Immuoexpression of estrogen receptor-β and progesterone receptor in prostate adenocarcinoma, does it inhibit neoplastic proliferation and invasion?

Kinjal N. Bera, Shakti K. Yadav, Om Prakash, Sompal Singh, Namrata Sarin

Departments of Pathology and Urology, North Delhi Municipal Corporation Medical College and Hindu Rao Hospital, Delhi, India

Address for correspondence:
Dr. Namrata Sarin, Department of Pathology, North Delhi Municipal Corporation Medical College and Hindu Rao Hospital, Delhi, India.
E-mail: dnamrata50@gmail.com

ABSTRACT

Context: The roles of estrogen and progesterone in human prostate carcinogenesis have been only recently recognized. Aims: This study was conducted to evaluate the expressions of estrogen receptor-beta (ER-β), progesterone receptor (PR), and Ki-67 in benign and malignant lesions of the prostate. Settings and Design: The study was conducted at a tertiary care hospital. It was an analytical cross-sectional study. Materials and Methods: We selected a total of 39 cases including 26 cases of benign prostatic hyperplasia and 13 cases of adenocarcinoma prostate. The proportion of cases showing expression for ER-β, PR, and Ki-67 was noted for both groups. A difference in immunoexpression between benign and malignant cases was evaluated. Association between receptor expression and Gleason grade was evaluated for malignant cases. Statistical Analysis Used: To compare the difference in expressions of ER-β, PR, and Ki-67 Mann–Whitney U test was used. Association between ER-β, PR, and Ki-67 expression and Gleason grade was analyzed using the Chi-square test. Results: ER-β expression was seen in all benign and malignant cases, whereas the majority of the malignant cases (61.54%) were negative for progesterone expression. Epithelial expressions of ER-β and PR were significantly higher in benign as compared with malignant lesions. Malignant cases showed a significantly higher expression of Ki-67. However, we did not find any association between the expressions of these markers with Gleason grade. Conclusions: The expressions of ER-β and PR were significantly higher in the epithelium in benign cases as compared with malignant cases. Ki-67 expression was significantly higher in the malignant group as compared with the benign group.

KEY WORDS: Estrogen receptor-beta, Ki-67, progesterone receptor, prostate carcinoma

INTRODUCTION

Prostate cancer is one of the leading causes of morbidity and mortality in men >50 years of age with varying incidence among different countries and ethnic groups.[1] The highest rates of prostate cancer are reported in Australia/New Zealand (104.2/1,000,000), Western and Northern Europe and North America.[2] The incidence of prostate cancer is variable among different Asian countries. The incidence ranges from the lowest 3.0/100,000 in Iran to the highest 20.3/100,000 in the Philippines.[3]

The prostate gland is made up of two components: epithelium and stroma. For proper prostate functioning and homeostasis, the interaction between epithelium and stroma is the crucial factor. Although the exact etiology for benign prostatic hyperplasia (BPH) is not established, a hormonal theory involving a disturbance in stromal–epithelial interaction is thought to be a responsible factor for it.[4,5] Old age, black ethnicity, and a positive family history of the disease are the risk factors most commonly associated with the prostate carcinoma.[6] The dependency of the prostate for maintaining its size and secretory function on androgens is well documented. The role of estrogen in the prostate and human prostate carcinogenesis has been only recently recognized.[7,8] Considering the expression of estrogen receptor-beta (ER-β), its localization in human prostate tissue is not well recognized. Although the precise biological function of ER-β is incompletely
defined, it has been suggested that the receptor, acting through estrogen, may protect the normal prostate epithelium from undergoing unscheduled cell proliferation, neoplastic transformation, and from oxidative injuries. \[9,10\] Progesterone belongs to the same receptor family as the androgen and ERs. However, there are conflicting results regarding the presence of progesterone receptor (PR) in prostate tissue. The importance of PR in the human prostate and prostate carcinogenesis has never been adequately explained. \[11–13\] Ki-67 is a well-known proliferation-specific nuclear antigen. Ki-67 gives a higher labeling index than other antibodies with good inter-reading reproducibility. \[14\]

In view of the paucity of Indian literature and controversies in various studies of ER-β and PR expressions in prostatic lesions, this study was conducted to evaluate the expressions of ER-β, PR, and Ki-67 in benign and malignant prostatic lesions.

**SUBJECTS AND METHODS**

In this study, 39 specimens of prostate enlargement were analyzed, out of which 26 were of benign nodular hyperplasia and 13 were of adenocarcinoma prostate. Both prostatic needle biopsy and chips of transurethral resection of the prostate specimens were taken. Hematoxylin and eosin staining was done for morphological evaluation.

All malignant lesions were categorized according to the Gleason grading system, \[13\] with grade 1 (score \&le; 6), grade 2 (score 3 + 4 = 7), grade 3 (score 4 + 3 = 7), grade 4 (score 8), and grade 5 (score >9). For immunohistochemical evaluation, representative sections were taken and stained with anti-ER-β immunostain (rabbit polyclonal antibody, clone: ERb88, Biogenex, USA), anti-PR immunostain (rabbit monoclonal antibody, clone: EP2, Biogenex, USA), and anti-Ki-67 immunostain (mouse monoclonal antibody, Biogenex, USA). To detect the association of these marker with Gleason grades, their immunoexpression was sub-grouped into categories. Level of ER-β expression was evaluated using a semi-quantitative hot-spot method and the percentage of positive cells was scored in five subgroups: 1 (0–10%), 2 (11–40%), 3 (41–60%), 4 (61–80%), and 5 (>80%). Similarly, PR expression was scored in four subgroups, 0 (0%), 1 (<5%), 2 (5–50%), and 3 (>50%), and Ki-67 was scored in five subgroups, 1 (<1%), 2 (1–5%), 3 (6–10%), 4 (11–20%), and 5 (>20%).

Differences of expressions of ER-β, PR, and Ki-67 between benign and malignant cases were analyzed using the Mann–Whitney U test. Association between ER-β, PR, and Ki-67 expressions and Gleason grade was analyzed by the Chi-square test. A \( P \) value of <0.05 was considered statistically significant. All statistical analyses were carried out by using IBM SPSS 25.

**RESULTS**

The mean age of patients in the benign group was 62.92 ± 0.789 (mean ± SD) years, of which a maximum number of cases were in the age group of 56–60 years. While the mean age of patients in the malignant group was 67.38 ± 0.774 (mean ± SD) years and the majority of cases were in the age group of 71–75 years.

ER-β expression was seen in benign and malignant cases in both stroma and epithelium [Figure 1]. Epithelial ER-β expression score was >3 in the majority of cases in the benign group, whereas it was low [2] in most (76.92%) malignant lesions. Epithelial ER-β expression was significantly higher in the benign group as compared with the malignant group [Table 1]. We found a significant association between stromal ER-β expression and Gleason grade [Table 2]. Stromal and epithelial PR expression score was 2 in the majority of benign cases (73.08%, 50%). A majority of malignant cases showed complete loss of PR in stroma as well as epithelium [Figure 2]. Epithelial PR expression was significantly higher in the benign group as compared with the malignant groups (\( P \) value <0.001). Stromal and epithelial Ki-67 expression score was lower [score 2] in the benign group. Epithelial Ki-67 expression was significantly higher in malignant lesions (\( P \) value < 0.001, Figure 3). We did not find any association between the expressions of PR and Ki-67 with Gleason grade [Table 2].

**DISCUSSION**

Most reports on ER-β expression agree that levels decline in localized prostate cancer with an increasing grade from PIN through low to high Gleason scores. This pattern fits with the proposed antiproliferative and pro-differentiation function of prostatic ER-β with its loss permitting unregulated growth and de-differentiation of the prostatic epithelium. \[14\]

| Table 1: Difference in stromal and epithelial expressions of ER-β, PR, and Ki-67 between benign and malignant groups |
| --- |
| **Immunostaining** | **Benign** (%) | **Malignant** (%) | **P** |
| **ER-β** | Stroma | 44.0 (18.19) | 31.08 (15.19) | 0.099 |
| | Epithelium | 62.46 (21.20) | 32.85 (13.20) | 0.001 |
| **PR** | Stroma | 21.31 (18.53) | 12.31 (14.59) | 0.50 |
| | Epithelium | 10.65 (14.33) | 1.69 (3.4) | <0.001 |
| **Ki-67** | Stroma | 3.54 (2.78) | 2.23 (1.36) | 0.119 |
| | Epithelium | 4.58 (3.65) | 14.77 (4.56) | <0.001 |

**Figure 1:** Photomicrograph showing ER-β expression in (a) BPH (DAB, 100×) (b) prostate cancer (DAB, 400×). ER-β: estrogen receptor-beta, BPH: benign prostatic hyperplasia
We found that benign and malignant cases showed positive expression for ER-β; the level of expression was much lower in malignant cases as compared with benign cases. In concordance with the results of our study, a study by Grover et al.\(^{17}\) also showed that all benign and malignant cases showed expression for ER-β but a majority of the benign cases (83.3\%) had the high expression score. Similarly, Grover et al.\(^{17}\) showed the statistically significant result in comparing the expression of ER-β between two groups (\(P\) value = 0.001). Another study by Fixemer et al.\(^{18}\) showed that ER-β was expressed in the epithelium in all cases of carcinoma prostate, but a majority of primary carcinoma (87\%) cases showed a high score of ER-β expression, which is contradictory to the results of our study. Gabal et al.\(^{19}\) also demonstrated diminished ER-β levels in carcinoma compared with benign hyperplasia like our study, but in contrast to our study, levels of ER-β expression were markedly reduced in their study. We found in our study that there was no association between ER-β expression in stroma and epithelium with Gleason grade. Similarly, Fixemer et al.\(^{18}\) also showed that there was no statistically significant association between ER-β expression and Gleason grade. The controversies on the expression of ER-β between various studies might be because of variable antibody specificity, different primary antibodies, variable antigen retrieval, or the presence of unknown isoforms of ER-β protein. If the hypothesis that ER-β is lost during carcinogenesis is true, then it will help in guiding therapy in prostate cancer prevention trials.

In this study, we evaluated the expression of PR in both stroma and epithelium in all cases. Among 26 benign cases, 96.15\% and 84.62\% cases showed expression for PR in the stroma and epithelium respectively. Among malignant cases, 76.92\% and 38.46\% cases showed expression for PR in the stroma and epithelium, respectively. So, in our study, we found that there was a loss of expression for PR in malignant cases. We found that there was a statistically significant difference in the expression for PR in epithelium between two groups (\(P\) value = 0.025). Our results were in the concordance with the results of a study done by Bonkhoff et al.\(^{20}\) which also showed that there was a reduced expression of PR in malignant cases. In a study, Kang et al.\(^{21}\) have shown that PR expression was seen in 100\% and 93.8\% cases in stroma and epithelium in BPH cases. In malignant cases, PR expression was 76.7\% and 93.3\% in stroma and epithelium, respectively. This study had shown high expression for PR in the epithelium in malignant cases, which is contradictory to our results.

We also studied the correlation between Gleason grade and PR expression in stroma and epithelium, and we found that there was no statistically significant association between them (\(P\) value = 0.522 and \(P\) value = 0.781 for stroma and epithelium, respectively). Our result was in concordance with the results of the study by Kang et al.\(^{21}\). Bonkhoff et al.\(^{20}\) also showed similar results.

In a recent study, expression of PR (β isoform) in prostate carcinoma cases was found to be associated with unfavorable outcomes.\(^{22}\)

Song et al. studied the expression of androgen receptor, ER, and PR in BPH patients. They found that ER-α was distributed mainly in stromal cells. ER-β expression was found in the basal layer of epithelium, and sporadically in epithelial and stromal cells, whereas PR expression was found in both epithelial and stromal cells. They concluded that there is activation of androgen and PR receptor while repression of ER-α in BPH, indicating an inhibitory role of ER-α and promoting role of androgen and PR in the pathogenesis of BPH.\(^{23}\)

The proliferation marker Ki-67 reflects the tumor cell proliferation rate as it correlates with progression, metastasis, and prognosis in many different malignancies.\(^{24-26}\) We found that benign cases showed a lower level of Ki-67 expression in stroma and epithelium (score 02) as compared with malignant cases which showed a higher level of expression (score 04) in the epithelium. Similar results were obtained in a study done by Grover et al.\(^{17}\)

### Table 2: Association between ER-β, PR, and Ki-67 expression and Gleason grade in malignant lesions of the prostate

| Immunoexpression | Stroma | Epithelium |
|------------------|--------|------------|
| ER-β expression  | \(P=0.014\) | \(P=0.612\) |
| PR expression    | \(P=0.552\) | \(P=0.781\) |
| Ki-67 expression | \(P=0.532\) | \(P=0.216\) |

ER-β: estrogen receptor-beta, PR: progesterone receptor
Another study by Verma et al.\textsuperscript{29} also showed that 64% of malignant cases were positive for Ki-67 expression as compared with only 10% of BPH cases. The difference in the expression of Ki-67 in epithelium between two groups was statistically significant in our study ($P$ value = 0.000) which is in concordance with the results of Grover et al.\textsuperscript{17} Association between Ki-67 expression and Gleason grade was not statistically significant in either stroma or epithelium.

**CONCLUSION**

In this study, the expressions of ER-\(\beta\) and PR were significantly higher in the epithelium in benign cases as compared with malignant cases. Ki-67 expression was found to be significantly higher in malignant cases as compared with benign cases. The expression of ER-\(\beta\) in stroma was found to be significantly associated with Gleason grade.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Cancel-Tassin G, Cussenot O. Genetic susceptibility to prostate cancer. BJU Int 2005;96:1380-5.
2. Lalitha K, Suman G, Pruthvish S, Mathew A, Murthy NS. Estimation of time trends of incidence of prostate cancer—an Indian scenario. Asian Pac J Cancer Prev 2012;13:6245-50.
3. Parkin D, Vatanasapt V. Cancer registration in Asia in the year 2000: Past present and future. Differentiation 2008;76:578-86.
4. Cunha GR. Mesenchymal-epithelial interactions: Past, present, and future. Differentiation 2008;76:578-86.
5. Nicholson TM, Ricke WA. Androgens and estrogens in benign prostatic hyperplasia: Past, present and future. Differentiation 2011;82:184-99.
6. Nelson WG, De Marzo AM, Isaacs WB. Prostate Cancer. N Engl J Med 2002;349:366-81.
7. Horvath LG, Henshall SM, Lee CS, Head DR, Quinn DI, Makela S, et al. Frequent loss of estrogen receptor-beta expression in prostate cancer. Cancer Res 2001;61:5331-5.
8. Taplin ME, Ho SM. Clinical review 134: The endocrinology of prostate cancer. J Clin Endocrinol Metab 2001;86:3467-77.
9. Leav I, Lau K, Adams J, McNeal J, Taplin M, Wang J, et al. Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. Am J Pathol 2001;159:79-92.
10. Weihsua Z, Warner M, Gustafsson JA. Estrogen receptor beta in the prostate. Mol Cell Endocrinol 2002;193:1-5.
11. Grindstad T, Andersen S, Al-Saad S, Donnem T, Kiselev Y, Nordahl Melbo-Jorgensen C, et al. High progesterone receptor expression in prostate cancer is associated with clinical failure. PLoS One 2015;10:e0116691.
12. Luetjens CM, Didolkar A, Klesch S, Paulus W, Jeibmann A, Bocker W, et al. Tissue expression of the nuclear progesterone receptor in male non-human primates and men. J Endocrinol 2006;189:529-39.
13. Yu Y, Liu L, Xie N, Xue H, Fazli L, Buttyn R, et al. Expression and function of the progesterone receptor in human prostate stroma provide novel insights to cell proliferation control. J Clin Endocrinol Metab 2013;98:2887-96.
14. Lindboe CF, Torp SH. Comparison of Ki-67 equivalent antibodies. J Clin Pathol 2002;55:467-71.
15. Epstein JI, Egevad L, Amin MB, Dehaut B, Siglery JR, Humphrey PA, et al. The 2014 international society of urological pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma: Definition of grading patterns and proposal for a new grading system. Am J Surg Pathol 2016;40:244-52.
16. Cheng J, Lee EJ, Madison LD, Lazennec G. Expression of estrogen receptor beta in prostate carcinoma cells inhibits invasion and proliferation and triggers apoptosis. FEBS Lett 2004;566:169-72.
17. Grover SK, Agarwal S, Gupta S, Wadhwa N, Sharma N. Expression of estrogen receptor beta and Ki 67 in benign & malignant human prostate lesions by immunohistochemistry. Pathol Oncol Res 2015;21:651-7.
18. Fixemer T, Remberger K, Bonkhoff H. Differential expression of the estrogen receptor beta (ERbeta) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. Prostate 2003;54:79-87.
19. Gabal SM, Habib FM, Helmy DO, Ibrahim ME. Expression of estrogen receptor-beta (ER-B) in benign and malignant prostatic epithelial cells and its correlation with the clinico-pathological features. J Egypt Natl Canc Inst 2007;19:239-48.
20. Bonkhoff H, Fixemer T, Hunsicker I, Remberger K. Progesterone receptor expression in human prostate cancer: Correlation with tumor progression. Prostate 2001;48:285-91.
21. Kang MS, Park SY, Yoon HK. Estrogen and progesterone receptor expressions in benign prostatic hypertrophy and prostatic adenocarcinoma. Korean J Pathol 1998;32:346-51.
22. Grindstad T, Richardsen E, Anderssen S, Skjefstad K, Rakae Khanenkenari M, Donnem T, et al. Progesterone receptors in prostate cancer: Progesterone receptor B is the isof orm associated with disease progression. Sci Rep 2018;8:11358.
23. Song L, Shen W, Zhang H, Wang Q, Wang Y, Zhou Z. Differential expression of androgen, estrogen, and progesterone receptors in benign prostatic hyperplasia. Bosn J Basic Med Sci 2016;16:201-8.
24. Inwald E, Klinkhammer-Schalke M, Hofstadter F, Zeman F, Koller M, Gerstenhauer M, et al. Ki-67 is a prognostic parameter in breast cancer patients: Results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat 2013;139:539-52.
25. Johannessen AL, Torp SH. The clinical value of Ki-67/MIB-1 labeling index in human astrocytomas. Pathol Oncol Res 2006;12:143-7.
26. Kankuri M, Söderström KO, Pelliniemi TT, Vahlberg T, Pyrhönen S. Estrogen and progesterone receptor expression in benign prostatic hyperplasia. BJU Int 2005;96:1380-5.
27. Li S, Feng X, Li T, Zhang S, Zuo Z, Lin P. The 2014 international society of urological pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma: Definition of grading patterns and proposal for a new grading system. Am J Surg Pathol 2016;40:244-52.
28. Münstedt K, von Georgi R, Franke FE. Correlation between MIB1-determined tumor growth fraction and incidence of tumor lymphoma, nasal type: A report of 73 cases at MD Anderson Cancer Center. Am J Surg Pathol 2013;37:14-23.
29. Verma R, Gupta V, Singh J, Verma M, Gupta G, Gupta S, et al. Significance of p53 and Ki-67 expression in prostate cancer. Urol Ann 2015;7:488-93.