International Journal of Cancer Research 1 (1-2): 5-9, 2005
ISSN 1811-9727
© 2005 Asian Network for Scientific Information

HER-2/neu Gene Amplification in Prostate Cancer from Egyptian Patients by Fluorescence *in situ* Hybridization

Magdy Sayed Aly
Department of Biology, Faculty of Science, Cairo University (Beni-Suef Branch), Cairo, Egypt

Abstract: Prostate cancer is the most frequent malignancy and the second leading cause of cancer deaths among males in the Western world. A study of 40 cases of prostate cancer is conducted in an attempt to identify prognostic biomarkers that can distinguish aggressive cases that must be treated immediately. HER-2/neu oncogene amplification was initially studied because amplification of this gene has been reported in many other types of cancer. In this study, HER-2 gene amplification was assessed by fluorescence *in situ* hybridization (FISH) using a HER-2/neu gene probe with a chromosome 17 centromere control probe. The study was performed on formalin-fixed, paraffin-embedded tissues. FISH successfully analyzed all cases. Only 5 out of 40 (12.5%) were found to be amplified. This frequency was lower than the frequency of amplification found in other in other cancers studied. The level of amplification observed was correlated with the pathological grade. Our data indicate that HER-2/neu gene amplification status can be determined by FISH on archival prostate cancer specimens, significantly correlates with high tumor grade and is more frequently encountered in tumors with advanced pathological stage. Also, FISH is a sensitive technique for detection of abnormalities in the HER-2/neu gene and further studies should be undertaken to determine whether a FISH-based HER-2/neu detection method may prove of importance in the prediction of prognosis and planning of therapy in prostate cancer patients.

Key words: Fish, fluorescence *in situ* hybridization, HER-2neu, oncogene amplification, prostate cancer

INTRODUCTION

Prostate Cancer (PC) is the most commonly diagnosed cancer in men in the Western world and the second leading cause of male cancer death[1,2]. Risk factors for PC include age, race, country of origin and familial history.

An identifying number of recurring chromosomal abnormalities, identified in various tumors during the last years, have resulted in major advances in the understanding of the pathogenesis of malignant transformation. Although prostate cancer is the most common malignant disease in men in Western countries, knowledge about cytogenetic data are still limited as compared to other solid tumors. This is due to the failure to obtain a sufficient number of dividing cancer cells *in vitro*, the low mitotic index of the original tumor in most cases and the overgrowth by normal cells of the cancer cells in culture[3].

No molecular markers are currently available to accurately predict clinical outcome or to discriminate between aggressive cancers that will grow quickly and kill and those that will grow slowly for several years without serious ill effects.

Oncogene amplifications are manifestations of genetic instability, which has been implicated in the pathogenesis of many cancers. It is a characteristic of cancer cells that allows an increased production of specific proteins used for the acquisition and maintenance of the malignant phenotype. For example, it is known that approximately 25-30% of breast and ovarian carcinomas have amplification of the HER-2/neu gene, although the prognostic value of HER-2/neu amplification or over expression in other cancers has been controversial[4-9]. The HER-2neu oncogene, which is located on chromosome 17q21, encodes a transmembrane tyrosine kinase receptor family[10]. The receptor's role in oncogenesis can be derived from its action on cellular cascades involving proliferation and differentiation of epithelial cells.

The present study exploits the availability of the fluorescence *in situ* hybridization (FISH) technology for studying the amplification of HER-2/neu oncogene in prostate cancer cases to determine a subgroup of patients

Corresponding Author: Dr. Magdy Sayed Aly, Department of Biological Sciences, Faculty of Science, King Khalid University, P.O. Box 9004 Abha, Saudi Arabia
Tel: +966507531152 Fax: +96672290165 E-mail: magdyaly@yahoo.com

who are at risk for rapid disease progression can be identified. Our ultimate goal is to define prognostic biomarkers that can predict clinical outcome. Biomarkers that are able to discriminate the fast progressing cancers from the slow growing cancers are most urgently needed not only to save on the cost of the therapy, but also to preserve the quality of life and to avoid unnecessary morbidity associated with therapy[11,12]. A routinely available method to successfully identified abnormal DNA markers will be important in modern oncology practice.

MATERIALS AND METHODS

The primary population for study was prostate cancer patients who underwent radical prostatectomy at National Cancer Institute, Cairo, Egypt. Formalin-a consulting pathologist identified fixed paraffin-embedded tissue blocks from 40 Egyptian patients. One case of these patients was classified as stage T_1, 15 cases as T_2, 23 were classified as stage T_3 and one case as T_4. The lymph node status on all patients was none (No). Histopathological evaluation was performed using the Gleason System[13]. Gene amplification was determined by FISH using the CEP-17/HER-2/neu dual-probe FISH protocol (Vysis Corp, Downer's Grove, IL). Briefly, formalin fixed, paraffin embedded tissue sections (4 μm thick) were deparaffinized in xylene, dehydrated in 100% ethanol and air dried. After a brief wash in phosphate buffer saline, the slides were dehydrated serially and air-dried in the dark. Hybridization with the Spectrum Green HER-2/neu DNA probe and Spectrum Orange CEP-17 DNA probe was carried out according to the manufacture's instructions, using codenaturation block, overnight hybridization and rapid wash procedures. Nuclei were counterstained with DAPI 1 g mL^{-1} in Vectashield mounting media (Vector Laboratories, Burlingame, CA). Details of the FISH protocol have been discussed extensively elsewhere[14-17]. The procedure for oncogene amplification has been previously described[16]. Briefly, the raw data on the number of HER-2/neu gene copy number and chromosome 17 centromeric numbers were entered on a two-way table. The average copy numbers for both were calculated separately using the marginal totals in the table. Division can then calculate the amplification ratio. Slides with amplification ratios of less than 1.5 will be classified as “nonamplified”. Slides with amplification ratios = 1.5 will be classified as “amplified”. Ratios between 1.5 and 2.0 will be considered as “low amplification”. Ratios between 2.1 and 4.0 will be considered as "moderate amplification”. Ratios exceeding 4.0 will consider as “high amplification”.

RESULTS

Forty Egyptian patients with prostate cancer were included in this study. The histopathologic grade and clinical stage of the tumors studied are summarized in Table 1. One case of these patients was classified as stage T_1, 15 cases as T_2, 23 were classified as stage T_3 and one case as T_4. The median age of the patients was 63 years (range 47-73 years). Gleason scores were available for all patients and were 2-4 in 4 patient, 5-7 in 29 patients and 8-10 in 7 patients. No lymph node involvement or metastasis at the time of transurethral prostatectomy was detected.

Table 1: Results of FISH analysis of HER-2/neu oncogene amplification

Case #	Age	Stage	Gleason score	Amplification ratio
1	66	T_3	7	3.00
2	48	T_3	6	1.20
3	73	T_3	7	1.43
4	67	T_3	7	2.86
5	71	T_4	9	4.15
6	66	T_3	6	1.27
7	65	T_2	7	1.38
8	66	T_3	7	1.36
9	67	T_3	6	1.40
10	68	T_2	7	1.60
11	63	T_2	3	1.12
12	61	T_3	5	1.43
13	71	T_3	7	1.30
14	58	T_3	5	1.46
15	70	T_2	8	1.31
16	70	T_3	7	1.44
17	67	T_3	7	1.29
18	71	T_1	7	0.90
19	66	T_2	8	1.30
20	56	T_2	4	1.30
21	71	T_3	5	1.39
22	79	T_3	9	1.45
23	70	T_3	8	1.45
24	62	T_3	5	1.44
25	67	T_3	7	1.40
26	68	T_2	6	1.31
27	63	T_2	3	1.30
28	61	T_3	5	1.42
29	71	T_3	7	1.39
30	58	T_3	8	1.27
31	70	T_2	5	1.12
32	70	T_3	7	1.20
33	54	T_3	5	1.20
34	59	T_3	8	1.29
35	61	T_2	6	1.29
36	70	T_2	6	1.25
37	47	T_2	5	1.31
38	50	T_2	7	1.75
39	65	T_2	4	1.22
40	55	T_2	5	1.10

Table 2: Relationship of HER-2/neu amplification to pathological grade

HER-2/neu amplification	Totals[a]	AMP[b]	Dipolid[c]
T_1	1	0	1
T_2	15	2	13
T_3	23	2	21
T_4	1	1	0

a: n=40, b: Amplification, c: Normal cell

HER-2/neu gene amplification, as determined by FISH, was performed on all cases studied. Among all the cases successfully analyzed by FISH, 5 (12.5%) showed HER-2/neu oncogene amplification and 35 (87.5%) showed no amplification (Table 1). The level of amplification observed was correlated with the pathological grade. The only one case with grade T_4 showed high-level amplification, 2 cases with grade T_3 showed moderate-level amplification and 2 cases with grade T_2 showed low-level amplification, according to previously defined criteria (Table 2).

DISCUSSION

The present study was conducted to determine whether HER-2/neu oncogene can serve as biomarker to predict patient prognosis for prostate cancer.

The HER-2/neu gene codes for a transmembrane 185 kDa class I tyrosine kinase receptor protein related to the epidermal growth factor receptor, the amplification or overexpression of which is implicated in the pathogenesis of many human cancers[18-20]. Currently available data suggest that there is an increased HER-2/neu expression in prostate cancer by Western and Southern blotting[21-23]. Moreover, the study of HER-2/neu and other oncogene amplifications using immunohistochemistry has not yielded unequivocal results. FISH technique may prove to be a superior technique to overcome this problem[24].

Our study is not in agreement to prior reports of frequent HER-2/neu gene amplification in patients with prostate carcinoma. Ross *et al.*[25, 26] previously reported that HER-2 overexpression in patients with prostate carcinoma by IHC was 29% and that gene amplification was observed in 41% of patients. Those authors also found that both overexpression and amplification were correlated with tumor grade. An earlier study by Kuhn *et al.*[27] reported from their studies that HER-2 overexpression is not a prognostic marker for prostate cancer. In contrast to breast cancer, FISH detects HER-2/neu amplification in a substantial proportion of prostate cancers that do not overexpress HER-2/neu by IHC[28]. Thus, further investigation is needed to clarify these apparently discordant results[29].

In the present study we evaluated 40 patient specimens for HER-2/neu oncogene amplification. All cases were successful. As noted above, the frequency of HER-2/neu gene amplification found in the present study is less than other previous studies of some of the other types of cancer (Table 1). Mark *et al.*[16] found 11% HER-2/neu amplification in rhabdomyosarcoma. In another study of 40 cases of stage I to stage IV breast cancer, the overall frequency of HER-2/neu oncogene amplification was 22.5%. Furthermore, the highest frequency of oncogene amplification was found to be associated with the highest stages. The highest level of amplification was also found in the higher stages. Based on these results, it is evident that a comparable frequency and level of HER-2/neu amplification in prostate cancer was found[30]. Moreover, Mark *et al.*[17] demonstrated that the frequency of gene amplification is much lower and the level of amplification is less intense in their study of prostate cancer.

Oxley *et al.*[31] concluded that the increased Her-2/neu oncogene copy number appears to be rare in clinically localized prostatic adenocarcinoma and is related to chromosome 17 polysomy rather than true amplification. As a result, it would not be a useful biomarker for identifying those patients who will have recurrences after radical prostatectomy.

It has been shown that HER-2 expression in archival tumor specimens in patients with prostate carcinoma that has been progressed to hormone-refractory prostate carcinoma is infrequent[32].

It was also demonstrated by Skacel *et al.*[33] that low-level amplification of HER-2/neu in prostate cancer was found in 26% of cases (3 to 5 signals per nucleus, corrected for chromosome 17 aneusomy). The presence of HER-2/neu amplification was associated with high tumor volume (>2.0 cm (3), $p = 0.004$). Also Edwards *et al.*[34] showed low levels of HER2 gene amplification (8, 7/89) using FISH technique and HER-2 protein expression (12%, 11/89) using IHC.

Nevertheless, high levels of Her-2/neu expression in androgen-independent tissue have not been observed universally. Morris *et al.*[35] reported that approximately 40% of 10 androgen-independent samples over-expressed Her-2 (compared with 14% of androgen-dependent samples) in their screening program for Phase II trastuzumab and paciltaxel trial. Reese *et al.*[36] using the Tab 250 antibody, reported an even lower rate of Her-2 positivity in patients with androgen-independent disease: Only 3 of 22 patients had intermediate to high expression levels (2+ or 3+). Other reports also suggest that HER2 protein over-expression and gene amplification are relatively uncommon in androgen-independent prostate cancer[37].

In conclusion, HER-2/neu gene amplification status can be determined by FISH on archival prostate cancer specimens, significantly correlates with high tumor grade and is more frequently encountered in tumors with advanced pathological stage. Also, FISH is more sensitive than IHC for detection of abnormalities in the HER-2/neu gene and further studies should be undertaken to determine whether a FISH-based HER-2/neu detection

method may prove of importance in the prediction of prognosis and planning of therapy in prostate cancer patients. Exploration of other biomarkers in prostate cancer using FISH is warranted.

REFERENCES

1. Parker, D.L., T. Tong, S. Bolden and P.A. Wingo, 1997. Cancer statistics 1997. C.A. Cancer J. Clin., 47: 5-27.
2. Landis, S.H., T. Murray, S. Bolden and P.A. Wingo, 1999. Cancer statistics 1999. C.A. Cancer J. Clin., 49: 8-31.
3. Micale, M.A., A. Mohamed, W. Sakr, I.J. Powell and R. Wolman, 1992. Cytogenetics of primary prostatic adenocarcinoma. Clonality and chromosome instability. Cancer Genet. Cytogenet, 61: 165-73.
4. Slamon, D.J., W. Godolphin, L.A. Jones, J.A. Holt, S.G. Wong, D.E. Keith, W.J. Levin, S.G. Stuart, J. Udove and A. Ullrich, 1989. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science, 244: 707-12.
5. Press, M.F., M.C. Pike, V.R. Chazin, G. Hung, J.A. Udove, M. Markowicz, J. Danyluk, W. Godolphin, M. Sliwkowski, R. Akita, M.C. Paterson and D.J. Slamon, 1993. Her-2/neu expression in node-negative breast cancer: Direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. Cancer Res., 53: 4960-70.
6. Gullick, W.J., S.B. Love, C. Wright, D.M. Barnes, B. Gusterson, A.L. Harris and D.G. Altman, 1991. C-erbB-2 protein overexpression in breast cancer is a risk factor in patients with involved and uninvolved lymph nodes. Br. J. Cancer, 63: 434-8.
7. Allred, D.C., G.M. Clark, A.K. Tandon, R. Molina, D.C. Tormey, C.K. Osborne, K.W. Gilchrist, E.G. Mansour, M. Abeloff, L. Eudey and W.L. McGuire, 1992. HER-2/neu in node-negative breast cancer: Prognostic significance of overexpression influenced by the presence of *in situ* carcinoma. Clin. Oncol., 10: 599-605.
8. Fournier, G., A. Latil, Y. Amet, J.H. Abalain, A. Volant, P. Mangin, H.H. Floch and R. Lidereau, 1995. Gene amplifications in advanced-stage human prostate cancer. Urol. Res., 22: 343-7.
9. Slamon, D.J., G.M. Clark, S.G. Wong, W.J. Levin, A. Ullrich and W.L. McGuire, 1987. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science, 235: 177-82.
10. Schechter, A.L., M.C. Hung, L. Vaidyanathan, R.A. Weinberg, T.L. Yang-Feng, U. Francke, A. Ullrich and L. Coussens, 1985. The neu gene: An erbB-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. Science, 229: 976-8.
11. Bland, K.I., M.M. Konstadoulakis, M.P. Vezeridis and H.J. Wanebo, 1995. Oncogene protein co-expression. Value of Ha-ras, c-myc, c-fos and p53 as prognostic discriminants for breast carcinoma. Ann. Surg., 221: 706-22.
12. Bland, K.I., 1995. The molecular pathology of colorectal and mammary carcinoma: Prognostic and therapeutic implications for surgeons. Ann. Surg., 222: II-VII.
13. Carter, H.B. and A.W. Partin, 2002. Diagnosis and staging of prostate cancer. In Campbell's Urology, 8th Edn., Walsh, P.C. *et al.*, Ed., Saunders, Philadelphia.
14. Afify, A.M., B.A. Werness and H.F. Mark, 1999. HER-2/neu oncogene amplification in stage I and stage III ovarian papillary serous carcinoma. Exp. Mol. Pathol, 66: 163-9.
15. Afify, A., K.I. Bland and H.F. Mark, 1996. Fluorescent *in situ* hybridization assessment of chromosome 8 copy number in breast cancer. Breast Cancer Res. Treat, 38: 201-8.
16. Mark, H.F., S. Brown, C.L. Sun, M. Samy and A. Afify, 1998. Fluorescent *in situ* hybridization detection of HER-2/neu gene amplification in rhabdomyosarcoma. Pathobiology, 66: 59-63.
17. Mark, H.F., D. Feldman, S. Das, H. Kye, S. Mark, C.L. Sun and M. Samy, 1999. Fluorescence *in situ* hybridization study of HER-2/neu oncogene amplification in prostate cancer. Exp. Mol. Pathol., 66: 170-8.
18. Costa, M.J., J. Walls and J.D. Trelford, 1995. c-erbB-2 oncoprotein overexpression in uterine cervix carcinoma with glandular differentiation. A frequent event but not an independent prognostic marker because it occurs late in the disease. Am. J. Clin. Pathol., 104: 634-42.
19. De Potter, C.R., 1994. The neu-oncogene: More than a prognostic indicator? Hum Pathol., 25: 1264-8.
20. Bacus, S.S., C.R. Zelnick, G. Plowman and Y. Yarden, 1994. Expression of the erbB-2 family of growth factor receptors and their ligands in breast cancers. Implication for tumor biology and clinical behavior. Am. J. Clin. Pathol., 102: S13-24.
21. Zhau, H.E., D.S. Wan, J. Zhou, G.J. Miller and A.C. von Eschenbach, 1992. Expression of c-erb B-2/neu proto-oncogene in human prostatic cancer tissues and cell lines. Mol. Carcinog, 5: 320-7.

22. Ross, J.S., T. Nazeer, K. Church, C. Amato, H. Figge, M.D. Rifkin and H.A. Fisher, 1993. Contribution of HER-2/neu oncogene expression to tumor grade and DNA content analysis in the prediction of prostatic carcinoma metastasis. Cancer, 72: 3020-8.
23. Sadasivan, R., R. Morgan, S. Jennings, M. Austenfeld, P. Van Veldhuizen, R. Stephens and M. Noble, 1993. Overexpression of Her-2/neu may be an indicator of poor prognosis in prostate cancer. J. Urol., 50: 126-31.
24. Persons, D.L., K.A. Borelli and P.H. Hsu, 1997. Quantitation of HER-2/neu and c-myc gene amplification in breast carcinoma using fluorescence *in situ* hybridization. Mod. Pathol., 10: 720-7.
25. Ross, J.S., C. Sheehan, A.M. Hayner-Buchan, R.A. Ambros, B.V. Kallakury, R. Kaufman, H.A. Fisher and P.J. Muraca, 1997. HER-2/neu gene amplification status in prostate cancer by fluorescence *in situ* hybridization. Hum. Pathol., 28: 827-33.
26. Ross, J.S., C.E. Sheehan, A.M. Hayner-Buchan, R.A. Ambros, B.V. Kallakury, R.P. Jr. Kaufman, H.A. Fisher, M.D. Rifkin and P.J. Muraca, 1997. Prognostic significance of HER-2/neu gene amplification status by fluorescence *in situ* hybridization of prostate carcinoma. Cancer, 79: 2162-70.
27. Kuhn, E.J., R.A. Kurnot, I.A. Sesterhenn, E.H. Chang and J.W. Moul, 1993. Expression of the c-erbB-2 (HER-2/neu) oncoprotein in human prostatic carcinoma. J. Urol., 150: 1427-33.
28. Liu, H.L., R. Gandour-Edwards, P.N. Jr Lara, R. de Vere White and J.M. LaSalle, 2001. Detection of low level HER-2/neu gene amplification in prostate cancer by fluorescence *in situ* hybridization. Cancer J.,7: 395-403.
29. Shi, X.B., P.H. Gumerlock and R.W. deVere White, 1996. Molecular biology of prostate cancer. World J. Urol., 14: 318-28.
30. Balcerczak, E., M. Mirowski, A. Sasor and R. Wierzbicki, 2003. Expression of p65, DD3 and c-erbB2 genes in prostate cancer. Neoplasma, 50: 97-101.
31. Oxley, J.D., M.H. Winkler, D.A. Gillatt and D.S. Peat, 2002. Her-2/neu oncogene amplification in clinically localised prostate cancer. J. Clin. Pathol., 55: 118-20.
32. Lara, P.N. Jr, F.J. Meyers, C.R. Gray, R.G. Edwards, P.H. Gumerlock, C. Kauderer, G. Tichauer, P. Twardowski, J.H. Doroshow and D.R. Gandara, 2002. HER-2/neu is overexpressed infrequently in patients with prostate carcinoma. Results from the california cancer consortium screening trial. Cancer, 94: 2584-9.
33. Skacel, M., A.H. Ormsby, J.D. Pettay, E.K. Tsiftsakis, L.S. Liou, E.A. Klein, H.S. Levin, C.D. Zippe and R.R. Tubbs, 2001. Aneusomy of chromosomes 7, 8 and 17 and amplification of HER-2/neu and epidermal growth factor receptors in Gleason score 7 prostate carcinoma: A differential fluorescent *in situ* hybridization study of Gleason pattern 3 and 4 using tissue microarray. Hum. Pathol., 32: 1392-7.
34. Edwards, J., R. Mukherjee, A.F. Munro, A.C. Wells, A. Almushatat and J.M. Bartlett , 2004. HER2 and COX2 expression in human prostate cancer. Eur. J. Cancer, 40: 50-5.
35. Morris, M.J., V.E. Reuter and W.K. Kelly *et al*., 2000. A Phase II trial of herceptin alone and with taxol for the treatment of prostate cancer. Proc. Am. Soc. Clin. Oncol., 19: 1298.
36. Resse, D.M., E.J. Small and F.N. Waldman *et al*., 2000. HER2 expression in androgen independent prostate cancer. Proc. Am. Soc. Clin. Oncol., 19: 1365.
37. Reese, D.M., E.J. Small, G. Magrane, F.M. Waldman, K. Chew and D. Sudilovsky, 2001. HER2 protein expression and gene amplification in androgen-independent prostate cancer. Am. J. Clin. Pathol., 116: 234-9.