Turkish Journal of Pathology 2008;24(2):76-83

Her-2/neu oncogene expression in prostate carcinoma: Evaluation of gene amplification by FISH method

Prostat kanserinde Her-2/neu onkogen ekspresyonu: Gen amplifikasyonunun FISH yöntemiyle değerlendirilmesi

Duygu KANKAYA[1], Ayşe SERTÇELİK[1], Gülşah KAYGUSUZ[1], Işınsu KUZU[1], Serpil DİZBAY SAK[1], Sümer BALTACI[2]

Ankara University, School of Medicine, Department of Pathology[1] and Urology[2], ANKARA

ABSTRACT

The variability in the biological behaviour of prostate cancer and the inadequacy of markers used for assessing prognosis cause difficulties in the management of patients. We conducted a study of 74 prostate carcinomas to identify the significance of Her-2/neu oncogene in the initiation and progression of prostate carcinoma. Tissue array blocks were constructed and overexpression of Her-2/neu protein was assessed in areas of carcinoma, low and high grade intraepithelial neoplasia and benign prostate hyperplasia by immunohistochemistry. Flourescence in-situ hybridization (FISH) method was used to investigate Her-2/neu gene amplification in areas of carcinoma of 56 cases. Her-2/neu protein was expressed in a cytoplasmic/membranous manner. The expression rate significantly increased from areas of benign prostate hyperplasia to prostatic intraepithelial neoplasia and reached the highest degree in areas of carcinoma. Her-2/neu overexpression was observed in 72.97% of prostate carcinomas while gene amplification was present in 4.05% of the cases. Polisomy or any other anomaly involving chromosome 17 was not detected. There was no relationship between the ratio of Her-2/neu protein expression or gene amplification and known prognostic parameters as Gleason grade, and stage of the tumor. The discordance between the expression and amplification rates suggested that expression may be the cause of some other factors rather than true gene amplification. According to the results of the present study involving mostly the clinically localized prostate carcinomas, it can be suggested that Her-2/neu oncogene does not seem to have any role in clinically localized prostate carcinoma, but no assessment could be made for advanced stage carcinomas and the progression to androgen independent disease.

Key words: Her-2/neu, prostate carcinoma, immunohistochemistry, flourescence in-situ hybridization

ÖZET

Prostat kanserinin biyolojik davranışındaki değişkenlik ve prognostik belirleyicilerin yetersizliği, hastaların takip ve tedavisinde zorluklara neden olmaktadır. Yetmiş dört prostat kanseri olgusunu içeren çalışmamızda, Her-2/neu onkogeninin kanser başlangıcı ve ilerlemesindeki rolünü araştırdık. Doku "array" bloğu hazırlandı ve aşırı Her-2/neu protein ekspresyonu karsinom, düşük ve yüksek dereceli intraepitelyal neoplazi ve benign prostat hiperplazisi alanlarında değerlendirildi. Elli altı karsinom olgusunda flöresan in-situ hibridizasyon (FISH) yöntemiyle Her-2/neu gen amplifikasyonu araştırıldı. Her-2/neu proteini sitoplazmik ve membranöz ekspresyon gösterdi. Ekspresyonun benign prostat hiperplazisi alanlarından prostatik intraepitelyal neoplazi alanlarına gidildikçe artış gösterdiği ve karsinom alanlarında en yüksek düzeye ulaştığı görüldü. Prostat kanseri vakalarında Her-2/neu ekspresyon oranı %72.97 iken, gen amplifikasyon oranı %4.05 olarak tespit edildi. Kromozom 17'de polizomi veya başka herhangi bir anomali izlenmedi. Her-2/neu protein ekspresyon ve gen amplifikasyon düzeyi ile Gleason skoru, tümör evresi gibi bilinen prognostik parametreler arasında herhangi bir ilişki izlenmedi. Ekspresyon ve amplifikasyon oranları arasındaki tutarsızlık, ekspresyonun gerçek bir gen amplifikasyonu dışı nedenlere bağlı olabileceğini düşündürdü. Büyük kısmını klinik lokalize prostat karsinomu olgularının oluşturduğu hasta grubunu içeren çalışmamızda sonuçlar, Her-2/neu onkogeninin klinik olarak lokalize evrede öneminin olmadığını düşündürmüş, ancak daha ileri evreler ve androjen bağımsız faza geçiş ile ilgili yorum yapılamamıştır.

Anahtar sözcükler: Her-2/neu, prostat karsinomu, immünhistokimya, flöresan in-situ hibridizasyon

Received: 20.02.2008
Accepted: 07.04.2008
Corresponding Author: Duygu Kankaya, M.D., Ankara University, School of Medicine Department of Pathology, Level 01, 06100, Sihhiye, Ankara, Turkey

INTRODUCTION

Prostate cancer is one of the leading malig-

nancies affecting men worldwide and the variability in the clinical and biological behaviour of this tumor, the inadequacy of markers used for assesing prognosis cause difficulties in the management of these patients. Searching for new prognostic parameters and therapeutic modalities has been the aim of many researches.

Oncogene amplifications are manifestations of genetic instability and have been shown to play a part in the pathogenesis of many cancers. As a result of gene amplification, increased production of specific proteins leads cancer cells to acquire and maintain malignant potential. Her-2/neu gene is a protooncogene which is a member of the EGF receptor family and encodes a transmembrane receptor protein with tyrosine kinase activity and involves in normal cell growth. Her-2/neu protooncogene shows oncogenic conversion via gene amplification and protein overexpression which leads to abnormal proliferation and agressive behaviour of tumor cells (1,2).

Recently there has been a growing interest in Her-2/neu oncogene, expression / amplification which has been shown to correlate with a shorter survival in a variety of epithelial malignancies (3-7).

Since new therapeutic modalities are now available that specifically target Her-2/neu oncogene, diagnostic tests for identifying Her-2/neu oncoprotein have been used as routine application in the management of breast cancer patients.

There are many studies reported in the literature investigating the role of Her-2/neu oncogene in prostate carcinomas which have provided contradictory results. This study was performed to test the hypothesis that Her-2/neu oncogene might have a role in the initiation and progression of prostate carcinomas.

MATERIALS and METHODS

Patient selection and tissue array construction:

The study included materials from 74 patients with prostate cancer who underwent radical prostatectomy with bilateral pelvic lymph node dissection at the urology department of Ankara University School of Medicine between 1993 and 2002. Hematoxylin and eosin stained histological sections from formalin fixed, paraffin embedded tissues were reviewed and histological grade was determined according to the Gleason system. Representative areas were selected for each tumor specimen and tissue array (TA) blocks were constructed by taking cores of 4 mm in diameter from these areas using punch biopsy needle. Two 6 cores (average 3.7 cores per case) were taken from each tumor specimen. Twenty three TA blocks, each containing 12 cores were obtained. Four-micrometer sections of these array blocks were cut and placed on charged slides. Three sections, one for Hematoxylin-eosin, one for immunohistochemistry, and one for flourescence in-situ hybridization were obtained from each TA block. Hematoxylin-eosin stained sections were used for histological verification of areas of benign prostate hyperplasia (BPH), low grade prostatic intraepithelial neoplasia (LGPIN), high grade prostatic intraepithelial neoplasia (HGPIN) and prostatic carcinoma. According to the Gleason grade system, one grade was determined in each core of the cases and the most frequent grade among the cores of the same case was defined as “dominant grade” and considered as the representative Gleason grade of the case. The highest Gleason grade among the cores was defined as the “maximum grade”.

Immunohistochemistry (IHC):

Sections (4 mμ) were deparaffinized and antigen retrieval was performed by immersing slides in 10 mmol/L citrate buffer (ph=6) and heating in a 95°C water bath for 20 minutes. After application of hydrogen peroxide for 5 minutes, the slides were incubated with primary antibody which was a polyclonal rabbit anti-human HER2/neu antibody recognizing an intracellular

domain of Her-2/neu oncoprotein (DAKO, Denmark, Code No: A 0485) (1/300 dilution) for 20 minutes. Then slides were incubated with biotin for 10 and with streptavidin for 10 minutes respectively. This was followed by immersion in substrate-chromogen solution for 8 minutes. Hematoxylin was used for counterstaining. Sections from a breast cancer specimen, which was known to be 3+ positive for Her-2/neu, were used as positive controls.

Both cytoplasmic and membranous staining were evaluated in the areas of cancer, LGPIN, HGPIN and BPH in each core and scored according to the intensity and the percentage of stained cells. Intensity was scored on a 0 to 3+ scale (0: no staining, 1: weak, 2: moderate, 3: strong staining). The percentage of stained cells was evaluated on a 0 to 3+ scale (for membranous staining: 0: no staining, 1+:1-5%, 2+:6-20% and 3+:21-100%; for cytoplasmic staining: 0: no staining, 1+:1-30%, 2+:31-80%, 3+:81-100%). Cytoplasmic staining score (CSS) and membranous staining score (MSS) were calculated separately by adding the intensity and percentage scores, and the total staining score (TSS) in each core was determined by adding CSS and MSS. The highest staining score (CSS: 0-6, MSS: 0-6, TSS: 0-12) among the cores of the same case was accepted as the representative staining score of the case.

Flourescence in-situ hybridization (FISH):

Her-2/neu gene amplification was investigated in 56 of 74 prostate carcinomas by FISH and for each case 1-3 cores were evaluated. Deparaffinisation, sequential acid incubation, denaturation, proteinase K digestion steps were followed respectively. After formamide denaturation and formalin fixation, the slides were dehydrated in graded ethyl alcohol and air dried. FISH was performed on pretreated slides using Q-BIOgene Chromosome 17p12 (HER2/NEU)/Alphasatellite 17 Cocktail (dual color) TM FISH probe (Cat. #: PONC1712). After hybridization, nuclei were stained with 6-diamidin 2-fenilindiol dihydrochloride antifade solution (DAPI) and the signals were visualized on a ZEISS Axiscope fluorescence microscope. Only the cells with at least one red and one green signal in their nuclei were evaluated. Red (Her 2) and green (Chromosome 17) signals were counted in 40 non-overlapping tumor nuclei in each core and the ratio of red and green signals was defined as the amplification score. Cores with an amplification score of >2 or with tight clustering signal pattern in ≥50% of counted cells were considered as positive for Her-2/neu gene amplification. The highest amplification score among the cores of the same case was accepted as the representative amplification score of the case.

Statistical Analysis:

"Kruskal Wallis Test", Mann Whitney test", "Student's T test", "Chi Square test", "Spearmen's Correlation test" were used for statistical analysis. All analyses were performed using Statistica software and a p value of <0.05 was considered as statistically significant.

RESULTS

Patients

The mean age of the patients was 62.41 (±5.6) years. 10, 42, 14, 8 patients were stages T2a, T2b, T3a, T3b, respectively. They were N0 except for 2 of them and 3 of them had bone metastasis. The mean preoperative PSA value was 12.48 ng/ml (±9.72). Follow up periods of the 74 patients ranged from 9 to 60 months. Biochemical recurrence occurred in 3 patients (defined as a serum PSA of greater than 0.4 ng/lt). Three of the patients with metastasis had received antiandrogen therapy at 1 month after the surgery. All of them responded initally but had developed androgen independent disease after the median time of 40 months. The preoperative PSA levels significantly correlated with the stage of the tumor (p=0.05) and Gleason grade (p=0.05).

Histopathology, Immunohistochemistry

and FISH:

The distribution of cases according to Gleason grade is shown in Table 1.

Of the 74 prostate cancers, 54 (%72.97) showed immunostaining, only in cytoplasmic (n=34; 45.94%), membranous (n=6; 8.1%) in mixed (cytoplasmic and membranous) patterns (n=14; 18.91%) respectively (Fig. 1). No correlation between immunostaining scores and stage of the tumor was found (p=2.91).

There was no statistically significant association between immunostaining scores and dominant or maximum Gleason grade and patients' ages or preoperative PSA levels as well.

Table 1. Distribution of cases according to the dominant and maximum Gleason grade.

Gleason Grade	3 (n, %)	4 (n, %)	5 (n, %)
Dominant grade	35 (47.3)	37 (50)	2 (7.2)
Maximum grade	23 (31.1)	42 (56.8)	9 (12.2)

Immunostaining was compared in areas of BPH, LGPIN, HGPIN and prostatic carcinoma. The percentage of cores with only cytoplasmic or mixed (cytoplasmic and membranous) immunostaining patterns significantly increased from areas of BPH to PIN and reached the highest degree in areas of prostatic carcinoma (Chi-Square test, p=0, p=0); whereas no such correla-

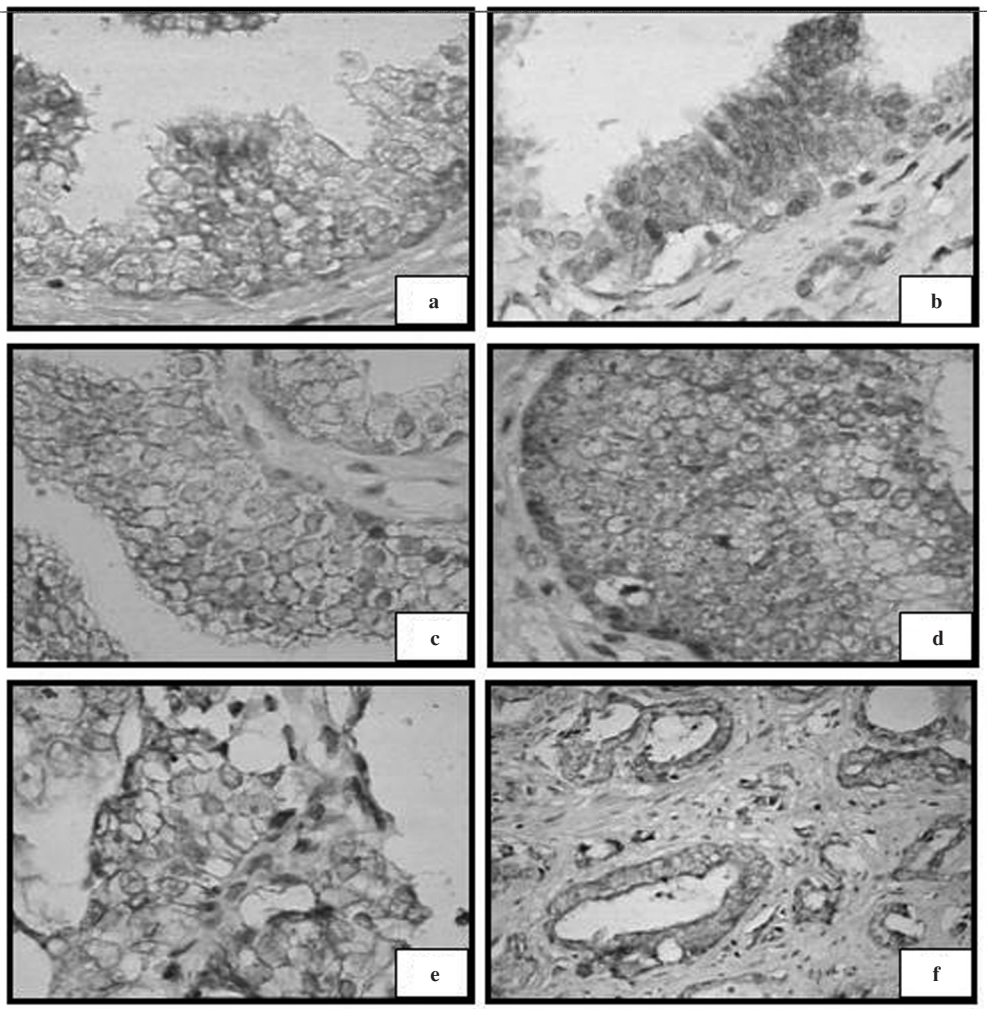

Figure 1. Examples of Her-2/neu expression. a. LGPIN, membranous (+1) (x100), b. LGPIN, cytoplasmic (+1) (x100), c. HGPIN, membranous (+2) (x100), d. HGPIN, cytoplasmic (+2) (x100), e. Gleason grade 3 prostate carcinoma, membranous (+2) (x100), f. Gleason grade 3 prostate carcinoma, cytoplasmic (+2) (x40).

Table 2. The percentage of immunostained cores in areas of BPH, PIN and carcinoma.

	Cytoplasmic staining (n, %)	Membranous staining (n, %)	Membranous and cytoplasmic staining (n, %)
Cancer (n=254 cores)	112 cores (44.09)	19 cores (7.48)	12 cores (4.72)
HGPIN (n=55 cores)	21 cores (38.18)	6 cores (10.9)	24 cores (43.6)
LGPIN (n=13 cores)	5 cores (38.46)	2 cores (15.38)	5 cores (38.46)
BPH (n=117 cores)	14 cores (11.96)	4 cores (3.41)	16 cores (13.67)
	p=0	p=0.168	p=0

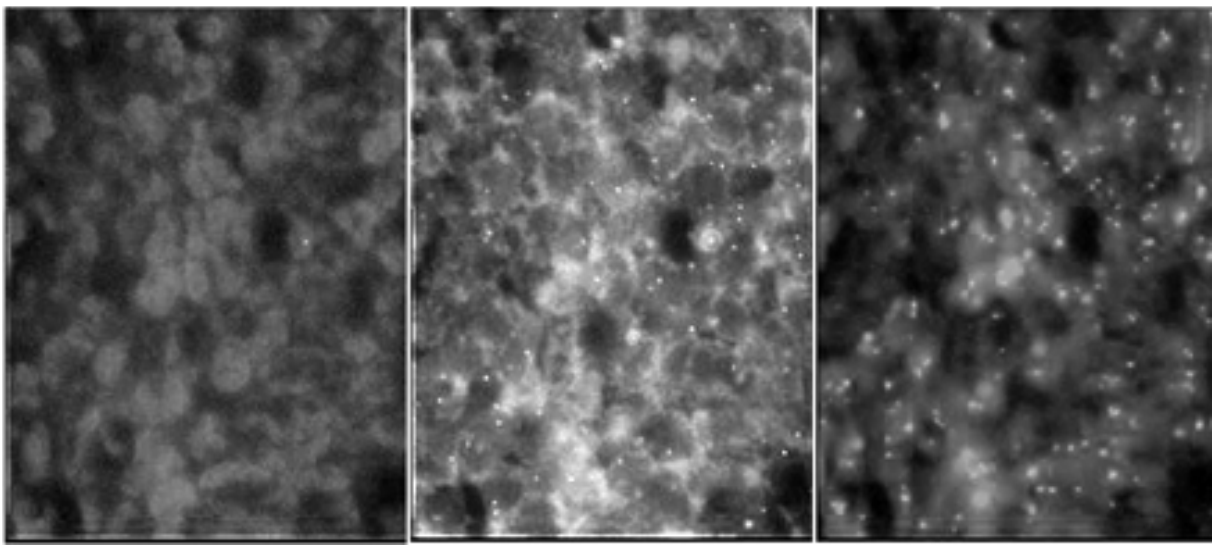

Figure 2. Her-2/neu gene amplification by FISH (green / red signals >2).

tion was found for cores with only membranous staining (Table 2).

The percentage of immunostaining (in cytoplasmic or membranous pattern) was compared between cores with different Gleason grades. The relationship between Gleason grade and the percentage of immunostained cores was investigated among the total number of 254 cores representing 74 patients. There was no relationship between Gleason grade and Her-2/neu protein expression.

The percentages of immunostained cores (in cytoplasmic or membranous pattern) were compared between groups with different Gleason grades. There was no relationship between Gleason grade and Her-2/neu protein expression.

In 56 of the 74 cases, a minimum of 40 tumor cells with at least one CEP17 and one Her2 Neu signal could be detected in one or more cores stained with FISH. Gene amplification was detected in only 3 tumors, one of which belonged to a patient with androgen independent disease (Fig. 2). Of the 3 FISH positive cases, in only one with a score of +1 was present There was no association between amplification score and dominant or maximum Gleason grades (p=0.291, p=0.513, respectively) or between amplification score and immunostaining scores (p=0.864, p=0.521, respectively).

DISCUSSION

Studies using immunohistochemistry to investigate Her-2/neu oncoprotein expression in prostate carcinomas have given contradictory and confusing results with a reported expression rate of 0% to 100% (8-11).

Some studies detected Her-2/neu overexpression in prostate carcinomas and found cor-

relation with Gleason grade, tumor stage and aneuploidy (9,12-14), whereas no such correlation was found in some of them (8,11).

Technical factors as the type of examined tissue, fixation, staining protocols, antigen retrieval procedures, the type of anti-Her2 antibodies, heterogeneity in case selection and scoring systems may be responsible for this disparity (15,16). The most frequently observed immunohistochemical staining pattern was cytoplasmic rather than membranous. The cytoplasmic immunostaining may be explained by the binding of antibody to the intracellular part of Her-2/neu transmembrane protein. Ross et al. (12), found significant correlation between Her-2/neu cytoplasmic immunostaining intensity and tumor grade or aneuploidy status. Differential staining of Her-2/neu in different subcellular organelles may be related to cancer stages and cell types (17). In the present study, the frequency of membranous immunostaining inversely correlated with Gleason grade and no membranous immunostaining was seen in the areas of grade 5 carcinoma. Compatibly, Kameda et al. (18) has previously reported that Her-2/neu immunoreactivity in cells of poorly differentiated gastric tumors was detected most often within the cytoplasm, whereas the immunoreactivity in well differentiated tumor cells was detected on the cell membranes. However, Her-2/neu expression detected by Western blotting was very low or none in the cytoplasmic positive tumor cells. This finding suggests that cytoplasmic staining may be a non-specific finding. Alternatively there may be an error preventing the membranous localization of the protein.

The significant increase of Her-2 overexpression from areas of BPH to PIN and carcinoma may indicate that Her-2/neu oncoprotein is involved in growth stimulation and oncogenic transformation of prostatic cells. However, the role of Her-2/neu expression in the progression of prostate carcinoma is argueable, since no correlation with known prognostic parameters such as Gleason grade, stage of tumor was detected. Our results are compatible with a previously published report which suggested that Her-2/neu oncoprotein may be involved in the initial neoplastic transformation but its contribution in tumor progression may probably be more limited (19).

According to the studies performed using FISH, one group has found amplification of the Her-2/neu oncogene in up to 44% of the cases, and this was associated with advanced pathological stage and higher Gleason score. However, more recent work by that group showed a lower amplification rate of 10-25% (20-22). Another study on different stages of prostate carcinoma- from localised to metastatic- found no amplification at any stage (23). The differences in amplification rates in these studies might be due to the definition of amplification used by the different investigators or increase in gene copy numbers due to chromosome 17 polysomy which might be mistaken for true amplification. In our series, most of the cases were clinically localized prostate cancer, Her-2/neu gene amplification was present only in 4.05%, in spite of an overexpression rate of 72.97%. Polisomy or any other anomaly involving chromosome 17 was not detected in any of cases which might be responsible for high Her-2/neu protein expression. The discordance between the expression and amplification of Her-2/neu oncogene suggests that Her-2/neu oncoprotein expression may not be associated with Her-2/neu gene amplification which makes it difficult to comment on the significance of Her-2/neu protein expression in the development of prostatic carcinoma. We are in the same opinion with Zhau et al. (17) who suggested that the underlying mechanism for higher Her-2/neu protein expression might be due to increased rate of Her-2/neu transcription, increased Her-2/neu mRNA stability, posttranscriptional processing, or all of them.

It has been suggested that Her-2/neu amplification was rare in clinically localized prostate carcinoma (24). Her-2/neu oncogene amplification/overexpression has been shown to be as-

sociated with the progression of prostate cancer from an androgen dependent to an androgen independent phenotype. Higher expression of Her-2/neu has been detected in androgen refractory and metastatic prostate carcinomas, in contrast to early stages (25-29). This finding led to the use of a monoclonal antibody targeting Her-2/neu in the treatment of androgen refractory carcinoma (30), however trials showed that it had a poor efficacy as a single agent (31). Because nearly all prostate carcinomas included in this study were clinically localized and only five of them were in hormone refractory state, we cannot comment on the significance of Her-2/neu oncoprotein in the progression of androgen dependent to androgen independent phenotype.

In conclusion, the significant increase of Her-2 overexpression from areas of BPH to PIN and carcinoma may indicate that Her-2/neu oncoprotein is involved in stimulation of the growth and oncogenic transformation of prostatic cells. However, the role of Her-2/neu expression in the progression of prostate carcinoma is argueable, since no correlation with known prognostic parameters such as Gleason grade, stage of tumor was detected. Furthermore, the discordance between the expression and amplification rates of Her-2/neu oncogene suggests that Her-2/neu oncoprotein expression may not be associated with true gene amplification which makes it difficult to comment on the significance of Her-2/neu protein expression in prostate carcinoma. We think that some other factors such as increased rate of Her-2/neu transcription, posttranscriptional processing may be the underlying mechanism for high Her-2/neu protein expression in prostate cancer. Our limitation was that most of the patients included in this study had clinically localized prostate cancer and no assesment could be made on the significance of Her-2/neu oncoprotein in the progression of carcinoma to the androgen independent phenotype. Therefore, studies on larger series of prostate carcinoma representing different stages are necessary to determine whether Her-2/neu gene has a role in prostate carcinoma.

REFERENCES

1. Hung MC, Schechter AL, Chevray PY, Stem DF, Weinberg RA. Molecular cloning of the neu gene: Absence of gross structural alteration in oncogenic alleles. Proc Natl Acad Sci USA 1986; 83:261-264.
2. Hynes NE, Stern DF. The biology of erbB-2/neu: Her-2 and its role in cancer. Biochem Biophys Acta Rev Cancer 1994; 1198:165-184.
3. Press MF, Bernstein L, Thomas PA, Zhou JY, Ma Y, Hung G, et al. Her-2/neu gene amplification characterized by flourescence in situ hybridization: poor prognosis in node-negative breast carcinomas. J Clin Oncol 1997;15: 2894-2904.
4. Saffari B, Jones LA, el-Naggar A, Felix JC, George J, Press MF. Amplification and overexpression of Her-2/neu (c-erbB2) in endometrial cancers: correlation with overall survival. Cancer Res 1995;55: 5693-5698.
5. Ranzani GN, Pellegata NS, Previdere C, Saragoni A, Vio A, Maltoni M, Amadori D. Heterogeneous protooncogene amplification correlates with tumor progression and presence of metastasis in gastric cancer patients. Cancer Res 1990; 50: 7811-7814.
6. Press MF, Pike MC, Hung G, Zhou JY, Ma Y, George J, et al. Amplification and overexpression of Her-2/neu in carcinomas of the salivary gland: correlation with poor prognosis. Cancer Res 1994; 54: 5675-5682.
7. Zhau HE, Zhang X, von Eschenbach AC, Scorsone K, Babaian RJ, Ro JY, Hung MC. Amplification and expression of the c-erbB2/neu proto-oncogene in human bladder cancer. Mol Carcinogen 1990;3:254-257.
8. Visokarpi T, Kallioniemi OP, Koivula T, Harvey J, Isola J. Expression of epidermal growth factor receptor and erbB2 (Her2/neu) oncoprotein in prostatic carcinomas. Mod Pathol 1992; 5: 643-648.
9. Sadasivan R, Morgan R, Jenning S, Austenfeld M, Van Veldhuizen P, Stephens R, Noble M. Overexpression of HER-2/neu may be an indicator of poor prognosis in prostate cancer. J Urol 1993; 1993:126-131.
10. Cohen RJ, Cooper K, Haffejee Z, Robinson E, Becker PJ. Immunohistochemical detection of oncogene proteins and neuroendocrine differentiation in different stages of protate cancer. Pathology 1995; 27: 229-232.
11. Lara PN Jr, Meyers FJ, Gray CR, Edwards RG, Gumerlock PH, Kauderer C, et al. HER-2 / neu is overexpressed infrequently in patients with prostate carcinoma. Results from the California Cancer Consortium Screening Trial. Cancer 2002 May 15; 94: 2584-2589.
12. Ross JS, Nazeer T, Church K, Amato C, Figge H, Rifkin MD, et al. Contribution of HER-2/neu oncogene expression to tumor grade and DNA content analysis in the prediction of prostatic carcinoma and metastasis. Cancer 1993; 72: 3020-3028.
13. Epstein JI, Amin M, Boccon-Gibod L, Egevad L, Humphrey PA, Mikuz G, et al. Prognostic factors and reporting of prostate carcinoma in radical prostatectomy and pelvic lymphadenectomy specimens. Scand J Urol Nephrol Suppl. 2005;216:34-63.

14. San Miguel Fraile P, Dos Santos JE, Pélaez Boismorand E, Ortiz Rey JA, Iglesias Rodríguez B, Cambronero Santos J, et al. Expression of the cerbB-2 (HER-2/neu) oncoprotein in prostatic adenocarcinoma. Actas Urol Esp 2005; 29:64-69.
15. Ware JL, Maygarden SJ, Koontz WW, Strom SC. Immunohistochemical detection of c-erbB-2 protein in human benign and neoplastic prostate. Hum Pathol 1991; 22: 254-258.
16. Sanchez KM, Sweeney CJ, Mass R, Koch MO, Eckert GJ, Geary WA, et al. Evaluation of HER-2/neu expression in prostatic adenocarcinoma: A request for a standardized, organ spesific methodology. Cancer 2002; 95:1650-1655.
17. Zhau HE, Wan DS, Zhou J, Miller GJ, et al. Expression of c-erbB2/ neu protooncogene in human prostatic cancer tissues and cell lines. Mol Carcinog 1992; 5:320-327.
18. Kameda T, Yasui W, Yoshida K, Tsujino T, Nakayama H, Ito M, et al. Expression of ERBB2 in human gastric carcinomas: relationship between p185ERBB2 expression and the gene amplification. Cancer Res 1990; 50: 8002-8009.
19. Gu K, Mes-Masson A, Gauthier J, Saad F. Overexpression of Her-2/neu in human prostate cancer and benign hyperplasia. Cancer Letters 1996; 99: 185-189.
20. Ross JS, Sheehan CE, Hayner-Buchan AM, Ambros RA, Kallakury BV, Kaufman RP Jr, et al. Prognostic significance of Her-2/neu gene amplification status by fluorescence in situ hybridization of prostate carcinoma. Cancer 1997; 79: 2162-2170.
21. Ross JS, Sheehan C, Hayner-Buchan AM, Ambros RA, Kallakury BV, Kaufman R, et al. Her-2/neu gene amplification status in prostate cancer by fluorescence in situ hybridization. Hum Pathol 1997;28: 827-833.
22. Kallakury BV, Sheehan CE, Ambros RA, Fisher HA, Kaufman RP Jr, Muraca PJ, Ross JS. Correlation of p34cdc2 cyclin-dependent kinase overexpression, CD44s downregulation, and Her-2/neu oncogene amplification with recurrence in prostatic adenocarcinomas. J Clin Oncol 1998;16:1302-1309.
23. Bubendorf L, Kononen J, Koivisto P, Schraml P, Moch H, Gasser TC, et al. Survey of gene amplifications during prostate cancer progression by high-throughput fluorescence in situ hybridization on tissue microarrays. Cancer Res 1999;59:803-806.
24. Oxley JD, Winkler MH, Gillatt DA, and Peat DS. Her-2/neu oncogene amplification in clinically localised prostate cancer. J Clin Pathol 2002; 55:118-120.
25. Arai Y, Yoshiki T, Yoshida O. C-erbB2 oncoprotein: a potential biomarker of advanced prostate cancer. Prostate 1997; 30: 195-201.
26. Morote J, de Torres I, Caceres C, Vallejo C, Schwartz S Jr, Raventos J. Prognostic value of immunohistochemical expression of the c-erbB2 oncoprotein in metastatic prostate cancer. Int J Cancer 1999; 84:421-425.
27. Craft N, Shostak Y, Carey M, Sawyers C: A mechanism for hormone independent prostate cancer through modulation of androgen receptor signaling by the Her2/neu tyrosine kinase. Nat Med 1999; 5:280-285.
28. Signoretti S, Montironi R, Manola J, Altimari A, Tam C, Bubley G, et al: Her-2/neu expression and progression toward androgen independence in human prostate cancer. J Natl Cancer Inst 2000; 92: 1918-1925.
29. Montironi R, Mazzucchelli R. HER-2 expression and gene amplification in high-grade PIN and prostate cancer. Arch Ital Urol Androl 2006;78:135-139.
30. Agus DB, Bunn PA Jr, Franklin W, Garcia M, Ozols RF. HER-2/neu as a therapeutic target in non-small cell lung cancer, prostate cancer, and ovarian cancer. Semin Oncol 2000; 27(6 Suppl 11):53-63; discussion 92-100.
31. Ziada A, Barqawi A, Glode LM, Varella-Garcia M, Crighton F, Majeski S, et al. The use of trastuzumab in the treatment of hormone refractory prostate cancer; phase II trial. Prostate 2004; 60:332-337.