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Original Article

The oncogenic gene fusion TMPRSS2: ERG is not a diagnostic or prognostic marker for ovarian cancer

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Abstract: TMPRSS2:ERG is a gene fusion resulting from the chromosomal rearrangement of the androgen-regulated TMPRSS2 gene and the ETS transcription factor ERG, leading to the over-expression of the oncogenic molecule ERG. This gene rearrangement has been found in approximately half of all prostate cancers and ERG overexpression is considered as a novel diagnostic marker for prostate carcinoma. However, little is known about the role of the TMPRSS2:ERG gene fusion in ovarian cancer. The purpose of this study was to test ERG expression in ovarian cancer and its potential as a diagnostic marker for ovarian carcinoma progression. A tissue microarray containing 180 ovarian cancer tissues of various pathological types and grades were examined by immunohistochemical analysis for expression of ERG. We also used 40 prostate carcinoma tissues and 40 normal tissues for comparison in parallel experiments. ERG-positive expression was detected in 40% of the prostate tumor cancer, as well as in internal positive control endothelial cells, confirming over-expression of ERG in prostate cancer at relatively the same rate observed by others. In contrast, all of the ovarian tumor patient tissues of varying histologic types were ERG-negative, despite some positivity in endothelial cells. These results suggest that the oncogenic gene fusion TMPRSS2:ERG does not occur in ovarian cancer relative to prostate cancer. Therefore, development of ERG expression profile would not be a useful diagnostic or prognostic marker for ovarian cancer patient screening.

Keywords: ovarian cancer, prostate cancer, immunohistochemistry, ERG; androgens, TMPRSS2:ERG

Introduction

Transmembrane protease, serine 2 is a serine protease enzyme encoded in humans by the TMPRSS2 gene [1]. TMPRSS2 intracellular localization is primarily at the plasma membrane due to its type II transmembrane, receptor class A, and scavenger-receptor cysteine-rich domains, in addition to its protease domain. The protease domain of TMPRSS2 has been shown to undergo catalytic cleavage and extracellular secretion in prostate cancer cells [2], although the exact biological function of this gene is unknown [3]. What is known conclusively is that TMPRSS2 is up-regulated in androgen-sensitive prostate cancer, down-regulated in androgen-independent prostate cancer, and that oncogenic function in prostate cancer relies on gene fusion yielding over expression of E-twenty six (ETS) transcription factors, including ERG and ETV1 [4]. As tumors are composed of normal and malignant cells, including various stromal components such as endothelial cells, fibroblasts, inflammatory immune cells, and mesenchymal stem cells that interact with cancer cells in an ETS/ERG-dependent manner to promote proliferation and metastasis [9], further investigation into the role of ETS-family transcription factors as proto-oncogenes in multiple tumor settings will likely yield additional prognostic tools.

Multiple studies have shown that ERG (21q.22.2), an Ets-related gene and transcription factor member, is an important proto-oncogene with increased activity in various human malignancies, such as Ewing's sarcoma and acute myeloid leukemia, and has a regulatory role in endothelial differentiation, vascular development, angiogenesis, and vasculogenesis

[5-7]. Deleterious mutational events affecting the activation of transcription factors such as ERG, which bind to DNA to activate or inhibit transcription of adjacent genes, is associated with malignant transformation of normal cells into cancerous cells [8], directly promoting tumorigenesis. Therefore, the over-expression of the transcription factor ERG directly promotes tumorigenesis. Indeed, the oncogenic TMPRSS2:ERG fusion gene accounts for ERG over expression in 40% - 80% of prostate cancers in humans, and contributes to the transition toward androgen-independent prostate cancer through disruption of androgen receptor signaling [4]. Prostate cancers expressing the TMPRSS2:ERG fusion may have a more aggressive phenotype with a higher risk for prostate cancer related death, perhaps due to the increased ERG expression [10]. The ERG oncoprotein is also a diagnostic and prognostic marker for prostate cancers, useful in determining patient response to therapies and treatments, as well as in ascertaining risk for recurrence. Therefore, clinical prognosis may be affected by the detection of this gene product in ovarian cancer patients.

Ovarian cancer is the leading cause of female reproductive cancer related mortality [11]. Although the etiology of ovarian cancer is unknown, there are several risk factors, including genetic defects, family history, and age, which can increase the chance of developing ovarian carcinoma. However, the role of androgens in the development of ovarian cancer is currently not understood. It has been shown that menopausal estrogenic stimulation occurs due in large part to estrogenic storage in adipose tissue stimulated by androgens of adrenal and ovarian origin [12]. In vitro analysis has indicated that androgenic stimulation of ovarian cancer cells can lead to p27 degradation and cell cycle promotion, implicating androgen treatment of post-menopausal patients as a potential risk factor for cancer development [13]. Moreover, androgen treatment significantly increased ovarian cancer cell survival and proliferation, inducing the expression, activity and phosphorylation of telomerase in a phosphatidylinositol 3-kinase-dependent fashion [14], implying a potential role for androgens in ovarian carcinogenesis.

As expression of the oncogenic TMPRSS2:ERG fusion gene is a useful prognostic marker in prostate cancers [15], we sought to extend this research to ovarian carcinoma by utilizing retrospective immunohistochemical detection of ERG in a large ovarian cancer cohort containing varying histologic types, relative to progressive prostate tissue arrays, to determine whether a) ERG over-expression in ovarian tumor cells occurs and b) this over-expression correlates to prognostic patient outcome.

Materials and methods

Patients, clinicopathologic data, and ovarian cancer and prostate cancer tissue microarrays

180 representative tumor samples and clinical data were obtained from women diagnosed with ovarian cancer that had undergone initial surgery at The University of Texas MD Anderson Cancer Center. These samples and data were analyzed in this retrospective cohort study. The average age of the patients was 58.16 years old. 10 patients were diagnosed with clear cell carcinoma, 10 patients were diagnosed with endometrioid carcinoma, 3 patients were diagnosed with MMMT (malignant mixed Müllerian tumor), 2 patients were diagnosed with mucinous carcinoma, 149 patients were diagnosed with serous carcinoma, 3 patients were diagnosed with transitional cell carcinoma, and 3 patients were diagnosed with undifferentiated/poorly differentiated carcinoma. These diagnoses were graded on a scale of 1 to 3 and categorized as high-grade or low-grade. The stage was ranked on a scale of 1 to 11 and was assigned a FIGO (according to the International Federation of Gynecology and Obstetrics staging system) stage of 1 to 4 as well. This study also analyzed prostate tissues samples provided by the University of California, Los Angeles from a progressive tissue array that contained 80 cases. The tissue array contained normal prostate tissues and prostate tumor tissues that were categorized as normal prostate tissue, Gleason 6 prostate carcinoma, or Gleason 7 and greater prostate carcinoma. The tissue microarray blocks were constructed by obtaining one representative paraffin-embedded block from each patient, taking one core representative of each block, and embedding these samples onto a slide.

Immunohistochemical staining and analysis

The tissue microarray slides were subjected to

Table 1. Comparison of ERG expression in prostate and ovarian cancer tissues

Tissue Type	Number cases with ERG-positive cells	Number of cases with ERG -negative cells	% of ERG-positive
Normal prostate tissue	0	40	0
Gleason 6 prostate carcinoma normal tissue region	0	20	0
Gleason 6 prostate carcinoma tumor tissue region	9	11	45
Gleason >7 prostate carcinoma normal tissue region	0	20	0
Gleason >7 prostate carcinoma tumor tissue region	7	13	35
Clear cell carcinoma	0	10	0
Endometroid adenocarcinoma	0	10	0
MMMT	0	3	0
Mucinous adenocarcinoma	0	2	0
Serous carcinoma	0	149	0
Transitional cell carcinoma	0	3	0
Undifferentiated carcinoma	0	3	0

immunohistochemical staining by the core lab according to the manufacturer's protocol (Bond Max, Norwell, MA, USA). Briefly, tissue sections were deparaffinized using Bond dewax solution for 30 minutes at 72°C, rinsed with an alcohol series, rehydrated with PBS, subjected to 30 minutes of antigen retrieval pretreatment with Tris/EDTA buffers, washed, hydrophobically outlined, treated with 3.0% hydrogen peroxide to block endogenous peroxidase activity, washed, incubated for 8 minutes at room temperature with a 1:30 dilution of an antibody against ERG [21] (clone 9FY, Biocare Medical, Concord, CA, USA), washed, incubated for 8 minutes at room temperature with an HRP-conjugated anti-mouse/anti-rabbit IgG, washed, developed with 3,3'-diaminobenzidine (DAB) for 10 minutes, rinsed, treated with a DAB enhancer for 5 minutes, washed, counterstained with hematoxylin for 8 minutes, washed, dehydrated, and mounted and covered with cytoseal. Strongly ERG-positive prostate tumor tissues, and internal endothelial cell staining, were used as positive controls. ERG immunohistochemical staining was analyzed by two gynecologic pathologists (J.L. and J.Z.). The degree of staining was not quantified with a grading system for the ovarian tissue samples because all of the ovarian cancer tissues appeared ERG-negative (see results section).

Results

Patient characteristics

The median overall survival (OS) time for the 180 ovarian cancer patients was 3.3 years (95% CI: 2.7-4.0 years), and the overall survival rates were 56% (95% CI: 0.52-0.60) at 3 years, 34% (95% CI: 0.30-0.38) at 5 years, and 19% (CI: 0.16-0.22) at 10 years. The average age of the ovarian cancer patients was 58.16 years old.

ERG is not over expressed in ovarian cancer patients

First, we evaluated the veracity of our immunostain protocol using prostate cancer specimens as a positive control. Elevated ERG expression was observed in 40.0% (16/40) of prostate tumor tissue samples tested, 7 of which had a Gleason score of 7 or higher (**Table 1**). Intense nuclear immunostaining was observed in prostate cancer cells (**Figure 1A**), supporting previous studies that over-expressed truncated ERG is predominantly located in the nucleus [16]. Additionally, some prostate tissue samples were negative for ERG in the tumor epithelium, but positive within endothelial cells (data not shown). This may be due to endoge-

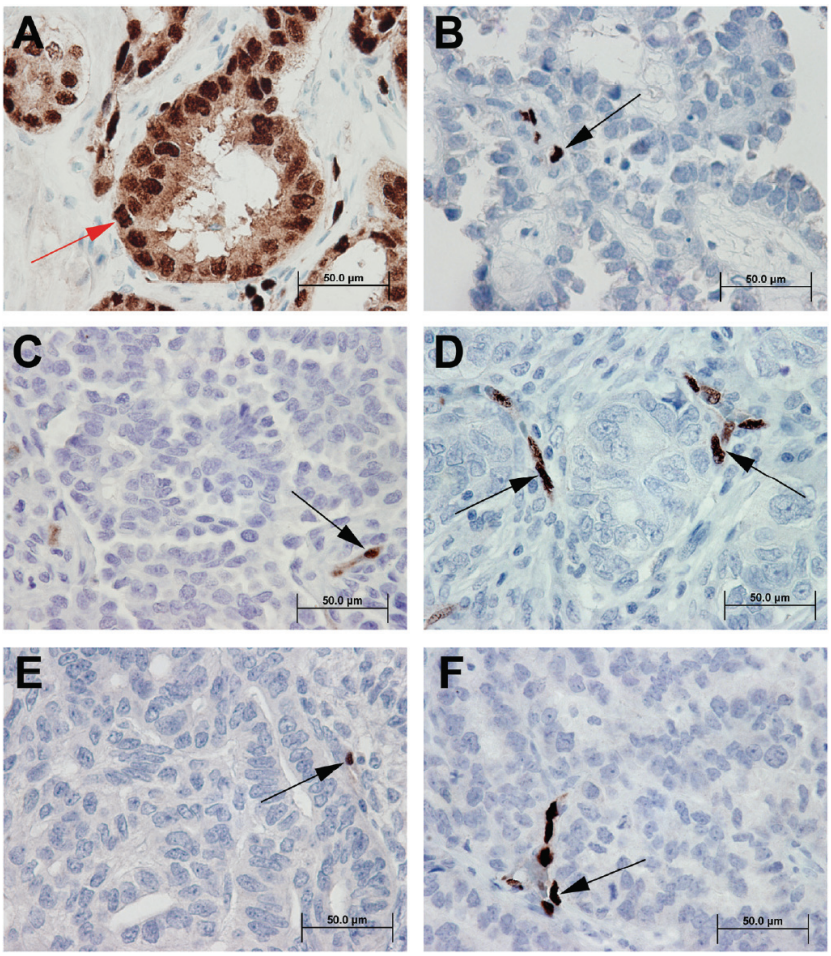

Figure 1 Immunoreactivity patterns of ERG in prostate carcinoma (**A**) and ovarian carcinomas (**B-F**). (**A**) Strong nuclear and weak cytoplasmic ERG-positive staining in prostate carcinoma. (**B-F**) Absence of ERG staining in clear-cell carcinoma (**B**), low-grade serous carcinoma (**C**), high-grade serous carcinoma (**D**), low-grade endometrioid carcinoma (**E**), and high-grade endometrioid carcinoma (**F**). Note the presence of ERG-positive staining in the endothelial cells of both prostate and ovarian tissue samples. Red arrow indicates cancer epithelial cells, black arrows indicate endothelial cells. Magnification is X400, scale bars = 50µm.

nous wild-type ERG that was also present in endothelial cells (vessels) and lymphocytes, where ERG is known to have a biologic function and is expressed during angiogenesis [17]. ERG detection in endothelial cells has been demonstrated, although its significance is unknown [18]. Notably, prostate cancer-associated fibroblasts were largely ERG-negative. In contrast, ERG immunodetection was negative in all ovarian cancer tissue samples, regardless of histotype, although some endothelial cells staining was detected (**Figure 1B-F**). There was no staining of benign epithelium in any of the cases.

Discussion

Our results indicate that the TMPRSS2:ERG gene fusion does not play a role in the progression of ovarian cancer and patient prognosis. Although the immunohistochemical detection of ERG is a useful tool for suggesting ERG rearrangement status and assisting in the diagnosis of prostate carcinoma, the absence of ERG expression in our ovarian cancer samples demonstrates that ERG cannot be used as a diagnostic marker for ovarian carcinoma.

Certain chromosomal translocations are related to the pathophysiologic development of disease. Several ETS transcription factors have been related to cancer through genetic defects such as gene fusion [19]. The ETS-family member ERG is highly expressed due to chromosomal rearrangements and fusion with the androgen-regulated TMPRSS2 gene [17]. ERG has been identified as the most over-expressed proto-oncogene in prostatic carcinoma and this over-expression is often caused by the fusion of the promoter region of the TMPRSS2 gene to ETS transcription factors [20]. The fusion gene TMPRSS2:ERG rearrangement caused by translocation and interstitial deletion (the more common mechanism) of DNA between the genes ERG and TMPRSS2 is present in approximately 50% of prostate cancers [21]. The TMPRSS2:ERG gene fusion is the most common gene rearrangement in prostate cancer and of the approximately 50% of prostate cancers that have this gene fusion, more than 90% over-express ERG [22]. This gene fusion affects the development of prostate cancer via the promotion of tumor cell invasion, activation of the C-MYC on-

cogene, and the abrogation of epithelial differentiation [22]. Others have shown that ERG oncoprotein overexpression also acts as a surrogate marker for androgen receptor signaling defects [23, 24]. TMPRSS2:ERG plays an important role in cancer progression by abrogating and disrupting lineage-specific differentiation of the prostate [3]. Therefore, the presence of this TMPRSS2:ERG gene fusion in prostate cancer is associated with increased risk for an aggressive phenotype during disease progression, and is an important diagnostic and prognostic marker that can be used to predict disease relapse for prostate cancer patients.

Expression of the ERG oncoprotein is correlated with the chimeric fusion transcript of TMPRSS2:ERG, and it is rarely expressed in normal epithelial tissues. However, both prostate cancer cells and tumor vasculature are positive for ERG expression, despite the absence of the TMPRSS2:ERG gene fusion in endothelial cells. This result may be explained by previous research which demonstrated that the TMPRSS2:ERG gene fusion product can bind to the ERG locus to drive the over-expression of wild-type ERG in prostate cancers [25]. Only 40% of the prostate tumor cases were ERG-positive in this study compared to the approximately 50% of ERG-positive prostate cancers reported by studies from other groups [21]. This lower frequency may be due to different prostate cancer tissue detection methods, differences in tumor location, as well as tumor heterogeneity [26]. Significant correlation between Gleason score and ERG-positive expression or TMPRSS2:ERG gene fusion status, and the relationship between the frequency of the gene fusion and the aggressiveness of the prostate cancer type, were not observed due limited information.

Androgens have been implicated in the development of several cancers, including prostate cancer, in which they can trigger genomic rearrangements. The TMPRSS2 gene contains androgen response elements in the promoter, and recent work shows that androgen exposure regulates the TMPRSS2:ERG gene fusion, and that prostate tumor cells with this fusion that are exposed to androgen increase ERG expression [21]. Conversely, other studies have shown that androgen ablation inhibits TMPRSS2:ERG expression. Androgen signaling plays an important role in prostate cancer survival and growth through ligand activation of the androgen receptor (AR). However, ERG can disrupt AR signaling by inhibiting AR expression; therefore, there is a correlation between TMPRSS2:ERG expression and AR [22]. AR is also one of the nuclear receptor transcription factors that mediates androgen behavior, influencing the development, maintenance, and progression of androgen-dependent tissues [27]. In summary, the fusion between the androgen-regulated TMPRSS2 gene and the members of the Ets transcription factor family (ERG, ETV1, ETV4) has been identified as a genomic aberration in prostate cancer.

Androgens also may play a role in the development of ovarian carcinoma. Androgens are major hormonal products of the ovary and serve as substrates for estrogen synthesis. Although androgenic-stimulation of AR has been shown to promote growth and differentiation of male urogenital structures, it is not essential for female reproductive organ development and fertility [28]. Normal ovarian epithelial cells secrete androgens and express surface AR, indicating that ovarian biology is influenced and regulated by androgenic stimulation as well as estrogenic. Thus, ovarian cancer risk may increase due to factors regulated by excessive androgenic stimulation of ovarian epithelial cells, and evidence supports the connection between the pathophysiology of ovarian cancer and androgen function [29]. High levels of androgens are associated with an increased risk for ovarian cancer, and the ovarian surface epithelium is an androgen responsive tissue, with androgens stimulating cell proliferation and decreased cell death, suggesting that androgens could be significant in the pathogenesis of ovarian cancer [28]. AR stimulation and function may influence follicular growth and maturation, atresia, and luteinization, further expanding its potential role in ovarian carcinogenesis [28]. However, our results suggest that TMPRSS2:ERG oncogenic gene fusion does not appear to play a role in ovarian cancer development.

In summary, all of the ovarian tumor cases that we tested were ERG-negative. Therefore ovarian cancer epithelial cells are unlikely to express the TMPRSS2:ERG fusion gene rearrangement, implying that ERG over-expression is not a useful diagnostic marker for ovarian cancer. Additionally treatments that target prostate tumor cells may not be effective against ovarian tumor cells, reinforcing the concept that the mecha-

nisms and pathways yielding cell transformation are not universal between disparate tissue settings.

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Conflicts of interest

The authors declare no conflicts of interest.

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