative reverse transcription PCR (QRT-PCR), to quantitate mRNA levels, was established. The expression of TGF-α and EGFR mRNA were analyzed in fresh ventral prostate from neonate to older ages and in isolated prostatic stromal and epithelial cells derived from 20-day-old rat ventral prostate.

Materials and Methods

Animals

Male Sprague-Dawley rats were purchased from Bantin-Kingman Inc. (Kent, WA); 1, 20, 60, and 100-day-old rats were used for the study of prostate development. The 60-day-old rats were castrated under anesthesia of Avertin, which consists of 25 g Tribromoethanol with 15.5 ml Tertiary amyl alcohol. Three days later, the prostate glands were removed. For culture of both prostatic epithelial and stromal cells, 20-day-old rats were used. All procedures were approved by the university animal care committee.

Culture of ventral prostate cells

Twenty-day-old SD rats were killed, and ventral prostates were removed. Tissue was incubated with HBSS (Gibco BRL, Gaithersburg, MD) containing 675 U collagenase activity/ml of Type II collagenase (Sigma, St. Louis, MO) and 0.04% deoxyribonuclease I (Sigma) at 37 C for 4 h. After incubation, tissue was separated by centrifugation. The mixture was spun at 30 × *g* for 4 min to pellet the epithelial cells. The remaining supernatant was centrifuged at 190 × *g* for 6 min to pellet the stromal cells. To ensure highly pure populations of cell types, both pellets were resuspended and spun at 30 × *g* for 4 min. The supernatant from stromal cells was removed and repelleted at 190 × *g* for 6 min. These isolated cell populations were then subsequently placed in 10% bovine calf serum F12 media. After 24 h, the cells were washed in serum-free F12 media for 24 h. The following day, the media was changed with F12, with or without the following treatments: 10^{-7} M testosterone (Sigma), 100 ng/ml EGF (Gibco BRL), 25 ng/ml KGF (Gibco BRL), 10 ng/ml human recombinant TGF-β1 (Gibco BRL), 10% bovine calf serum (Hyclone, Logan, UT), combination of TGF-β1 and testosterone 10^{-7} M, EGF or 10% bovine calf serum. The treatments were for a period of 72 h. On the sixth day of culture, cells were harvested for RNA extraction. Cell purity has been studied in previous reports (24) and was analyzed using immunohistochemistry of both cell populations.

RNA preparation

Total RNA was obtained using TRI REAGENT (Sigma). Briefly, tissue or cells were lysed in TRI REAGENT (1 ml per 50–100 mg of tissue, 1 ml per 10 cm^2 of culture plate). After adding 0.2 ml chloroform per ml of TRI REAGENT, the mixture was centrifuged at 12,000 × *g* for 15 min at 4 C. Colorless upper aqueous phase was transferred to a fresh tube, and 0.5 ml isopropanol per ml of TRI REAGENT was added to pellet RNA. The mixture was centrifuged at 12,000 × *g* for 10 min at 4 C. The RNA pellet was washed with 75% ethanol and resuspended in diethylpyrocarbonate (DEPC) H_2O. RNA was stored at −80 C until analysis.

RT

Complementary DNA (cDNA) was synthesized in a total vol of 20 μl containing 1 μg total RNA, 0.05 μM of specific 3′-primers (TGF-α: 5′-CAG AGT GGC AGC AGG CAG TC-3′, EGFR: 5′-GAG TGG TGG GCA GGT GTC TT-3′, cyclophilin: 5′-ATT TGC CAT GGA CAA GAT GCC-3′), 4 μl of 5× first standard buffer (Life Technologies, Gaithersburg, MD), 10 mM dithiothreitol (Life Technologies), 0.125 mM deoxynucleotide triphosphates, 100 U murine leukemia virus transcriptase (Life Technologies), 20 U inhibitor (Promega, Madison, WI), and DEPC-H_2O. Initially, RNA was denaturated and annealed in the presence of each primer and DEPC-H_2O at 65 C for 15 min. The tube was placed on ice for 5 min. The other reagents were added to the tube and incubated at 42 C for 2 h. To decrease high background signal, a higher RT reaction temperature (42 C) was used (25). The mixture was incubated at 95 C for 5 min to inactivate murine leukemia virus transcriptase. The mixture was diluted by UV-treated H_2O containing Bluescript plasmid (Stratagene, La Jolla, CA) as carrier DNA. Final concentrations of cDNA and Bluescript plasmid were 1 ng/μl and 10 ng/μl, respectively. This concentration of Bluescript plasmid was included in all samples and standards.

Quantitative PCR

As a standard, PCR products of TGF-α, EGFR, and cyclophilin (amplified by each specific primer) were subcloned into Bluescript plasmid (Stratagene). Each subclone was sequenced in both directions and confirmed to be rat TGF-α, EGFR, and cyclophilin. The size of the PCR product generated was as follows; 138 bp (TGF-α), 208 bp (EGFR), 105 bp (cyclophilin). Plasmid DNA containing TGF-α, EGFR, or cyclophilin subclones were used to generate standard curves ranging from 0.1 fg/μl to 1.0 pg/μl (cyclophilin: 10 fg/μl to 100 pg/μl). PCR was performed on a Perkin-Elmer GeneAmp PCR System 9600 and carried out in a total 25-μl reaction vol containing 5 μl of the plasmid DNA or RT reaction, 0.4 μM of 3′ primer as shown above, 0.4 μM of 5′ primer (TGF-α: 5′-TGG CTG TCC TCA TTA TCA CC-3′, EGFR: 5′-CTG CTG GGG AAG AGG AGA GGA GAA C-3′, cyclophilin: 5′-ACA CGC CAT AAT GGC ACT GG-3′), 2.5 μl of ×10 GeneAmp PCR buffer (containing 1.5 mM $MgCl_2$, Perkin-Elmer, Branchburg, NJ), 25 μM deoxynucleotide triphosphates, 0.5 U of *Taq* DNA polymerase (Perkin-Elmer), 1 μCi of [α -^{32}P] deoxycycidine triphosphate (Amersham Life Science, Inc. Arlington Heights, IL).

The reaction cycle sequence comprised 5 min at 95 C followed by 25 cycles comprising 1 min at 95 C, 2 min at 55 C, 3 min at 72 C for TGF-α (EGFR: 30 cycles); and 25 cycles comprising 1 min at 95 C, 1 min at 60 C, 2 min at 72 C for cyclophilin.

After PCR, the PCR products were separated by electrophoresis on 6% acrylamide gel. After running the gel, it was dried and analyzed by a Phosphoimager (Molecular Dynamics, Sunnyvale, CA). Each gene was assayed in separate PCR reactions from the same RT samples. Equivalent steady-state mRNA levels for each gene were determined by comparing each sample with the appropriate standard curve. TGF-α and EGFR were normalized for cyclophilin. All samples were simultaneously measured in duplicate with an intraassay variability of 5.3% (TGF-α), 12.2% (EGFR), and 9.2% (cyclophilin).

^{3}H -Thymidine incorporation assay

Cell growth was analyzed by quantitating ^{3}H-thymidine incorporation into newly synthesized DNA. Stromal and epithelial cells were placed at subconfluent densities (less than 1 million cells/cm^2) in 0.5 ml DMEM containing 0.1% calf serum. Cells were plated for 24 or 48 h then treated for an additional 24 h. After treatment, 0.5 ml DMEM containing 2 μCi ^{3}H-thymidine was added to each well, and cells were incubated for 4 h at 37 C, followed by sonication. The quantity of ^{3}H-thymidine incorporated into DNA was determined as previously described (26). Data were normalized to total DNA per well using an ethidium bromide procedure, described previously (27). Under these subconfluent culture conditions, approximately 0.5–1.5 μg DNA was detected per well. Values of ^{3}H-thymidine incorporation were generally greater than 2×10^3 cpm/μg DNA.

Immunocytochemistry

Ventral prostate sections were obtained from 20-day-old Sprague-Dawley rats. Tissue specimens were immediately fixed in Bouin's solution for 3–6 h. Tissue was sectioned at 5-μm thick, deparaffinized, rehydrated, and treated with an aqueous solution of 3.0% H_2O_2 to quench endogenous peroxidase. After several washes in Tris-buffered saline (TBS) buffer, sections were then incubated in TBS containing 1% BSA for 2 h at 37 C to reduce nonspecific staining. After rinsing three times in TBS, a sheep antirat TGF-α antibody (East Acres Biological, South Bridge, MA) or a monoclonal EGFR antibody (Sigma) was added

at a 1:500 and 1:100 dilution, respectively, and incubated overnight at 37 C, followed by 2 h at 4 C. After three rinses in TBS, the primary antibody was detected with antisheep or antimouse biotinylated secondary antibody, and the biotin was detected with an avidin-biotin peroxidase kit (ABC-Elite, Vector Laboratories, Burlingame, CA). Diaminobenzidine tetrachloride was used as a chromagen, and serial sections were lightly counterstained with hematoxylin.

Statistical analysis

All data were analyzed by a JMP 3.1 statistical analysis program (SAS Institute Inc., Cary, NC). All values are expressed as mean ± SEM Statistical analysis was performed using one-way ANOVA. Significant differences were determined using the Dunnett's test for comparison with control and using the Tukey-Kramer HSD (honestly significant difference) test for multiple comparisons. Statistical difference was confirmed at $P < 0.05$.

Results

Characterization of the QRT-PCR of TGF-α and EGFR

A reproducible and accurate procedure for QRT-PCR was established. The linearity of the PCR reaction, relative to cycle number, was determined (28). The PCR product for TGF-α increased at 21 cycles and reached a plateau at 30 cycles. This suggested that 25 cycles was an appropriate cycle number for QRT-PCR (Fig. 1). For EGFR the PCR, product continued to amplify at 39 cycles. Thirty cycles was midpoint on the linear amplification of the PCR product and was judged appropriate for analysis (Fig. 1). In regard to cyclophilin, we have previously reported that 25 cycles is appropriate (24). Products of both standard plasmid DNA, which contained a subcloned PCR product containing rat cDNA and cDNA produced from an unknown sample, were analyzed. PCR products of diluted plasmid DNA and diluted unknown cDNA were compared. These two curves were parallel and indicated that the QRT-PCR is reliable for quantitative analysis (Fig. 2, A and B). The coefficient of variation for the intraassay and interassay variability was found to be between 5 and 10%.

Analysis of freshly isolated and cultured stromal and epithelial cells

The purity of the isolated cell populations was determined with immunocytochemistry and analysis of the cultures. For

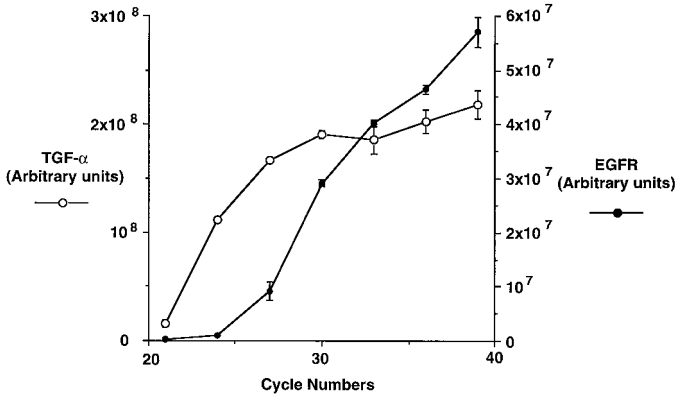

FIG. 1. Relationship between cycle numbers and arbitrary units of PCR products of TGF-α and EGFR. Values are expressed as mean ± SEM (n = 3). Linearity relationship with cycle number and PCR product are shown for TGF-α and EGFR.

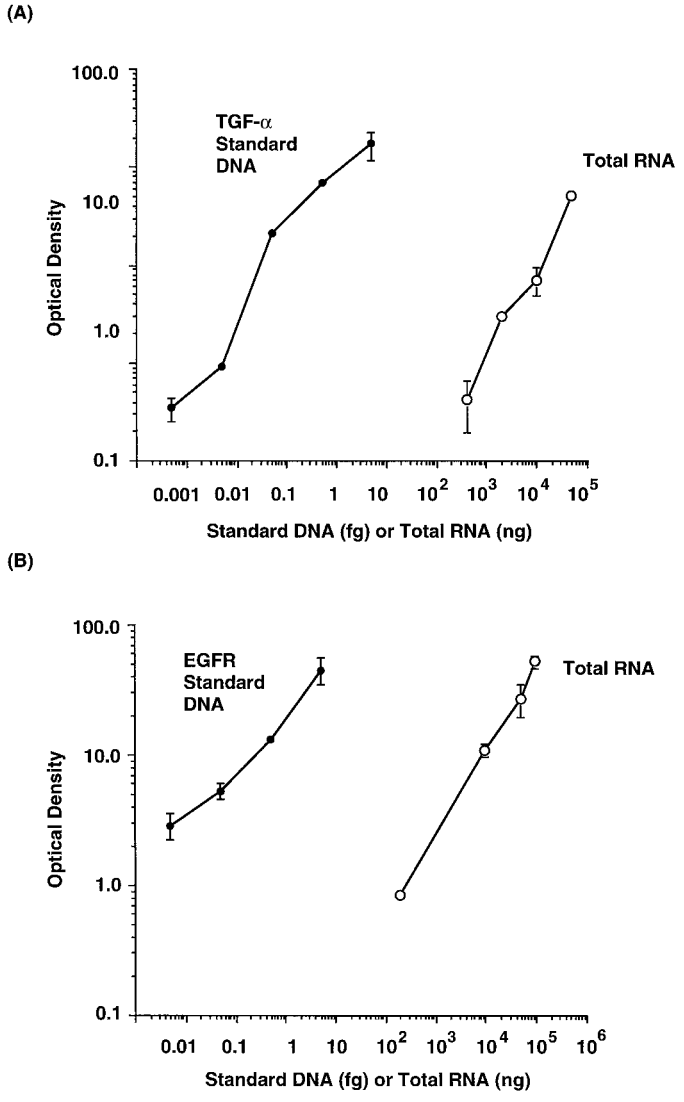

FIG. 2. Dilution curve of both plasmid DNA (standard) and total RNA for TGF-α (A) and EGFR (B). Values are expressed as mean ± SEM (n = 2). Parallel relationships in PCR products between plasmid DNA and total RNA are shown. The quality of PCR product is expressed as optical density; the amount of plasmid DNA, in femtograms; and RNA, in nanograms.

the epithelial cells, keratin staining identified the cells; and for the stromal vimentin cells, staining was used. The purity of the cell preparations was routinely monitored and found to be 93 ± 1% purity for the epithelial cells and 86 ± 4% purity for the stromal cells. This will be noted in any data interpretations to be discussed.

The QRT-PCR was used to quantitate mRNA expression of TGF-α and EGFR in both stromal and epithelial cells of rat ventral prostate. Differences in expression between freshly isolated cells and cultured cells were examined. In fresh samples (20-day-old rat prostate), TGF-α expression normalized by cyclophilin ranged from $0.001–0.004 \times 10^{-3}$. These values were lower than EGFR, which ranged from $0.006–0.028 \times 10^{-3}$ (Fig. 3). EGFR and TGFα expression was significantly higher ($P < 0.01$) in cultured epithelial cells, compared with freshly isolated cells. Cultured and freshly

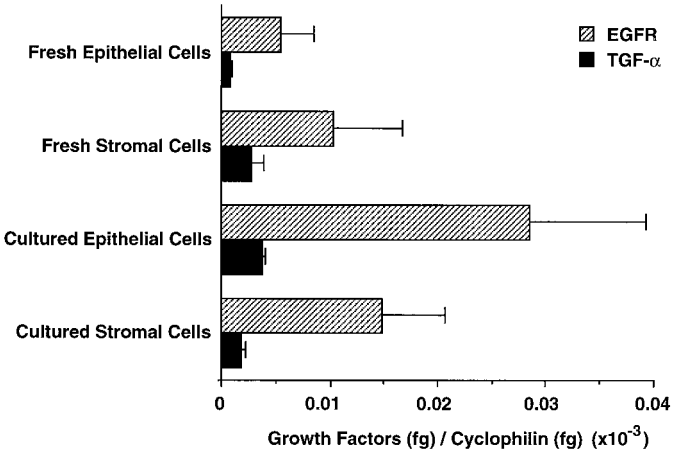

FIG. 3. Expression of mRNA for TGF-α and EGFR in both epithelial cells and stromal cells. Fresh cells (which are separated into epithelium and stroma from 20-day-old rat ventral prostate) and cells after 6-day culture are analyzed. Values are normalized by cyclophilin and expressed as mean ± SEM (n = 4).

isolated stromal cells showed comparable levels of expression with no statistically significant difference.

Developmental regulation of TGF-α and EGFR

To investigate the changes in TGF-α and EGFR mRNA levels during rat prostate development from a neonatal period (1-day-old) to an older age (100-day-old), ventral prostates were removed from rats aged 1, 20, 60, and 100 days of age, and the mRNA levels were measured by QRT-PCR. The effects of androgen depletion on TGF-α and EGFR expression were analyzed by castration of 60-day-old rats. All the expression data for TGF-α and EGFR were normalized by cyclophilin expression. Relative mRNA levels, compared with 20-day-old mRNA levels set as 1.0, are presented for comparative purposes. TGF-α expression did not vary during development (Fig. 4). After castration of 60-day-old rats, TGF-α mRNA levels significantly increased to 4.7-fold that of 60-day-old rats. EGFR expression was increased in a midpubertal stage and tended to decrease with age. After castration, EGFR expression elevated to 3.7-fold that of 60-day-old rats; however, not significantly different from 20-day-old levels of expression (Fig. 4). Except for the castration data, the error associated with individual developmental points was relatively low, and it represents the mean ± SEM from 3–5 different experiments.

Hormonal regulation of TGF-α and EGFR

To elucidate the effects of testosterone and growth factors on TGF-α and EGFR expression (*i.e.* mRNA levels), both stromal and epithelial cells from ventral prostate of 20-day-old rats were cultured and treated. EGF and KGF were used as stimulatory growth factors, and human recombinant TGF-β1 was used as an inhibitory growth factor. TGF-β1 was combined with testosterone or EGF to study potential suppressive effect. All values were normalized by cyclophilin and expressed as relative expression, compared with control nontreated cells.

EGF significantly stimulated (4.2-fold) TGF-α expression in stromal cells, and the EGF effect was not inhibited by TGF-β1 (Fig. 5A). Combination of EGF and TGF-β1 stimulated TGF-α expression 2.8-fold but was not significant. Testosterone (10^{-7} M) had no effect on the expression of TGF-α. Similar observations were made with 10^{-6} M and 10^{-8} M concentrations of testosterone (data not shown). KGF and TGF-β1 alone also showed no effect on TGF-α mRNA levels. TGF-β1 did not influence the effect of testosterone on TGF-α expression. Interestingly, in epithelial cells, no treatment was found to alter the expression of TGF-α (Fig. 5A).

In stromal cells, no treatment altered the expression of EGFR (Fig. 5B). In epithelial cells, both KGF and EGF significantly stimulated EGFR expression. This stimulation by EGF was suppressed by TGF-β1. TGF-β1 alone did not affect EGFR expression (*i.e.* mRNA levels). Testosterone (10^{-7} M) significantly inhibited EGFR expression. A combination of testosterone and TGF-β1 had the same suppression as testosterone alone. Therefore, the only effect of TGF-β1 was an inhibition of EGF on EGFR mRNA expression in epithelial cells.

Cell growth assays

Cultured 20-day-old rat ventral prostate cells were used to examine the effects of testosterone, EGF, KGF, and TGF-β1 on cell growth. A ^{3}H-thymidine incorporation assay was performed. A 10% bovine calf serum positive control treatment significantly stimulated (about 4- to 5-fold) DNA synthesis in both stromal and epithelial cells. Testosterone and EGF stimulated (2.5- to 3-fold) DNA synthesis in both stromal and epithelial cells (Fig. 6). Anti-TGF-α antibody alone showed no effect on DNA incorporation in either stromal or epithelial cells. However, anti-TGF-α antibody significantly suppressed the effect of testosterone in both stromal cells and epithelial cells. Therefore, the effects of testosterone on tritiated thymidine incorporation are, in part, mediated by TGF-α. The TGF-α antibody has previously been shown not to cross-react with EGF, basic fibroblast growth factor, heregulin, or TGF-β (data not shown). Although the effects of the TGFα antibody completely inhibited androgen action, multiple factors are likely needed to regulate prostate cell growth and androgen actions.

Immunocytochemistry

Tissue sections from freshly isolated 20-day-old rat prostate were obtained for analysis (Fig. 7). Sections were incubated with a TGF-α or EGFR antibody (Fig. 7). Controls with nonspecific IgG also are shown. Staining of both TGF-α and EGFR was localized to the luminal side of selected epithelial cells of the prostate ducts. The expression pattern was similar in different regions of the ventral prostate [Fig. 7, A and B *vs.* (C and D)]. Minimal staining was detected in the stromal cells. Results suggest that the prostate epithelial cells express higher levels of TGF-α and EGFR protein than stromal cells.

Discussion

The high level of EGF in human prostatic fluid suggests that EGF may have an important role in prostatic function (29). In rat ventral prostate, EGF was detected and shown to

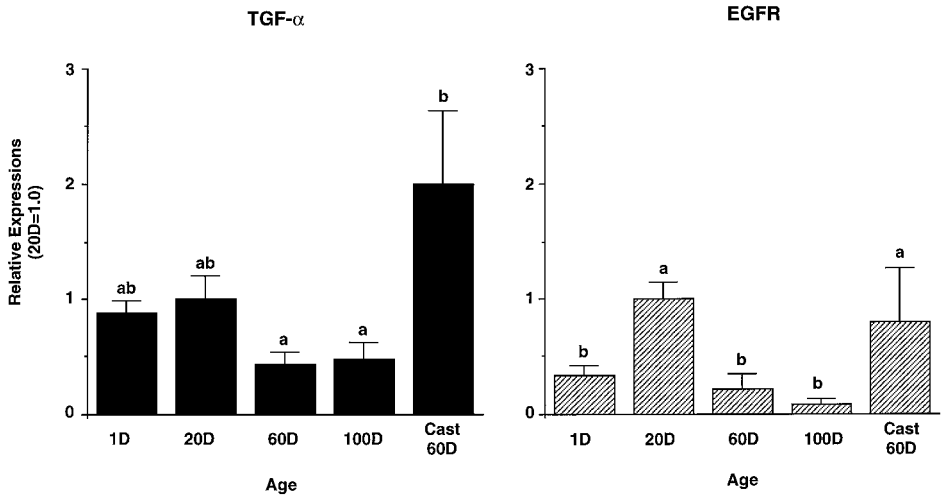

FIG. 4. Changes in the relative expression of TGF-α and EGFR during development of rat ventral prostate. Normalized values were compared with those of 20-day-old rat, as relative expression, and expressed as mean ± SEM (n = 3–5). Statistical analyses were performed by Dunnet's test, to compare with 20-day-old rat; and Tukey-Kramer HSD test, for multiple comparisons. Statistical difference is indicated by different *letter superscripts* ($P < 0.05$).

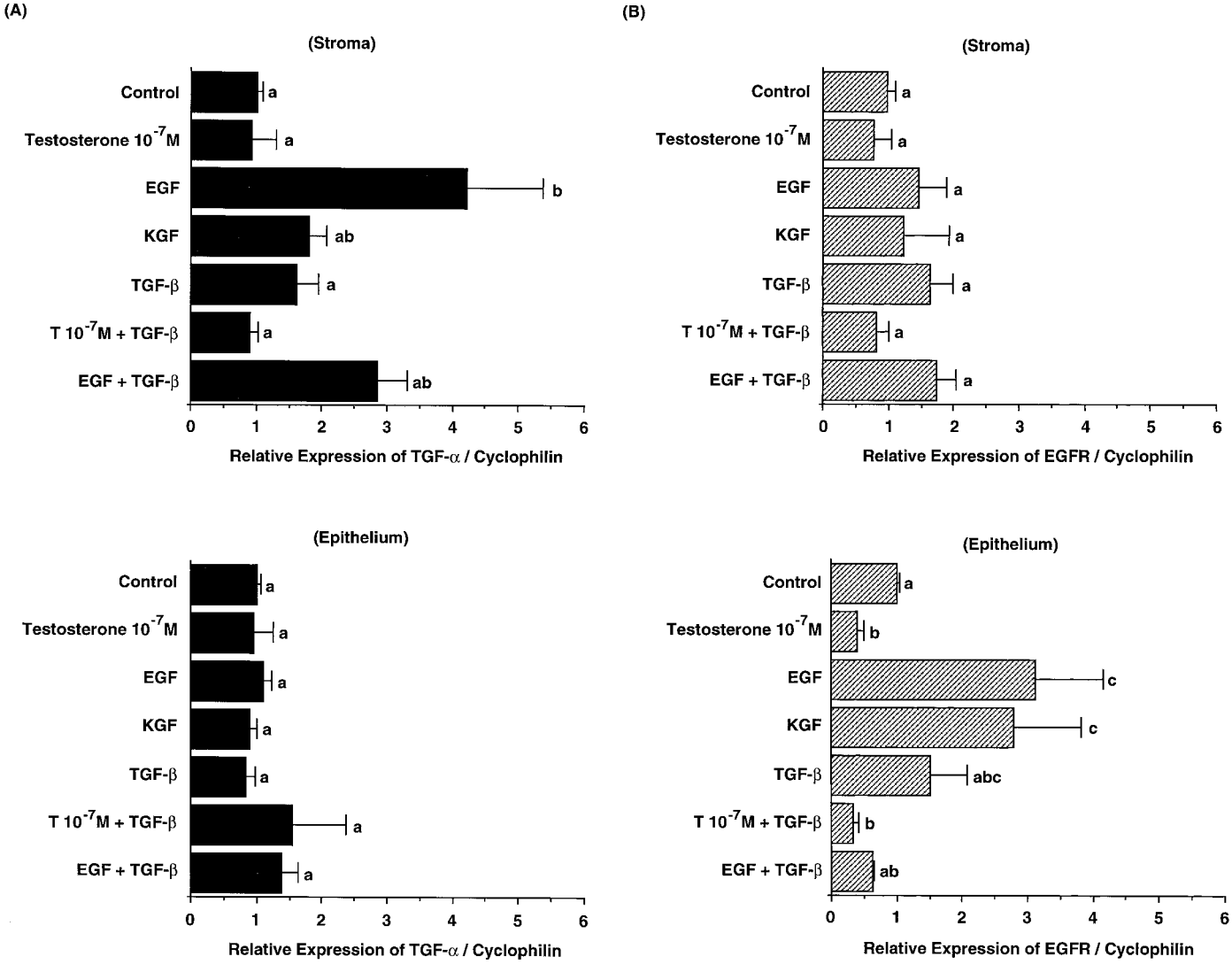

FIG. 5. Relative expression of TGF-α (A) and EGFR (B) in cultured 20-day-old rat ventral prostate stroma and epithelium with or without treatment. Values were normalized by cyclophilin and compared with those of nontreated control cells. Values are expressed as mean ± SEM (n = 4–12). Statistical analyses were performed by Dunnet's test and Tukey-Kramer HSD test for multiple comparisons. Statistical difference is indicated by different *letter superscripts* ($P < 0.05$).

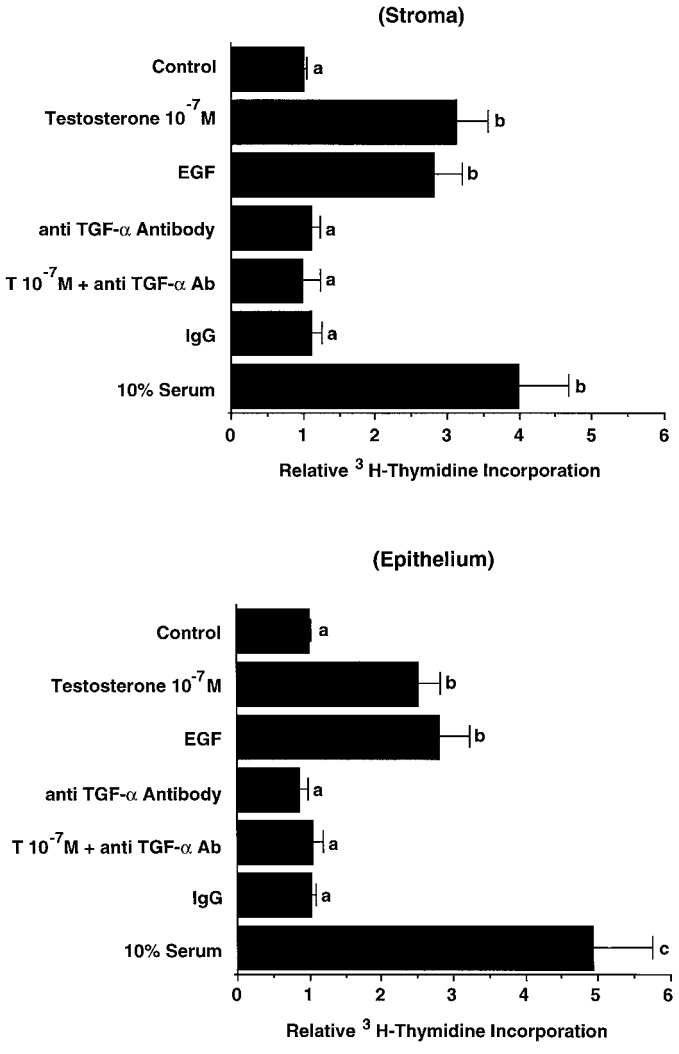

FIG. 6. The results of ^{3}H-thymidine incorporation assay in cultured rat ventral prostate. Relative values are expressed as mean ± SEM (n = 3) and compared with control nontreated cells. Statistical analyses were performed by Dunnet's test and Tukey-Kramer HSD test for multiple comparisons. Statistical difference is indicated by different *letter superscripts* ($P < 0.05$).

be regulated by androgen (13, 16, 17, 30). TGF-α is structurally and biochemically similar to EGF. The role of TGF-α in the rat ventral prostate remains to be elucidated. In the current study, TGF-α and EGFR mRNA levels during development of prostate and in isolated epithelial and stromal cells were investigated. TGF-α and its receptor protein were previously shown in rat ventral and lateral prostate by an immunohistochemical study (18). Protein for TGF-α was only detected in the epithelial cells after 28 days of age in the rat (18). In the current study, both proteins were localized in epithelial cells in 20-day-old rat prostate. Moreover, TGF-α and EGFR mRNA were found to be expressed in both epithelium and stromal cells using fresh and cultured samples. The results of localization of mRNA and protein does not directly correspond. The sensitivity of the detection procedure for protein, compared with mRNA detection, is likely a factor to be considered. In addition, the stromal-derived TGFα may act on epithelial cells that results in localization of protein but not mRNA.

A stimulatory effect of TGF-α/EGF on the prostate was suggested, because EGF stimulated DNA synthesis in epithelial and stromal cells (31–33). Interestingly, anti-TGF-α antibody inhibited the effect of testosterone on tritiated thymidine incorporation into prostatic cells. These observations suggest that the effects of testosterone on prostatic cells are mediated, in part, by TGF-α. Although the TGF-α antibody completely blocked androgen stimulated growth, the actions of androgen likely involved multiple factors. Interestingly, testosterone did not show any effect on TGF-α expression (*i.e.* mRNA levels) *in vitro*. Whether the *in vitro* conditions inhibited androgen actions or that multiple factors are needed to mediate androgen action remains to be elucidated. After castration of 60-day-old rats, TGF-α and EGFR mRNA expression was elevated about 4-fold. Both binding capacity of ligand to EGFR and EGFR mRNA in rat ventral prostate were confirmed to be increased after castration by ligand binding study and Northern blot analysis, respectively (7, 19). The current study showed the same increase in EGFR mRNA. From these observations, TGF-α and EGFR expression seems to be regulated by testosterone *in vivo*; however, its actions may not be direct, and they may be mediated by other factor(s).

During rat prostatic development, TGF-α mRNA expression was not changed, whereas EGFR mRNA expression was increased at midpuberty and decreased with age. Thomson *et al.* (34) reported changes in TGF-α and EGFR mRNA in rat ventral prostate during development (from 2-day-old to adult) by ribonuclease protection assay. Results were similar to those of the current study. They also reported that the expression of an epithelial marker remained constant during development (34). This observation suggests that the stroma/epithelium ratio remains constant during development of the prostate. It is also suggested that the changes in growth factor expressed were not caused by changes in the stroma/epithelium ratio. Using immunocytochemistry, Taylor *et al.* (18) showed that protein for TGF-α was expressed in the apical region of rat ventral prostatic epithelial cells after 28-days. They concluded that TGF-α may promote differentiation rather than proliferation of the epithelial cells of rat ventral prostate. On the other hand, normal human prostatic epithelial cells expressed EGFR but were unreactive for TGF-α, whereas stromal cells expressed TGF-α but not EGFR protein (35). Other reports suggested an autocrine loop of TGF-α/EGFR, because TGF-α transcription was detected in prostatic carcinoma but not in benign prostatic hyperplasia (36–38). Results presented in the current study indicate an apical/luminal localization in selected epithelial cells but minimal expression in stromal cells (Fig. 7). The significance of the TGF-α, EGF, and EGFR system still remains to be elucidated in the prostate. The differences among species, age, and disease state may change expression of mRNA and protein of these growth factors and receptor. Further study is required to elucidate the growth factor network in the prostate.

In cultured epithelial cells, the expression of EGFR (*i.e.* mRNA levels) was suppressed by testosterone. This observation suggests that testosterone down-regulates EGFR ex-

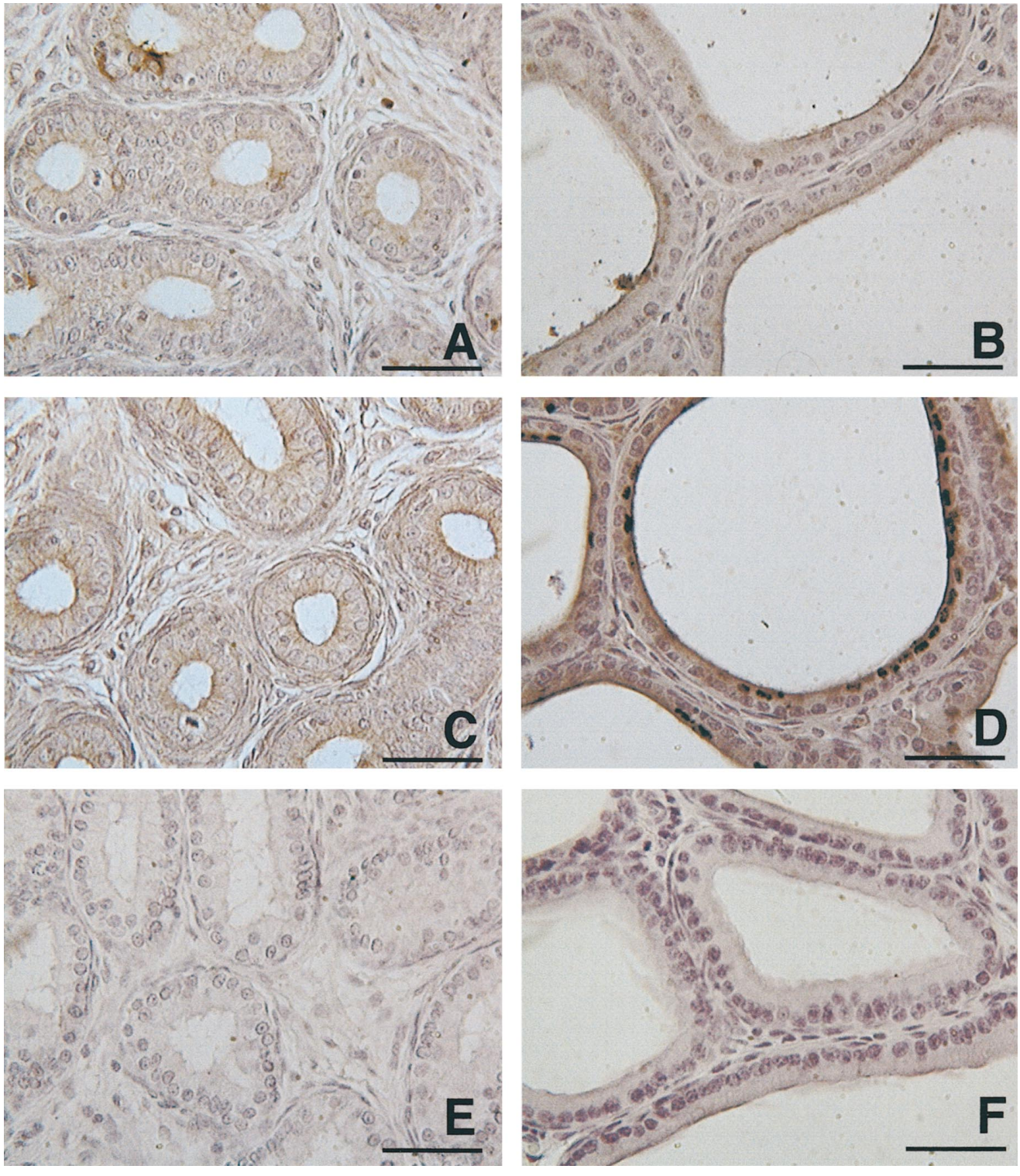

FIG. 7. Immunocytochemical analysis of TGF-α (A and B) and EGFR (C and D) of different sized ductal regions in 20-day-old rat ventral prostate. Sections were incubated with TGF-α antibody (A and B), EGFR antibody (C and D), or nonspecific IgG for control (E and F). Micrographs are shown at $\times$400 magnification. Replicate micrographs are presented from three different experiments in replicate. Each *inset bar* indicates 5 μm.

pression. In normal rat ventral prostate, androgens down-regulate EGF binding capacity by 6-fold (19). In contrast, in a prostatic carcinoma cell line, androgens up-regulate EGFR expression (39). At the midpubertal stage, EGFR was found to be elevated. The prostate is rapidly growing at this stage, and it is likely that an increase of EGFR is required for cell proliferation and differentiation. However, once prostate growth is complete, EGFR is suppressed. The cultured cells used in the current study were derived from 20-day-old rats to correlate to the growth period of the prostate. Although

the relationship between androgen and the expression of TGF-α and EGFR mRNA was not obvious in rat prostatic development, the expression of TGF-α and EGFR was increased in 60-day-old rats after castration. After castration, many growth factors are elevated (7, 10, 24). It is speculated that elevation of TGF-α and EGFR, after castration, is induced by dramatic changes in local growth factors. The altered expression of these growth factors after castration will have a role in inducing prostate cell death and decreasing prostate weight. The cultured cells used in the current study contain low levels of contaminating cell populations, which needs to be considered in any data interpretation involving the cultured cells.

In regard to the relationships between growth factors, TGF-α mRNA expression was stimulated by EGF in stromal cells, whereas EGFR mRNA was regulated by testosterone, EGF, and KGF in epithelial cells. These observations are interesting, and they suggest potential stromal-epithelial interactions. The expression of TGF-α and EGFR were confirmed in both stromal and epithelial cells; however, the regulation of TGF-α and EGFR is distinct in stroma and epithelium. In carcinoma and benign prostate hyperplasia, the EGF/TGF-α and EGFR generate an autocrine loop (35–39). EGF has a stimulatory effect on the expression of TGF-α and EGFR. EGF up-regulation suggests potential autocrine and paracrine interactions. This is the first report of a role of KGF in the expression of TGF-α and EGFR in the rat prostate gland. KGF stimulated EGFR mRNA expression. This observation suggests that KGF has a stimulatory effect on the EGF/TGF-α-EGFR system. However, EGF and KGF also stimulated TGF-β expression, which is one of the inhibitory factors of prostatic growth (24). The fact that EGF and KGF both have stimulatory and inhibitory effects suggests that there is a network of growth factors to maintain homeostasis of cell proliferation and death of prostatic cells.

Acknowledgments

We thank Linda Miyashiro and Eugene E. Herrington for technical assistance, and Susan Cobb for assistance with the preparation of the manuscript. We also thank Drs. Andrea S. Cupp and Jeff A. Parrott for critically reading the manuscript.

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