The Significance of ERG and Androgen Receptor Expression in Adenocarcinoma Prostate

Husain I,[1] Sagar P,[2] Shukla S,[3] Babu S,[4] Singhai A,[4] Sankhwar SN,[4] Husain N[3]

[1]Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh.

[2]ONGC Hospital, Dehradun.

[3]Dr. Ram Manohar lohia, Institute of Medical Sciences, Lucknow, Uttar Pradesh.

[4]King George's Medical University, Lucknow.

Corresponding Author

Saumya Shukla

Dr. Ram Manohar lohia

Institute of Medical Sciences,

Lucknow, Uttar Pradesh, India.

E-mail: saumyavarun@gmail.com

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ABSTRACT

Background

Fusions of transmembrane protease, serine 2 (TMPRSS2) with erythroblast transformation specific transcription factors have been found in prostate cancer. The v-etserythroblastosis virus E26 oncogene homologue (ERG) is a proto-oncogene of the erythroblast transformation specific transcription factor family. TMPRSS2-ERG fusion is the most common molecular alteration present in about 50% of prostatic adenocarcinomas. Androgen receptor (AR) plays a key role in prostate development and is involved in the progression of prostate cancer.

Objective

To evaluate the significance of combined ERG and AR expression in cases of prostatic adenocarcinoma.

Method

The study was conducted at Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. Formalin fixed-paraffin embedded archival prostatic tissue specimens were obtained. A total of 10 cases of prostatic adenocarcinoma were included in the study. Immunohistochemistry for Androgen receptor was done by the standard protocol. Multiplex immunohistochemical staining was done for ERG+CK5 using a primary antibody cocktail of mouse and rabbit antibodies.

Result

Specific AR immunostaining was exclusively nuclear and was present in all 10 cases in varying intensity. Specific ERG immunostaining was nuclear and was present in seven cases (70%) and absent in three cases (30%). The three cases that were negative for ERG had a Gleason score of ≤ 6 and the AR staining was strong and present in about 90% of the cells. Gleason score was directly related to the ERG staining while AR staining was inversely related to the ERG staining.

Conclusion

The prognostic value of combined ERG and AR over-expression, its associated genes should be further investigated as potential therapeutic targets in prostate cancer progression. Preliminary data is being presented. Larger prospective studies with survival analysis are essential for prognostic significance.

KEY WORDS

Androgen Receptor, Prostate Adenocarcinoma, v-etserythroblastosis virus E26 oncogene homologue

INTRODUCTION

The incidence of prostate cancer in India is relatively lower when compared with the western population. Androgen receptor (AR) plays a key role in prostate development and is involved in the progression of prostate cancer. Transmembrane protease, serine 2 (TMPRSS2) is an androgen regulated gene.[1] Fusions of TMPRSS2 with erythroblast transformation specific (ETS) transcription factors have been found in prostate cancer. The ETS family has numerous transcription factor proto-oncogenes, the commonest is the v-etserythroblastosis virus E26 oncogene homologue (ERG). TMPRSS2-ERG fusion is the most common molecular alteration present in about 50% of prostatic adenocarcinomas.[1,2] TMPRSS2-ERG fusion was first reported in 2005 by Tomlins et al. Recently, rare fusions of TMPRSS2 with other ETS variant (ETV) family members such as ETV1, ETV4, ETV5 and FLI1 have also been reported in prostatic adenocarcinomas. It is believed that TMPRSS2-ERG fusion is an early event in prostate oncogenesis and progression that results from either a small deletion on chromosome 2 or as a result of translocation. As a consequence of this fusion, the ERG gene becomes androgen regulated and is over-expressed in prostatic epithelium and in precursor prostatic intra-epithelial neoplastic lesions.[2,3]

The objective of the study was to evaluate the significance of combined ERG and AR expression in cases of prostatic adenocarcinoma.

METHODS

The study was conducted at Dr. Ram Manohar Lohia institute of Medical Sciences, Lucknow, Uttar Pradesh, India. Formalin fixed-paraffin embedded (FFPE) archival prostatic tissue specimens were obtained. A total of 10 cases of prostatic adenocarcinoma were included in the study. The study was performed after obtaining approval from the Institutional Ethical Committee of Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow Uttar Pradesh, India. A written informed consent was taken from all the participants of the study. The confidentiality was maintained and all further research related testing was performed on the formalin fixed paraffin embedded tissue only after the histopathological diagnosis was established. No additional intervention was performed.

The FFPE tissue blocks were sectioned at 3-4 µm, using a microtome (Leica, Germany), mounted on tissue bond-coated slides (Biocare, USA) and stained with hematoxylin and eosin (H&E) for histopathological evaluation and Gleason scoring was done.

Immunohistochemistry (IHC) for Androgen receptor was done by the standard protocol. The tissue section on coated slides was fixed overnight at 60°C in a dry oven, deparaffinized in xylene and rehydrated through graded ethanol series. Sections were blocked with 3% hydrogen peroxide in methanol for 30 min to quench any endogenous peroxidase activity, if present, and were then processed for antigen retrieval in Pascal (DAKO Cytomation, California) by placing in sodium citrate buffer (pH-6.0). Sections were incubated for an hour with anti-AR-pan, rabbit monoclonal (Biogenex, Hyderabad F39.4.1), followed by treatment with polymer-based secondary antibody kit (Dakopatts, Envision kit, Denmark). Bound antibody was visualized using diaminobenzidine, according to the manufacturer's instructions. Sections were counter-stained with hematoxylin and mounted. Positive and negative (by omitting primary antibody) controls were run with all batches.

The number and intensity of immunoreactive nuclei were assessed. Owing to the heterogeneous content of positive staining cells especially in cases of carcinoma, each slide was scanned at x 50 to identify the area with the highest staining. The intensity of staining was evaluated on a scale of 0-3, where 0= no staining, 1=weak staining (+), 2=moderate staining (++) and 3=strong staining (+++). The intensity grades were reported independently by two pathologists.

Multiplex immunohistochemical staining was done for ERG+CK5 (9FY+EP42, Biocare medical, USA) using a primary antibody cocktail of mouse and rabbit antibodies. The tissue section on coated slides was fixed overnight at 60°C in a dry oven, deparaffinized in xylene and rehydrated through graded ethanol series. Sections were blocked with 3% hydrogen peroxide in methanol for 30 min to quench any endogenous peroxidase activity, if present, and were then processed for antigen retrieval in Pascal (DAKO Cytomation, California) by placing in sodium citrate buffer (pH-6.0). Sections were incubated for 30min with the primary antibody ERG. The detection was performed using separate secondary antibodies for specific species. The horseradish peroxidise (HRP) detection system was followed by a denaturation step to avoid cross-reactivity of the second detection system. This was followed by incubation with the second primary antibody CK 5 for 30 min and detection was done using the alkaline phosphatase (AP) detection system. Visualisation was done using diaminobenzidine and red chromogen, followed by counterstaining with hematoxylin. Control samples were run with every batch. Benign prostatic tissue was used as a control for CK5 while the endothelial lining of blood vessels was used as a control for ERG. ERG stained the nuclei in cases of prostatic adenocarcinoma as brown while CK 5 stained the cytoplasm of the basal cells in normal prostatic tissue red (fig. 1)

Statistical analysis was performed using the IBM-Statistical Package for Social Sciences (SPSS, International Business Machines Corporation, New York, USA) analysis software, version 16. All P were calculated with two-sided tests and $p \leq 0.05$ was considered significant and highly significant when $p \leq 0.01$.

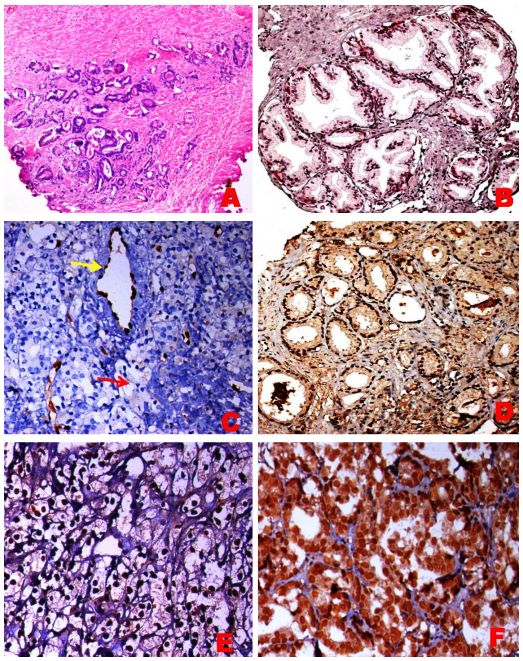

Figure 1. (A): Prostatic adenocarcinoma with small sized neoplastic glands and absence of basal cells (H&Ex100), (B) Benign Prostatic Hyperplasia with ERG+CK 5 dual stain highlighting the basal layer with red chromogen and absence of ERG staining (x100), (C): Absence of ERG+CK5 dual staining in prostatic adenocarcinoma [Red arrow] with positive staining for ERG in the endothelial lining of blood vessel [Yellow arrow] (x200), (D): Positive staining for ERG with absence of staining for CK5 in prostatic adenocarcinoma (x100), (E): Strong nuclear staining for AR in prostatic adenocarcinoma (DABx200), (F): Moderate nuclear staining for AR in prostatic adenocarcinoma (DABx200)

RESULTS

Specific AR immunostaining was exclusively nuclear and was present in all 10 cases in varying intensity. The intensity of AR staining in prostatic adenocarcinoma was moderate in four cases (40%) and strong in six cases (60%). Specific ERG immunostaining was nuclear and was present in seven cases (70%) and absent in three cases (30%). CK5 staining was absent in all cases of prostatic adenocarcinoma. The three cases that were negative for ERG had a Gleason score of ≤6 and the AR staining was strong and present in about 90% of the cells. This implies that the Gleason score was directly related to the ERG staining; this finding was statistically significant (p= 0.033) while AR staining was inversely related to the ERG staining (Table 1 and 2). However, this was not significant statistically. (p=0.200)

Table 1. Co-relation between Gleason score and ERG expression in cases of adenocarcinoma prostate

Gleason Score	ERG positive	ERG negative
≤ 6	**1**	**3**
≥ 7	6	0

p=0.033 using the Fisher Exact test.

Table 2. Statistical co-relation between AR and ERG expression in cases of adenocarcinoma prostate

AR staining strength	ERG positive	ERG negative
Strong staining	3	3
Moderate staining	4	0

p=0.200 using the Fisher Exact test.

DISCUSSION

TMPRSS2-ERG fusion is the most frequently identified chimeric gene and has been associated with undifferentiated and invasive phenotypes. TMPRSS2-ERG has also been detected in about 20% prostatic intraepithelial neoplasia (PIN) lesions and rarely in benign prostatic hyperplasia (BPH) regions mainly in areas adjoining prostatic adenocarcinoma.[4] In the study conducted by Berg, in the year 2016, the author stated that ERG status in prostatic adenocarcinoma in important in terms of prognosis and tumour progression.[5]

In our study, the expression of ERG directly correlated with the Gleason score. This finding was in concordance with the results of Lee et al. the authors stated that lower Gleason grade demonstrated higher rates of TMPRSS2-ERG fusion compared with high-grade tumours, including those demonstrating a large cribriform glands pattern.[6] Prostate cancer with large cribriform glands revealed rare TMPRSS2-ERG gene fusion.[6]

In the study conducted by Navaei et al. 77.3% cases harboured the co-expression of AR and ERG; and majority of theses case has a low Gleason score.[7] In certain studies, the expression of ERG and positive immunostaining inversely co-related with the Gleason score and was associated with higher tumour metastatic potential, early progression and bilateral disease. Rajput et al demonstrated that TMPRSS2-ERG fusion was more frequently present in poorly and moderately differentiated tumours when compared with well differentiated tumours.[8-10] While Kelly et al. observed no significant co-relation between ERG expression and the Gleason score.[3]

The association between Gleason score and ERG expression needs to be validated with studies that include large cohorts. Survival analysis and disease progression needs evaluation for proper co-relation. In this study, the cases of prostatic adenocarcinoma which were negative for ERG, demonstrated strong immunostaining for AR and had a Gleason score of ≤ 6. This finding is contradictory to the results of Huang et al. who stated that ERG positive/ AR positive cases had higher rates of prostatic cancer associated mortality.[11]

Rosenbaum et al. stated that AR expression was higher in cases with advanced and metastatic disease as compared to localised disease in both ERG positive and ERG negative

cases.[1] Bastus et al. reported that treatment with androgen can induce TMPRSS2-ERG fusion in both malignant and non-malignant prostate epithelial cells.[12] Although the fusion could be detected in malignant cells following 24 hours treatment, prolonged exposure to androgen was required to detect fusion transcript in non-malignant cells.[13]

Hence, it is essential to study the combined expression of ERG and AR in cases of prostatic adenocarcinoma prior to androgen deprivation therapy. The examination and correlation of ERG and AR have diagnostic significance and may be useful in assessing the biological character of the prostate cancer as well as selecting the best treatment with therapeutic molecular targets.[7] Multiple other miscellaneous factors that include diet, genetics and environmental factors play an essential role in the expression of both AR and ERG in cases of prostatic adenocarcinoma.[14] Additionally the co-expression of ERG/AR in prostate cancer could indirectly suggest that the patient harbors a gene fusion between ERG and a 5'-partner driven by androgen signalling such as TMPRSS2, SLC45A3 or NDRG1.[7]

The limitations of the study include the small sample size. The prognostic value of combined ERG and AR over-expression, its associated genes should be further investigated as potential therapeutic targets in prostate cancer progression. Interesting preliminary data has been communicated. Larger prospective studies with survival analysis are essential for prognostic significance.

CONCLUSION

The prognostic value of combined ERG and AR over-expression, its associated genes should be further investigated as potential therapeutic targets in prostate cancer progression. Preliminary data is being presented. Larger prospective studies with survival analysis are essential for prognostic significance.

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