Gynecologic Oncology 130 (2013) 174–180

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Trefoil factor family 3 (TFF3) expression and its interaction with estrogen receptor (ER) in endometrial adenocarcinoma ☆

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HIGHLIGHTS

- TFF3 seems to be a novel gene pathway in the pathogenesis of endometrioid adenocarcinoma.
- There is strong association of TFF3 and ER in the estrogen-dependent endometrioid carcinoma.
- TFF3+ seems to forecast a good prognosis in EAC.

ARTICLE INFO

Article history:
Received 9 January 2013
Accepted 30 March 2013
Available online 8 April 2013

Keywords:
Trefoil factor family-3
Endometrial adenocarcinoma
Estrogen-receptor
Prognostic value

ABSTRACT

Objectives. TFF3 has been found to be up-regulated at the gene and protein levels in endometrioid adenocarcinoma (EAC) when compared to uterine serous carcinoma (USC) and normal endometrium. In addition, TFF3 has been proven to be an estrogen-responsive gene and its expression level positively correlated to estrogen-receptor (ER) status in breast cancer cell culture. The aims of this study are to determine the expression and the prognostic value of TFF3 in a large series of human endometrial cancer and its relation with ER.

Methods. We evaluated 328 endometrial carcinomas using TFF3 and ER antibody on paraffin-embedded tissue. 74% were type I (EAC), and 26% were type II (USC, CCC and carcinosarcoma).

Results. In type I carcinomas, $TFF3^+$ expression was associated with no lympho-vascular invasion ($p = 0.0131$), disease status ($p = 0.0132$), recurrence-free survival ($p = 0.0424$) and overall survival ($p = 0.0018$). There was a positive association between TFF3 and ER ($p < .0001$). The combination of $TFF3^+/ER^+$ was associated with low FIGO grade ($p = .0122$), early FIGO stage ($p = .0062$), absence of recurrence ($p = .0037$), absence of LVI ($p = .0011$), no lymph node involvement ($p = .0116$) and disease status ($p = .0107$). TFF3 appeared to be an independent prognostic marker in predicting recurrences ($p = .046$). In type II carcinomas, TFF3 failed to have a prognostic value.

Conclusion. 1-TFF3 seems to be a novel pathway in the pathogenesis of type I endometrial carcinomas. 2-The strong association of TFF3 and ER in the estrogen-dependent endometrioid carcinoma could explain the reason for its frequent expression by this tumor type. 3-$TFF3^+$ seems to forecast a good prognosis in type I endometrial carcinomas. Based on our data, TFF3 expression in endometrial cancer deserves further investigation.

Introduction

Endometrial adenocarcinoma (EC) is the most common gynecologic malignancy in developed countries. There are approximately 42,000 cases diagnosed annually in the United States, resulting in almost 8000 deaths [1]. EC has been classified into two types depending on morphology, pathogenesis, behavior and treatment: Type-I (endometrioid (EAC) and mucinous carcinomas), and type-II (uterine serous carcinoma (USC), clear cell carcinomas (CCC) and carcinosarcoma). Type- I carcinomas are usually low-grade and present at early stages upon presentation. They

☆ All authors declare that there are no financial disclosures, nor conflicts of interest.

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http://dx.doi.org/10.1016/j.ygyno.2013.03.030

are related to unopposed estrogen stimulation. Type II carcinomas are usually high grade and often present at advanced stages. High tumor grade, advanced stage disease, presence of lympho-vascular involvement (LVI), deep myometrial invasion (MI) and type II carcinoma constitute a high-risk group for recurrence and aggressive outcome in comparison to a low-risk group defined by low tumor grade, tumor confined to uterine corpus at presentation, absence of LVI, superficial MI, and type I adenocarcinoma [2–5]. The pathogenesis of endometrial cancer development is a multistep process of oncogene activation and tumor suppressor gene inactivation and specific molecular alterations are present in type I and type II carcinomas. Type I carcinomas are characterized by PTEN and KRAS mutations, and defects in DNA mismatch repair genes reflected by a microsatellite instability phenotype. Type II carcinomas are characterized by Her/neu-2 and P53 mutations [6,7].

Recent discoveries of genetic pathways and tumor biomarkers in endometrial carcinomas have opened new advances for the use of some of these biomarkers as targets for emerging therapies. Recently, the Gynecologic-Oncology Group (GOG) conducted a phase II clinical trial on patients with recurrent and persistent endometrial cancer using a targeted therapy such as bevacizumab, an angiogenesis inhibitor, resulting in what seemed to be promising survival data [8]. However, other drug trials which target these biomarkers and search for new biomarkers using cDNA microarrays and quantitative real-time polymerase chain reaction (qRT-PCR) are ongoing and it is likely that these findings can be translated to clinical use. Following this rationale, and using cDNA microarray, we previously found *TFF3* (trefoil family protein) to be up-regulated in EAC in comparison to USC which was confirmed at the RNA level using qRT-PCR [9].

TFF3 is located on chromosome 21q22.3 [10]. It belongs to the human trefoil factor family which consists of three small secreted proteins (TFF1, TFF2, TFF3) that are expressed by mucous-secreting epithelia. The normal function of TFF proteins is to maintain the integrity and repair of mucosal surfaces. TFF3 has been reported to be overexpressed at the gene and the protein level in human neoplasms such as prostate, breast, and colon cancer [11–14]. Previously, patients with EAC- grade 3 (G3) endometrial carcinomas were found to have higher TFF3 serum concentrations when compared to healthy patients [15]. An interaction between estrogen-receptor (ER) and TFF3 was found in breast cancer cell cultures where ER activation up-regulated TFF3 expression and TFF3 stimulation increased ER transcriptional activity. Therefore, suggesting that TFF3 is an estrogen-responsive gene and its expression is positively correlated with ER status [16,17]. However, TFF3 expression and its role in endometrial cancer are still widely unknown. Therefore, the aims of this study are to evaluate the expression and the prognostic value of TFF3 in a large sample of human endometrial cancers and to determine the interaction between ER and TFF3.

Materials and methods

Patient population

After obtaining IRB approval, the pathology archives were searched for endometrial carcinoma cases from January 2000–December 2009. Data was extracted from clinical charts including patients' age, surgical stage, post-operative treatment, site of recurrence and cause and time of death. All patients underwent surgical staging with a total hysterectomy and bilateral salpingo-oophorectomy (TAH + BSO), and pelvic washings. Pelvic and para-aortic lymphadenectomy was performed for patients with advanced stage disease and high grade tumors. Patients were treated according to the National Comprehensive Cancer Network guidelines (www.cancer.gov).

Histological evaluation

Tumor grade was assessed using the International Federation of Gynecology and Obstetrics (FIGO) system [18]. FIGO grading was determined as follows; tumors with <5% solid areas were grade 1 (G1), tumors with 5%–50% solid areas were grade 2 (G2) and tumors with >50% solid areas were grade 3 (G3). Nuclear grade was determined by the variation in nuclear size and shape, chromatin distribution and size of the nucleoli. Tumor stage was assigned based on 1988 FIGO surgical staging guidelines (FIGO, 1989) [19]. All slides were examined by an expert gynecologic pathologist for confirmation of the histologic type, tumor size, tumor grade, depth of MI and presence of LVI.

Tissue microarray and immunohistochemistry

Paraffin-embedded tissues from endometrial adenocarcinoma cases were used to construct a tissue microarray as described previously [20,21]. Punch biopsies of normal endometrial tissue from 23 (n = 10 proliferative phase; n = 7 secretory phase, n = 6 post-menopausal) patients were also taken for the array as normal controls. Briefly, carefully choosing the morphologically representative region on the chosen individual paraffin-embedded blocks (donor blocks), a core tissue biopsy of 0.6 mm was punched and transferred to the donor paraffin-embedded block (receiver block). To overcome tumor heterogeneity and tissue loss, 3 core biopsies were performed from different areas of each tumor. One section was stained with hematoxylin and eosin to evaluate the presence of the tumor by light microscopy.

For immunohistochemical analysis, 4 μm thick sections were deparaffinized with xylene, and washed with ethanol. Sections were cooled 20 min then incubated 10 min with 3% H_2O_2 to quench endogenous peroxidase activity. Blocking was performed using serum-free protein block, Dakocytomation (Carpenteria, CA) for 30 min. The sections were pretreated with an EDTA buffer saline solution and microwaved for 20 min, and then sections were incubated with ER (clone 6 F11, monoclonal, 1:100 dilution, Novacastra-UK) and TFF3 (monoclonal, 1:400 dilution, LifeSpan-USA) for 1 h at room temperature. The diaminobenzidine complex was used as a chromogen. Breast adenocarcinoma and normal colon tissue were used as positive controls for ER and TFF3 respectively. Negative control slides omitting the primary antibody were included in all assays. The stain was nuclear for ER, and cytoplasmic for TFF3. The extent of immunohistochemical reactivity was graded based on intensity as follows: 0 (negative), 1+ (weak), 2+ (moderate), 3+ (strong). For the statistical analysis, negative stains were grouped as group I (negative) and weak, moderate and strong stains as group II (positive). The Allred scoring system was used for ER evaluation [22]. The proportion of positive cells (scored on a scale of 0–5) and staining intensity (scored on a scale of 0–3) were assessed. The sum of intensity and percentage was done and produced total scores from 0 to 8. For statistical analysis, any score > 3 was considered as positive.

Statistical analyses

Statistical analyses were performed by R (http://www.r-project.org/). The clinical parameters used for modeling were age, tumor size, histologic subtypes, and depth of MI, LVI, FIGO grade, lymph node positivity, recurrence, disease status, recurrence time and survival time. To test the association between the biomarker, the clinical parameters, and ER status, Fisher's exact test was performed for categorical parameters and logistic regression model was used for the continuous ones. Multivariate analysis was performed by using the logistic regression model. Cox proportional hazards models were used to check the association between TFF3 and the overall survival and recurrence free survival. All reported p values are two sided. Only a p value of <0.05 was considered as significant.

Results

TFF3 and ER were positive in 62.36% and 63.55% of all 328 endometrial cancer cases, respectively. TFF3 was negative in 20 cases of