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Biochemical and Biophysical Research Communications 342 (2006) 92–100
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EGFR signaling pathway negatively regulates PSA expression and secretion via the PI3K-Akt pathway in LNCaP prostate cancer cells ☆

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Received 7 January 2006
Available online 2 February 2006

Abstract

Epidermal growth factor (EGF) and its receptor (EGFR) are involved in hormone-refractory growth and poor prognosis of a subgroup of human prostate cancer. In this communication, we investigated the regulation of PSA by the EGFR signaling pathway using LNCaP C-81 prostate cancer cells. Administration of EGF stimulated the growth of LNCaP C-81 cells, however, PSA expression and secretion were suppressed. An EGFR inhibitor, AG1478, abrogated the PSA suppression effect by EGF, in concurrence with the suppression of tyro-phosphorylation levels of EGFR. Interestingly, the AR level was also decreased in EGF-treated LNCaP C-81 cells. Moreover, LY294002, but not PD98059, inhibited the PSA and AR suppression effect by EGF in concurrence with the suppression of phosphorylation levels of Akt. In conclusion, our results strongly suggest the existence of a novel androgen-independent PSA regulatory mechanism, i.e., the EGFR signaling pathway negatively regulates PSA expression which may be induced by the alteration of AR expression via the PI3K-Akt pathway in LNCaP C-81 cells.

Keywords: PSA; EGF receptor; PI3K-Akt pathway; Androgen receptor; LNCaP C-81 cells

For advanced prostate cancer, primary androgen deprivation therapy is a mainstay due to its androgen-dependent tumor growth. Although most cases initially respond to androgen deprivation, eventually this therapy fails and the patient dies of recurrent androgen-independent prostate cancer (AIPC). Currently, there is no effective therapeutic option for this form of the disease. The precise molecular mechanisms of AIPC development are poorly understood, however, to elucidate such mechanisms is the first step towards improving treatment outcomes of AIPC. Moreover, appropriate evaluation of disease status and treatment efficacy is also required. For more than a decade, prostate-specific antigen (PSA) has been extensively used as the most reliable biomarker to screen for prostate cancer and is also used as a surrogate marker to assess response to therapy for prostate cancer [1,2].

The expression of PSA is primarily activated by androgens and regulated by the androgen receptor (AR) at the transcription level. Numerous studies indicate that the serum PSA level correlates directly with advancing clinical and pathological stage or that PSA is directly related to the volume of cancer. However, in the case of AIPC, PSA regulation is quite complicated and its description is unspecified. For example, some patients have normal serum PSA level with high Gleason grade and/or aggressive disease. The prognosis of this subgroup of patients is extremely poor because most patients' cancers already have androgen-independent characteristics at initial diagnosis [3–5]. Thus, for such patients, PSA may not be a reliable biomarker. To elucidate the androgen-independent PSA regulation is imperative for accurate assessment of prostate cancer status and selection of appropriate therapy for AIPC.

☆ *Abbreviations:* EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; PSA, prostate-specific antigen; AR, androgen receptor; PI3K, phosphatidylinositide-3-OH kinase; AIPC, androgen-independent prostate cancer.

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doi:10.1016/j.bbrc.2006.01.106

Some studies have suggested the existence of androgen-independent regulation mechanisms of PSA during progression to AIPC [6–8]. Recent evidence indicates that a number of growth factors and cytokines such as forskolin (FSK), insulin-like growth factor I (IGF-I), epidermal growth factor (EGF), and interleukin 6 (IL-6) stimulate AR signaling [9–11] and regulate PSA gene transcription in the absence of androgen [12,13]. However on another front, several investigations have suggested that AR is downregulated by some polypeptide growth factors such as basic fibroblast growth factor (bFGF) or EGF [14–16]. Thus, under some conditions, there may be a possibility that some growth factors negatively regulate PSA through downregulation AR expression. However, data are lacking regarding which factors have a positive or negative effect on actual PSA secretion and expression.

Among the ErbB family, EGFR and ErbB-2 in particular play a key role in numerous processes that affect tumor development and progression in various tumors [17–19]. In prostate cancer, several lines of evidence indicate that expression of EGF and EGFR is involved in androgen-independent growth and poor prognosis of a subgroup of human prostate cancer [20–22]. In another investigation, both EGFR and ErbB-2 immunoreactivity has been shown to be associated with an unfavorable prognosis in a homogeneous series of hormonally untreated cancer patients, in particular if these two receptors are combined [23]. Thus, EGFR and/or ErbB-2 may play a crucial role in the development of AIPC. Regarding androgen-independent PSA regulation, previous observations have demonstrated that not the EGFR, but the ErbB-2, signaling pathway via MAP kinases (ERK1/2) plays a critical role in upregulating the secretion of PSA by LNCaP cells in an androgen-depleted environment [17]. However, currently, data are lacking regarding the functional role of EGFR and its ligands in androgen-independent PSA secretion and expression in human prostate cancer. In this communication, to elucidate the role of EGFR and its ligands in prostate cancer progression, we investigated the regulation of PSA by the EGFR signaling pathway using subline of LNCaP prostate cancer cells.

Materials and methods

Materials. RPMI 1640 culture medium, monoclonal anti-β-actin antibody, and EGF were purchased from Sigma (St. Louis, MO, USA). RPMI 1640 culture medium (phenol red free), fetal bovine serum (FBS), L-glutamine, trypsin–EDTA, and gentamicin were obtained from Gibco-BRL (Gaithersburg, MD). Charcoal/dextran-treated FBS (c-FBS) was from Hyclone (Logan, UT, USA). Acrylamide was obtained from National diagnostics (Atlanta, GA, USA). An enhanced chemiluminescence (ECL) reagent kit, the ECL plus reagent kit, and protein molecular weight standard marker were purchased from Amersham (Arlington Heights, IL, USA). AG 1478, PD98059, LY294002, and TGFα were from CARBIOCHEM (San Diego, CA, USA). Polyclonal anti-PSA antibody (C-19), polyclonal anti-EGF receptor antibody (1005), polyclonal anti-AR antibody (N-20), anti-mouse immunoglobulin G, anti-goat immunoglobulin G, and anti-rabbit immunoglobulin G were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal anti-phospho-EGF receptor (Tyr1173) antibody was from Upstate Biotechnology (Lake Placid, NY, USA). Anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody, anti-p44/42 MAP kinase antibody, anti-phospho-Akt (Ser473) antibody, and anti-Akt antibody were purchased from Cell Signaling Technology (Beverly, MA, USA).

Cell culture and preparation for analysis. The human prostate carcinoma cell line, LNCaP-FGC [24], originally purchased from the American Type Culture Collection (Rockville, MD, USA), was used to develop LNCaP C-81 cells, resembling hormone-refractory prostate cancer, which exhibit androgen-independent growth and a higher PSA secretion level compared to the parental LNCaP C-33 cells, as reported previously [17,25]. Cells were routinely maintained in the regular culture medium, that is, phenol red-positive RPMI 1640 medium supplemented with 5% FBS, 1% glutamine, and 0.1% gentamicin. For EGF, TGFα, and inhibitor treatment, a steroid-reduced medium, that is phenol red-free RPMI 1640 medium containing 5% charcoal/dextran-treated, heat-inactivated certified FBS, was used [26], mimicking an androgen-depleted environment, to evaluate androgen-independent regulation of PSA and another proteins.

To investigate the effect of EGF, TGFα, and various inhibitors on LNCaP cells, cells were seeded at a density of 4.0×10^4 cells per 1 cm^2 culture flask in regular culture medium. After 3 days, one set of attached cells was harvested and cell numbers were counted as day 0. The remaining attached cells were fed with fresh steroid-reduced medium and cultured for an additional 48 h before reagent administration. After 48 h of steroid depletion, starved cells were fed with the steroid-reduced medium supplemented with EGF, TGF-α, and/or inhibitors at the appropriate concentration. Cells and conditioned media were harvested and cell numbers were counted at 24 and 48 h after treatment.

Western blot analysis. Cultured cells were harvested by scraping and were lysed as previously described [26,27]. Briefly, pelleted cells were lysed in ice-cold hypotonic cell lysis buffer containing protease inhibitors. An aliquot of total cellular lysate (40–60 μg/lane) in an SDS–polyacrylamide gel was electrophoresed and transferred to nitrocellulose membranes. After blocking, the membrane was incubated with primary antibody for 3 h at room temperature. After washing with TBS-T three times, the membrane was incubated with the appropriate secondary antibody for 1 h at room temperature. The specific protein bands were detected by ECL or ECL plus reagent kit. The relative level of protein expression was semi-quantified by laser densitometer.

Immunohistochemistry. The archival specimens of prostates from 27 men were obtained by radical prostatectomy. All specimens were histopathologically diagnosed for prostatic adenocarcinoma. Immunohistochemical staining was performed on sections of formalin-fixed and paraffin-embedded specimens. Tissue sections (5 μm thick) were mounted on pre-coated slides and were deparaffinized with xylene and rehydrated by graded ethanol. Antigen retrieval was performed by autoclaving at 95 °C for 40 min in 0.01 M sodium citrate buffer (pH 6.0). The slides were then incubated for 30 min in 3% H_2O_2 in methanol to block endogenous peroxidase. After blocking, sections were incubated with anti-PSA (1:1000), anti-EGF (1:50), or anti-ErbB-1 (1:200) at 4 °C overnight. After rinsing, sections were incubated with appropriate biotinylated secondary antibody, and then the bound antibodies were detected by the avidin-biotin complex technique utilizing the ABC staining kit (Zymed, CA, USA). The sections were counterstained with hematoxylin.

All of the analyses of immunohistochemical staining were assessed by light microscopy within the tumor area. PSA, EGF, and EGFR were scored according to our previous report [23]. Briefly, immunoreactivity was scored by estimating the percentage of positive tumor cells as follows: score 0, no immunoreactive cell; score +1, positivity in <5% cancer cells; score +2, positivity in 5–50% of cancer cells; and score +3, positivity in >50% of cancer cells. Specimens were considered positive for PSA, EGF, and EGFR expression when the score was +2 or +3.

Statistical analysis. All statistical analysis was performed using the Kruskal–Wallis test on StatView for Windows (version 5.0).

Results

Effect of EGF on the growth of LNCaP C-81 cells

At first, to investigate the effect of EGF on the growth of LNCaP cells, EGF was added to C-81 cells under steroid-reduced culture conditions. As shown in Fig. 1A, a 48-h administration of 10 ng/ml EGF stimulated the growth of C-81 cells by ≈1.5-fold, compared to that of control cells. As shown in Fig. 1B, the tyro-phosphorylation level of EGFR was elevated at 10 min and then diminished at 30 min by EGF treatment. The protein level of EGFR was decreased by EGF treatment, and this was thought to represent receptor endocytosis and degradation. The effect of TGFα, another ligand of EGFR, was similar to that of EGF, and EGFR was also phosphorylated by TGFα (data not shown). Thus, the growth stimulation of C-81 cells by EGF can be manifested via EGFR activation.

Effect of EGF on androgen-independent PSA expression and secretion in LNCaP C-81 cells

To investigate the effect of EGF on androgen-independent PSA expression of C-81 cells, we analyzed the change in PSA expression at different EGF doses. Interestingly, the expression of cellular PSA was clearly decreased by administration of EGF in a dose-dependent manner. As shown in Fig. 2A, expression of PSA by C-81 cells treated with 10 ng/ml of EGF was suppressed by over 50%, to lower than that of the control cells at 48 h. The effect of EGF on androgen-independent PSA secretion was then analyzed. Conditioned media were harvested at 24 and 48 h after EGF treatment and then cell numbers were counted. As shown in Fig. 2B, secreted PSA by C-81 cells treated with 10 ng/ml of EGF was suppressed by over 50%, to lower than that of control cells at 48 h. We also analyzed the effect of TGFα on the expression and secretion of PSA using the same experimental design and found that the effect of TGFα was almost identical to that of EGF (data not shown).

Thus, these results suggest that the tyro-phosphorylation of EGFR was induced by treatment of its ligands, which led to a decrease in not only cellular PSA expression, but also PSA secretion by LNCaP C-81 cells under androgen-depleted conditions.

Effect of EGFR inhibitor on androgen-independent PSA secretion and expression in LNCaP C-81 cells

To further determine the role of EGFR in androgen-independent PSA secretion and expression in LNCaP C-81 cells, the effect of EGFR inhibitors, i.e., AG1478, on PSA expression was analyzed. As shown in Fig. 3A, the growth stimulation effect of EGF was clearly abrogated by AG1478 treatment. The EGFR of this set was phosphorylated by EGF treatment and clearly blocked by AG1478 (Fig. 3B). Simultaneously, cellular and secreted PSA were analyzed by immunoblotting. As shown in Figs. 3C and D, both cellular and secreted PSA were suppressed by EGF by over 50%, to lower than that of control cells at 48 h. Interestingly, these suppressive effects were clearly blocked by AG1478, and both secreted and cellular PSA were restored to levels similar to control levels.

Therefore, the results shown in Fig. 3 indicate that EGFR signaling is again involved in regulating androgen-independent PSA secretion and expression in LNCaP C-81 cells.

Effect of EGF treatment on the expression of AR in LNCaP C-81 cells

To further investigate the mechanism of PSA suppression by EGFR signaling, we analyzed the effect of EGF on AR expression. Surprisingly, as shown in Fig. 4A, expression of AR was decreased to over 50% lower than that of the control cells at 48 h after EGF treatment under

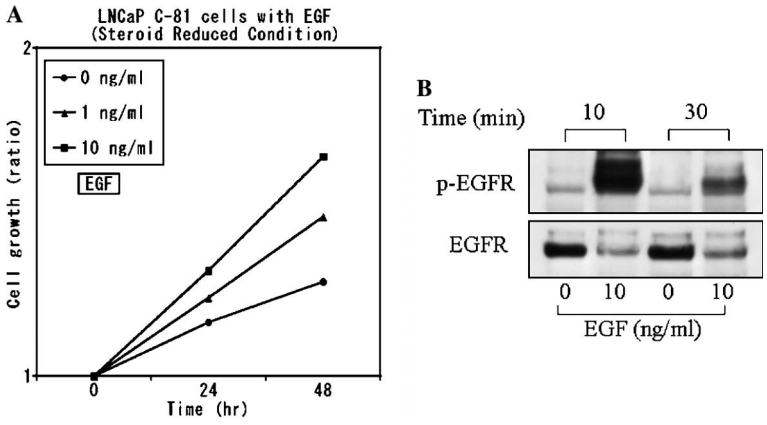

Fig. 1. Effect of EGF on C-81 cells. (A) Effect of EGF on the growth of C-81 cells in steroid-reduced conditions. Growth effect of EGF was dose-dependent. Similar results were obtained in three sets of independent experiments. (B) Effect of EGF on the expression of EGFR. Tyro-phosphorylated EGFR (upper panel) and EGFR protein (lower panel) were detected on the same membrane by Western blot analysis using corresponding specific antibodies. EGFR was strongly phosphorylated by EGF treatment at 10 min.

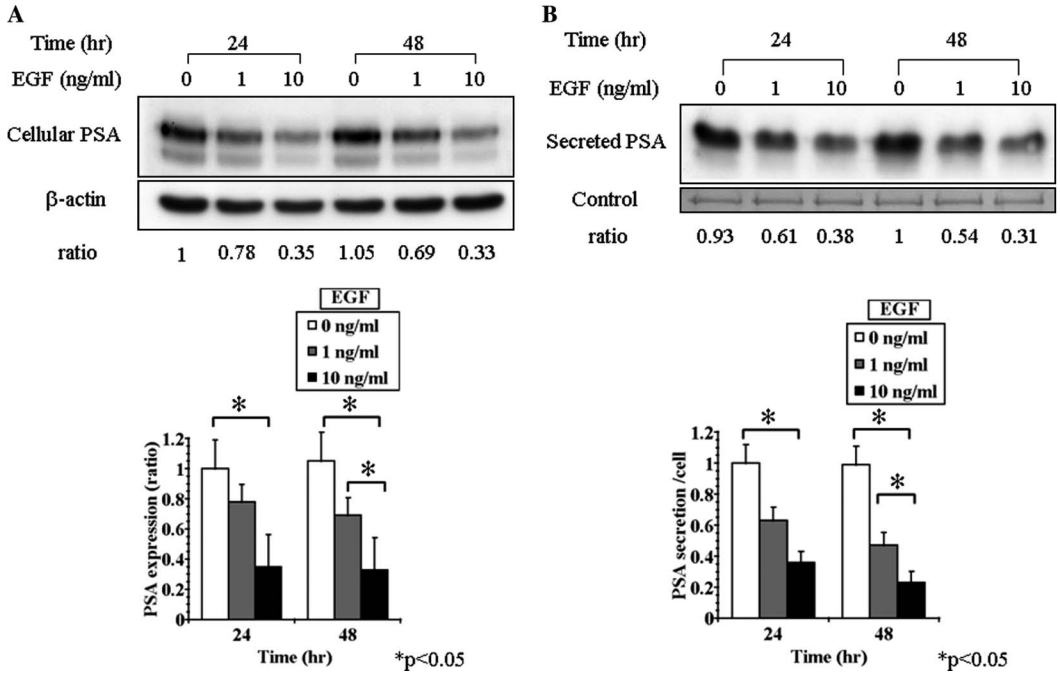

Fig. 2. Effect of EGF on androgen-independent PSA expression and secretion in C-81 cells. (A) Effect of EGF on cellular PSA. Cell lysates were analyzed by immunoblotting, followed by densitometric analysis, and then the ratio was calculated with β-actin as loading control. Expression of cellular PSA by C-81 cells treated with 10 ng/ml EGF was suppressed by over 50%, to lower than that of control cells at 48 h. (B) Effect of EGF on secreted PSA in conditioned media. A Coomassie-blue-stained protein band on the nitrocellulose membrane was used as the loading control. The secreted level of PSA by C-81 cells was semiquantified by densitometric analysis and normalized to their respective cell numbers. Secretion of PSA by C-81 cells treated with 10 ng/ml EGF was suppressed by over 50%, to lower than that of control cells at 48 h.

androgen-depleted conditions. Moreover, this AR suppression was clearly abrogated by AG1478 (Fig. 4B). Thus, these results imply that EGF can negatively regulate AR expression in an androgen-independent manner, and that this effect may be manifested via EGFR signaling. Furthermore, negative regulation of PSA by EGF may be the result of activating androgen-independent EGFR–AR interaction.

Effect of PD98059 on androgen-independent PSA secretion and expression in LNCaP C-81 cells

To better understand the processes of PSA suppression by EGFR-AR signaling, we investigated the effect of PD98059, MAP kinase (MEK) inhibitor, on androgen-independent PSA secretion and expression. As shown in Fig. 5A, both secreted and cellular PSA were suppressed by EGF treatment by over 50%, to lower than that of control cells at 48 h. Also shown in Fig. 5A, both secreted and cellular PSA were suppressed by 10 mM PD98059 treatment by over 50%, to lower than that of control cells at 48 h. This effect of PD98059 was thought to occur through the blockade of ErbB-2 signaling [17]. Different to AG1478, PD98059 did not inhibit the suppression of PSA expression and secretion induced by EGF, while similar to PSA, AR expression was suppressed by EGF but this effect of EGF was not abrogated by PD98059 (Fig. 5A). Therefore, the results shown in Fig. 5 indicate that the suppression of PSA expression and secretion induced by EGFR–AR signaling is not manifested via the EGFR-MAP kinase signaling pathway.

Effect of LY294002 on androgen-independent PSA secretion and expression in LNCaP C-81 cells

Subsequently, we lined up the PI3K-Akt signaling pathway as a candidate for the PSA suppressing pathway which might mediate the interaction between EGFR and AR. Similar to the suppression effect of AG1478 on the EGF induced growth of C-81 cells, LY294002, PI3K inhibitor, abrogated the EGF induced growth stimulation of C-81 LNCaP cells (Fig. 6A). However, as shown in Fig. 6B, LY294002 could not suppress PSA expression and secretion. Interestingly, the suppression of PSA expression and secretion by EGF was obviously blocked by 20 mM LY294002, and both secreted and cellular PSA levels were almost similar to those of control levels at 48 h. Correspondingly, AR suppression induced by EGF was clearly blocked by LY294002. The phosphorylated level of Akt induced by EGF was higher than those of control cells, and Akt phosphorylation was clearly blocked by LY294002 (Fig. 6C). In addition, EGFR and MAPK of this set were phosphorylated by EGF treatment, however the phosphorylation of EGFR and MAP kinase was not inhibited by LY294002 (Fig. 6B).

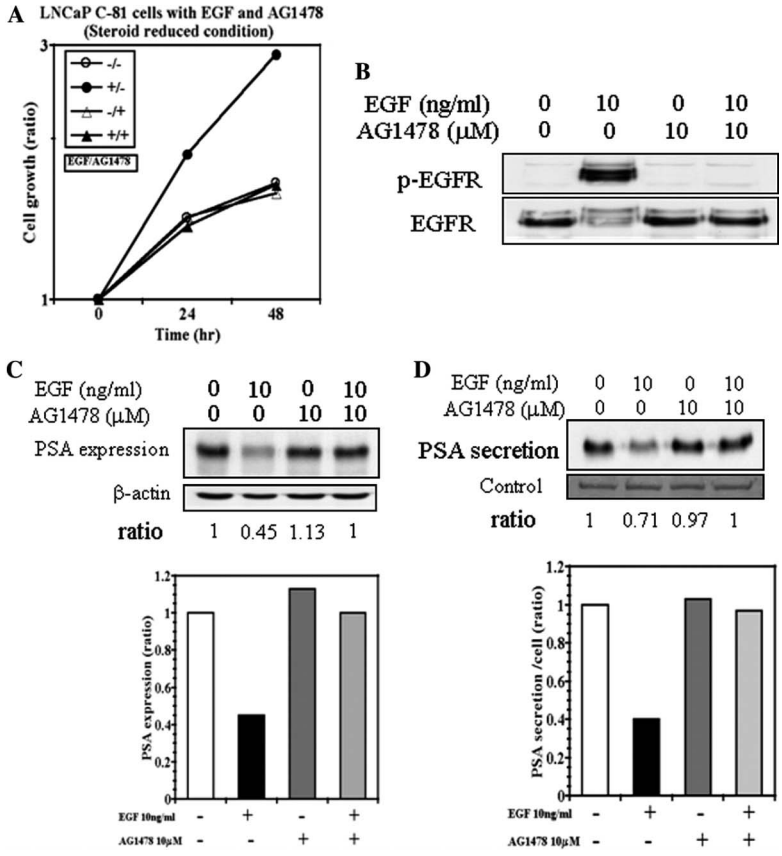

Fig. 3. Effect of EGFR inhibitor on androgen-independent PSA expression and secretion in C-81 cells. (A) Effect of AG1478 on the growth of C-81 cells. The growth effect of EGF was almost completely blocked by AG1478. Similar results were obtained in three sets of independent experiments. (B) The effects of EGF and AG1478 on the phosphorylation level of EGFR. Strong phosphorylation of EGFR occurred by EGF and was almost completely blocked by AG1478. (C) Effects of EGF and AG1478 on cellular PSA. Suppression of cellular PSA induced by EGF was clearly blocked by AG1478, and PSA was restored to control levels at 48 h. (D) Effects of EGF and AG1478 on PSA secretion in conditioned media. Suppression of PSA secretion induced by EGF was clearly blocked by AG1478, and PSA was restored to control levels at 48 h.

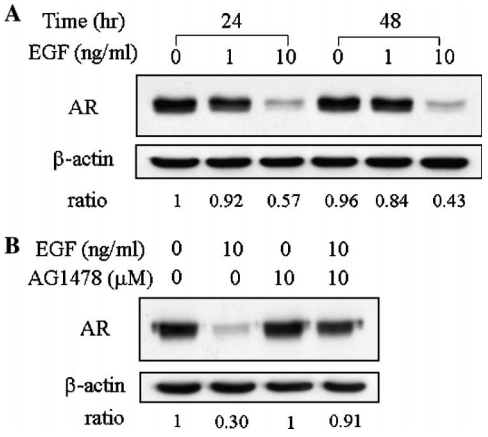

Fig. 4. Effect of EGF on the expression of AR in C-81 cells. Cell lysate was analyzed by immunoblotting, as described in Materials and methods. (A) Expression of AR was clearly decreased by EGF in a dose-dependent manner. (B) Effects of AG1478 and EGF on the expression of AR. Suppression of AR by EGF was clearly inhibited by AG1478.

Therefore, the results shown in Fig. 6 imply that PSA suppression induced by EGFR-AR signaling may be manifested by the suppression of AR through the EGFR-PI3K/Akt signaling pathway.

Immunohistochemical localization of PSA, EGF, and EGFR in human prostate cancer

To delineate the actual relationship between PSA, EGF, and EGFR in human prostate cancer, we performed immunostaining to analyze the localization of these proteins using archival specimens. In prostatic cancer tissue sections, the EGF immunoreactivity of the cancer cells was more intense than in normal prostate epithelium and weaker than in hyperplastic glands. Interestingly, PSA and EGF expression clearly showed inverse correlation in prostate cancer areas (Figs. 7A and B). In the prostate cancer specimens, the immunoreactivity of EGFR was detected in a wider area than in normal and hyperplastic tissue. Moreover, the expression of EGFR in prostate cancer appeared to correlate with EGF (Fig. 7C). As shown in the Table 1, the proportion of cells staining for PSA tended to decrease with the Gleason grade, and the difference was statistically significant ($p < 0.0001$). On the other hand, the expression of EGF and EGFR significantly increased with the Gleason score ($p < 0.0001$ and $p = 0.0022$, respectively).

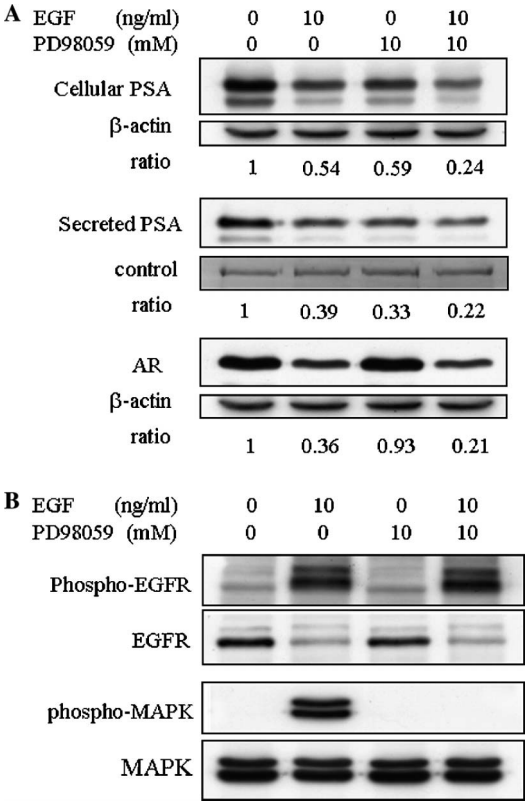

Fig. 5. Effect of PD98059 on the androgen-independent regulation of PSA and AR in C-81 cells. (A) PD98059 could not inhibit the suppression of PSA expression and secretion induced by EGF. Similar to PSA, expression of AR was suppressed by EGF but this effect of EGF was not blocked by PD98059. (B) Another set of LNCaP cells was fed with fresh steroid-reduced medium in the absence or presence of 10 mM PD98059 with or without 10 ng/ml EGF for 15 min after 48 h of steroid depletion. Strong phosphorylation of EGFR and MAPK occurred by EGF, and only phosphorylation of MAPK was blocked by PD98059.

Discussion

The expression of PSA is primarily activated by androgens and regulated by the AR at the transcriptional level through androgen responsive elements (AREs). However, levels of serum PSA eventually escape from androgen regulation and increase during androgen deprivation therapy, even with castration levels of serum testosterone. This androgen-independent increase in PSA is known as biochemical recurrence, which is an indicator for clinical progression to AIPC. Currently, the details of the molecular mechanisms of this phenomenon remain to be established. The key to understand the mechanism of progression to AIPC is delineating the molecules involved in the activation of AR-regulated genes or proteins, such as PSA. Several lines of evidence have shown that the PKA and ErbB-2 signaling pathways can upregulate the activity of PSA promoter in an androgen-independent manner [10,12,28] and that ErbB-2 signaling via the MAP kinase signal pathway is involved in regulating androgen-independent PSA secretion by LNCaP C-81 cells [17]. Other investigations have indicated that various growth factors and cytokines stimulate AR signaling in the absence of androgen [9–11]. Thus, growth factor receptors, such as the ErbB family, may play a crucial role in androgen-independent PSA regulation. However, to the best of our knowledge, little attention has been given to involvement of EGFR regarding androgen-independent regulation of PSA in human prostate cancer. Thus, we were interested in the role of EGF/EGFR on androgen-independent PSA expression and secretion, and we investigated using LNCaP C-81 prostate cancer cells.

Previous studies determined that EGFR does not play a critical role in upregulating androgen-independent secretion of PSA by LNCaP cells [17]. Our results in this communication support this notion that EGFR may not be a key player in upregulating PSA, but may play a crucial role as a negative regulator of PSA in an androgen-independent manner. In addition, we also confirmed the results using human prostate cancer archival specimen that the PSA immunoreactivity decreased with Gleason grade, and EGF/EGFR expression clearly showed inverse correlation with PSA expression at same lesion.

To further analyze the mechanism of the EGF effect on negative PSA regulation, we focused on the changes in AR expression in our experimental system. Interestingly, the expression of AR was clearly decreased by administration of EGF in a dose-dependent manner, and the suppressive effect of AR by EGF was obviously inhibited by AG1478. Our data strongly suggest that the suppression of PSA secretion and expression by EGF could be the result of AR downregulation induced by the tyro-phosphorylation of EGFR. Previous studies have indicated that AR may be stimulated in an androgen-independent manner by many factors, including androgen antagonists, growth factors, and cytokines [7]. Among these studies, some reports described that EGF downregulates AR mRNA or protein in LNCaP cells, which supports our observations [15,16].

From the results of our investigation, an interaction between AR and EGFR is speculated. Several studies have shown an inverse correlation between AR expression and phosphotyrosine levels of EGFR [26,29]. These alterations in AR signaling are believed to indicate a close link between prostate cancer cell growth, invasiveness, and acquisition of androgen-independent phenotype. However, the detailed mechanisms are not fully understood. One possibility is that AR is a direct target for Mdm2-mediated ubiquitylation and proteolysis, and additionally, interaction between EGFR and Mdm-2 has been reported [30]. Furthermore, another study has reported that PTEN modulates Mdm-2 transcription and isoform selection by negatively regulating its P1 promoter [31]. PTEN tumor suppressor protein dephosphorylates phosphatidylinositol $(3,4,5)P_3$, the product of PI3 kinase [31]. On the basis of the results of the above investigations, we hypothesized AR suppressing interplay between EGFR and the PI3K-Akt signaling pathway. The MAP kinase and PI3K-Akt pathways are two major signaling pathways and have been speculated to mediate the interaction between EGFR and AR. However,

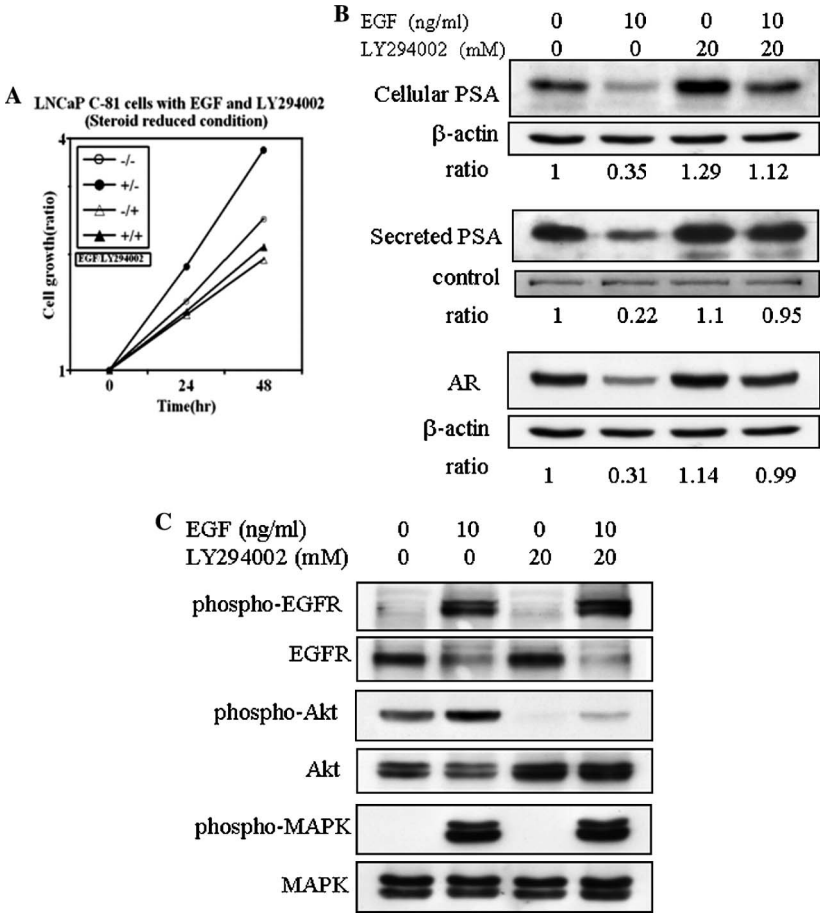

Fig. 6. Effect of LY294002 on the androgen-independent regulation of PSA and AR in C-81 cells. (A) Effect of LY294002 on the growth of C-81 cells. The growth stimulation effect of EGF was suppressed by LY294002. Similar results were obtained in three sets of independent experiments. (B) Suppression of cellular and secreted PSA induced by EGF was clearly blocked by LY294002, and PSA was restored to control levels at 48 h. Similar to PSA, expression of AR was suppressed by EGF, and this effect of EGF was clearly blocked by LY294002. (C) Another set of C-81 cells was fed with fresh steroid-reduced medium in the absence or presence of 20 mM LY294002 with or without 10 ng/ml EGF for 15 min after 48 h of steroid depletion. Strong phosphorylation of Akt, EGFR, and MAPK occurred by EGF, and only phosphorylation of Akt was blocked by LY294002.

to the best of our knowledge, little attention has been given to involvement of these signaling pathways from the viewpoint of androgen-independent PSA regulation by EGFR in human prostate cancer. Our results clearly show that the PI3K-Akt pathway, but not the MAP kinase pathway, plays a crucial role as a negative regulator of PSA through mediating EGFR-AR interaction. Previous observations have shown an increase in phospho-Akt in parallel with a decline in AR staining in the MNU model [32]. Moreover, another investigation demonstrated that AR is an additional substrate for Akt, and that the PI3K-Akt pathway was able to phosphorylate AR in vivo, then inhibited AR transactivation [33]. Our results support this notion. However, another report concluded that Akt is an activator of AR which is required for HER-2/neu signaling of androgen-independent survival and growth of prostate cancer [34]. Currently, the precise mechanism of different effects of EGFR and ErbB-2 in regulating androgen-independent PSA expression is not fully elucidated. The difference of homo- or heterodimerization pattern of EGFR, ErbB-2, or ErbB-3 may be one explanation for this question. Further research is required.

Taken together, our results strongly suggest that in human prostate cancer under androgen reduced environment, activation of EGFR signaling may lead to a decrease in the expression and secretion of PSA in an androgen-independent manner. This effect may be demonstrated by activation of the PI3K-Akt pathway through downregulation of AR expression. Several investigations have suggested that EGFR and its ligands were over-expressed in AIPC compared to androgen-sensitive prostate cancer [20–23]. Thus in AIPC patients, it is highly possible that EGFRs are phosphorylated and then PSA expression is suppressed in some part of cancer tissue. Since expression of EGFR often exhibits great individual differences and heterogeneity of tissue distribution, this interpretation may not apply to all patients. It is well documented that a subgroup of advanced prostate cancer cases exhibit lower serum PSA [3–5]. In such cases, the over-expression of EGFR activation may lower PSA

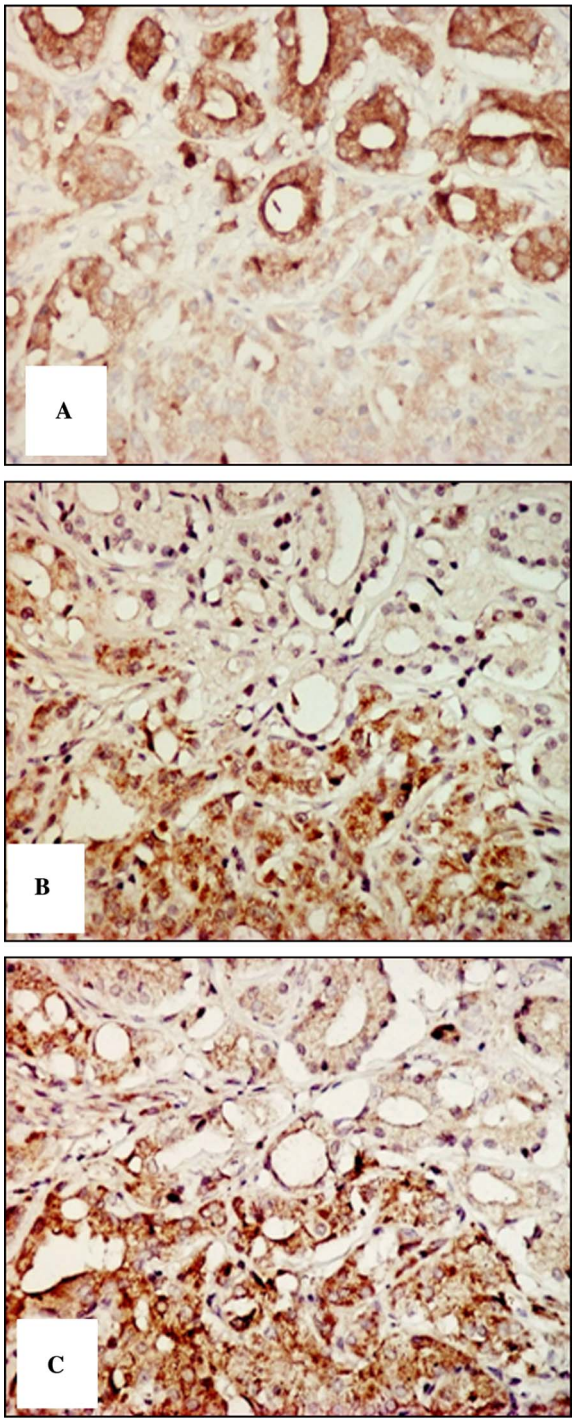

Fig. 7. Immunolocalization of PSA, EGF, and EGFR in human prostate cancer tissue. Staining was performed using the immunoperoxidase method, as described in Materials and Methods. Immunolocalization of PSA (A) was more intense in the lower grade area of prostate cancer. Immunolocalization of EGF (B) was more intense in the higher grade area of prostate cancer. This observation was similar to EGFR localization (C). PSA and EGF expression clearly showed inverse correlation in prostate cancer areas.

expression, which indicates that PSA may not be a useful marker with which to diagnose or assess response to therapy in this subgroup of patients.

Table 1
Staining intensity of PSA, EGF, and EGFR according to Gleason grade in human prostate cancer specimen

Gleason grade	3	4	5	p value
n	17	12	8	
PSA intensity	2.5 ± 0.6	1.4 ± 0.5	1.0 ± 0	<0.01
EGF intensity	1.1 ± 0.3	2.2 ± 0.4	2.5 ± 0.5	<0.01
EGFR intensity	1.4 ± 0.6	1.8 ± 0.7	2.6 ± 0.5	0.0022

Currently, PSA is the most reliable biomarker of prostate cancer but it is not infallible. The precise molecular mechanisms of androgen-independent PSA regulation are still unclear. Understanding these mechanisms of PSA regulation will be the next step towards developing more appropriate evaluation of prostate cancer condition and therapeutic effect.

Acknowledgments

We thank Dr. Ming-Fong Lin and Dr. Ta-Chun Yuan at University of Nebraska Medical Center (Omaha, United States) for critical comments and editorial assistance. We also thank Mr. Takumi Shimogama and Mr. Yoshikazu Tsuji for excellent technical assistance. This work was supported by grants from Japan Society for Promotion of Science (15591699, 17591684).

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