HER-2/neu (p185neu) Protein Expression in the Natural or Treated History of Prostate Cancer

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Abstract

Purpose: Amplification of HER-2/neu gene and overexpression of its encoded product, the p185neu (HER-2/neu) tyrosine kinase membrane receptor, have been associated with tumor progression in certain neoplasms. We conducted this study to investigate patterns of HER-2/neu protein expression in prostate cancer, analyzing different points in the natural and treated history of the disease.

Experimental Design: Radical prostatectomy cases (83) and 20 metastatic lesions were studied for the association between HER-2/neu protein overexpression detected by immunohistochemistry and clinicopathological parameters, including time to prostate-specific antigen (PSA) relapse.

Results: HER-2/neu protein overexpression, defined as complete membrane staining in >10% of tumor cells using the Food and Drug Administration-approved Dako kit, was found in 9 of 45 (20%) of evaluable hormone naïve primary tumors and 23 of 34 (67%) primary tumors after androgen-deprivation therapy (P = 0.0001). Of the 20 metastatic lesions, positivity was noted in 16 (80%) of the cases. On univariate analysis, HER-2/neu overexpression was associated with pretreatment PSA (P = 0.011) and time to PSA relapse (P = 0.02). After controlling for pretreatment PSA, the association between hormone treatment and HER-2/neu overexpression was still observed. No association was found between HER-2/neu overexpression and Gleason score, capsular invasion, and tumor proliferative index determined by Ki67.

Conclusions: These data suggest that there is significant HER-2/neu overexpression in primary tumors that persist after androgen deprivation. It also emphasizes the importance of characterizing tumors at determined points in the natural or treated history of prostate cancer when targeting treatment to specific biological processes.

Introduction

HER-2/neu protein is a transmembrane tyrosine kinase receptor with high homology to the epidermal growth factor receptor (1). Amplification of the HER-2/neu gene and overexpression of its encoded protein have been observed in certain tumor types, including breast, ovary, and lung carcinomas (2–4). In prostate cancer, several studies have reported HER-2/neu gene amplification or protein overexpression to variable degrees in cell lines (5) and xenografts (6), as well as in primary tumor samples (7, 8). Little consideration in the literature has been given to the point in the history of disease that the tumor sample being analyzed represents. The distinction between diagnostic biopsies and radical prostatectomy specimens, the specifics of prior therapy administered, and whether the tumor was obtained from the prostate or metastatic lesion are not reported consistently in different series. Disparate levels of reported HER-2/neu expression in prostate cancer may also be attributable to the lack of standardization of the immunohistochemical assays used, the antibody used to assess HER2 status, distinct antigen recovery strategies, and scoring methodologies with different definitions of “abnormal” (9–13).

The current study focused on the pattern of HER-2/neu protein expression in prostate cancers representing two distinct clinical stages (14) assessed with a standardized immunohistochemical assay localized disease (pre and postandrogen ablation) and androgen-independent metastatic tumors. The associations between patterns of HER-2/neu protein expression and standard clinicopathological parameters of poor outcome were also examined.

Materials and Methods

Clinical and Pathological Data. A cohort of 103 patients with prostate cancer was studied, including 83 cases with localized tumors and 20 different patients with metastatic lesions. The 83 localized tumors were obtained for the study by radical prostatectomy performed between 1990 and 1991 at Memorial Sloan-Kettering Cancer Center, and patients were followed up at the center. Metastatic lesions (20) from patients with progressive androgen-independent disease were also analyzed. Samples were formalin-fixed paraffin-embedded tissue specimens. Representative H&E stained sections of each paraffin block were examined microscopically to confirm the presence of tumor, as well as to evaluate the pathological grade and...
stage of the primary tumors analyzed. Thirty-four of the 83 patients with clinically localized tumors received preoperative neoadjuvant complete androgen ablation therapy, which composed of the combination of a luteinizing hormone-releasing hormone agonist and antiandrogen. The remaining 49 patients were hormone naïve. Forty-five of the 49 hormone-naïve primary lesions with sufficient tumor representation on tissue sections were assigned a histological grade. Cases were grouped as low Gleason score (<7, n = 28) and high Gleason score (≥7, n = 17). Postandrogen deprivation samples were not graded (15). According to pathological stage, cases were grouped into organ-confined tumors (pT2, n = 50) or tumors extending beyond prostatic capsule (T ≥ 3, n = 33). The response variable time to PSA relapse was defined as the time from radical prostatectomy to the time of the first detectable (nonzero) PSA. Three consecutive increases of PSA were required to confirm PSA relapse; however, the time of relapse was taken as the time of the first detectable PSA value. Three patients who did not achieve a nonmeasurable PSA after radical prostatectomy were excluded from the analysis. The 20 metastatic androgen-independent cases were all bone lesions.

**Immunohistochemical Analysis of HER-2/neu Expression.** The Dako Herceptest Immunohistochemical kit (Dako Corp., Carpinteria, CA) was used as described previously (16, 17). Membrane immunoreactivities for HER-2/neu protein were categorized as undetectable or "zero" to +3 category, as defined by the developers of the commercial kit and compared with the supplied positive and negative controls. Score "zero" is defined as undetectable staining or membrane staining in <10% of the tumor cells. Score +1 is defined as faint membrane staining detected in >10% of the tumor cells. Score +2 was considered as weak to moderate complete membrane staining observed in >10% of the tumor cells. Finally, score +3 was defined as a moderate to strong complete membrane staining observed in >10% of the tumor cells. HER-2/neu protein expression was classified into two categories defined as follows: negative (scores 0 and 1) and positive (scores 2 and 3). The cutoff point was used based on reported studies in breast cancer (16, 17) and as approved for use by the United States Food and Drug Administration.

**Immunohistochemical Analysis of Ki67 Expression.** Tumor proliferative activity was assessed by the anti-Ki67 monoclonal antibody MIB1 (1:50 dilution; Immunotech Corp., Luminy, France). Clone M1g8-Kpl1, a mouse monoclonal antibody of the same subclass as MIB1, was used as a negative control at similar working dilution (PharMingen Laboratories, San Diego, CA). Sections were immersed in boiling 0.01% citric acid (pH 6.0) for 15 min to enhance antigen retrieval, allowed to cool, and incubated with the primary antibody overnight at 4°C. Biotinylated horse antimouse IgG antibodies were applied for 1 h (1:500 dilution; Vector Laboratories, Burlingame, CA), followed by avidin-biotin peroxidase complexes for 30 min (1:25 dilution; Vector Laboratories). Diaminobenzidine was used as the final chromogen, and hematoxylin was used as the nuclear counterstain. Nuclear immunoreactivities were classified into two categories: negative (<20% of tumor cells displaying nuclear staining) and positive (≥20% tumor cells displaying nuclear staining). Ki67 proliferative index was considered high when ≥20% of tumor cells displayed a positive MIB1 nuclear staining pattern (18, 19).

**FISH.** We conducted FISH analyses using a HER-2/neu gene copy number in 66 primary cases using a unique sequence HER-2/neu probe (Ventana Medical System, Tucson, AZ). The assay was considered to detect gene amplification if more than four copies of the HER-2/neu gene were identified in ≥40 tumor cells.

**Statistical Analyses.** The baseline variables examined were PSA (units) at time of diagnosis, Gleason score (divided into two mutually exclusive categories: <7 or ≥7), T stage of disease (pT2 or ≥pT3), and HER-2/neu membrane expression patterns (negative or positive, see above). Statistical analyses were conducted to explore: (a) the association between immunophenotypic variables and clinicopathological parameters, such as tumor grade, tumor stage, preoperative PSA, and hormonal status; and (b) the association between HER-2/neu phenotypes and PSA relapse free survival. The Fisher’s exact test was used to assess the associations among the different variables, and results were considered significant if the P was <0.05 (20). The FREQ procedure in SAS was used for this study (21). The LOGISTIC procedure in SAS, using the Wald test, was used to assess the univariate association between preoperative PSA and HER-2/neu phenotypes considering PSA as a continuous variable (21). The univariate associations between preoperative PSA and hormonal status were also explored, whereas the univariate associations between time to PSA relapse and HER2 immunophenotype were evaluated using the Log-rank test (22). Survival distributions were generated using the Kaplan-Meier estimate (23). The Cox proportional hazards model was used to examine the relationship between time to PSA relapse and HER-2/neu protein overexpression after controlling for pretreatment PSA and hormone treatment (24). A Wald test was used to test for an association between hormonal status and HER-2/neu overexpression controlling for pretreatment PSA (21).

**Results**

Table 1 summarizes immunohistochemical data in relation to clinicopathological parameters. Fig. 1 illustrates the immunohistochemical patterns of HER-2/neu protein expression in representative tumors with different staining scores compared with the control. In normal prostate samples, as well as in normal and benign hyperplastic glands, we observed HER-2/neu expression in basal cells, which served as internal controls for the evaluation of HER-2/neu immunostaining. In contrast, luminal (or secretory) cells in the normal glands were unreactive to HER-2/neu antibodies.

HER-2/neu membrane overexpression was observed in 32 of 83 (38.5%) of the radical prostatectomy cases (Table 1). Twenty-two (26.5%) cases were scored as zero, 29 (35%) cases were scored as +1, and 32 cases (38.5%) were considered +2 (Fig. 1). This last value was used to define the cutoff point for the nuclear counterstain. Nuclear immunoreactivities were classified into two categories: negative (<20% of tumor cells displaying nuclear staining) and positive (≥20% tumor cells displaying nuclear staining). Ki67 proliferative index was considered high when ≥20% of tumor cells displayed a positive MIB1 nuclear staining pattern (18, 19).
HER-2/neu protein overexpression. We did not observe staining score of +3 as per the Dako kit control in this cohort of patients.

Twenty-three of 34 (67%) patients who received neoadjuvant androgen ablation therapy were found to have HER-2/neu protein overexpression, versus 9 of 45 (20%) patients who were hormone naïve at the time of radical prostatectomy. This association was statistically significant (P = 0.0001). We also observed an association between baseline PSA and receipt of neoadjuvant hormones (P = 0.0004). This association, coupled with the observed association between pretreatment PSA and HER-2/neu protein overexpression (P = 0.011), suggests that pretreatment PSA may be a confounding factor in the relationship between hormone treatment and HER-2/neu expression. However, after controlling for pretreatment PSA, the association between hormone treatment and HER-2/neu expression still exists (P = 0.0003). We also found that 16 of the 20 (80%) androgen-independent metastatic cases displayed HER-2/neu protein overexpression. Three cases (15%) were scored as zero, 1 case (5%) was scored as +1, 10 cases (50%) were scored as +2, and 6 cases (30%) were considered +3.

No association was observed between HER-2/neu protein overexpression and tumor stage (P = 0.36) or Ki67 proliferative index (P = 0.74). The same observation was seen analyzing hormone-naïve group, as no association was observed between HER-2/neu protein overexpression and tumor stage (P = 0.265), Gleason score (P = 0.071), or Ki67 (P = 0.583). An association was found between HER-2/neu protein overexpression and time to PSA failure after radical prostatectomy (P = 0.02); however, after adjusting for pretreatment PSA and hormone treatment, the association between HER-2/neu protein overexpression and time to PSA relapse was not significant (P = 0.94).

FISH procedures revealed that only 2 of 66 cases analyzed had HER-2/neu gene amplification. The two cases displaying HER-2/neu gene amplification also showed a HER-2/neu protein overexpression phenotype.

### Discussion

This study shows the importance of evaluating tumors representing specific points in the natural history of prostate cancer (14). In untreated hormone-naïve primary tumors, HER-2/neu expression was infrequent (20%). In contrast, overexpression was observed in 80% of metastatic primary tumors surviving after androgen ablation. These patterns of HER-2/neu expression in prostate cancer should be regarded as “deregulated” rather than “ectopic,” because HER-2/neu is detected in the basal cells of normal prostate glands.

Overall, HER-2/neu membrane overexpression was observed in 38% of the radical prostatectomy specimens. However, with reported rates of HER-2/neu overexpression in prostate cancer ranging from 0 to 94%, it is difficult to determine a precise estimate (25–30). An evaluation of methodological differences between the situations helps to clarify the significance of the outcomes. These include the nature of the material studied, as well as the method of assessment. In this regard, we used a standardized kit and established cutoff values, albeit validated for breast cancer. We observed a correlation between score +2 overexpression and PSA relapse. This is the same cutoff value used in the context of breast cancer studies (16, 17). We have also noted that HER-2/neu immunostaining in primary prostate cancer specimens is heterogeneous and focal. None of the primary tumors was scored as +3; however, 30% of metastatic lesions had a score of +3. In addition, we found that 80% (16 of 20) of tumors representing androgen-independent metastatic disease overexpressed HER-2/neu protein. This shows the contribution of prior therapy and clinical stage to overall outcomes.

HER-2/neu protein overexpression in primary tumors was compared with gene amplification using FISH in 66 cases. None of the HER-2/neu-negative tumors showed amplification, whereas two of the HER-2/neu-positive cases revealed amplification signals. Similar observations were recently reported by Signoretti et al. (31). HER-2/neu overexpression in the absence of gene amplification may be attributable to transcriptional and posttranslational mechanisms (32), a phenomenon that is also clinically frequent for other oncogenes, such as cyclin D1 and mdm2 (33, 34).

A recent study on prostate cancer reported that 8 of 86 (9%) cases displayed HER-2/neu gene amplification; however, only one of these eight tumors had a “moderate” amplification signal, whereas the remaining seven tumors were all described to have “low” amplification signals (35). It appears that, despite several earlier studies using a FISH-based assay that found higher gene amplification rates (28, 36), the frequency of HER-
2neu amplification in primary prostate carcinoma is generally lower than that reported in other tumor types, including ovary and larynx cancer (3, 37). The most significant association was observed between HER-2/neu overexpression and prior androgen deprivation, a factor often not considered as a prognostic factor. Even after controlling for the confounding effect of pretreatment PSA, this association was still observed.

Recent reports in which specific interactions between HER-2/neu and the androgen receptor have been described (38, 39). Forced overexpression of HER-2/neu in androgen-dependent prostate cancer cells allowed ligand-independent growth. In this setting, HER-2/neu was able to activate the androgen receptor signaling even in the absence of the ligand, namely androgens. This “cross-talk” is probably bi-directional, meaning that androgen ablation, known to down-regulate androgen receptors, could also up-regulate HER-2/neu in response to cellular stress.

An alternative explanation is that the higher frequency of positivity in neoadjuvant-treated tumors is simply a function of disease extent. The association observed between HER-2/neu overexpression and higher pretreatment PSA is consistent with this postulate. Similarly, the association between HER-2/neu overexpression and time to PSA relapse in this cohort may also reflect the more advanced nature of the tumor treated in this cohort. Against this is the fact that the association between HER2 status and prior hormone exposure persisted after controlling for baseline PSA.

The present study emphasizes the importance of characterizing the clinical state of the patient from which the tumor is studied. On the basis of our observation, we believe that detailed information regarding prior hormone exposure is essential when designing clinical trials targeting HER2 and other signaling molecules associated with prostate cancer progression. In that regard, xenograft studies with targeted therapy to HER-2/neu demonstrated disparate responses dependent on the androgen status of the prostate cancers (40).

References

Fig. 1 Immunohistochemical staining of control cell lines provided by the standardized Dako kit and primary prostatic carcinomas with anti-HER-2/neu monoclonal antibody using the Dako kit. A, intense and homogeneous membrane staining in HER-2/neu overexpressing control cells. B, representative field of moderately intense control cells expressing low to intermediate HER-2/neu levels. C, representative area of HER-2/neu nonexpressing control cells, showing negative immunohistochemical staining profile. D, primary tumor displaying a strong membrane staining in tumor cells. Note the lack of immunoreactivities observed in the stroma elements. E, nonexpressing control cells, showing negative immunohistochemical staining profile. D, neu levels. F, primary tumor with undetectable levels of HER-2/neu, representative of the negative phenotype cases. Original magnifications: A, B, C, D, and F, ×400; E, ×200.


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