Her-2-neu Expression and Progression Toward Androgen Independence in Human Prostate Cancer

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Background: Human prostate cancers are initially androgen dependent but ultimately become androgen independent. Overexpression of the Her-2-neu receptor tyrosine kinase has been associated with the progression to androgen independence in prostate cancer cells. We examined the expression of Her-2-neu in normal and cancerous prostate tissues to assess its role in the progression to androgen independence. Methods: Prostate cancer tissue sections were obtained from 67 patients treated by surgery alone (UNT tumors), 34 patients treated with total androgen ablation therapy before surgery (TAA tumors), and 31 patients treated with total androgen ablation therapy after surgery (TAA tumors), and 18 patients in whom total androgen ablation therapy failed and who developed bone metastases (androgen-independent [AI] disease). The sections were immunostained for Her-2-neu, androgen receptor (AR), prostate-specific antigen (PSA), and Ki-67 (a marker of cell proliferation) protein expression. Messenger RNA (mRNA) levels and gene amplification of Her-2-neu were examined by RNA in situ hybridization and fluorescent in situ hybridization (FISH), respectively, in a subset of 27 tumors (nine UNT, 11 TAA, and seven AI). All statistical tests were two-sided. Results: Her-2-neu protein expression was statistically significantly higher in TAA tumors than in UNT tumors with the use of two different scoring methods (P = .008 and P = .002). The proportion of Her-2-neu-positive tumors increased from the UNT group (17 of 67) to the TAA group (20 of 34) to the AI group (14 of 18) (P < .001). When compared with UNT tumors, tumor cell proliferation was higher in AI tumors (P = .014) and lower in TAA tumors (P < .001). All tumors expressed AR and PSA proteins. Although Her-2-neu mRNA expression was high in TAA and AI tumors, no Her-2-neu gene amplification was detected by FISH in any of the tumor types. Conclusions: Her-2-neu expression appears to increase with progression to androgen independence. Thus, therapeutic targeting of this tyrosine kinase in prostate cancer may be warranted. [J Natl Cancer Inst 2000;92:1918–25]

Her-2-neu is a receptor tyrosine kinase that belongs to the epidermal growth factor receptor family. Her-2-neu overexpression, which is seen in 20%–30% of breast and ovarian cancers, results from gene amplification and is associated with poor prognosis (1–4). Recently, Her-2-neu has become a therapeutic target in breast cancer with the advent of antibodies generated against its extracellular domain (5). In breast cancer clinical trials, one such antibody, trastuzumab (Herceptin; Genentech Inc., South San Francisco, CA), has been shown to be effective when coadministered with other chemotherapeutic agents (6,7).

In prostate cancer, the assessment of Her-2-neu overexpression has been more problematic, and the results are controversial (8–19). The controversy is due both to procedural differences, including variability of tissue fixation protocols and use of antibodies from different sources, and to biologic differences, including the heterogeneity of prostate cancers. Also, contradictory results about whether the gene is amplified have been reported from fluorescent in situ hybridization (FISH) analysis of the Her-2-neu locus in both primary and metastatic prostate cancers (20–25).

Although unequivocal evidence of Her-2-neu overexpression in human prostate cancer has not been available, evidence suggests that Her-2-neu may be important in prostate cancer progression. First, in the severe combined immunodeficient (SCID) mouse human prostate cancer xenograft LAPC (i.e., Los Angeles Prostate Cancer), Her-2-neu is overexpressed in androgen-independent sublines (i.e., grown in castrated hosts) compared with parental (androgen-dependent) LAPC cells (26). Second, induced overexpression of Her-2-neu in androgen-dependent prostate cancer cells activates the androgen receptor (AR) in a ligand-independent fashion, conferring androgen-independent growth to these cells (26). Because of these observations and the availability of U.S. Food and Drug Administration (FDA) approval to use therapeutic anti-Her-2-neu antibodies for breast cancer therapy, nonrandomized clinical trials of Herceptin have been started in prostate cancer.

We undertook this study with the following aims: 1) to determine whether Her-2-neu protein is expressed in human prostate cancers; 2) to assess whether Her-2-neu protein expression increases with progression toward androgen independence using two scoring methods; and 3) to address the mechanism of Her-2-neu overexpression by analyzing, in a subset of human prostate tumors, genomic copy number by FISH and messenger RNA (mRNA) levels by in situ hybridization (ISH).

Patients and Methods

Patient Population

This study was performed after approval by the Institutional Review Board of the Dana-Farber Cancer Institute and Brigham and Women’s Hospital, Boston, MA. Archival paraffin-embedded tissue blocks of prostate cancer from patients treated during the period from 1991 through 1996 were retrieved from the Departments of Pathology from Beth Israel Deaconess Medical Center, Boston, from the University of Ancona, Italy, and from the University of California, Los Angeles.

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versity of California, Los Angeles. Prostate cancer tissue sections were analyzed from three groups of patients: 1) patients who did not receive any treatment before surgery (UNT tumors, from 67 patients), 2) patients who were treated with total androgen ablation for 3 months before surgery (goserelin [Zoladex] depot every 28 days plus bicalutamide [Casodex] at a dose of 50 mg/day for 12 weeks) (TAA tumors, from 34 patients), and 3) patients who developed bone metastases after hormonal therapy failure (androgen-independent [AI] tumors, from 18 patients). Stage (27) and Gleason grade (28) were available for all primary prostate cancers. Levels to prostate specific antigen (PSA) recurrence and survival times were available for 69 prostatectomy cases (38 UNT and 31 TAA). The median follow-up was 3 years.

**Immunohistochemistry**

**Methodology.** Immunostaining was performed on all tissue sections with the use of the following primary antibodies: Her-2-neu (AO485; Dako Corp., Carpinteria, CA) at a 1:200 dilution, AR (Upstate Biotechnology, Lake Placid, NY) at a 1:50 dilution, PSA (BioGenex, San Ramon, CA) at a 1:50 dilution, and Ki-67 (Immunotech, Westbrook, ME) at a 1:50 dilution. To accurately identify prostate basal cells, we used the A4A antibody, directed against the p63 basal cell marker (29,30), at a 1:50 dilution. All antibodies were diluted in phosphate-buffered saline (PBS). The anti-Her-2-neu antibody used in this study is the same as the one that is included in the Herceptest kit (Dako, Inc.) approved by the FDA for the evaluation of Her-2-neu expression in breast carcinomas.

Sections (5 μm thick) were placed on slides, deparaffinized, rehydrated, and microwaved in 10 mM citrate buffer (pH 6.0) (BioGenex, San Ramon, CA) at 750 W for 15 minutes for Ki-67 and p63 immunostaining and for 30 minutes for AR immunostaining. For Her-2-neu immunostaining, sections were microwaved at 750 W for 5 minutes and at 375 W for an additional 15 minutes. Slides were cooled at room temperature for 30 minutes. Sections were then incubated for 10 minutes with 10% normal goat serum (Vector Laboratories, Inc., Burlingame, CA) diluted in PBS. The primary antibody was applied at room temperature in an automated stainer (OptiMax Plus 2.0 bc; BioGenex). Staining was detected with the use of the MultiLink-HRP kit (BioGenex). Stained sections were counterstained with either Meyer’s hematoxylin or methyl green.

Paraffin sections of the LNCaP and PC3 prostate cancer cell lines (American Type Culture Collection, Manassas, VA) and of the androgen-independent subline of the LAPC4 xenograft (26) (gift of Charles L. Sawyer, University of California, Los Angeles) were used for immunostaining controls and to standardize the Her-2-neu staining expression level (see below). Omission of the primary antibody with PBS served as a negative staining control.

**Scoring methods.** Two pathologists (S. Signoretti and M. Loda) read and scored the slides independently. Scoring was performed by two different methods, a relative scoring system and an absolute scoring system.

In the relative scoring system, the staining intensity of the tumor cells was evaluated in comparison to the staining intensity of the basal cells of the adjacent normal glands (internal positive control). The internal control was used to overcome the potential variability of immunoreactivity among the different tissue specimens. Her-2-neu expression in tumor cells was scored as equal to, higher than, or less than the expression in adjacent basal cells. Only tumor cells expressing Her-2-neu at a level equal to or higher than that in basal cells were scored as positive. The percentage of positive cells was assessed in each tumor section and was used as a continuous variable in the statistical analysis. In addition, a cutoff of 50% Her-2-neu-positive cells was applied to separate high (≥50%) and low (<50%) expressors. Maximaly selected chi-square methodology (31) was used to demonstrate that 50% was a good cutoff point (data not shown). Before analyzing the entire series of tumors, we confirmed the validity of the relative scoring system by image analysis in a subset of 20 prostate cancer tissue samples (10 high and 10 low Her-2-neu expressors). Overall, nine were UNT, and 11 were TAA. Densitometric quantitation of antibody staining per cell was done as described previously (32). Selected areas that included both tumor cells and adjacent normal basal cells were acquired with the use of imaging software provided with a Quantomet Q570 computerized imaging/microscopy system (Leica, Northvale, NJ). Because Her-2-neu staining was heterogeneous in each tumor sample, tumor cells analyzed were representative of the predominant population (i.e., expressing more or less Her-2-neu than adjacent basal cells). The staining densities in the tumor and normal areas were calculated for each tumor or normal field by dividing the total integrated optical density (i.e., total staining) in the field by the area of the mask, which is a computer-generated region that precisely demarcates the target cells to be quantified in the field. To confirm that basal cells represented an adequate internal control, we observed no statistical difference in the staining intensity of basal cells in UNT and TAA tumors (Student’s t test, P = .15). Specifically, the mean staining density for basal cells was 1.35 (95% confidence interval [CI] = 1.17–1.53) in the UNT tumors and 1.19 (95% CI = 1.06–1.33) in the TAA tumors. Subsequently, to assess Her-2-neu expression levels in the tumors, we calculated a staining density ratio distribution between the high and low Her-2-neu expressors (P<.001). The staining density ratio ranged from 0.31 to 1.56. To specify a cutoff, we selected a point a priori that was two standard deviations above the mean for low expressors (staining density ratio = 0.793). The rationale for selecting this cutoff point was to avoid false negatives; if the low expression levels were normally distributed, only 2.5% of low expressors would have levels higher than this cut-point. With the use of this cutoff point and the image analysis method, the relative scoring system resulted in one false positive and no false negatives (sensitivity of 100% and specificity of 92%), thus validating the use of this scoring methodology in the analysis of the entire series of tumors.

The absolute scoring method was used to evaluate Her-2-neu expression in tissue sections that lacked the internal basal cell controls, such as bone metastases. Immunostains were evaluated according to the scoring method used for Her-2-neu expression in breast cancer (33). Briefly, tumor sections were considered Her-2-neu positive only if complete weak to moderate (2+) or strong (3+) membrane staining was observed in at least 10% of the tumor cells. We used the cell lines PC3 (2+ in <10% of cells; negative standard) and LNCaP (2+ in >90% of cells; positive standard, weak to moderate intensity) and the xenograft LAPC4 (3+ in >90% of cells; positive standard, strong intensity) as reference standards for the evaluation of Her-2-neu expression in the human tumors (Fig. 1, A).

**Scoring AR, PSA, and Ki-67 immunostains.** Sections stained for PSA and AR were scored as either positive or negative for PSA and AR expression, respectively. The rates of tumor cell proliferation were calculated as the percentage of nuclei in each section that stained positive for Ki-67. For statistical analyses, the proliferation rate was analyzed as a continuous variable, and a proliferation rate of less than 1% was scored as nonproliferating.

**In Situ Hybridization**

The subset of 20 tumors previously analyzed by image analysis and seven additional AI metastatic tumor samples were analyzed by ISH to detect Her-2-neu mRNA. To generate the complementary RNA (cRNA) probe for ISH, we used a 5′-329-base-pair segment of Her-2-neu complementary DNA as template for in vitro transcription. 3′-labeled (New England Nuclear, Boston, MA) antisense and sense cRNA probes were in vitro transcribed with T7 and T3 RNA polymerase (Promega Corp., Madison, WI), respectively. ISH was performed as described previously (34).

The tumor sections were scored for Her-2-neu mRNA expression in a manner similar to the relative scoring system for immunohistochemistry. Specifically, we determined the number of autoradiographic grains in both normal and tumor cells by counting 100 cells in each subset and obtaining an average number of grains per cell. Her-2-neu mRNA expression in tumor cells was scored as equal to, higher than, or less than Her-2-neu mRNA expression in adjacent basal cells. The percentage of tumor cells expressing Her-2-neu at a level equal to or higher than that in basal cells was assessed for each tumor. High-expressing tumors were defined as those in which more than 50% of the tumor cells expressed Her-2-neu at a level equal to or higher than that in basal cells. Her-2-neu mRNA in AI tumors was simply scored as present or absent.

**Fluorescent In Situ Hybridization**

FISH was performed on the same subset of 27 tumors analyzed by ISH and on the LAPC4 xenograft. Paraffin-embedded tissue sections were deparaffinized and digested with proteinase K to remove proteins that might block access to the DNA target. Target DNA in the tissue sections and the biotinylated DNA HER-2-neu probe (Ventana Medical Systems, Inc., Tucson, AZ) were codenatured at 90 °C for 12 minutes and then hybridized with the probe overnight at 37 °C. Tissue sections were washed with 0.5x standard saline citrate to remove unhybridized probe. The hybridized probe was detected by incubation with fluorescein-labeled avidin...
RESULTS

Her-2-neu Protein Expression in Normal and Neoplastic Human Prostates

We first analyzed the expression of Her-2-neu protein in normal prostate tissue adjacent to tumor in the same sections. Her-2-neu expression was universally detected by immunohistochemistry in the normal basal cells from both UNT and TAA specimens. Thus, we used basal cell Her-2-neu expression as an internal control for the analysis of Her-2-neu expression in the tumor cells. Her-2-neu expression was not detected in secretory cells from UNT tumors. In contrast, Her-2-neu expression, as characterized by diffuse membrane staining, was detected in secretory cells from TAA tumors (Fig. 1, B).

All of the primary prostate cancers expressed Her-2-neu in at least 1% of cells. Variability in Her-2-neu expression was observed both in the intensity of immunostaining and in the percentage of positive tumor cells. With the use of both the relative and the absolute scoring methods, Her-2-neu expression was statistically

Statistical Analysis

Student’s t test was used to compare the percentage of Her-2-neu-positive tumor cells as a continuous variable between TAA and UNT tumors and to compare the staining densities of the basal cells in the two groups of tumors. To validate the relative scoring system using image analysis, we first generated descriptive statistics for the 20 tumors in the validation set. The Wilcoxon rank sum test (35) was used to verify differences in staining density ratio obtained in a blinded fashion among cases previously categorized as low and high Her-2-neu expressors. Fisher’s exact test (36) was used to test for an association between Her-2-neu expression levels

(35) - (A, B) Her-2-neu standards for immunohistochemistry scoring. i: Formalin-fixed, paraffin-embedded PC3 prostate carcinoma cells showing complete membrane staining in less than 10% of cells (negative standard) (3,3-diaminobenzidine [DAB] detection with hematoxylin counterstain). Original magnification x400. ii: LNCaP prostate carcinoma cells showing crisp 2+ membrane staining in the majority of cells (DAB detection with hematoxylin counterstain). Original magnification x400. iii: LAPC4 prostate carcinoma xenograft showing strong (3+) membrane staining in all cells (DAB detection with hematoxylin counterstain). Original magnification x400. iv: Immunohistochemical staining of normal prostate and prostate from a patient treated by total androgen ablation therapy. i: Her-2-neu is expressed in the basal cells of the normal prostate (DAB detection with hematoxylin counterstain). Original magnification x400. ii: In a serial section of that analyzed in i, basal cells are identified by p63 nuclear staining (DAB-nickel chloride detection with methyl green counterstain). Original magnification x400. iii: Her-2-neu is expressed in the basal and secretory cells in the prostate from a patient treated by total androgen ablation therapy (DAB detection with hematoxylin counterstain). Original magnification x400. iv: In a serial section of that analyzed in iii, basal cells are identified by p63 nuclear staining (DAB-nickel chloride detection with methyl green counterstain). Original magnification x400. (Ventana Medical Systems, Inc.). Nuclei were counterstained with the DNA-binding dye 2-(4-aminophenyl)-6-indolecarbamidinedihydrochloride (DAPI) (Ventana Medical Systems, Inc.). Paraffin-embedded breast cancers with and without known Her-2-neu gene amplification were used as positive and negative controls in each experiment.

Sections were examined with an epifluorescence microscope equipped with dual and single filters to detect the fluorescein and DAPI fluorochromes. Two fields of 20 tumor nuclei were evaluated, and the number of HER-2 signals per nucleus was determined. A tumor was considered to be positive for HER-2/neu gene amplification if the average number of signals per nucleus was greater than four.
significantly higher in TAA than in UNT primary prostate cancers. Specifically, with the use of the relative scoring method, there was a statistically significant difference in the percentage of Her-2-neu-positive cells (continuous variable) between TAA and UNT tumors \( (P = .008) \). When the cutoff of 50\% was used to categorize high and low expressors, 34 (51\%) of 67 UNT cases versus 27 (79\%) of 34 TAA cases expressed high Her-2-neu levels \( (P = .011) \). With the use of the absolute scoring method, 17 (25\%) of 67 UNT cases versus 20 (59\%) of 34 TAA cases \( (P = .002) \) were scored as positive (Table 1, A and B; Fig. 2).

Because AI tumors lack basal cells in the metastases, we used the absolute scoring method to assess Her-2-neu expression in these tumors. There was a statistically significant higher percentage of Her-2-neu-positive cases (14 [78\%] of 18) in AI tumors than in UNT tumors \( (P<.001) \) (Table 1, B). Finally, the percentage of Her-2-neu-positive cases showed a statistically significant increase from UNT to TAA to AI tumors \( (P<.001) \) (Table 1, B; Fig. 2).

Although Gleason grade is usually overestimated following androgen ablation treatment \( (40) \), both UNT and TAA tumors were assigned a Gleason grade to determine if there was an association between Her-2-neu expression and tumor

**Table 1. Her-2-neu expression**

<table>
<thead>
<tr>
<th></th>
<th>UNT (n = 67)</th>
<th>TAA (n = 34)</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>A) Relative scoring method</strong></td>
<td></td>
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<tr>
<td>Mean percentage of Her-2-neu-positive cells (95% confidence interval)</td>
<td>50.1 (42.3–57.9)</td>
<td>67.7 (57.8–77.5)</td>
<td>Student’s t test: ( P = .008 )</td>
</tr>
<tr>
<td>Percentage of Her-2-neu high expressors, applying a 50% cutoff (95% confidence interval)</td>
<td>51 (39–64)</td>
<td>79 (62–91)</td>
<td>Fisher’s exact test: ( P = .011 )</td>
</tr>
<tr>
<td><strong>B) Absolute scoring method</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of Her-2-neu-positive cases (95% confidence interval)</td>
<td>25 (16–37)</td>
<td>59 (41–75)</td>
<td>78 (52–94)</td>
</tr>
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*UNT = tumors from patients not treated with total androgen ablation therapy; TAA = tumors from patients treated with total androgen ablation therapy; AI = tumors from patients that failed to respond to total androgen ablation therapy and developed androgen-independent disease metastatic to the bone.
†Fisher’s exact test: UNT versus TAA, \( P = .002 \); UNT versus AI, \( P<.001 \); TAA versus AI, \( P = .227 \).
Overexpression of Her-2-neu confers androgen-independent growth to the LNCaP prostate cancer cell line in vitro through the activation of the AR in a ligand-independent fashion (26). Therefore, to determine if increased expression of Her-2-neu in human prostate cancers was associated with expression and activation of the AR, we assessed the expression of the AR and of its known downstream target, PSA, in all of the tumors. All of the tumors were positive for both AR and PSA, as detected by immunohistochemistry (Fig. 3, ii and iii), except for three AI metastatic tumors, which expressed AR but not PSA. The lack of PSA expression in these three tumors may be a result of the scant fragments of tumor tissue available for analysis.

Both the biologic and clinical progression of prostate cancer to androgen-independent disease following androgen ablation therapy can be broken down into two distinct phases. The tumor initially escapes dependence on androgen for survival, and it subsequently escapes dependence on androgen for growth (41). To determine whether prostate cancers of the TAA and AI types belonged to the survival or growth stage, we assessed the proliferation rate in the tumor sections by Ki-67 immunostaining. The proliferation rate was statistically significantly lower in TAA tumors than in UNT tumors (P<.001), whereas Her-2-neu expression was higher in TAA tumors than in UNT tumors. In contrast, the proliferation rate of AI metastatic prostate cancers was statistically significantly higher than in both UNT (P = .014) and TAA (P<.001) tumors (Fig 2, A).

Genomic and mRNA Analysis of Her-2-neu

With the use of ISH, Her-2-neu mRNA was detected in 17 of 20 prostatectomy specimens, both in tumor cells and in the adjacent normal prostate glands. The other three tumors appeared to lack Her-2-neu mRNA, most likely because the RNA had degraded. Of the 17 positive tumors, 12 were found to express high levels of Her-2-neu mRNA (Fig. 3, iv). Seven of these 12 tumors also expressed high levels of Her-2-neu protein. Five tumors that expressed low levels of Her-2-neu mRNA also expressed low levels of Her-2-neu protein. Six of seven AI metastatic tumors tested by ISH expressed Her-2-neu mRNA. All of these tumors expressed high Her-2-neu protein levels. Thus, there was concordance between Her-2-neu protein and mRNA expression. No hybridization signal was detected with the control sense signal (Fig. 3, v).

Fig. 3. Serial sections from a TAA prostate cancer (i.e., cancer from a patient who received total androgen ablation therapy) analyzed by immunohistochemistry for Her-2-neu, androgen receptor, and prostate-specific antigen (PSA) protein expression, by in situ hybridization (ISH) for Her-2-neu messenger RNA expression, and by fluorescent in situ hybridization (FISH) for analysis of the Her-2-neu locus. i: Complete membrane staining for Her-2-neu in the tumor cells (3,3-diaminobenzidine [DAB] detection with hematoxylin counterstain). Original magnification ×400. ii: Nuclear immunostaining for the androgen receptor (DAB detection with hematoxylin counterstain). Original magnification ×400. iii: Positive cytoplasmic immunostaining for PSA (DAB detection with hematoxylin counterstain). Original magnification ×400. iv: ISH with the antisense 35S-labeled Her-2-neu probe generates a specific signal in neoplastic cells (hematoxylin–eosin counterstain). Original magnification ×400. v: ISH with the sense 35S-labeled Her-2-neu probe. No specific signal is detected (hematoxylin–eosin counterstain). Original magnification ×400. vi: FISH for the Her-2-neu locus shows no gene amplification [2-(4-amidinophenyl)-6-indolecarbamidinehydrochloride counterstain]. Original magnification ×1000.
We also analyzed the same subset of tumors for evidence of genomic amplification. Of the 28 tumor samples analyzed by FISH, seven (five primary carcinomas and two metastases) did not produce an adequate result because of either tissue overdigestion or high background. Genomic amplification of the HER-2-neu locus was not detected in any of the prostate tumors or in the LAPC4 xenograft. All scorable tumor samples (n = 21), including the LAPC4 xenograft, had a Her-2-neu FISH score of less than 4 (Fig. 3, vi). The breast carcinoma used as the positive control showed amplification of the Her-2-neu locus (FISH score >10), while the one used as the negative control showed no amplification.

**DISCUSSION**

Tumor progression to androgen-independent growth eventually occurs in prostate cancer patients treated with androgen ablation therapy. No modes of therapy are currently available for AI tumors. Molecular characterization of the progression of prostate cancer to androgen independence is of paramount importance in understanding the biologic mechanisms underlying this process.

The majority of AI prostate tumors express both AR (32,42) and androgen-dependent genes, indicating that the AR-signaling pathway is functional in the absence of androgen. However, the mechanism by which the pathway is activated in the absence of androgen is unknown. AR mutations (43–48) or AR gene amplification (49) and alterations in kinases (50–55), growth factors (52,56), and nuclear receptor coactivators (57) have all been proposed to modulate AR signaling and may, therefore, play key roles in the mechanism of androgen independence in prostate cancer.

One possible player in the development of androgen independence is Her-2-neu. Craft et al. (26) demonstrated that overexpression of Her-2-neu confers androgen-independent growth to the androgen-dependent LNCaP prostate cancer cell line. Furthermore, in the absence of androgen, overexpression of Her-2-neu activates the transcription of PSA. It is interesting that, unlike other kinases that are able to activate the AR pathway in the absence of the ligand (50), Her-2-neu-mediated PSA activation requires the AR but is not inhibited by the antiandrogen drug Casodex. Thus, Her-2-neu overexpression in prostate cancer cells activates the AR pathway in the absence of the ligand, simulating androgen independence of prostate cancer in vivo.

Further evidence that Her-2-neu may play an important role in the progression to AI prostate cancer is our observation of a progressive increase in Her-2-neu expression in UNT, TAA, and AI prostate cancers. In addition, primary prostate carcinomas treated with TAA and AI metastases coexpressed AR and PSA, which is important because coexpression of Her-2-neu with AR and PSA is consistent with activation of the AR pathway by Her-2-neu in a ligand-independent fashion.

During the natural history of prostate cancer progression, both human and experimental, there is a latent, relatively quiescent period when tumor cells survive but do not proliferate in an environment devoid of androgen (40,41,58). Subsequently, a second period ensues during which highly proliferating tumor cells expand and metastasize. Our data show that, although there was an increase in Her-2-neu expression in primary tumors treated by androgen ablation, there was a decrease in proliferation rates relative to those of untreated prostate tumors. In contrast, there was a dramatic increase in the proliferation rates of androgen-independent bone metastases. A possible explanation for the differences in proliferation rates is that Her-2-neu initially confers survival to tumor cells by activating the AR pathway in an androgen-independent fashion. These same cells, however, may still require androgens for proliferation. This hypothesis is supported by the observation that, although the majority of secretary cells undergo apoptosis upon androgen withdrawal (59,60), residual secretary cells present in the treated prostate express high levels of Her-2-neu protein. Increased expression of Her-2-neu may, therefore, be a prostate-specific rather than a tumor-specific mechanism for cell survival in an androgen-depleted environment.

Although genomic amplification of the HER-2-neu locus is responsible for Her-2-neu protein overexpression in breast and ovarian carcinomas (2), the mechanism for Her-2-neu overexpression in prostate cancer is unknown, and, in fact, overexpression itself in the prostate is still controversial. Our findings show that the Her-2-neu protein is expressed at statistically significantly higher levels in prostate cancers treated by androgen deprivation (TAA and AI) than in untreated cancers (P<.005) and that high protein expression is accompanied by high mRNA expression but is not accompanied by HER-2-neu gene amplification. Thus, steady-state Her-2-neu transcripts (and, consequently, Her-2-neu protein) may be progressively increased by androgen ablation. Alternatively, tumor cells with initial high levels of Her-2-neu may be selected for during androgen deprivation through the elimination of the androgen-sensitive, low-Her-2-neu-expressing tumor cell subpopulation. Indeed, in untreated primary prostate carcinomas, positive immunostaining for Her-2-neu was associated with decreased tumor cell differentiation, i.e., a higher Gleason grade (59,60). The response to androgen therapy appears to be inversely related to Gleason grade (40). Thus, the Her-2-neu-expressing tumor component with high Gleason grade may not be responsive to androgen ablation therapy and might, therefore, be selected for survival. However, our data indicate that the increased Her-2-neu expression in treated tumors relative to untreated tumors is independent of Gleason grade. Thus, increased Her-2-neu expression may be a necessary mechanism for prostate tumor cells to survive in an androgen-depleted environment, independent of the differentiation status of tumor cells.

There is evidence that determining the functional status of Her-2-neu may not be a requirement in order to target the molecule therapeutically. Protein levels of Her-2-neu do not necessarily reflect its functional activity. However, in a human xenograft model of prostate cancer, Herceptin dramatically inhibited growth in androgen-dependent tumors that had been characterized purely on the basis of immunohistochemical overexpression of Her-2-neu. Herceptin was also effective against AI tumors when it was used in combination with the chemotherapy agent paclitaxel (61). In addition, as a single agent, a different anti-Her-2-neu antibody that is directed against a more proximal epitope of the extracellular domain of the Her-2-neu protein strongly inhibited Her-2-neu-positive, AI xenograft tumors (62). Taken together, these data support the approach of therapeutic targeting of Her-2-neu in both androgen-dependent and androgen-independent stages of prostate cancer on the basis of protein expression alone.

The demonstration that a population of Her-2-neu-positive tumor cells gradually
increases with progression toward AI prostate cancer further justifies targeting Her-2-neu in androgen-independent disease. In addition, because Her-2-neu positive tumor cells are present in untreated tumors and increase in relative number during the early phases of androgen deprivation, combining hormonal ablation with Her-2-neu targeting may help eradicate those tumor cells that are able to survive in an androgen-depleted environment and are destined to become androgen independent for growth.

Finally, it has recently been shown that PEA3, a member of the efs family of transcription factors, specifically targets a DNA sequence on the Her-2-neu promoter, repressing its activity (63). Because we have shown that the levels of Her-2-neu mRNA parallel protein expression in prostate carcinomas, future development of transcriptional inhibitors of Her-2-neu may also represent a valid therapeutic option.

In summary, we have shown that progression of prostate cancer toward androgen independence is characterized by a gradual increase in Her-2-neu expression by the tumor cells. We propose that Her-2-neu may function by initially permitting prostate cancer cell survival in an androgen-depleted environment. We also speculate that, over time, reactivation of proliferation occurs in Her-2-neu-positive cells, probably in association with additional genetic events. Her-2-neu targeting in advanced AI prostate cancers is, therefore, justified. More important, our data suggest that the combination of androgen ablation and Her-2-neu targeting could be effective in androgen-dependent tumors.

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