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Evaluation of c-erbB-2 Expression, Amplification, and Prognostic Value in Primary and Metastatic Prostate Adenocarcinomas

ORIGINAL INVESTIGATION

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ABSTRACT

Objective: The c-erbB-2 gene codes for a membrane receptor protein that is homologous to the epidermal growth factor receptor. It has been established as a prognostic factor in breast and other cancers. The prognostic role of c-erbB-2 expression and gene amplification in prostate carcinoma is still controversial. In this study, we searched for c-erbB-2 overexpression and gene amplification in primary and metastatic prostate carcinomas and their correlation with tumor grade, age, and survival.

Material and Methods: c-erbB-2 protein overexpression was analyzed on 59 prostate carcinomas, including 44 primary and 15 metastatic prostate carcinomas, by immunohistochemistry and fluorescence in situ hybridization analysis.

Results: When divided into two Gleason grade categories, there were 20 low-grade and 24 high-grade cases in the primary prostate carcinoma cases. C-erbB-2 overexpression was detected in 13.6% of primary and in 60% of metastatic prostate carcinomas. The overexpression was correlated with Gleason score (p=0.001) and clinical outcome but not with age (p>0.05). None of the cases showed HER-2 amplification by FISH.

Conclusion: In this study, c-erbB-2 protein overexpression was associated with high grade and advanced pathological stage. Additionally, it was also associated with metastatic prostate tumors. These findings support that this marker indicates a potentially aggressive clinical course and metastasis. Therefore, this marker may be helpful for planning therapy.

Keywords: c-erbB-2 expression, prostate carcinoma, prognostic parameter

INTRODUCTION

Proto-oncogenes, including the c-erbB-2 (HER-2/neu) oncogene, located at chromosome 17 q, represent a family of normal cellular genes that are involved in cell growth and differentiation. The c-erbB-2 gene encodes a 185-kd transmembrane glycoprotein that has homology to the epidermal growth factor receptor. HER 2 is homologous to, but distinct from, other members of the erbB family, which includes epidermal growth factor receptor 1 (erbB-1), 3 (erbB-3), and 4 (erbB-4). The binding of growth factors to these receptors activates tyrosine receptor kinases, which causes cell growth, proliferation, and differentiation. Today, it is clear that alterations in gene structure, amplification, or overexpression play a role in the pathogenesis of some human cancers (1, 2). The amplification of the c-erbB-2 gene results in elevated levels of c-erbB-2 messenger RNA and protein that can be detected by immunohistochemistry (IHC) (1-3). Overexpression of this gene is detected in 20%-30% of breast and ovarian cancers. Overexpression occurs by a gene amplification mechanism and is associated with a poor prognosis.

Although several studies evaluated the prognostic influence of c-erbB-2 amplification and overexpression in breast cancer, previous studies about this gene and protein in prostate cancer have been limited (4-7). In light of this knowledge, we aimed to evaluate the immunohistochemical expression and amplification of c-erbB-2 in primary prostate carcinomas and in bone metastasis. In addition, expression results were compared with Gleason grade, age, and survival.

MATERIALS and METHODS

Prostate samples

A cohort of 59 patients with prostate cancer was studied. Forty-four men who underwent either transurethral resection or radical retropubic prostatectomy with a diagnosis of adenocarcinoma of the prostate were randomly selected from the pathology files and urologic services of Çukurova University, Medical Faculty Hospital. Metastatic lesions (15 cases) from patients with progressive disease were also analyzed. The microscopic slides from each case were reviewed, and the tumors were graded according to the Gleason system. The 15 metastatic cases were all bone lesions.

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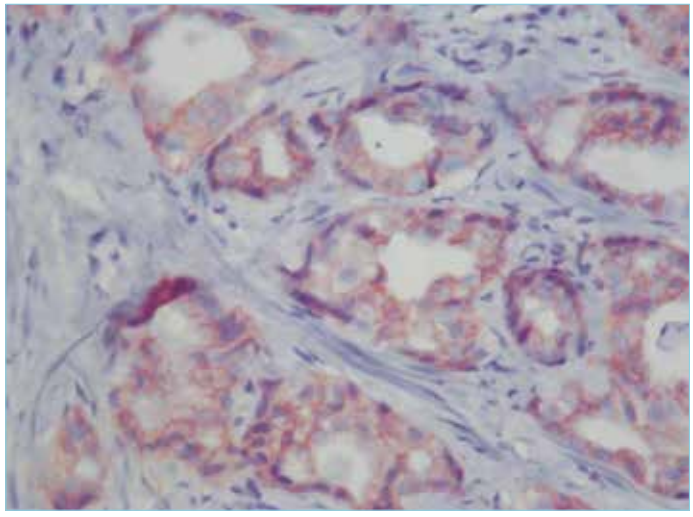

Figure 1. Membranous 2+ positivity of c-erbB-2 expression in primary prostate carcinoma (immunohistochemistry X400)

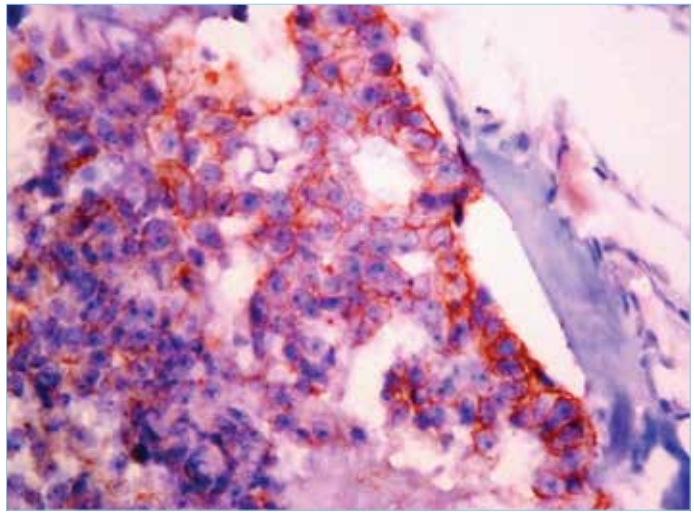

Figure 2. Diffuse 3+ membranous positivity of c-erbB-2 in metastatic prostate carcinoma (immunohistochemistry X200)

Immunohistochemistry

Five-micron-thick sections of formalin-fixed, paraffin-embedded tissue samples were deparaffinized and rehydrated through a series of graded alcohols. Antigen retrieval was carried out in a microwave oven with "peroxidase blocking reagent" for 8-9 minutes. The slides were kept in citrate buffer for 20 minutes at room temperature and then rinsed in PBS. Sections were incubated for 20-30 minutes with acid-urea solution, washed, and then incubated for 20-30 minutes with goat serum. After washing, the sections were incubated with anti-c-erbB-2 (polyclonal rabbit antibody, DAKO, Denmark) at room temperature for 90 minutes, washed, and incubated for 30 minutes with biotinylated horse anti-mouse IgG immunoglobulin (DAKO, Denmark). After washing, the sections were incubated for 30 minutes with streptavidin peroxidase reagent and washed again. The immunoperoxidase was visualized with AEC (3 amino 9 ethylcarbazole), (DAKO, USA). The sections were counterstained with Mayer's hematoxylin and then coverslipped.

Evaluation of c-erbB-2 expression by IHC

Only membrane staining intensity and pattern were evaluated using a 0 to 3+ scale (0: completely negative, 1+: faint membranous positivity in >10% of tumor cells, 2+: moderate complete membranous positivity in >10% of tumor cells, 3+: strong completed circumferential membranous positivity in > 10% of tumor cells). Scores of 2+ and 3+ membranous staining were considered positive.

Evaluation of c-erbB-2 amplification by fluorescent in situ hybridization (FISH)

FISH was performed by using the PathVysion HER-2 DNA Probe and Paraffin Pretreatment Reagent kit (ABBOTT, Vysis, Depamiks). HER-2 was labeled with Spectrum Orange, and the CEP17 was labeled with Spectrum Green. Nuclei were counterstained with DAPI in phenylenediamine dihydrochloride. The slides were analyzed by fluorescent microscopy. Hybridization signals were counted in 60 nuclei/specimen. Individual signals for HER-2 and CEP 17 were recorded, and the results were reported as the HER copy number divided by the CEP 17 copy number; ratios ≥2.0 signified HER-2 amplification.

Statistical Methods

SPSS for Windows, ver. 10.0 was used for statistical analyses. Differences in c-erbB-2 levels between the groups were evaluated by chi-square or Fisher's exact test. The Kaplan-Meier method was used to estimate cumulative survival, and log-rank test was applied to compare stratified survival functions. Data were expressed as mean±SD (standard deviation), n (number of cases), and percent (%). A p value less than 0.05 was considered significant.

RESULTS

The 59 men ranged in age from 52 to 82 (mean, 67) years. There were 31 transurethral resections, 13 radical prostatectomy specimens, and 15 metastatic bone lesions. Twenty of the 44 primary prostate tumors were low-grade (Gleason score 6 and lower), and 24 of them were high-grade (Gleason score 7 and higher). C-erbB-2 protein membrane overexpression was detected in 6 (13.6%) of 44 primary prostate tumors. Twenty-eight (63.3%) cases were scored as 0, 10 (22.2%) cases were scored as +1, and 6 (13.6%) were considered +2. We did not observe a staining score of +3 in this series. We also found out that 9 out of 15 (60%) metastatic cases displayed c-erbB-2 protein expression. Four cases (26.6%) were scored as 0, 2 cases (13.3%) were scored as +1, 3 cases (20%) were scored as +2, and 6 cases (40%) were considered +3 (Figure 1, 2). Table 1 summarizes the clinicopathological characteristics of the 59 patients.

DISCUSSION

The dramatic increase in the diagnosis of prostate cancer has required new prognostic markers that are even applicable to needle biopsies that would allow clinicians to stratify the patients into groups for receiving significantly different therapies. Overexpression of the c-erbB-2 oncoprotein is a common occurrence in breast cancer and was one of the first oncogene assays to gain clinical use as a prognostic marker (1-5). In prostate cancer, the assessment of c-erbB-2 overexpression has been more problematic, and the results are controversial.

Techniques that are used to detect c-erbB-2 abnormalities have included gene-based assays, such as Southern and slot blot testing,

Table 1. Clinicopathological Characteristics of Patients.

Patient Characteristics	Patient Data n=59
Age (years)	
Mean±SD	67.0±6.9
Median	67
Min-Max	52-82
Operation n, %	
Transurethral	31 (52.5)
Radical Prostatectomy	13 (22.0)
Bone metastasis	15 (25.4)
c-erbB-2 n, %	
0-1+	33 (55.9)
2+	20 (33.9)
3+	6 (10.2)
Grade n, %	
Low	20 (45.5)
High	24 (54.5)
Status n, %	
Alive	21 (35.6)
Dead	32 (54.2)
Unknown	6 (10.2)
Follow-up duration (months)	
Mean±SD	18.6±12.0
Median	12
Min-Max	3-48

polymerase chain reaction (PCR) methods, and in situ fluorescent hybridization (FISH) techniques. Qualitative and quantitative measurements have been performed with IHC on frozen and formalin-fixed paraffin-embedded tissues, western blot testing, and enzyme-linked immunosorbent assay (ELISA) (1-7).

Study of the c-erbB-2 gene in prostate carcinoma with various techniques has been confounded by conflicting results. McCann et al. studied 23 prostate carcinomas and could not find c-erbB-2 oncoprotein expression by using IHC (1). Sadasivan et al. found that 7 of 25 prostate cancers had expression of c-erbB-2 by IHC in fresh samples (8). Berner et al. (9) could not find expression in 94 specimens. On the other hand, Kuhn et al. demonstrated c-erbB-2 oncoprotein expression with a ratio of 34% in prostate carcinomas, but they were unable to demonstrate c-erbB-2 gene amplification by polymerase chain reaction technique (10). Besides, Ross et al. assessed 113 cases of prostate cancer by fluorescence in situ hybridization and found out that 41% of them showed c-erbB-2 gene amplification (11). Montironi et al. (12) showed gene amplification in a small proportion of nuclei and some prostate adenocarcinoma cases. These contradictory results about c-erbB-2 gene amplification in prostate carcinoma might be associated with increased surface receptor expression without gene amplification. This apparently involves some type of transcriptional or posttranscriptional regulation, like in some breast carcinomas. Also, the heterogeneity of prostate cancer and the methodological differences can be other possible explanations for these variable results. Factors other than technical ones, such as the relation with the type of treatment given, patient selection, and use of androgen ablation treatment, may explain the variability of reported cases (13).

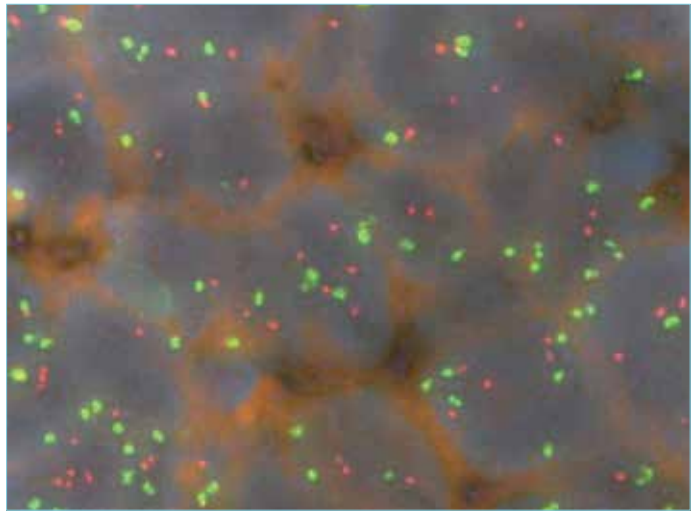

Figure 3. No amplification was detected at the c-erbB-2 gene by FISH analysis (CEP-17/HER2 dual probe [Spectrum Orange-labeled HER2 DNA probe and Spectrum Green CEP-17 DNA probe; Vysis Corp])

Most of the previous studies have been conducted in androgen-dependent and androgen-independent cancer (12, 14). The development of prostate cancer and the progression from normal prostate epithelium to androgen-dependent and to hormone-refractory prostate cancer is a multistep process. The molecular mechanisms responsible for the development of androgen independence are not yet clear (15, 16). One possible factor in the development of androgen independence is c-erbB-2. Recently published data pointed out that overexpression of c-erbB-2 confers androgen-dependent growth to androgen-independent prostate cancer, and the ratio up to 60% positivity in hormone-refractory prostate carcinoma supports this idea (12-18). We also found out that 60% of metastatic tumors overexpressed c-erbB-2 protein. We were not able to get satisfactory information about these metastatic lesions on whether they were androgen-independent or not. But, we think that the relationship between c-erbB-2 and androgen-independent tumors should be searched in large series with long follow-up.

Koeppen et al. (19) and Lara MP et al. (16) performed a c-erbB-2 IHC test using the Hercept test and found 2+ positivity only in a small portion of the cases. However, none of the cases was graded 3+, remarkably similar to the current study. Compared to breast cancer, c-erbB-2 protein is weakly expressed in prostate carcinoma. In our study, none of the primary tumors was scored 3+; however, 40% (6 of 15) of metastatic tumors overexpressed c-erbB-2 as +3. Our data suggest that progression of prostate cancer is characterized by an increase in c-erbB-2 expression by tumor cells. This expression probably has metastasis-promoting action. Recently, Leung et al. pointed out that expression of ERBβ2 is an inde-

Table 2. Mean and Median Cumulative Survival (Months) for Patients (n=59) According to Grade and c-erbB-2 Expression

		Mean (median)	Dead/ Total n	p value (log-rank test)
Grade	Low	40.0 (-)	3/16	0.007
	High	24.0 (18)	16/22	
c-erbB-2	Negative	33.7 (-)	11/29	0.000
	Positive (2+)	16.3 (15)	15/18	
	Positive (3+)	7.8 (8)	6/6	
Overall		24.9 (18.0)	32/53	

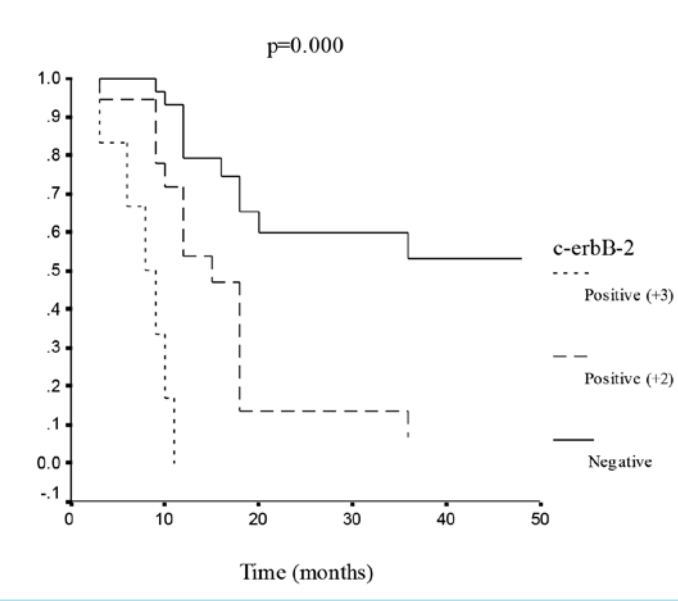

Figure 4. Cumulative survival of patients according to c-erbB-2 expression

pendent prognostic marker, and additionally, expression of ERβ2 and ERβ5 identified the group of patients with the worst clinical outcome (20). These ERBβ isoforms can be searched in advanced prostate carcinomas. The prognosis of patients with prostate cancer depends largely on the grade and stage of the tumor. Other prognostic factors, like aneuploidy and S phase, are unreliable and difficult to perform (15-20). However, detection of c-erbB-2 is easy and can be readily used in formalin-fixed paraffin-embedded tissue in a routine laboratory.

In this study, we detected c-erbB-2 protein expression in 13.6% of the primary prostate adenocarcinoma cases and in 60% of metastatic tumors. Our results indicate that c-erbB-2 expression is more frequent in tumors of advanced stage and in tumors that have a high Gleason score. Those cases that express c-erbB-2 and other prognostic factors, such as high tumor grade, indicate a poorer prognosis. Statistical analysis of survival shows that expression of c-erbB-2 is associated with significantly worse survival. In this regard, overexpression of c-erbB-2 may be useful for assessing the biologic behavior of prostate carcinoma. Additionally, a monoclonal antibody directed against the extracellular domain of c-erbB-2 can be an alternative therapy for metastatic prostate carcinomas.

CONCLUSION

In conclusion, identification of c-erbB-2 overexpression and its relation with other parameters will likely provide valuable insights into the prognosis, treatment, and management of prostate carcinoma.

HER-2 amplification was not detected in any primary or metastatic tumor (Figure 3).

C-erbB-2 expression was significantly associated with Gleason score (p=0.01) in primary prostate tumors but not with age (p>0.05). We lost 6 patients to follow-up. Follow-up ranged from 18 to 48 months. A statistically significant correlation was also observed between survival and c-erbB-2 overexpression both in primary and metastatic tumors (Figure 4) (Table 2).

Ethics Committee Approval: This retrospective study was not directly realized on human subject but on human tissue samples archieved in the collection of the Department of Pathology of Medical Faculty in Cukurova University so Ethics Committee Approval was not indicated.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Authors' Contributions: Conceived and designed the experiments or case: AA, ŞE, Vİ. Performed the experiments or case: AA, ŞE, Vİ. Analyzed the data: EB, ZT, GŞ, SZ, GG. Wrote the paper: AA. All authors read and approved the final manuscript: yes

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. McCann A, Dervan PA, Johnston PA, Gullick WJ, Carney DN. c-erbB-2 oncoprotein expression in primary human tumors. Cancer 1990; 65(1): 88-92. [CrossRef]
2. Disis ML, Cheever MA. HER-2/neu protein: a target for antigen-specific immunotherapy of human cancer. Adv Cancer Res 1997; 71: 343-71. [CrossRef]
3. Ross JS, Fletcher JA. Her-2/neu (c-erbB-2) gene and protein in breast cancer. Am J Clin Pathol 1999; 112(Suppl): 53-67.
4. Sütterlin MW, Haller A, Gassel AM, Peters K, Caffier H, Dietl J. The correlation of c-erbB-2 oncoprotein and established prognostic factors in human breast cancer. Anticancer Res 2000; 20(6): 5083-8.
5. Tetu B, Brisson J. Prognostic significance of HER-2/neu oncoprotein expression in node-positive breast cancer. The influence of the pattern of immunostaining and adjuvant therapy. Cancer 1994; 73(9): 2359-65. [CrossRef]
6. Uehara T, Kaneko Y, Kanda N, Yamamoto T, Higashi Y, Nomoto C, et al. c-erbB-2 and c-erbB-1 (ear-1) gene amplification and c-erbB-2 protein expression in Japanese breast cancers: their relationship to the histology and other disease parameters. Cancer Res 1990; 81 (6-7): 620-4.

7. Perren TJ. c-erbB-2 oncogene as a prognostic marker in breast cancer. Br J Cancer 1991; 63(3): 328-32. **[CrossRef]**
8. Sadasivan R, Morgan R, Jennings S, Austenfeld M, Van Veldhuizen P, Stephens R, et al. Overexpression of HER-2/NEU may be an indicator of poor prognosis in prostate cancer. J Urol 1993; 150(1): 126-31.
9. Berner A, Nesland JM, Waehre H, Silde J, Fossa SD. Hormone resistant prostatic adenocarcinoma. An evaluation of prognostic factors in pre-and post-treatment specimens. Br J Cancer 1993; 68(2): 380-4. **[CrossRef]**
10. Kuhn EJ, Kurnot RA, Sesterhenn IA, Chang EH, Moul JW. Expression of the c-erbB-2 (HER-2/neu) oncoprotein in human prostatic carcinoma. J Urol 1993; 150(5 Pt 1): 1427-33.
11. 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(11): 2162-70. **[CrossRef]**
12. Montironi R, Mazzucchelii R, Barbisan F, Stramazzotti D, Santinelli A, Scarpelli M, et al. HER2 expression and gene amplification in pT2 Gleason score 6 prostate cancer incidentally detected in cystoprostatectomies: comparison with clinically detected androgen-dependent androgen-independent cancer. Human Pathol 2006; 37(9): 1137-44. **[CrossRef]**
13. Fossa A, Lilleby W, Fossa SD, Gaudernack G, Torlakovic G, Berner A. Independent prognostic significance of HER-2 oncoprotein expression in pNO prostate cancer undergoing curative radiotherapy. Int J Cancer 2002; 99(1): 100-5. **[CrossRef]**
14. Calvo BF, Levine AM, Marcos M, Collins QF, Iacocca MV, Caskey LS, et al. Human epidermal receptor-2 expression in prostate cancer. Clin Cancer Res 2003; 9(3): 1087-97.
15. Di Lorenzo G, Tortora G, D'Armiento FP, De Rosa G, Staibano S, Autorino R, et al. Expression of epidermal growth factor receptor correlates with disease relapse and progression to androgen-independence in human prostate cancer. Clin Cancer Res 2002; 8(11): 3438-44.
16. 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; 94(10): 2584-9. **[CrossRef]**
17. 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(23): 1918-25. **[CrossRef]**
18. Hernes E, Fossa SD, Berner A, Othes B, Nesland JM. Expression of the epidermal growth factor receptor family in prostate carcinoma before and during androgen-independence. Br J Cancer 2004; 90(2): 449-54. **[CrossRef]**
19. Koeppen HK, Wright BD, Burt AD, Quirke P, McNicol AM, Dybdal NO, et al. Overexpression of HER2/neu in solid tumours: an immunohistochemical survey. Histopathology 2001; 38(2): 96-104. **[CrossRef]**
20. Leung YK, Lam HM, Wu S, Song D, Levin L, Cheng L, et al. Estrogen receptor β2 and β5 are associated with poor prognosis in prostate cancer, and promote cancer cell migration and invasion. Endocr Relat Cancer 2010; 17(3): 675-89. **[CrossRef]**