Dual EGFR/HER2 Inhibition Sensitizes Prostate Cancer Cells to Androgen Withdrawal by Suppressing ErbB3

Liqun Chen¹², Benjamin A. Mooso¹², Maitreyee K. Jathal¹², Anisha Madhav¹², Sherra D. Johnson¹², Elyse van Spyk¹², Margarita Mikhailova³, Alexandra Zierenberg-Ripoll², Lingru Xue², Ruth L. Vinall², Ralph W. deVere White², and Paramita M. Ghosh¹²

Abstract

Purpose: Patients with recurrent prostate cancer are commonly treated with androgen withdrawal therapy (AWT); however, almost all patients eventually progress to castration resistant prostate cancer (CRPC), indicating failure of AWT to eliminate androgen-sensitive prostate cancer. The overall goal of these studies is to determine whether dual inhibition of the receptor tyrosine kinases epidermal growth factor receptor (EGFR) and HER2 would prolong the effectiveness of this treatment in prostate cancer.

Experimental Design: We used androgen-dependent LNCaP cells and its CRPC sublines LNCaP-AI and C4-2. Additional data were collected in pRNS-1-1 cells stably expressing a mutant androgen receptor (AR-T877A), and in nude mice harboring CWR22 tumors. Studies utilized EGFR inhibitors erlotinib and AG1478, and HER2 inhibitors trastuzumab and AG879.

Results: Dual EGFR/HER2 inhibition induced apoptosis selectively in androgen-sensitive prostate cancer cells undergoing AWT, but not in the presence of androgens, or in CRPC cells. We show that AWT alone failed to induce significant apoptosis in androgen-dependent cells, due to AWT-induced increase in HER2 and ErbB3, which promoted survival by increasing Akt phosphorylation. AWT-induced ErbB3 stabilized the AR and stimulated PSA, while it was inactivated only by inhibition of both its dimerization partners EGFR and HER2 (prostate cancer cells do not express ErbB4); but not the inhibition of any one receptor alone, explaining the success of dual EGFR/HER2 inhibition in sensitizing androgen-dependent cells to AWT. The effectiveness of the inhibitors in suppressing growth correlated with its ability to prevent Akt phosphorylation.

Conclusion: These studies indicate that dual EGFR/HER2 inhibition, administered together with AWT, sensitize prostate cancer cells to apoptosis during AWT. Clin Cancer Res; 17(19); 6218–28. ©2011 AACR.

Introduction

Androgen withdrawal therapy (AWT) is currently the standard of care for men with advanced prostate cancer (1); however, it was found that in most patients its effects typically last 18 to 24 months, after which the patient developed resistance to such therapy (castration resistant prostate cancer; CRPC). Although some groups reported increased apoptosis in prostatic tissue following AWT (2, 3), others found no increase in apoptotic indices in the majority of tumors (4, 5), although proliferation indices were consistently suppressed (2, 4). These studies, therefore, concluded that "androgen deprivation may act through suppression rather than ablation of prostatic cancers" (5, 6). These reports indicate that failure to undergo apoptosis during AWT maybe a major cause of resistance of prostate cancer cells to this therapy. Surviving cells likely undergo growth arrest and lie dormant following AWT, but will revive when an alternate growth stimulant comes to release it from this growth arrest, as was shown in a CWR22 xenograft model (7, 8). Therefore, adjuvant therapy that causes apoptosis during AWT would impede the onset of CRPC.

Here, we investigate the role of ErbB inhibitors in this effect. The ErbB family of 4 closely related type 1 transmembrane tyrosine kinase receptors include the epidermal growth factor receptor (EGFR/HER1/ErbB1), and related family members ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4; ref. 9). The ErbB receptors are activated by ligand binding, dimerization, and phosphorylation. EGFR, ErbB2, ErbB3, but not HER2, have specific ligands, such as EGF for EGFR and heregulins (HRG1-4) for ErbB3 and ErbB4 (9). However, ErbB3 lacks significant kinase activity; hence both HER2 and ErbB3 require heterodimerization,
Directed RNA inhibition duly restored the proapoptotic sine kinase inhibitor (TKI) treatment (24). Indeed, ErbB3 signaling precisely in response to EGFR/HER2-directed tyrosine phosphorylation and activation. Significantly, prostate cancer cells typically lack ErbB4 expression, but express high levels of ErbB3 (10, 11).

EGFR and HER2 are known to regulate cell proliferation, differentiation, angiogenesis, and survival (12); however, in clinical trials for patients with CRPC, studies using selective and specific inhibitors of individual receptors did not show any significant effect (13–17). In recent times, a number of dual EGFR/HER2 inhibitors have been developed, and were found to be more effective against prostate cancer cells and animal models compared with the single inhibitors (18, 19). Tyrosine phosphorylation of HER2 and ErbB3, transactivation of the androgen receptor (AR), and cell proliferation induced by heregulin were more potently inhibited by the EGFR/HER2 dual tyrosine kinase inhibitor GW572016 (lapatinib) than the EGFR-specific inhibitor gefitinib (20, 21). Despite the success of the preclinical studies, in phase II single-agent clinical trials, lapatinib was fairly well-tolerated and resulted in stable disease for 12 weeks but evidenced no decrease in prostate-specific antigen (PSA), an AR transcriptional target, in patients with hormone sensitive prostate cancer (22) or in unselected patients with CRPC, as measured by PSA (23).

Here, we concentrate on the effects of dual EGFR/HER2 inhibitors and the conditions under which they are effective. It is known that AR function at low levels of androgen is mediated not by EGFR, but by the heterodimerization of HER2 with ErbB3 (18). Sergina and colleagues showed that ErbB3 was upregulated and provided compensatory signaling precisely in response to EGFR/HER2-directed tyrosine kinase inhibitor (TKI) treatment (24). Indeed, ErbB3-directed RNA inhibition fully restored the proapoptotic effects of TKIs (24). These reports suggested that the failure of EGFR and HER2 inhibitors may be due to the activation of ErbB3 in these tumors. Studies conducted in vitro (25, 26), in animal models (6), and in clinical specimens (27) indicate an increase in Akt phosphorylation during AWT which promotes cell survival. On the basis of these reports, we investigated whether dual EGFR/HER2 inhibitors were effective when they downregulated ErbB3 and/or Akt phosphorylation, and whether they impede prostate cancer progression to CRPC by inducing cell death during AWT.

Materials and Methods

Cell culture and pharmacologic treatments

Androgen-dependent LNCaP prostate cancer cells were purchased from American Type Culture Collection, and C4-2 cells were obtained from UroCor. Castration resistant clones of LNCaP cells (LNCaP-AI cells) have been described by us elsewhere (11, 25). pRNS-1-1 cells were also described earlier (11, 28). Recombinant human epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) were obtained from Invitrogen, recombinant human heregulin 1 (HRG1) was from PeproTech INC. AG1478 and AG879 were from Calbiochem, EMD Chemicals, Inc. Erlotinib (Tarceva) was provided by OSI Pharmaceuticals, Inc., and also was obtained from LC Laboratories, while trastuzumab (Herceptin) was a gift from Genentech, Inc. Bicalutamide (Casodex) was kindly provided by AstraZeneca, while lapatinib was purchased from LC Laboratories. Rabbit polyclonal EGFR, HER2, ErbB3, β-actin, and AR antibodies were from Santa Cruz Biotechnology. Rabbit polyclonal anti-phospho-Akt (Ser 473), anti-phospho–EGFR (Y1068), anti-phospho–HER2 (Y1248), phospho–ErbB3 (Y1289), α-tubulin, and Akt antibodies were from Cell Signaling Technology. Transfections and plasmids used have been described earlier (11). Human Akt1 siRNA was obtained from Santa Cruz Biotechnology, against the sequence: 5’-ACGAGGAGAGUACAUCAAGAC-3’.

Mouse studies

Four- to 5-week-old Balb/c athymic nude-Foxn1nu (nu/nu) male mice were obtained from Harlan Sprague Dawley, Inc. Suspensions of CWR22 cells were mixed in 50% Matrigel solubilized basement membrane (BD Biosciences) and xenografts were established by s.c. injections of 2.5 × 10^6 cells/site into the flanks. When palpable tumors were observed, animals were treated with (i) vehicle or (ii) a combination of erlotinib (0.8 mg/Kg, 100 µL per dose, 5 times per week by oral gavage) and trastuzumab (20 mg/Kg, 90 µL per dose, 2 times per week by intraperitoneally injection), dissolved in a solution of PBS and 0.5% Tween 20. Three days after start of drug regimen, the animals were castrated by bilateral scrotal excision, following isoflurane-anesthesia. Control animals were sham-operated by opening the animals surgically, but no tissues were removed. Drug administration was continued post-surgery, but after 8 days, the mice were euthanized, tumors were collected, and divided into sections for

Translational Relevance

The goal of these studies is to identify therapeutic strategies that prolong the effectiveness of androgen withdrawal therapy (AWT) in patients with metastatic prostate cancer. Inhibitors of ErbB kinases such as erlotinib, lapatinib, and trastuzumab have been tested in patients with castration resistant prostate cancer (CRPC) and in hormone-naive patients, with little effect. Here, we present novel data showing that, instead, dual ErbB inhibitors sensitize prostate cancer to AWT, and are thereby likely to prolong its effects. We show that during AWT, HER2 and ErbB3 levels increase, resulting in significant ErbB-dependent survival advantage that allows progression to CRPC. However, dual epidermal growth factor receptor (EGFR)/HER2 inhibition, which inhibits their dimerization partner ErbB3 as well, induced apoptosis in cells undergoing AWT, despite ineffectiveness in hormone-naive cells and in cells that have already progressed to CRPC. Our data indicate that administration of dual EGFR/HER2 inhibitors in prostate cancer patients undergoing AWT may impede the onset of CRPC.
paraffin embedding and snap freezing in liquid nitrogen. Mice were weighed and blood was collected periodically and PSA levels measured by a standard ELISA kit (Fitzgerald Industries Intl.).

Immunohistochemistry and statistical analysis

We used rabbit polyclonal anti-ErbB3 (C-17; 1:100 dilution) antibodies from Santa Cruz Biotechnology, Ki67 was from DAKO, while TUNEL kit was from Millipore. For negative controls, we used a Universal Rabbit IgG control (DAKO) in place of the primary antibody. Diaminobenzidine was used as a chromogen, and counterstaining was with hematoxylin. Only the epithelial cells were scored. The extent of staining was scored 0 to 3, where 0 represented no staining, +0.5 represents low (<20% staining), +1 represent intermediate (30%–50%), +1.5 (50%–70%) and +2 represent high staining (>80%). To evaluate the differences in staining expression in the 3 diagnostic groups, we used t tests with a Welch approximation. Columns represent the mean ± SD of samples from each group.

Flow cytometry and MTT assay

MTT and flow cytometric analysis was carried out as described earlier (11, 29, 30). Proliferation was estimated in propidium iodide stained ethanol-fixed cells by MOD-Flow cytometry and MTT assay group.

Indicators of AR activity in the prostate, were analyzed in C4-2 cells stably transfected with vector only, but not those expressing AR(T877A), an androgen-sensitive active mutation found in LNCaP cells (Fig. 1B). However, in CSS, where AR was inactive, this treatment inhibited growth, despite the presence of the AR(T877A) mutant (Fig. 1B). These results indicate that AR activity suppresses the effects of ErbB inhibitors.

Androgen withdrawal stimulates, whereas dual EGFR/HER2 inhibition suppresses, ErbB3 levels

Forty-eight-hour treatment with erlotinib (10 μmol/L), but not trastuzumab (21 μg/mL) inhibited EGF-stimulated EGFR phosphorylation, whereas trastuzumab, but not erlotinib, affected the expression of HER2 (Fig. 2A, left). On the other hand, the combination, but not the individual drugs, inhibited ErbB3 phosphorylation, and reduced ErbB3 levels (Fig. 2A, right) also (Supplementary Fig. S3A). Since prostate cancer cells do not express ErbB4 (Supplementary Fig. S3B; ref. 10), we examined the effects of AWT on the levels of the other ErbB receptors. There was no significant change in EGRF levels upon culture in CSS, however, both HER2 and ErbB3 levels increased significantly as AR levels declined (Fig. 2B, top; also Supplementary Fig. S3C). Consistent with previous findings (6, 26), we saw a concomitant increase in Akt phosphorylation (Ser 473) in LNCaP (Fig. 2B, top). However, AWT caused no change in ErbB3 in LNCaP cells, which expressed both higher AR (11) and ErbB3 (Fig. 2B, bottom). Comparison of LNCaP versus LNCaP-AI showed that the latter expressed higher levels of HER2 and ErbB3, and also higher ErbB3 phosphorylation (Fig. 2C). Taken together, these results indicate that in LNCaP cells, but not its CRPC subline, ErbB3 levels increase during AWT whereas it is suppressed by dual EGFR/HER2 inhibition.

Dual EGFR/HER2 inhibition suppresses ErbB3 and PSA levels in CWR22 xenografts in nude mice

CWR22 xenografts were established in 4- to 5-month-old male nude mice, and when the tumors were palpable, the animals were treated with vehicle only or with erlotinib (0.8 mg/kg, 5 times per week) and trastuzumab (20 mg/kg, 2 times per week) in combination. The animals were castrated, or sham operated, 3 days after the drugs were started, but drug treatments were continued until the end. The animals were divided as: (i) vehicle only, sham operated (n = 6), (ii) vehicle only, castrated (n = 6), and (iii) drug treated, castrated (n = 6). CWR22 tumors shrink rapidly following castration, hence to obtain sizable tumors that can be analyzed; the animals were sacrificed 8 days after the procedure. Serum levels of PSA, a clinical indicator of AR activity in the prostate, were analyzed in blood drawn (i) at the beginning of the study, (ii) on the day of castration/sham operation, and (iii) at the end of the study (Fig. 3A, top). In vehicle-treated, sham-operated animals, PSA levels increased significantly with time (P = 0.049), whereas in castrated animals, the change in
PSA was not significant. In those treated with the drug combination, PSA levels decreased 3-fold. At the end of the study, the difference between PSA levels from castrated animals that were vehicle treated (16.3 ± 8.3 ng/mL) versus drug treated (4.3 ± 3.2 ng/mL) was significant (P = 0.02), whereas the difference between sham operated (29.8 ± 7.9 ng/mL) versus control animals were not (P > 0.05).

Staining for ErbB3 in the formalin-fixed and paraffin-embedded sections showed weak staining in the sham-operated mice (n = 6) whereas the castrated and vehicle-treated mice showed strong staining (n = 6), which was eliminated in the castrated mice treated with the drug combination (n = 5; one of the tumors was too small for analysis; Fig. 3B). Quantitation of the staining levels showed a significant increase in ErbB3 levels from sham operated, vehicle treated (0.63 ± 0.43) to castrated, vehicle-treated tumors (1.33 ± 0.26; P = 0.009), which was reduced 40% in tumors treated with the drugs in castrated animals (0.8 ± 0.45) (P = 0.05; Fig. 3C). Castration suppressed proliferation and induced apoptosis in these animals, as indicated by Ki67 and TUNEL staining (Supplementary Fig. S4), respectively, whereas both effects were enhanced by treatment with the drug combination (Fig. 3D). These results confirm that dual EGFR/HER2 inhibition reduces ErbB3 levels and reduces serum PSA levels.

ErbB3 overexpression stabilizes androgen receptor levels and promotes castration resistant cell growth mediated by Akt

LNCaP cells overexpressing ErbB3 grew at a much faster rate compared with parental LNCaP cells (Fig. 4A, top) and were not growth inhibited by the AR antagonist bicalutamide (Casodex) even at 10 µmol/L (Fig. 4A, middle) indicating androgen-independent cell growth. Flow cytometric analysis revealed this to be due to an increase in the percentage of cells entering the cell cycle (increased...
S-phase) which was not impeded by bicalutamide (Fig. 4A, bottom). Although culture in CSS-containing medium causes a decrease in the levels of the AR in LNCaP cells, increased expression of ErbB3 in the same cells maintained AR levels (Fig. 4B). Because ErbB3 is a known inducer of Akt phosphorylation (29), we examined the role of Akt in ErbB3-mediated cell growth. Increased ErbB3 stimulated Akt phosphorylation (Fig. 4C), while downregulation of Akt expression by siRNA suppressed ErbB3-induced proliferation in LNCaP cells (Fig. 4D), thereby indicating that Akt phosphorylation mediated the regulation of LNCaP cell growth by ErbB3.

Resistance to growth inhibition by dual EGFR/HER2 inhibition correlates with the ability of the inhibitors to suppress Akt phosphorylation

LNCaP-AI cells expressed higher levels of Akt phosphorylation compared with parental LNCaP cells (Fig. 5A, top). Treatment with the combination of trastuzumab and erlotinib, but not the individual drugs, significantly inhibited heregulin 1B (HRG1)-induced Akt phosphorylation in LNCaP cells, but not in LNCaP-AI (Fig. 5A, bottom). Similarly, the same combination inhibited Akt phosphorylation in parental pRNS-1-1 cells which lack a functional AR, whereas in cells that express AR(T877A), the drug combination failed to inhibit Akt activity (Supplementary Fig. S5A). These results correlate Akt phosphorylation with the growth inhibitory effects of the combination of trastuzumab and erlotinib.

Figure 2. ErbB3 inhibition by the combination of erlotinib and trastuzumab, and its stimulation by AWT, in LNCaP cells. A, Western blots showing the effect of erlotinib (10 μmol/L) and trastuzumab (21 μg/mL) on ErbB receptor tyrosine kinases. Left, LNCaP cells were serum starved in the presence of erlotinib and/or trastuzumab for 48 hours, followed by further treatment with 10 ng/mL EGF for 5 minutes. Lysates were blotted with anti-phospho-EGFR (Y1068; 1st), anti-EGFR (2nd), or anti-HER2 (3rd) antibodies. Right, alternatively, the cells were stimulated with 50 ng/mL HRG1 to induce ErbB3 phosphorylation, and immunoblotted with anti-phospho-ErbB3 (1st) and anti-ErbB3 (2nd). B, Western blots showing that AWT causes increased HER2 and ErbB3 expression and phosphorylation of Akt. LNCaP cells were cultured in FBS-containing medium up to 75% confluence and then switched to CSS-containing medium for the indicated period of time. Cell lysate was collected and immunoblotted with antibodies to anti-AR (1st), anti-EGFR (2nd), anti-HER2 (3rd), anti-ErbB3 (4th), anti-phospho-Akt (Ser 473; 5th), anti-Akt (6th), and anti-β-actin (7th). In contrast to LNCaP, its CRPC subline LNCaP-AI did not experience a similar increase in ErbB3 following AWT (8th). C, comparison of the activation and expression of the ErbB receptors expressed in LNCaP cells and its CRPC subline LNCaP-AI. The cells were serum starved for 48 hours and then EGF (10 ng/mL; left) or HRG (50 ng/mL; right) were added for the times indicated.
effect on cell growth in FBS-containing medium (Fig. 5C). On the other hand, LNCaP-AI cells were not growth arrested by the latter combination (Supplementary Fig. S5B). These results indicate that suppression of cell growth by the drug combination correlates with inhibition of Akt phosphorylation.

Suppression of Akt phosphorylation sensitizes castration resistant prostate cancer cells to dual EGFR/HER2 inhibition

Finally, we investigated methods of overcoming the resistance of prostate cancer cells to ErbB inhibitors. Because LNCaP-AI are not sensitive to dual inhibition of EGFR and HER2, and expressed higher ErbB3 compared with LNCaP, we investigated whether the increase in ErbB3 contributed to this resistance. Similar to the effects of a combination of erlotinib and trastuzumab, the combination of AG1478 and AG879 impeded the increase in cell numbers but did not reduce them below initial levels in LNCaP cells cultured in FBS (Fig. 6A, top), indicating growth arrest but not cell death. However, when the same cells were cultured in CSS, there was a 50% decrease in cell numbers indicating cell death (Fig. 6A, bottom). On the other hand, culture in CSS failed to have a similar effect in LNCaP cells overexpressing ErbB3 (Fig. 6B), indicating that ErbB3 increase...
induced resistance to this drug combination. In support of a role for Akt phosphorylation in this process, LNCaP cells cultured in CSS experienced increasing Akt phosphorylation more than a period of 5 days when exposed to vehicle alone whereas when they were exposed to the combination of AG1478 and AG879, Akt phosphorylation was significantly impeded (Fig. 6C, top). On the other hand, in LNCaP-AI cells resistant to this drug combination (Supplementary Fig. S5B), the increase in Akt phosphorylation in response to CSS exposure was not affected (Fig. 6C, bottom). The fact that Akt phosphorylation increased upon CSS treatment in LNCaP-AI cells whereas ErbB3 levels did not (Fig. 2B) indicates that other factors also contribute to Akt phosphorylation in CRPC. Our results indicated that, failure of dual EGFR/HER2 inhibition to induce apoptosis resulted from a failure of the same drugs to downregulate Akt phosphorylation. In support, AG1478 and AG879 in combination was not effective in inducing apoptosis in LNCaP-AI cells in the presence of control siRNA (9.89% in control siRNA vs. 13.25% in control siRNA + AG1478 + AG879), whereas Akt siRNA alone induced a significant increase in Annexin V staining (28.28%) which was further increased in the presence of the drugs (44.65%; Fig. 6D).
Previous studies showed that the dual EGFR/HER2 inhibitor lapatinib evidenced no decrease in PSA in patients with hormone sensitive prostate cancer (22) or in unselected patients with CRPC (23). The goal of this study was to determine whether dual EGFR/HER2 inhibition has any role in the prevention of disease progression in prostate cancer.

We show that androgen-dependent prostate cancer cells with low ErbB activity do not show substantial response to ErbB inhibitors, whereas during AWI, ErbB2, and ErbB3 levels increase, which regulates Akt phosphorylation and also cell survival. Hence, during this period, if the increase in these receptors is inhibited by dual EGFR/ErbB2 inhibition, which also inhibits ErbB3 phosphorylation, the increase in Akt phosphorylation and survival can be prevented.

**Figure 5.** Akt phosphorylation at Ser 473 correlates with the ability of ErbB inhibitors to impede cell growth. A, top, LNCaP-Al cells experience increased levels of Akt phosphorylation (Ser 473) compared with LNCaP. LNCaP-Al cells were serum starved and then treated with 10 ng/mL IGF-1 for various times as shown. Note the increase in Akt phosphorylation at Ser 473 with time. Bottom, Western blots showing the effect of erlotinib (10 μmol/L) and trastuzumab (21 μg/mL) on LNCaP and LNCaP-Al cells. Cells were grown to 75% confluence, and then serum starved for 48 hours in the presence of erlotinib or trastuzumab or both. The cells were then further treated with 50 ng/mL HRG1 for 15 minutes, to stimulate Akt phosphorylation downstream of ErbB3 activation, cell lysates collected and immunoblotted with antibodies to anti-phospho–Akt (Ser 473; 1st, 3rd), and total Akt (2nd, 4th). B, top, Western blots showing the specificity and selectivity of AG1478 and AG879 on the activation of EGFR and HER2, respectively. Serum starved LNCaP cells were treated with vehicle (DMSO), 5 μmol/L AG1478, or 2 μmol/L AG879 for 48 hours followed by further treatment with PBS or 10 ng/mL EGF for 5 minutes. EGF induced the phosphorylation of both EGFR (Tyr1068) and HER2 (Tyr1248). Bottom, LNCaP cells cultured in FBS or CSS were treated with the 2 drugs for 3 or 5 days. Western blotting shows that in the presence of FBS, there was no effect of the drugs, alone or in combination, on Akt phosphorylation, whereas in CSS, Akt phosphorylation at Ser 473 was significantly affected. C, MTT assay was used to determine the cell growth rate with the combination of AG879 (2 μmol/L) and AG1478 (5 μmol/L) of pRNS1-1 cells stably transfected with a T877A mutant AR grown in medium containing FBS (left) or medium containing CSS (right). Data represent mean ± SD of 3 independent experiments.
However, once ErbB3 levels have increased, the same drugs fail to affect the levels of Akt phosphorylation, thereby indicating that they can inhibit \textit{de novo} activation of ErbB3 but cannot dephosphorylate the receptor after it is activated. Although individual EGFR and HER2 inhibitors had differential effects on prostate cancer cells, the overall effect of dual inhibition was similar. The difference between various inhibitors of the same receptor may be attributed to the strength of the binding of these inhibitors to the receptor. We see that in both cases, the drug combinations resulted in a decrease in Akt phosphorylation. Because ErbB4 is lost in prostate cancer, the ErbB dimers formed in this disease include EGFR homodimers and EGFR-HER2, HER2-ErbB3, and EGFR-ErbB3 heterodimers (discussed in details in ref. 31). All contribute to survival of prostate cancer cells; hence inhibition of only 1 receptor will not prevent downstream signaling. Our data show that inhibition of both EGFR and HER2 is required to prevent ErbB3 signaling, likely by preventing its dimerization. Because only ErbB3 but not EGFR or HER2 have p85 PI3K-binding sites (9), the majority of the Akt signaling may be downstream of ErbB3 dimerization with EGFR or HER2, which will be inhibited only upon dual inhibition. ErbB3 monoclonal antibodies such as MM-121 are currently in development (32), and are also likely to succeed in combination with other ErbB inhibitors such as lapatinib.
We show that in cells expressing high AR, either hormone-naïve cells never exposed to AWT, or in CRPC cells that have high AR transcriptional activity, dual ErbB inhibition is unable to inhibit Akt phosphorylation and cell survival. In a previous study, we had shown that in hormone-naïve cells, the AR suppresses ErbB3 levels by transcriptionally regulating the ErbB3 inhibitor Nrdp1 (11). Because ErbB3 is capable of inducing AR-independent cell growth, this is likely an attempt by the AR to suppress AR-independent signaling. Hence, in androgen-dependent cells growing in the presence of high androgen levels, cell survival is AR-dependent and not ErbB3-dependent. Therefore, inhibition of ErbB3 or its binding partners will not affect cell growth or survival. On the other hand, when AR levels decreased during AWT, ErbB3 levels rebound and cell growth becomes dependent on signal transduction downstream of this receptor. Therefore, if at this time, ErbB3 signaling is suppressed, cell survival is impacted.

ErbB3 increase during AWT likely as an attempt to prevent AR decrease. In this study, we show that ErbB3 stabilize AR levels; thereby preventing its decrease in low-androgen medium. Further studies are required to see whether this is the mechanism by which ErbB3 promotes androgen-independent cell growth, but if so, it will explain why, in some CRPC cells, growth is still AR dependent, but not androgen-independent, as has been shown by other labs (33, 34). Despite this, it seems that the ErbB3-stabilized AR is incapable of downregulating ErbB3 (which is reasonable, if it requires that ErbB3 to stabilize it), as we previously showed (11). Furthermore, once the cell progresses to a CRPC phenotype, it is no longer capable of responding to dual EGFR/HER2 inhibition to downregulate Akt phosphorylation downstream of ErbB3. Hence, dual EGFR/HER2 inhibition does not affect cell survival or even cell growth in CRPC cells.

In CRPC cells, the effects of ErbB receptors and the AR are compounded by high Akt phosphorylation (29). Akt is induced by other factors including IGF, hence in CRPC cells, which are associated with multiple changes in cell signaling pathways (see ref. 35 and references within), it is likely that the cells have become adept at kinase switching, resulting in activation of multiple cell survival pathways. As a result, in these cells, dual EGFR/HER2 inhibition will not prevent all aberrant Akt phosphorylation. Therefore, our goal is to prevent the increase in aberrant Akt phosphorylation, and PSA progression, indicative of relapse, following AWT, by using the dual inhibitors during and not after this treatment. The clinical and therapeutic consequences of such a treatment could be quite profound. A 2009 study of 1,078 patients with hormone-sensitive prostate cancer enrolled in SWOG trial 9346, where PSA progression (PSA-P) was defined as an increase of 25% or more over nadir, median subsequent overall survival was shown to be 10 months in patients experiencing PSA-P within 7 months of hormone treatment, versus 44 months for those who did not have PSA-P during this period (36). Therefore, it is likely that if coadministration of dual EGFR/HER2 inhibitors delays PSA-P beyond 7 months, we would see a significant increase in PSA progression.

In conclusion, our data indicate that dual EGFR/HER2 inhibition is an effective tool for sensitizing androgen-dependent prostate cancer cells to apoptosis during AWT, likely preventing prostate cancer progression to CRPC following AWT treatment, but is not effective in CRPC cells expressing high Akt phosphorylation. However, this strategy may find utility with the advent of new therapeutic agents such as abiraterone acetate, a CYP17 inhibitor that blocks steroid biosynthesis (37), and MDV3100, a more potent AR inhibitor (38). In post-docetaxel patients, abiraterone increased survival by 3.9 months over controls (37) and it would be of interest to see whether this leads to an increase in ErbB3/HER2 as well, and whether prevention of this increase, if any, would further prolong survival. It is clear from this study, that the window of opportunity for using ErbB inhibitors in prostate cancer is when ErbB3 is rising and not when it is stable. The study also shows that potentially effective drugs if utilized in the wrong clinical setting may be prematurely judged to be ineffective.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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