Androgen Receptor Expression in Prostate Cancer Cells Is Suppressed by Activation of Epidermal Growth Factor Receptor and ErbB2

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Abstract
Prostate cancers (PCa) that relapse after androgen deprivation therapies [castration-resistant PCa (CRPC)] express high levels of androgen receptor (AR) and androgen-regulated genes, and evidence from several groups indicates that ErbB family receptor tyrosine kinases [epidermal growth factor (EGF) receptor (EGFR) and ErbB2] may contribute to enhancing this AR activity. We found that activation of these kinases with EGF and heregulin-β1 rapidly (within 8 hours) decreased expression of endogenous AR and androgen-regulated PSA in LNCaP PCa cells. AR expression was similarly decreased in LAPC4 and C4-2 cells, but not in the CWR22Rv1 PCA cell line. The rapid decrease in AR was not due to increased AR protein degradation and was not blocked by phosphatidylinositol 3-kinase (LY294002) or MEK (UO126) inhibitors. Significantly, AR mRNA levels in LNCaP cells were markedly decreased by EGF and heregulin-β1, and experiments with actinomycin D to block new mRNA synthesis showed that AR mRNA degradation was increased. AR mRNA levels were still markedly decreased by EGF and heregulin-β1 in LNCaP cells adapted to growth in androgen-depleted medium, although AR protein levels did not decline due to increased AR protein stability. These findings show that EGF and ErbB2 can negatively regulate AR mRNA and may provide an approach to suppress AR expression in CRPC. [Cancer Res 2009;69(12):5202–9]

Introduction
Androgen receptor (AR) plays a central role in prostate cancer (PCa), with androgen deprivation therapies being the standard initial systemic treatment, but tumors eventually recur despite castrate androgen levels. These castration-resistant PCas (CRPC) express high levels of AR mRNA, AR protein, and androgen-regulated genes, indicating that AR transcriptional activity has been reactivated. One mechanism contributing to this reactivation is increased intratumoral androgen synthesis, but it seems clear that PCa adapts to androgen deprivation through multiple mechanisms that generate adequate AR activity despite castrate levels of circulating androgens (1–5). Evidence from several groups indicates that the ErbB family receptor tyrosine kinases ErbB1 [epidermal growth factor (EGF) receptor (EGFR)] and ErbB2 (HER2, Neu) contribute to enhancing AR activity in CRPC. Studies in PCa cell line and xenograft models have found increased EGFR or ErbB2 expression in tumors that relapse after castration, although this is not a consistent finding in patient samples and these receptors may also be enhanced by increased expression of ErbB ligands (6–14).

EGF can increase AR transactivation at low androgen levels, which may be mediated by increased expression or phosphorylation of the transcriptional coactivator protein TIF2/GRIP1 (15–18). The Ras-Raf-mitogen-activated protein kinase (MAPK) pathway and e-Src, which are activated downstream of EGFR, may also enhance AR responses to low levels of androgen (19–21). ErbB2 expression was increased in the LAPC4 xenograft model of CRPC, and a dual EGFR/ErbB2 inhibitor could reduce AR transcriptional activity and inhibit PCA xenograft growth after castration (6, 22). In CWR22 xenograft-derived CWR-R1 cells, heregulin stimulation of ErbB2 enhanced AR activity and cell growth (23). Other studies have shown that ErbB2 can enhance AR stability and that ErbB2 inhibition decreases AR DNA binding activity at low levels of androgen levels by a phosphatidylinositol 3-kinase (PI3K)–dependent, Akt-independent mechanism (6, 24, 25). In contrast, some studies indicate that ErbB2 enhances AR activity through the MAPK pathway or Akt (26, 27).

ErbB signaling also has been reported to negatively regulate AR expression and activity. In one study, EGF decreased AR mRNA and expression of androgen-regulated genes in LNCaP cells (28). In other studies, heparin binding EGF (HB-EGF) was found to decrease AR protein expression through activation of mTOR and decreased AR mRNA translation (29, 30). EGF also decreased PSA expression and secretion via the PI3K/Akt pathway in androgen-independent LNCaP-C81 cells (31). Finally, Akt in LNCaP cells may phosphorylate AR and enhance its ubiquitination by Mdm2 and degradation, but this seems to be dependent on cell passage number (32–36). Due to the significance of ErbB signaling in PCa, this study further examined how both EGF and heregulin-β1 regulate AR expression and activity in PCa cells.

Materials and Methods
Cell culture. LNCaP, LAPC4, C4-2, and CWR22-Rv1 cells were cultured in RPMI 1640/10% fetal bovine serum (FBS). HeLa and PC3-AR cells were cultured in DMEM/10% FBS. For DHT treatment, cells were grown to 50% confluence in RPMI 1640/10% FBS. HeLa and PC3-AR cells were cultured in DMEM/10% FBS. For DHT treatment, cells were grown to 50% to 60% confluence in medium with 5% charcoal/dextran-stripped serum (CSS; Hyclone) for 2 d before treatment.

Real-time reverse transcription–PCR. Primers and probes for quantitative real-time reverse transcription–PCR (RT-PCR) amplification were as follows: PSA forward, 5'-GATGAAACAGGCTGTGCCG-3'; PSA reverse, 5’-CGAGTTGTGCTTGCCAG-3'; PSA probe, 5-FAM-CAGGAACAAAAGCGTGAGTCTTGCGTGGG-3; AR forward, 5'-GGAATTCCTGTGCATGAAA-3; AR reverse, 5’-GGAATTCCTGTGCATGAAA-3; AR probe, 5-FAM-CAGGAACAAAAGCGTGAGTCTTGCGTGGG-3; For forward, 5'-GGAATTCCTGTGCATGAAA-3'; AR reverse, 5’-GGAATTCCTGTGCATGAAA-3; AR probe, 5’-FAM-CATTATGCCAGTCTTTTG-3'. Each reaction used 50 ng RNA and was normalized

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by coamplification of 18S or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA.

**Immunoblotting.** Cell extracts were prepared by boiling for 15 min in 2% SDS buffer. Blots were probed with anti-PSA (1:3,000, polyclonal, BioDesign), anti-AR (1:2,000, polyclonal, Upstate), anti-FLAG (1:3,000, monoclonal, Sigma), anti-EGFR (1:1,000, polyclonal, Cell Signaling), anti-phosphorylated EGFR (Tyr1285; 1:1,000, polyclonal, Cell Signaling), anti-phosphorylated ErbB3 (Tyr1286; 1:1,000, polyclonal, Cell Signaling), anti-phosphorylated AKT (Ser\(^{473}\); 1:1,000, polyclonal, Cell Signaling), anti-phosphorylated ERK (Thr\(^{202}\)/Tyr\(^{204}\); 1:1,000, polyclonal, Cell Signaling), anti-\(\beta\)-tubulin (1:2,000, monoclonal, Chemicon), or anti-\(\beta\)-actin (1:5,000, monoclonal, Abcom). Blots were developed with 1:5,000 antirabbit or antimouse secondary antibodies (1:1,000, polyclonal, Cell Signaling), anti-FLAG (1:3,000, monoclonal, Sigma), anti-EGFR (1:1,000, polyclonal, Cell Signaling), anti-AR (1:2,000, polyclonal, Upstate), anti-HER2 (1:1,000, monoclonal, Cell Signaling), or anti-\(\beta\)-tubulin (1:2,000, monoclonal, Cell Signaling), or anti-\(\beta\)-actin (1:5,000, monoclonal, Abcom). Blots were developed with 1:5,000 antirabbit or antimouse secondary antibodies (1:1,000, polyclonal, Cell Signaling).

**Results**

**ErbB signaling decreases endogenous AR protein expression and represses AR transcriptional activity in LNCaP cells.**

EGFR and ErbB2 signaling have been shown to increase AR activity, but most work has been done on transfected AR or using inhibitors, and it is unclear whether activation of ErbB receptors increases endogenous AR activity in PCa cells. As expected, AR protein and activity in LNCaP cells were significantly induced by DHT, based on increased expression of androgen-regulated PSA (Fig. 1A). In contrast, EGFR and ErbB2 activation with EGF and heregulin-\(\beta\)1, respectively, markedly suppressed PSA induction by DHT (Fig. 1A, left and right, respectively). Moreover, AR protein in the absence or presence of DHT was greatly reduced by EGF or heregulin-\(\beta\)1 (Fig. 1A). The activation of ErbB2 by heregulin-\(\beta\)1 was confirmed based on phosphorylation of ErbB3 (Fig. 1A, right). EGFR phosphorylation was not seen after 24 h of EGF treatment (Fig. 1A, left), consistent with its known rapid degradation after activation (see Fig. 1D). Confirming that EGF and heregulin-\(\beta\)1 were suppressing PSA transcription, androgen-induced PSA mRNA was markedly decreased by EGF and heregulin-\(\beta\)1 (Fig. 1B). These results indicated that activation of EGFR and ErbB2 were decreasing AR protein expression, leading to decreased AR activity (although both growth factors could stimulate proliferation in the absence or presence of androgen; data not shown).

To support this hypothesis, we next examined a range of EGF and heregulin-\(\beta\)1 concentrations. EGF at 20 ng/mL, which maximally stimulated EGFR activation (data not shown), markedly decreased AR protein at 24 hours in hormone-depleted medium (Fig. 1C, left) or in FBS medium (Fig. 1C, right), with a corresponding decrease in PSA protein. Heregulin-\(\beta\)1 similarly decreased AR expression, with the concentration required for maximal ErbB2 activation (40 ng/mL based on ErbB3 phosphorylation, data not shown) being consistent with the concentration that decreased AR and PSA protein (Fig. 1C). In time course experiments, EGFR activation (based on Tyr\(^{1285}\) phosphorylation) could be detected after 0.1 hour but not at later times due to receptor down-regulation (Fig. 1D, left; data not shown). Robust ErbB3 phosphorylation was similarly detected at 0.1 hour but persisted for 24 hours (Fig. 1D, right). AR protein levels started to decline at ~2 hours, markedly decreased at 8 hours, and remained low after 24 hours.

**ErbB signaling decreases AR in other PCa cell lines.** To determine whether this repression of AR is LNCaP cell specific, we tested additional cells. LAPC4 cells have a wild-type AR and their growth is stimulated by androgen, but in vitro they express minimal PSA. Both EGF and heregulin-\(\beta\)1 in these cells slightly decreased the low levels of AR detected in the absence of DHT and greatly reduced AR in the presence of DHT (Fig. 2A). LAPC4 cells have a wild-type AR and their growth is stimulated by androgen, but in vitro they express minimal PSA. Both EGF and heregulin-\(\beta\)1 in these cells slightly decreased the low levels of AR detected in the absence of DHT and greatly reduced AR in the presence of DHT (Fig. 2A). LAPC4 cells have a wild-type AR and their growth is stimulated by androgen, but in vitro they express minimal PSA. Both EGF and heregulin-\(\beta\)1 in these cells slightly decreased the low levels of AR detected in the absence of DHT and greatly reduced AR in the presence of DHT (Fig. 2A). LAPC4 cells have a wild-type AR and their growth is stimulated by androgen, but in vitro they express minimal PSA. Both EGF and heregulin-\(\beta\)1 in these cells slightly decreased the low levels of AR detected in the absence of DHT and greatly reduced AR in the presence of DHT (Fig. 2A). LAPC4 cells have a wild-type AR and their growth is stimulated by androgen, but in vitro they express minimal PSA. Both EGF and heregulin-\(\beta\)1 in these cells slightly decreased the low levels of AR detected in the absence of DHT and greatly reduced AR in the presence of DHT (Fig. 2A).
DHT, but EGF and heregulin-β1 had no clear effect on AR protein (Fig. 2C).

As expected, the irreversible EGFR/ErbB2 inhibitor PD168393 effectively blocked both EGFR (pTyr\(^{845}\)) and ErbB3 (pTyr\(^{1289}\)) activation in response to EGF and heregulin-β1, respectively (Fig. 2D, bottom). Moreover, EGFR- and heregulin-β1-mediated repression of AR expression in LNCaP cells was abrogated by PD168393 (Fig. 2D, top). Interestingly, PD168393 increased androgen-induced PSA expression in the absence of growth factor stimulation, possibly due to the inhibition of basal EGFR or ErbB2 activity. Collectively, these data show that EGF, as well as heregulin-β1, markedly decrease both unliganded and liganded AR protein expression in several (but not all) AR-positive PCa cells.

**ErbB signaling does not decrease expression of transfected AR.** The results above are in contrast to some previous results with transfected AR (15, 18). Therefore, we used a triple-Flag tagged AR cDNA driven by a cytomegalovirus promoter to examine transfected AR in LNCaP and HeLa cells. In contrast to the above results with endogenous AR in LNCaP cells, EGF dramatically increased transiently transfected Flag-AR protein expression in the absence or presence of DHT (Fig. 3A). Heregulin-β1 also enhanced AR expression, but to a lesser extent than EGF. Similar results were obtained in HeLa cells (Fig. 3B). Because

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**Figure 2.** Effects of ErbB signaling on AR in other PCa cell lines. A–C, LAPC4, C4-2, or CWR22Rv1 cells in 5% CSS medium were treated with DHT (0–100 nmol/L) in the absence or presence of EGF or heregulin-β1 for 24 h, and equal amounts of protein were then immunoblotted for AR and PSA protein expression. D, bottom, LNCaP cells in 5% CSS medium were treated with EGF or heregulin-β1 in the absence or presence of PD168393 (10 μmol/L) for 0, 5, 15, or 30 min and then immunoblotted for phosphorylated EGFR (P-EGFR; Tyr\(^{845}\)) or phosphorylated ErbB3 (P-ErbB3; Tyr\(^{1289}\)) expression; top, LNCaP cells were treated with different combinations of PD168393, ethanol vehicle (0.1%), DHT (10 nmol/L), EGF (20 ng/mL), or heregulin-β1 (40 nmol/L) for 24 h and then immunoblotted for AR or PSA expression.

**Figure 3.** ErbB signaling does not decrease expression of transfected AR. A, LNCaP cells in 5% CSS medium were transfected with 0.25 μg Flag-AR for 24 h and then treated with EGF or heregulin-β1 in the absence or presence of DHT (10 nmol/L) for 24 h, and equal amounts of extracted proteins were immunoblotted for Flag (transfected AR) or total AR protein expression. B, HeLa cells in 5% CSS medium were transfected with 0.25 μg Flag-AR for 24 h, then treated with EGF or heregulin-β1 in the absence or presence of DHT (10 nmol/L) for 24 h, and immunoblotted for AR protein expression. C, PC-3 cells that stably express transfected AR (PC-3-AR) were grown in 5% CSS medium for 2 d, then treated with different concentration of EGF or heregulin-β1 in absence or presence of DHT (10 nmol/L) for 24 h, and immunoblotted for AR, β-Tubulin was used as loading control. D, AR mRNA expression in PC-3-AR cells treated as indicated for 24 h.
transiently transfected cells express high levels of AR protein that may not be regulated by physiologic mechanisms, we also examined PC3 cells (an AR-negative PCa cell line) that were stably transfected with the AR expression vector. AR expression in these cells was modestly increased by EGF, and expression in the absence of DHT was markedly increased by heregulin-\(\beta_1\) (Fig. 3C). Significantly, AR mRNA levels in the PC3-AR cells were not markedly altered by these growth factors, indicating that AR protein translation or stability were being increased (Fig. 3D). In any case, as these data showed that endogenous versus transfected AR respond differently to ErbB pathway activation, we continued to focus on mechanisms regulating endogenous AR expression.

EGF decreases AR expression independently of PI3K and Erk activation, whereas PI3K contributes to AR down-regulation by heregulin-\(\beta_1\). We next examined whether the PI3K/Akt or Ras/Raf/Erk pathways, both of which can modulate AR function, were required for the EGF- or heregulin-\(\beta_1\)-induced decrease in AR expression. LNCaP cells are PTEN deficient, so PI3K pathway activation is evidenced by high basal phosphorylated Akt, which was further enhanced by EGF (Fig. 4A, left and middle). Heregulin-\(\beta_1\) more strongly increased phosphorylated Akt levels, reflecting the robust recruitment and activation of PI3K by phosphorylated ErbB3 (Fig. 4A, right). The PI3K inhibitor LY294002 completely blocked the basal and EGF-stimulated Akt phosphorylation in LNCaP cells but did not prevent the marked decrease in AR protein in response to EGF (Fig. 4A, left and middle). In contrast, LY294002 substantially prevented the decrease in AR protein by heregulin-\(\beta_1\), despite only partially suppressing PI3K activation based on Akt phosphorylation (Fig. 4A, right).

Whereas EGF did not markedly enhanced PI3K activity in LNCaP cells, it very strongly activated the Ras/Raf/Erk pathway as evidenced by immunoblotting for phosphorylated Erk1/2 (Fig. 4B, middle). The MEK inhibitor UO126 blocked Erk activation in response to EGF but did not prevent the decrease in AR, indicating that EGF is not suppressing AR expression through Erk activation (Fig. 4B, middle). Heregulin-\(\beta_1\) only weakly stimulated Erk, and UO126 similarly did not block its ability to decrease AR expression (Fig. 4B, right).

As the above experiments examined AR after 24 hours, we next examined whether PI3K was contributing to the rapid decline in AR protein that can be clearly observed by 8 hours. Significantly, LY294002 did not prevent the marked decline in AR protein mediated by EGF or heregulin-\(\beta_1\) at 8 hours (Fig. 4C). We conclude that PI3K contributes to the decline in AR protein at 24 hours but that a distinct PI3K independent mechanism is mediating the rapid decline in AR protein between 2 and 8 hours in response to EGF and heregulin-\(\beta_1\).

AR protein degradation is not increased by EGF or heregulin-\(\beta_1\). To determine whether EGF or heregulin-\(\beta_1\) were increasing AR degradation, we used cycloheximide to inhibit new protein synthesis and assess AR protein stability. Cells in steroid-depleted medium (minus or plus DHT) were treated with cycloheximide alone or in conjunction with EGF or heregulin-\(\beta_1\), which were added 2 hours before the cycloheximide. This 2-hour pretreatment with growth factors was selected as AR protein expression is starting to decline at this time, and longer pretreatment results in much
lower baseline levels of AR that make half-life comparisons problematic. However, it should be noted that effects due to proteins that are induced by androgen after 2 hours may be missed. Cells were harvested at time 0 (immediately before cycloheximide addition) and at 4 to 24 hours. As seen in Fig. 5A, neither EGF nor heregulin-β1 substantially increased the rate of AR protein degradation at up to 8 hours, although degradation at 24 hours was increased. These results indicate that increased AR protein degradation does not account for the decline in AR protein levels that are observed within 8 hours of EGF or heregulin-β1 (see Fig. 1D) but may contribute to a further decline at later times.

**EGF and heregulin-β1 increase degradation of AR mRNA.** As AR protein degradation was not markedly increased by EGF or heregulin-β1 after up to 8 hours, we next assessed effects on AR mRNA. EGF markedly decreased endogenous AR mRNA by up to ~80% at 24 hours, whereas heregulin-β1 decreased AR mRNA by ~60% (Fig. 5B, left). These decreases occurred in the absence or presence of androgen. Moreover, they were observed within 4 hours, consistent with the rapid decline in AR protein (Fig. 5B, right). Significantly, AR mRNA levels were decreased by EGF and heregulin-β1 over a broad range of DHT concentrations, indicating that these growth factors are overriding mechanisms that enhance AR mRNA expression in response to androgen deprivation and low AR protein levels (37).

A regulatory element that represses AR gene transcription has been identified in the 5’ untranslated region (UTR), and it has been reported that a complex of Purα and hnRNPk binds this element and represses AR mRNA transcription (38–42). However, we did not detect increased expression of Purα or hnRNPk in response to EGF or heregulin-β1 (data not shown). Although this did not rule out posttranslational modifications in Purα or hnRNPk or decreased AR transcription by other mechanisms, we next examined AR mRNA stability. LNCaP cells (grown in medium minus or plus DHT) were pretreated with growth factors or vehicle for 8 hours, and actinomycin D was then added to block the new mRNA synthesis. In the absence of DHT or growth factors, AR mRNA had a half-life of ~8 hours, which was substantially decreased to ~4 hours in the presence of EGF or heregulin-β1 (Fig. 5C, right). EGF and heregulin-β1 similarly decreased AR mRNA half-life in the presence of DHT (Fig. 5C, right). It should be noted that the rate of AR mRNA degradation in the untreated cells increases abruptly after ~4 hours, which may

![Figure 5.](https://cancerres.aacrjournals.org/content/69/12/5206/F5.large.jpg)
reflect an actinomycin D–induced degradative pathway and result in an underestimation of AR mRNA stability in the untreated cells. In any case, the data indicate that increased mRNA degradation contributes to the decline in AR mRNA in response to EGF and heregulin-β1.

EGF and heregulin-β1 increase AR protein stability in LNCaP cells adapted to growth in androgen-depleted medium. Studies using patient samples and xenograft models have shown that AR mRNA levels are high in CRPC and are increased relative to primary untreated PCa (4, 43–45). Therefore, as EGFR and ErbB2 activities may be increased in CRPC, we considered whether EGF and heregulin-β1 would still suppress AR mRNA levels in PCa cells adapted to grow under androgen-deprived conditions. To test this hypothesis, we changed the growing condition of LNCaP cells from medium with normal FBS to medium with steroid-depleted CSS. Short-term culturing (1 week) in CSS medium did not significantly affect the suppression of AR protein by EGF or heregulin-β1 (data not shown), but a longer-term culture (∼4–6 weeks) in CSS medium did alter this response. As shown in Fig. 6A, AR protein levels in the LNCaP-CSS cells (cells grown in CSS medium for ∼4–6 weeks), in the absence or presence of DHT, were not decreased by EGF or heregulin-β1 (Fig. 6A). Immunoblotting for EGFR (which is rapidly down-regulated in response to activation) and pErkB3 confirmed that both the LNCaP and LNCaP-CSS cells were stimulated by EGF and heregulin-β1. Interestingly, in the LNCaP-CSS cells, heregulin-β1 stimulated the expression of PSA in the absence of added DHT (Fig. 6A, right), consistent with the conclusion that ErbB2 stimulation can, under some conditions, enhance AR transcriptional activity in the absence of androgens or at low androgen levels (6, 23–25).

Significantly, AR mRNA levels were markedly increased in the LNCaP-CSS versus the parental LNCaP cells and rapidly declined in response to DHT (Fig. 6B). Therefore, as these growth factors were still decreasing AR mRNA but not AR protein, we examined AR protein stability in the LNCaP versus LNCaP-CSS cells (pretreated for 2 hours with EGF or heregulin-β1 before addition of cycloheximide at time 0). AR protein was less stable (half-life ∼1 hour) in the LNCaP-CSS cells grown in CSS medium than in the parental LNCaP cells in the same medium (half-life ∼2.0 hours; Fig. 6C), indicating that the LNCaP-CSS cells adapted to androgen deprivation primarily by increasing AR mRNA levels. However, in contrast to the parental LNCaP cells (see above), both EGF and heregulin-β1 increased AR protein half-life in LNCaP-CSS cells from ∼1 to ∼3 hours (Fig. 6C, quantified in the right). This result indicates that increasing AR protein stability through activation of EGFR or ErbB2 is a mechanism that may contribute to maintaining AR protein expression in CRPC, particularly if it can become uncoupled from the down-regulation of AR mRNA.

Figure 6. EGF and heregulin-β1 increase AR protein stability in LNCaP cells adapted to growth in androgen-depleted medium. A and B, LNCaP cells were cultured in either 10% CSS medium (LNCaP-CSS) or 10% FBS medium for ∼4 to wk. The LNCaP-CSS and control LNCaP cells were then grown in 5% CSS medium for 2 d and then treated for 24 h with 0, 1, or 10 nmol/L DHT in the absence or presence of EGF (20 ng/ml) or heregulin-β1 (40 ng/ml). A, equal amounts of protein were immunoblotted for AR, PSA, EGFR (left), or phosphorylated ErbB3 (P-ErbB3; Tyr1289; right) expression. B, equal amounts of RNA were analyzed for AR mRNA by real-time RT-PCR (normalized using internal GAPDH control). C, control LNCaP and LNCaP-CSS cells in 5% CSS medium were treated with cycloheximide (10 ng/ml), minus or plus EGF or heregulin-β1, for 0, 1, 2, 4, or 8 h, and equal amounts of protein were then immunoblotted for AR. Right, quantification of AR normalized to β-tubulin.
Discussion

Previous studies indicate that stimulation of EGFR and ErbB2 can enhance AR stability and transcriptional function and may contribute to AR activity in CRPC (6, 15, 18, 22–25, 27). We initially examined LNCaP PCa cells to further define the molecular basis for these effects on AR and found that stimulation with both EGF and heregulin-β1 rapidly decreased expression of AR protein and the androgen-regulated PSA gene over a broad range of DHT concentrations. This decrease in AR protein was also observed in LAPC4 and C4-2 cells but not in CWR22Rv1 cells. Consistent with the latter result, AR protein in another CWR22-derived cell line (CWR-R1) was not changed in response to EGF or heregulin (18, 23). The rapid AR down-regulation in response to EGF and heregulin-β1 was not prevented by U0126 or LY294002, indicating that it was not mediated through the Erk or PI3K pathways. Moreover, AR protein degradation was not rapidly enhanced by EGF or heregulin-β1. In contrast, AR mRNA levels were rapidly decreased by both EGF and heregulin-β1 over a broad range of DHT concentrations. Decreased AR transcription likely contributes to this decrease, but AR mRNA degradation was also increased in response to EGF and heregulin-β1. Taken together these findings show that EGFR and ErbB2 activation, while having multiple effects on AR activity through diverse mechanisms, markedly decrease AR mRNA expression and increase AR mRNA degradation.

The AR has a long 3′ UTR, which contains a highly conserved UC-rich region implicated in the regulation of mRNA stability (46). Therefore, EGFR or ErbB2 may regulate expression of RNA binding proteins that interact with this UC-rich region (47). Decreased AR mRNA transcription also likely contributes to the marked decrease in AR mRNA levels in response to EGF and heregulin-β1. AR transcription may be regulated by multiple factors, including a suppressor element in the AR 5′ UTR (40, 48–52). Further studies are clearly needed to define the precise mechanisms by which EGF and heregulin-β1 are enhancing AR mRNA degradation and to assess their effects on AR mRNA transcription.

Previous studies indicated that EGF could enhance AR activity and that ErbB2 could enhance AR stability and responses to low levels of androgen (6, 15–18, 23–27). However, other studies in LNCaP cells found that EGF or HB-EGF decrease AR expression, consistent with the findings in the current study (28–31). One factor that may contribute to differences between studies is that results in some cases are based on transfected AR (15, 18). Another factor is the use of EGFR/ErbB2 inhibitors in some studies to examine the effects of basal growth factor receptor activity on the endogenous AR versus the use of EGF and heregulin-β1 to examine the response to EGFR/ErbB2 activation in the current study (22, 24, 25). Whereas one might conclude that decreased AR activity in response to EGFR/ErbB2 inhibitors would predict increased AR activity in response to EGF and heregulin-β1, this may not be the case as the rapid high-level stimulation with EGF/hereregulin-β1 may be eliciting distinct responses. Therefore, whereas the results in this study identify a novel mechanism by which EGFR and ErbB2 can suppress AR expression, the overall response to activation or inhibition of these receptors in vivo may be variable and not readily predictable due to interactions between multiple downstream pathways.

As noted above, EGFR and ErbB2 activate multiple downstream pathways that may directly or indirectly modulate AR expression and function. One example in this study was that EGF treatment caused a strong increase in DHT-stimulated PSA expression in C4-2 cells despite a decrease in AR protein. This is consistent with a previous study showing that EGF can increase phosphorylation and activity of the p160 transcriptional coactivator SRC-2/TIF2/GRIP1 (18). A second example was the ability of the PI3K inhibitor LY294002 to partially block the heregulin-β1–stimulated decline in AR protein at 24 hours (but not 8 hours), which is consistent with a previous study showing that mTOR activation in response to HB-EGF caused a decrease in AR translation (30). A third example is that heregulin-β1 increased AR protein stability and stimulated PSA expression in the LNCaP-CSS cells in the absence of added DHT. These effects are similar to those observed in LAPC4 cells adapted to grow under castrate conditions, although their molecular basis remains to be defined (24). Importantly, the LNCaP-CSS cells also adapted to androgen deprivation by increasing their AR mRNA levels in response to EGF/hereregulin-β1 but did not increase AR protein levels. This will be important to determine in CRPC patients whether the mechanisms that decrease AR mRNA in response to EGF/hereregulin-β1 are uncoupled from mechanisms that enhance AR transcriptional activity and to determine whether these former mechanisms can be targeted by drugs to prevent the increase in AR mRNA levels that occurs in CRPC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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