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Original Article

Unlike prostate, ERG is not expressed in endometrial lesions

Peggy S Sullivan, Peter Shintaku, Madhuri Wadehra, Yalda B Naeini, Neda A Moatamed

Department of Pathology and Laboratory Medicine, School of Medicine, UCLA David Geffen, Los Angeles, CA, USA

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Abstract: Avian v-ets erythroblastosis virus E26 oncogene homolog (ERG) is highly sensitive and specific for endothelial neoplasms. ERG overexpression is considered a novel diagnostic marker for prostate carcinoma. However, little is known about its role in endometrial cancer. In the current study, we examined the potential of ERG transcription factor as an immunohistochemical marker in endometrial tissues. Formalin-fixed paraffin-embedded endometrial tissues obtained from 535 cases were used to constructan endometrial tissue microarray. The tissue microarray included a range of histopathologies including benign endometrium (n=231), simple hyperplasia (n=105), complex hyperplasia (n=36), simple atypical hyperplasia (n=10), complex atypical hyperplasia (n=44), and endometrial carcinoma (n=109). All of the endometrial tissues were ERG-negative, despite the strong positivity in endothelial cells.These results suggest that, unlike prostate cancer, ERG does not play a role in endometrial carcinogenesis. Therefore, ERG expression would not be a useful diagnostic or prognostic marker for endometrial cancer patient screening.

Keywords: ERG, endometrium, hyperplasia, carcinoma

Introduction

Avian v-ets erythroblastosis virus E26 oncogene homolog (ERG) is a member of the ETS family of transcription factors. Its overexpression has high sensitivity and specificity for endothelial cells and high specificity for prostate carcinoma [1, 2]. The *ERG* gene, located on chromosome 21q22.3, fuses with transmembrane protease, serine 2 (*TMPRSS2),* and results in androgen receptor-induced overexpression of the ERG transcription factor in 40-70% of prostate carcinoma cases [3, 4]. TMPRSS2-ERG translocations are commonly seen with inactivation of the phosphatase and tensin homolog (PTEN) gene and recent studies suggest that these genetic alterations may be linked in prostate carcinogenesis [4-6].

Endometrial cancer is the most common cancer of the female reproductive organs in the United Stateswith an estimated 10,170 deaths per year [7]. Patients often present with postmenopausal bleeding and undergo endometrial biopsies that may reveal benign endometrium or hyperplasia before the development of cancer. Early detection approaches for endometrial cancer are lacking. Furthermore, studies have shown the poor reproducibility of endometrial carcinoma and its precursor lesions by routine histomorphology [8-10]. By identifying potential diagnostic IHC markers, earlier and reliable detection of carcinoma may be achieved.

ERG is a putative prognostic marker in prostate carcinoma [11, 12]. More recent studies demonstrate the use of ERG as an aid to distinguish high grade prostatic intraepithelial neoplasia from intraductal carcinoma [13, 14]. Like prostate cancer, certain types of endometrial carcinomas (e.g., endometrioid subtype) may express androgen receptor [15] and have PTEN genetic alterations [16], however little is known about ERG expression in endometrial carcinoma. ERG expression has been studied in a limited number of endometrial cancer cases [1, 2]. None to the authors' knowledge, however, have examined ERG expression in premalignant lesions such as endometrial hyperplasia. We

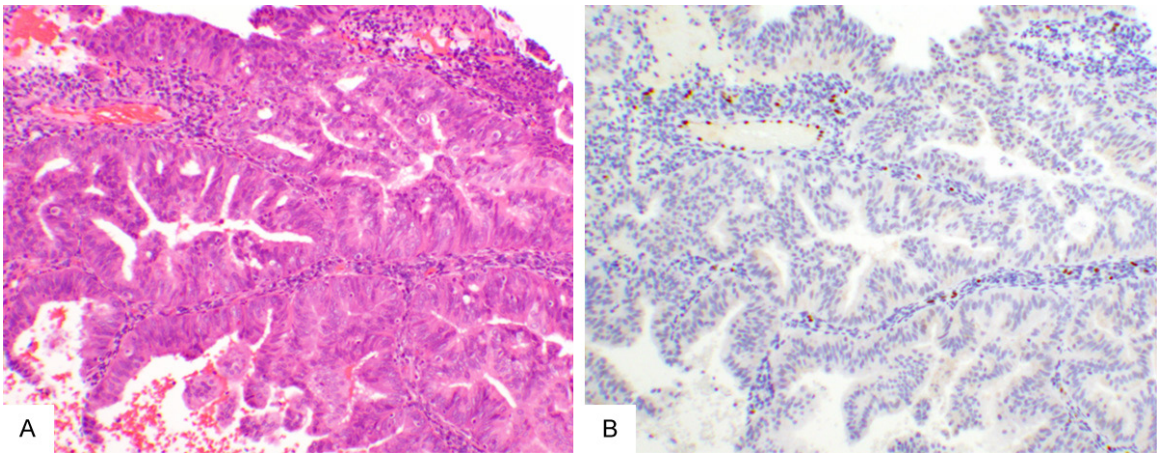

Figure 1. A. Shows the hematoxylin and eosin stained section of an endometrial carcinoma. B. Shows ERG immunohistochemistry of the same core showing negative stain in epithelial glands with positive nuclear staining of the vessels and focal positivity of the lymphoid cells (10× Objective).

sought to determine the incidence of ERG expression in a large number of benign, premalignant, and malignant endometrial tissues and evaluate its potential as a diagnostic and prognostic marker for endometrioid carcinoma.

Materials and methods

Tissue microarray

We used an early endometrioid carcinoma progression tissue microarray (TMA), as previously described [17, 18]. Briefly, 535 formalin-fixed, paraffin-embedded archival endometrial tissue samples were obtained from 207 patients by curettage, biopsy, or hysterectomy procedures dating from 1982 to 2002 (Department of Pathology and Laboratory Medicine, UCLA Medical Center). Institutional review board approval was obtained. Multiple specimens were collected from 150 patients during separate procedures, for a total of 457 'serial' specimens. Progression to endometrial cancer was observed in 46 patients with at least 1 year of follow-up. Three 1.0 mm diameter cores were sampled from each case for tissue microarray construction, as available, and primary histology was verified by a gynecologic pathologist (PSS). The primary histology included benign endometrium (n=231), simple hyperplasia (n=105), complex hyperplasia (n=36), simple atypical hyperplasia (n=10), complex atypical hyperplasia (n=44), and primary endometrial carcinoma (n=109). Benign endometrial cases included atrophic, weakly proliferative, menstrual, proliferative, secretory, disordered proliferative, polypoid endometrium, and endometrium with exogenous hormone effect (e.g., progestational therapy). All endometrial carcinoma cases were adenocarcinomas of endometrioid type.

Immunohistochemistry

Immunohistochemistry was performed on a Leica Bond III autostainer, using Leica Bond ancillary reagents/supplies and REFINE DAB detection system. Procedure involves antigen retrieval with Bond ER2 high pH epitope retrieval solution for 20 minutes at 95°C and mouse monoclonal anti-ERG (1:50 dilution, clone 9FY, Biocare Medical, #CM421, Concord, CA, USA) for 15 minutes. Subsequently, sections were incubated sequentially with post-antibody and horseradish peroxidase-based polymer for 8 minutes each. The chromogenic substrate was applied for 10 minutes followed by hematoxylin for 10 minutes. In addition to a positive control slide, vascular endothelial cells were used as an internal positive control. All TMA slides were reviewed by a gynecologic pathologist (NAM) for any nuclear or cytoplasmic ERG staining of lesional endometrial glandular epithelial tissue. Expression of ERG in the adjacent endometrial tissue was also evaluated.

Results

We examined the expression of ERG in a variety of endometrial specimens using TMA. Of the 535 tissue cores examined, none of the endo-

metrial glands showed expression for the ERG immunohistochemical stain, including all benign endometrium, hyperplasia with or without atypia, and endometrial carcinoma specimens. The spiral arteries and small vessels of the endometrial stromal showed strong diffuse nuclear staining in all endothelial cells. Scattered cells within the endometrial stroma (favor lymphocytes) showed strong nuclear staining as well. An example of the findings is shown in **Figure 1**.

Discussion

ERG is an important protooncogene with increased activity in various human malignancies such as Ewing's sarcoma and acute myeloid leukemia (AML) [19-21]. It has a regulatory role in endothelial cells and may be used as a diagnostic tool for vascular neoplasms [1, 2]. Recent attention has focused on ERG expression as a surrogate marker for the TMPSS2:ERG translocation and diagnostic marker for fusion-specific prostate carcinoma and its precursor lesions [11, 12, 22].

In our study, we have shown in a large number of cases that ERG protein expression is absent in endometrioid-type endometrial adenocarcinoma, its precursor lesions and normal endometrial tissue.

Our results suggest that ERG protein is not expressed in endometrial glandular epithelium and does not play a role in endometrial carcinogenesis.

Past studies with a smaller cohort of endometrial and other gynecologic tissues support these findings. Miettinen et al. studied ERG protein expression detected with immunohistochemistry using a mouse anti-ERG monoclonal antibody. Of the 14 differentiated endometrial adenocarcinomas, none had ERG expression. The same was true for 9 sarcomatous endometrial adenocarcinomas, normal adult endometrium and myometrium (unknown number of cases), and 29 ovarian/peritoneal carcinomas [1]. Yaskiv et al. studied ERG expression using a rabbit anti-ERG antibody and did not find any ERG expression in 4 endometrial endometrioid carcinomas and 5 ovarian papillary serous carcinomas [2]. Huang et al. reported a lack of ERG expression in 180 ovarian carcinomas [23]. Additionally, Scheble et al. did not shown a rearrangement of the ERG locus (either insertion or deletion) in 68 endometrial carcinomas, 42 uterine leiomyomata, 74 ovarian carcinomas or any epithelial neoplasm, other than prostate carcinoma [24].

ERG-associated translocations have been observed in cervical carcinoma cell lines [25]; however past studies have shown only rare expression in epithelial neoplasms other than prostate.

Miettinen et al. showed ERG expression in 1 of 42 pulmonary large cell undifferentiated carcinomas and 1 of 27 pleural epithelial tubulopapillary mesotheliomas. Cytoplasmic ERG staining was seen in occasional ductal carcinomas of the breast and membranous staining of thyroid papillary carcinomas. Miettinen et al. also showed positive staining in developing fetal mesenchymal tissue, blastic extramedullary myeloid tumors, and 2 of 29 Ewing sarcomas [1]. Yaskiv et al. demonstrated nuclear ERG staining in 1 of 65 urothelial carcinomas within the sarcomatoid component of the tumor. They also reported speckled nuclear staining in one thyroid medullary carcinoma, parathyroid adenoma, and small cell carcinoma of lung. For non-epithelial tumors, 5 of 6 fibrous meningiomas and 1 of 2 transitional meningiomas were also positive for ERG [2].

In summary, our study does not support the use of ERG expression as an immunohistochemical marker for endometrioid endometrial carcinoma, its precursor lesions, or normal endometrial tissue. Our findings suggest that ERG protein expression may not play a role in endometrial carcinogenesis. It further supports the specificity of the tumor in certain tissue types (e.g., vascular neoplasms and prostate cancer) as suggested in previous studies. Further studies are warranted to explore other endometrial carcinoma-specific immunohistochemical markers.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Neda A Moatamed, Department of Pathology and Laboratory Medicine, 10833 Le Conte Avenue, BOX 951732, 1P-241 CHS, Los Angeles, CA 90095-1732, USA. Tel: 310-825-

0581; Fax: 310-825-2483; E-mail: nmoatamed@mednet.ucla.edu

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