

Growth Inhibitory Effects of the Dual ErbB1/ErbB2 Tyrosine Kinase Inhibitor PKI-166 on Human Prostate Cancer Xenografts¹

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ABSTRACT

Experiments with human prostate cancer cell lines have shown that forced overexpression of the ErbB2-receptor tyrosine kinase (RTK) promotes androgen-independent growth and increases androgen receptor-transcriptional activity in a ligand-independent fashion. To investigate the relationship between ErbB-RTK signaling and androgen in genetically unmanipulated human prostate cancer, we performed biochemical and biological studies with the dual ErbB1/ErbB2 RTK inhibitor PKI-166 using human prostate cancer xenograft models with isogenic sublines reflecting the transition from androgen-dependent to androgen-independent growth. In the presence of low androgen concentrations, PKI-166 showed profound growth-inhibitory effects on tumor growth, which could be partially reversed by androgen add-back. At physiological androgen concentrations, androgen withdrawal greatly enhanced the ability of PKI-166 to retard tumor growth. The level of extracellular signal-regulated kinase activation correlated with the response to PKI-166 treatment, whereas the expression levels of ErbB1 and ErbB2 did not. These results suggest that ErbB1/ErbB2 RTKs play an important role in the biology of androgen-independent prostate cancer and provide a rationale for clinical evaluation of inhibitors targeted to this pathway.

INTRODUCTION

Carcinoma of the prostate is the most common malignancy affecting males and causes enormous morbidity and mortality in the United States and Western Europe. About one-third of men relapse after radical prostatectomy surgery because of previously undetected metastatic disease. Metastatic prostate cancer responds for a variable period of time to androgen-deprivation therapy but eventually resumes growth despite castrate levels of androgen. This state of disease, termed “androgen-independent” prostate cancer, is characterized by expression of the *AR*³ and AR-regulated genes, suggesting that the AR pathway is reactivated in a “ligand-independent” fashion. Several mechanisms have been proposed to explain the phenomenon of AR reactivation in the setting of castrate levels of ligand. These include mutations in *AR* that alter its ligand-binding affinity, overexpression of AR because of gene amplification, and/or increased recruitment of intracellular signal transduction pathways, which activate AR through ligand-independent mechanisms (1).

ErbB RTKs have been implicated as one such pathway that may play a role in androgen-independent prostate cancer progression. Experimental support for this concept comes from the observation that: (a) activation of ErbB1 and/or ErbB2 RTKs by EGF or forced

overexpression of ErbB2, respectively, results in androgen-independent activation of AR transcriptional activity in prostate cancer cell lines (2–4); (b) forced overexpression of ErbB2 promotes androgen-independent growth of prostate cancer cells (2, 3); and (c) androgen-independent prostate cancers express increased levels of ErbB2 receptor protein (2, 5–7). However, it is still uncertain whether ErbB1- and ErbB2-mediated signals contribute to the progression of human prostate cancer, which, unlike breast cancer (8), rarely shows amplification of *ErbB*-gene loci. Using xenograft models of human prostate cancer, we show here a striking interplay of AR and ErbB signaling pathways. Growth inhibition by the ATP site-specific ErbB1/ErbB2 RTK inhibitor PKI-166 was greatest in androgen-independent tumors, significantly augmented by simultaneous AW, and partially rescued by androgen supplementation. Growth inhibition by PKI-166 was positively correlated with the basal activation state of the ERKs ERK1/2. Our findings suggest that ErbB1/ErbB2 RTKs play an important role in the biology of human prostate cancer and may be a viable therapeutic target for novel kinase inhibitors (9).

MATERIALS AND METHODS

Reagents. The LAPC4 cell line was established from the LAPC4 human prostate cancer xenograft (10), A431 cells were kindly provided by Dr. Harvey Herschman (UCLA), and LNCaP cells were purchased from American Type Culture Collection. PKI-166 was obtained from Novartis Pharma AG, Basel, Switzerland. EGF and standard chemical reagents were obtained from Sigma. Antibodies against ErbB1 (*sc-101* and *sc-03*), ERK1/2 (*sc-94*), TGF- α (*sc-9043*), and prostate-specific antigen (*sc-7638*) were obtained from Santa Cruz Biotechnology, against ErbB2 from Oncogene Sciences (*OP-15*), against phosphotyrosine from Upstate (*4G10*), against activated ErbB1 from Chemicon (*Mab3052*), against phospho-ERK (Thr183/Tyr185) from Promega (*V8031*), and against phospho-Akt (Ser473) from Cell Signaling Technology (*Ab9271*).

Pulse-Chase Experiments and Immunoblotting. A431 cells were labeled for 12 h in methionine-free DMEM with 0.2 mCi [³⁵S]methionine/cysteine (Translabel; ICN Biomedicals) per 10-cm plate. Cells were subsequently washed three times in serum-free DMEM. Serum-free DMEM with 15 mg/liter unlabeled methionine (“chase-medium”) was added to each plate in the presence or absence of 5 μ M PKI-166. Ten min later 100 ng/ml EGF was added where indicated in Fig. 1B. Cells were lysed immediately after the third wash in serum-free DMEM (baseline sample) and at various intervals after the addition of chase-medium. Cell lysis, immunoprecipitation, SDS-PAGE-electrophoresis, autoradiography, and immunoblotting was performed following standard protocols (11). Protein concentration was determined in all of the lysates by Bio-Rad, and equal amounts of protein were loaded per lane. Quantification of immunoblot bands was performed using ImageQuant software.

Animal Experiments. SCID (C.B.-17 *Scid/Scid*) mice were bred and maintained in a laminar flow tower in a defined flora colony as described previously (12). All of the manipulations with the animals were performed in a laminar flow hood with sterile techniques following the guidelines of the UCLA Animal Research Committee. For preparation of single cell suspensions, LAPC4 (10) and LAPC9 (13) tumors continuously passaged in SCID mice were minced in serum-free Iscove’s medium (Life Technologies, Inc. Grand Island, NY), washed, digested with 0.1% Pronase E (EM Science, Gibbstown, NJ), washed again, and filtered through a 200 μ m nylon mesh (BioDesign Inc. of New York, Carmel, NY). The cells were plated overnight, resuspended in PrEGM, and injected with Matrigel into the right flank of SCID mice. Tumor size was determined with calipers, and mice were randomized to

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³ The abbreviations used are: AR, androgen receptor; RTK, receptor tyrosine kinase; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; UCLA, University of California Los Angeles; TGF, transforming growth factor; SCID, severe combined immunodeficient; AW, androgen withdrawal; MAPK, mitogen-activated protein kinase.

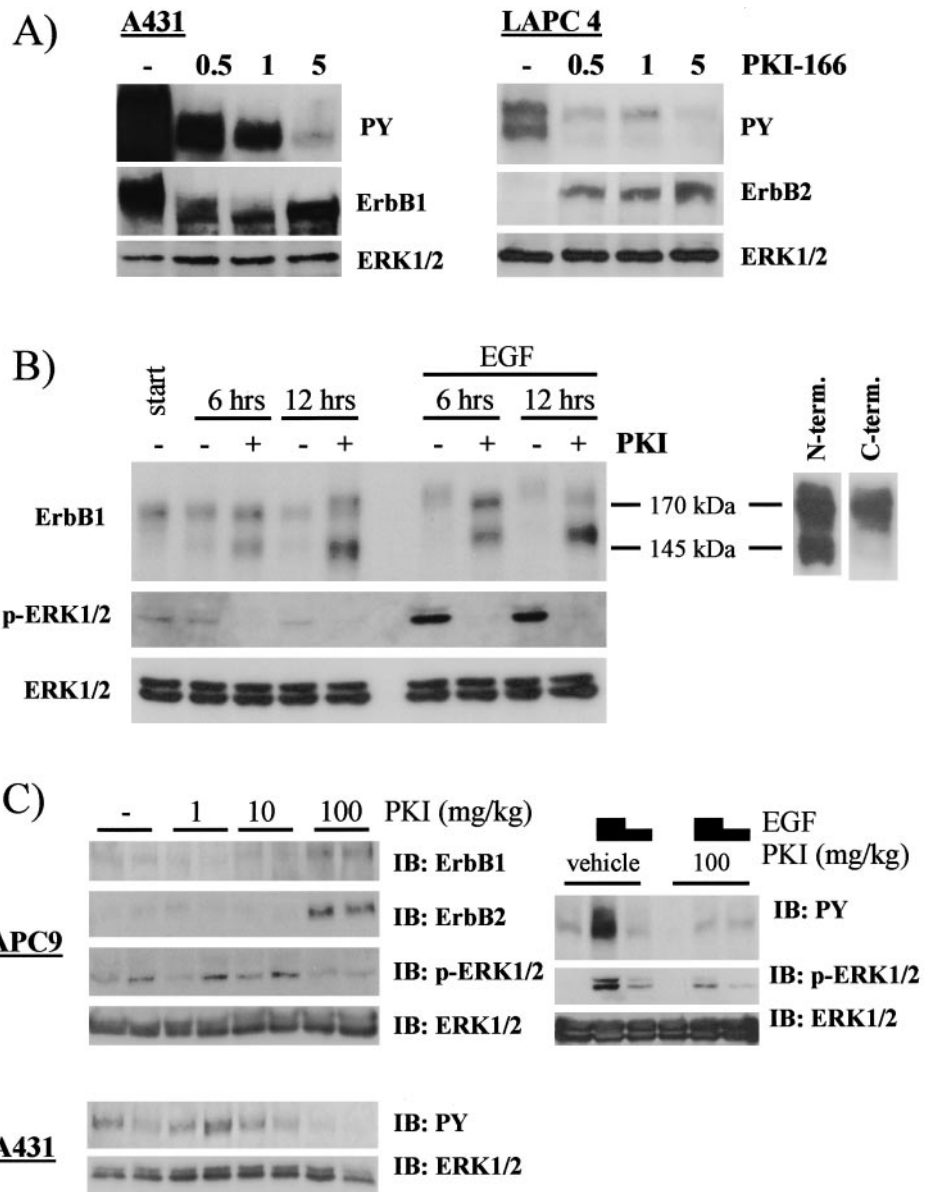


Fig. 1. PKI-166 inhibits ErbB1 and ErbB2 receptor signaling and degradation. *A*, immunoblots of A431 (*left panel*) and LAPC4 (*right panel*) cells treated for 12 h with various concentrations of PKI-166 (in μM) before stimulation with EGF (100 ng/ml) for 10 min. *B*, *left panel*, autoradiograph of immunoprecipitated ErbB1 (*top row*) and immunoblots (*middle and bottom row*) of lysates from A431 cells metabolically labeled for 12 h with [^{35}S]methionine and chased in the presence of PKI-166 (5 μM) and/or EGF (100 ng/ml). PKI-166 was added 10 min before EGF where indicated. *Right panel*, immunoblot of A431 cells treated with PKI-166 for 12 h using a ErbB1 antibodies directed against NH_2 -terminal and COOH -terminal epitopes. *C*, immunoblots of lysates from LAPC9 (*left top panel and right panel*) or A431 (*left bottom panel*) xenograft tumors harvested 1 h after the fifth daily dose of PKI-166 (2 tumors per condition). The *right panel* shows lysates of LAPC9 tumors from mice injected i.p. with PBS, 0.1 mg EGF, or 0.01 mg EGF 1 h after the fifth daily dose of PKI-166. Tumors were harvested 5 min after EGF injection.

treatment groups when tumor size reached $\sim 100 \text{ mm}^3$. Mean tumor volumes (in mm^3) at treatment begin was equal between groups. PKI-166 was administered daily by gavage with an 18-gauge animal feeding needle (VWR, San Dimas, CA). Testosterone pellets (Innovative Research of America) were implanted s.c. Tumor growth data are expressed as fold tumor volume compared with day 1 (Fig. 2, *A* and *C*) or as ratio between the fold increases in tumor volume for PKI-166 and vehicle treated mice ("T/C"; Figs. 2*B* and 3*B*). Statistical analyses comparing fold increases between groups were performed on the natural logarithms of the tumor volumes corrected for baseline volumes. Student's *t* test was used for comparison of two groups. ANOVA using the Tukey Studentized range method was used for multigroup comparisons.

RESULTS

PKI-166 Blocks ErbB1/ErbB2 Activity in Prostate Cancer Cells. We first examined the effects of PKI-166 on EGF-induced signal transduction through ErbB1 and ErbB2 RTKs in the human prostate cancer cell lines LAPC4 and LNCaP, both of which express the AR. The human vulvar carcinoma cell line A431, which expresses high levels of *ErbB1* because of amplification of the *erbB1* locus, was used as a positive control. In A431 cells treated with EGF, immuno-

blotting with a phosphotyrosine antibody showed a dominant band of $M_r \sim 170,000$ representing the phosphorylated ErbB1 receptor (Fig. 1*A*). In EGF-treated LAPC-4 cells, which express considerably less ErbB1 than A431 cells but more ErbB2 (data not shown), tyrosine phosphorylation of a $M_r 170,000$ and a $M_r 185,000$ band was observed representing phosphorylated ErbB1 and ErbB2, respectively. Dose-dependent inhibition of receptor autophosphorylation was noted after pretreatment with PKI-166, with estimated IC_{50} values of 0.5 μM for ErbB1 and 5 μM for ErbB2. Similar doses of PKI-166 have been reported to inhibit phosphorylation of ErbB1 and ErbB2 RTKs but not other tyrosine or serine/threonine kinases in nonprostatic human cancer cell lines (14–16). We also noted that PKI-166 treatment resulted in a dose-dependent increase in the level of ErbB1 protein in A431 cells and ErbB2 protein in LAPC4 cells. The increase in ErbB2 expression in LAPC4 cells was apparent at 0.5 μM PKI-166, a concentration that predominantly inhibits phosphorylation of ErbB1.

Previous work has indicated that ErbB1 protein is degraded after receptor activation by ligand, raising the possibility that the increased levels of ErbB1 and ErbB2 in PKI-166-treated cells are a reflection of

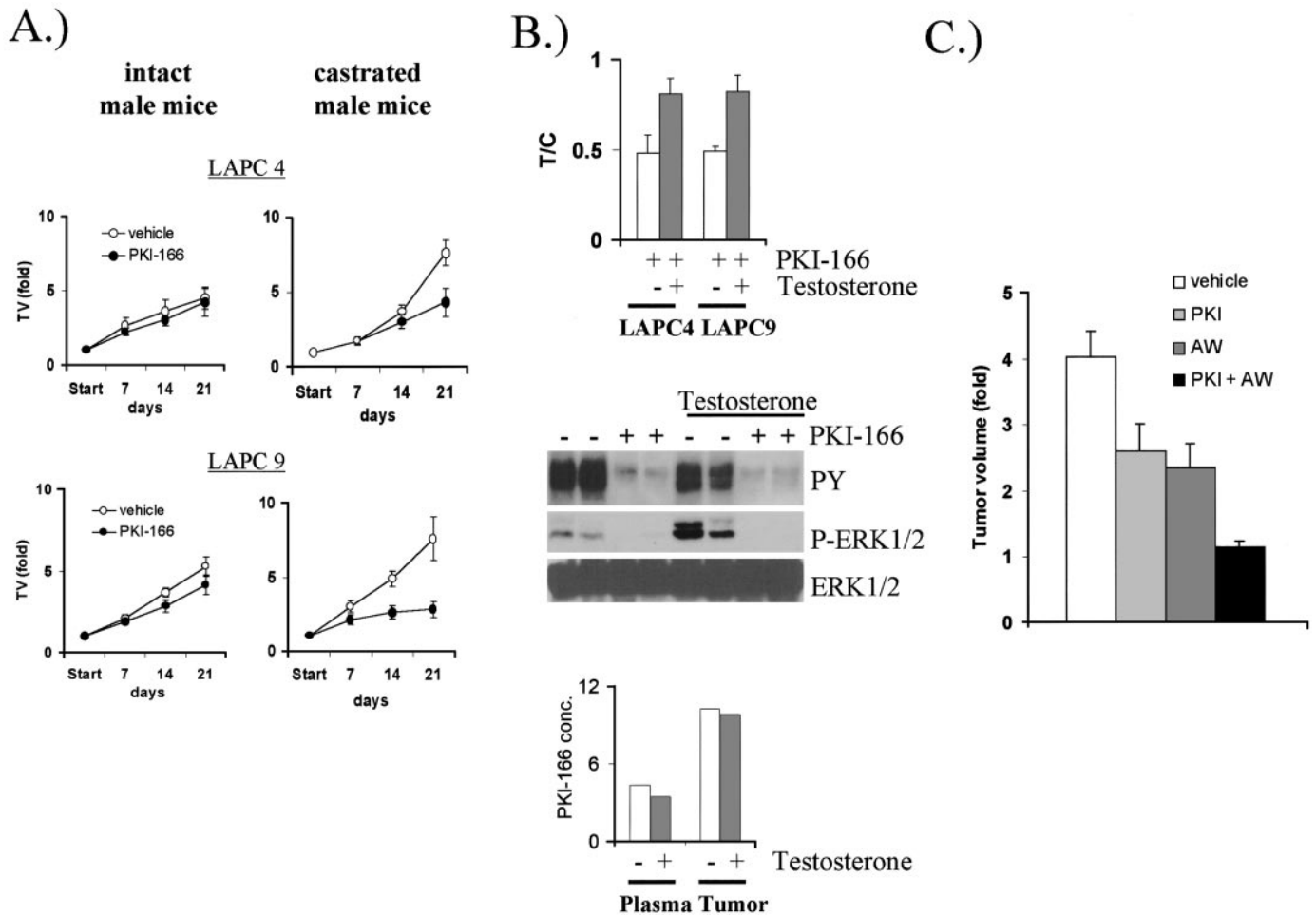


Fig. 2. Androgen modifies the growth response to PKI-166. **A**, PKI-166 inhibits the growth of human prostate cancer xenografts growing in castrated mice. Mice bearing LAPC4 or LAPC9 xenograft tumors were treated daily for 3 weeks with 100 mg/kg PKI-166 *versus* vehicle ($n = 6-9$ per group). Data are expressed as fold tumor volume compared with day 1; bars, \pm SE. **B**, *top panel*, testosterone rescues growth inhibition by PKI-166 in castrated animals. Castrated male mice bearing androgen-independent xenografts were randomized to four treatment groups (vehicle, PKI-166, vehicle plus testosterone, and PKI plus testosterone). Data are expressed as ratio between the fold increase in tumor volume for PKI-166 treated mice and the fold increase in tumor volume for vehicle treated mice (T/C). Each treatment group consisted of 8-9 mice. *Middle panel*, testosterone does not impair the ability of PKI-166 to inhibit ErbB1/ErbB2 signaling. Castrated male mice bearing LAPC9 xenograft tumors were treated with PKI-166 +/- testosterone for 21 days, and EGF (0.1 mg) was administered *i.p.* 1 h after the last PKI-166 dose. Tumors ($n = 2$ per treatment condition) were harvested 5 min later. Displayed are immunoblots for PY, ERK1/2, and total ERK1/2. *Bottom panel*, testosterone does not affect PKI-166 bioavailability in mice. PKI-166 levels in plasma (μM) and LAPC9 tumors (nmol/g) were determined by reversed-phase high-performance liquid chromatography in castrated mice after 21 days of treatment with PKI-166 (100 mg/kg) +/- testosterone. Blood and tumors were collected 1 h after the last dose of PKI-166 and displayed as mean values ($n = 3$ per group). **C**, AW augments inhibitory effect of PKI-166 on LAPC4 xenografts in intact male mice. Surgical castration was performed on the same day as PKI-166 treatment was started. Data are expressed as fold tumor volume compared with day 1. Six mice were treated per group; bars, \pm SE.

kinase inhibition. To determine whether pharmacologic inhibition of RTK activity delays receptor degradation, we measured the effect of PKI-166 on immunoprecipitated, [^{35}S]methionine/cysteine radiolabeled ErbB1 receptors in A431 cells (Fig. 1B, *left panel*). In the absence of PKI-166 and EGF, the receptor half-life of ErbB1 was between 6 and 12 h, consistent with the half-life of ~ 9 h published previously (17). Stimulation of A431 cells with EGF resulted in phosphorylation of ErbB1, as evidenced by retarded electrophoretic mobility compared with the unphosphorylated receptor, and shortening of the receptor half-life to < 6 h. PKI-166 impaired the degradation of ErbB-1 receptor protein in both the presence and absence of EGF. These data support the concept that kinase activity is required for receptor degradation, consistent with prior work showing increased receptor half-life in ErbB1 alleles containing point mutations within the ErbB1 ATP-binding site (18, 19). We also noted accumulation of a lower molecular weight protein in the ErbB1 immunoprecipitates from PKI-166-treated cells. This M_r 145,000 protein was immunoprecipitated by an ErbB1 antibody directed against a cell-surface epitope but not by an antibody directed against a COOH-terminal epitope (Fig. 1B, *right panel*), indicating that it is likely to be

a COOH-terminal truncation of the receptor. Similarly sized bands have been observed previously after treatment of A431 cells with the lysosomal inhibitor methylamine (17) and presumably represent an intermediate step in receptor degradation.

Because many growth factor signals are transmitted to the nucleus through ERKs (20), we also measured the effect of PKI-166 on ERK1/2 activation. At a dose that inhibits ErbB1 phosphorylation, PKI-166 completely blocked basal and EGF-induced ERK1/2 activation in A431 cells (Fig. 1B). Similar results were obtained in LAPC4 and LNCaP prostate cancer cells (data not shown). These data establish the biochemical activity of PKI-166 against ErbB1/ErbB2 RTKs *in vitro*, including effects on receptor autophosphorylation, receptor degradation, and additional signal transduction.

PKI-166 Blocks ErbB1/ErbB2 Signal Transduction in Tumors in Mice. We next examined the effects of PKI-166 treatment on ErbB1/ErbB2-mediated signaling *in vivo*. SCID mice bearing tumors from the human prostate cancer xenografts LAPC4 (10) and LAPC9 (13) or from the A431 cell line were treated for 5 days with 0, 1, 10, and 100 mg/kg of PKI-166, and tumor tissue was harvested 1 h after the last dose was administered. Lysates from A431 tumors displayed

constitutive phosphorylation of ErbB-1 that was inhibited by treatment of mice with 100 mg/kg PKI-166. Similar analysis of ErbB phosphorylation in prostate cancer xenografts was not informative because of low basal levels of phosphotyrosine (data not shown). However, we did observe decreased ERK1/2 activation and increased levels of total ErbB1 and ErbB2 protein in the prostate cancer xenografts from mice treated with 100 mg/kg of PKI-166, providing indirect evidence for ErbB1/ErbB2 RTK inhibition at this dose (Fig. 1C, left panel). To obtain direct evidence of ErbB1/ErbB2 blockade in PKI-166-treated mice, we induced receptor activation by systemic administration of EGF (21). Two different doses of EGF were injected i.p. and resulted in dose-dependent receptor phosphorylation and ERK1/2 activation in LAPC9 tumors (Fig. 1C, right panel). PKI-166 given p.o. at a dose of 100 mg/kg markedly blunted this activation (Fig. 1C, right panel). Similar results were obtained in mice bearing LAPC4 or A431 xenografts (data not shown).

PKI-166 Blocks the Growth of Prostate Cancers in Mice in an Androgen-dependent Fashion. Having defined the dose of PKI-166 required to inhibit ErbB1/ErbB2 RTKs *in vivo*, we were now able to examine the role of these RTKs in the growth of human prostate cancer. We chose the LAPC xenograft model to address this question because of its similarity to clinical prostate cancer (10) and the convenience of monitoring drug effects on s.c. tumor volumes. Tumors derived from the A431 cell line were used as a positive control and were completely growth arrested by PKI-166 (data not shown). Androgen-independent sublines of the prostate cancer xenografts grown in castrated host mice were consistently more sensitive to growth inhibition by PKI-166 than androgen-dependent sublines of the same xenograft growing in intact male ($P < 0.005$). This observation was confirmed in multiple experiments and noted in both LAPC4 and LAPC9 xenografts (Fig. 2A).

The trend toward enhanced activity of PKI-166 in the absence of androgen was reminiscent of our previous data showing more dramatic effects of forced ErbB2 overexpression on prostate cancer growth in castrated *versus* intact male mice (2). At that time we postulated that the major effects of ErbB1/ErbB2 pathway activation might be mediated through AR but that these effects were most relevant in the setting of low (castrate) levels of androgen. To examine this hypothesis, we asked if the suppression of growth by PKI-166 in castrated male mice could be rescued by androgen supplementation, which was administered by s.c. implantation of slow release testosterone pellets. In both LAPC4 and LAPC9 xenografts (Fig. 2B, top panel), androgen add-back partially rescued the growth inhibitory effects of PKI-166 ($P < 0.05$). One potential explanation for this result is that androgen impairs the ability of PKI-166 to inhibit ErbB1/ErbB2 RTKs. To examine this possibility, we treated eight castrated male mice bearing LAPC tumors with PKI-166 in the presence or absence of supplemental testosterone, and measured ErbB receptor and ERK1 activation in tumor lysates after systemic administration of EGF. Androgen supplementation did not impair the ability of PKI-166 to inhibit EGF-induced signal transduction (Fig. 2B, middle panel) nor did androgen affect the bioavailability of PKI-166 as shown by similar mean plasma and tumor drug levels in castrated and androgen-supplemented mice (Fig. 2B, bottom panel). These data indicate that the rescue of PKI-166-induced growth suppression by androgen supplementation cannot be explained by a failure of PKI-166 to inhibit its target. Rather, our findings suggest that ErbB1/ErbB2 signaling is not required for prostate cancer growth when androgen is present at high levels.

If a threshold level of circulating androgen exists below which ErbB1/ErbB2 RTKs are required for prostate cancer growth, acute AW by surgical castration should increase the growth-inhibitory effects of PKI-166. To test this hypothesis, we randomized intact male

SCID mice bearing the LAPC4 xenograft to four treatment groups. Compared with vehicle-treated mice, AW by surgical castration slowed the growth of LAPC4 tumors ($P < 0.05$), as expected (10). Growth inhibition by PKI-166 given at 100 mg/kg daily did not reach statistical significance. However, the combination of PKI-166 with AW resulted in nearly complete growth suppression (Fig. 2C). The difference between the combined treatment group and any of the other three treatment groups was highly statistically significant ($P < 0.001$).

Sensitivity to Growth Inhibition by PKI-166 Is Correlated with ERK Activation. The LAPC-4 and LAPC-9 xenografts have been passaged in mice over multiple generations, and various androgen-dependent and androgen-independent subclones derived from the original parental lines have been maintained independently. In the course of these studies we noted that subclones derived from the same parental line occasionally displayed differences in their sensitivity to PKI-166. These sublines provide an opportunity, within an isogenic system, to examine variables in the ErbB1/ErbB2 signaling pathway that might determine response to ErbB1/ErbB2 inhibition. To address this question, we performed biochemical analysis on six LAPC xenograft clones, of which four were grown in intact male mice (clones 1 and 2 for both LAPC 4 and LAPC 9) and two in castrated male mice (clones 3 for both LAPC 4 and LAPC 9). We examined not only the expression levels of the direct PKI-166 targets ErbB1 and ErbB2, but also the activation state of the Ras/MAPK and phosphatidylinositol 3'-kinase/Akt-pathways. Both pathways are considered central effectors of the ErbB signaling network (20) and have been implicated in the progression of human prostate cancer (3, 22–24). Expression of the ErbB-1 ligand TGF- α was included in the analysis because of its suggested role as an autocrine growth factor in androgen-independent prostate cancer (25). To be able to correlate for each subline the expression of these biochemical parameters with the growth response to PKI-166, we quantified the relevant immunoblot bands (Fig. 3A) by densitometry. Despite differences in the magnitude of ERK1/2-activation between the LAPC4 and LAPC9 xenograft models, we found within each model a positive correlation between the degree of growth-inhibition by PKI-166 and the level of ERK1/2-activation (Fig. 3B). No such correlation was found for expression levels of ErbB1, ErbB2, TGF- α , or activation level of Akt. Whereas the number of available sublines for each xenograft was not sufficient to perform a multivariate analysis, our results in an isogenic system suggest that expression levels of ErbB-1 or ErbB-2 are not sufficient to determine sensitivity to PKI-166 and are consistent with studies using the ErbB-1 inhibitor ZD1839 (26) or the anti-ErbB-2 monoclonal antibody Mab 4D5 (27).

We also noted that androgen-independent tumors (clones 3) showed increased activation of ERK1/2 when compared with tumors grown in intact male mice (Fig. 3A). This observation was confirmed by serial analysis of androgen-dependent LAPC4 xenografts at various times postcastration during the evolution to androgen independence (Fig. 3B). This data are consistent with results of immunohistochemical analyses of human prostate cancer specimens using phosphospecific antibodies to ERK1/2 (28).

DISCUSSION

In conclusion, our study shows that ErbB1/ErbB2 RTKs contribute to the growth of human prostate cancer and that this contribution is greatest when levels of androgen are limiting. The nature of the interaction between AR pathway and ErbB1/ErbB2 RTKs remains to be defined. It is conceivable that ErbB-RTKs provide growth and survival signals for prostate cancer cells, which are completely independent of AR and only biologically relevant under the selective pressure of androgen deprivation. Alternatively, ErbB-RTKs might

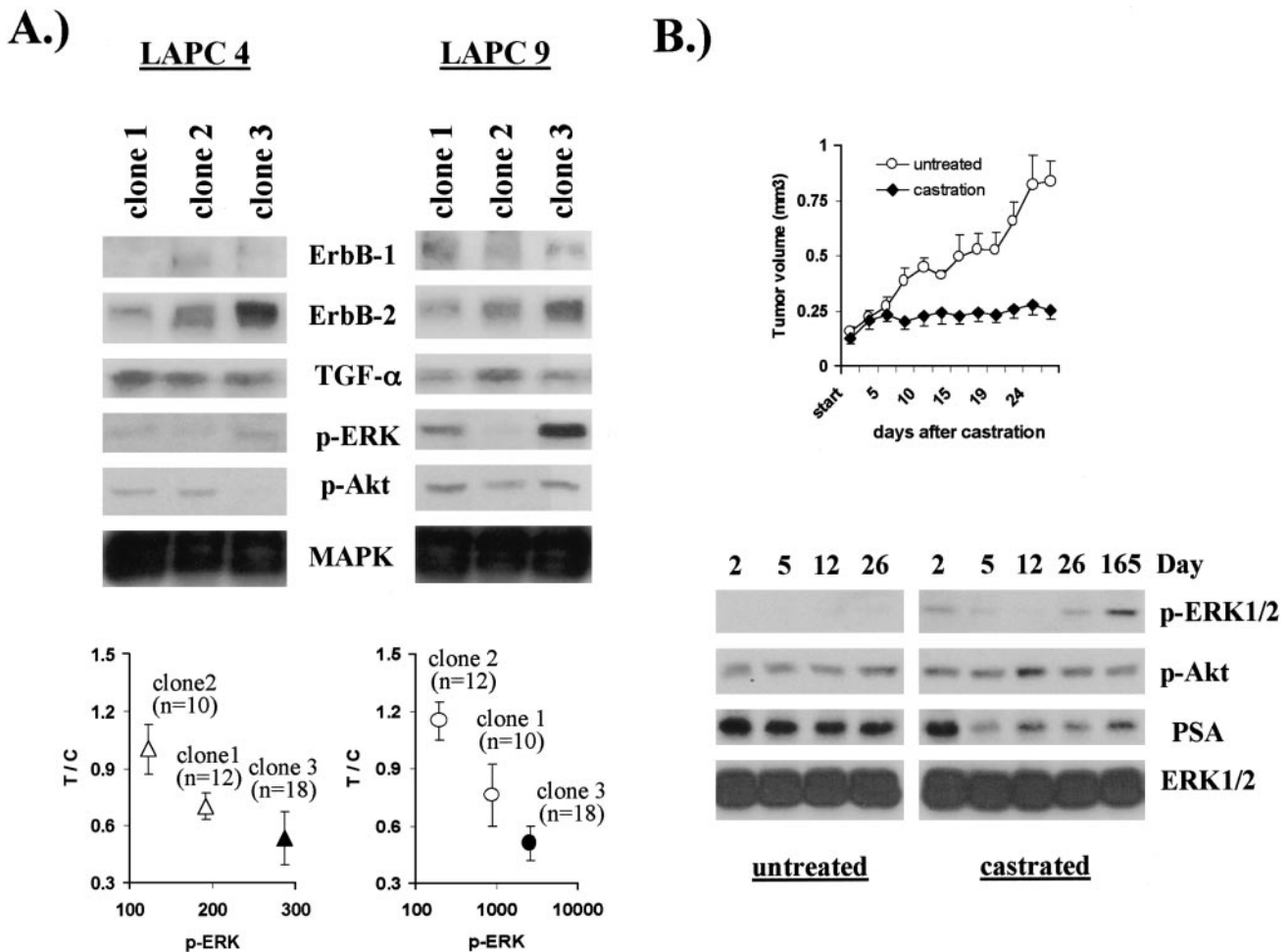


Fig. 3. Activity of p-ERK correlates with growth-inhibition by PKI-166 *in vivo* and is increased in androgen-independent tumors. *A, top panel*, immunoblot of tumor lysates from six different LAPC sublines; *bottom panel*, correlation between growth inhibition by PKI-166 and levels of activated ERK for six LAPC clones. Immunoblot bands were quantified by densitometry, and the absolute value under the peak was plotted on the X-axis. The Y-axis shows the ratio between the fold increase in tumor volume for PKI-166 treated mice and the fold increase in tumor volume for vehicle treated mice (*T/C*). *Open symbols* represent xenograft clones grown in intact mice; *closed symbols* represent xenograft clones growing in castrated animals. *B*, effect of castration on p-ERK levels in LAPC4 xenograft tumors harvested at various time points after castration; *bars*, \pm SE.

promote prostate cancer growth through ligand-independent activation of AR (2, 3). MAPKs, the primary effectors of the ErbB/Ras/Raf signaling pathway, have been suggested to link growth factor receptors and steroid hormone receptors, possibly by direct phosphorylation of the latter (29). In that context, our observation that increased ERK1/2 activation is positively correlated with response to ErbB1/ErbB2 pathway inhibition is particularly intriguing and warrants additional investigation.

A growing number of small molecule inhibitors of ErbB1 and/or ErbB2 are making their way into clinical trials and show slightly different potencies in relative inhibition of ErbB1 *versus* ErbB2 RTKs (9). Because these compounds appear to have therapeutic effects in subsets of patients, the question of which kinase is important for targeting, ErbB1 or ErbB2, is frequently raised. The dose of PKI-166 (100 mg/kg) used in most of our experiments inhibited both ErbB1 and ErbB2 RTKs, as evidenced by biochemical studies on tumor lysates, comparison of drug levels in mice with *in vitro* drug concentrations, and recent data from other mouse models of cancer treated with PKI-166 (15). Interestingly, the ErbB-2 RTK monoclonal antibody Herceptin failed to inhibit the growth of androgen-independent sublines of the CWR22 human prostate cancer xenograft model (30). Whereas this difference may reflect the use of different cell lines, it raises the possibility that inhibition of the ErbB-1 RTK is required to halt androgen-independent growth.

Despite the difficulty in dissecting the relative contribution of individual ErbB-receptor family members in naturally arising tumors, our data provide rationale for testing ErbB1/2-RTK inhibitors in clinical trials of human prostate cancer. It may also offer some guidance for the design of such studies. Whereas most current trials study the effects of ErbB-inhibitors in patients who failed hormonal therapy, our data suggest a role for combining ErbB-RTK inhibition with AW for patients with early-stage disease. Our findings also raise the possibility that the activation state of the Ras/MAPK pathway in clinical specimens might serve as a biomarker to identify tumors that "depend" on this pathway and may be more likely to respond to treatment with ErbB1/ErbB2 inhibitors.

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