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Synuclein-γ (SNCG) protein expression is associated with poor outcome in endometrial adenocarcinoma

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ABSTRACT

Objective. Synuclein-γ (SNCG) is a marker for adverse and aggressive disease in breast cancer. In previous study, we found SNCG mRNA to be overexpressed in uterine serous carcinoma compared to uterine endometrioid adenocarcinoma. The aim of this study is to explore the prognostic value of SNCG in patients with endometrial cancer.

Methods. 279 endometrial cancer patients were retrieved from the archives. The tissue paraffin blocks were stained for SNCG antibody and its expression was correlated with clinicopathological prognostic factors.

Results. There was a positive association between $SNCG^+$ immunoexpression and tumor grade, tumor stage, type II carcinomas, deep myometrial invasion and lymphovascular invasion. A correlation between $SNCG^+$ and adverse outcomes, such as shorter overall survival (OS) and disease free survival (DFS), was also detected. Following adjuvant therapy (radiation and chemotherapy or chemotherapy alone), we observed a difference in 5 years DFS rate between $SNCG^+$ (41.6%) and $SNCG^-$ patients (59.5%).

Conclusion. Overexpression of SNCG seemed to be a predictor biomarker for aggressive tumor behavior and adverse outcome in patients with endometrial cancer. Future exploration of SNCG as a potential therapeutic target for selected patients could be of interest.

Introduction

Endometrial carcinoma is the most common gynecologic malignancy in the United States [1]. Endometrioid adenocarcinomas (EAC) account for more than 80% of cases, and they tend to present as low grade, early stage tumors with favorable outcomes. While uterine serous carcinomas (USC) represent a minority (3–10%) of total endometrial cancer cases, these are usually high grade tumors with deep myometrial invasion, lymphovascular involvement, and a more aggressive clinical course [2]. USC is responsible for a disproportionate number of deaths due to the fact that most of these tumors have already spread outside the uteri corpus. The 5-year survival rate for stages I–II EAC is estimated to range between 75 and 87%, and between 44 and 50% for stages I–II USC [3–4]. Numerous prognostic parameters have been implicated in endometrial carcinoma including tumor grade, tumor subtypes, tumor stage, presence of lymphovascular invasion (LVI), and depth of myometrial invasion [5,6,7]. High tumor grade, advanced stage disease, presence of LVI, deep myometrial invasion (outer third) and type II (USC and clear cell subtypes) endometrial adenocarcinoma constitute a high-group risk for recurrence and aggressive outcome in comparison to low-risk group defined by low tumor grade, tumor confined to uterine corpus at presentation, absence of LVI, superficial myometrial invasion (inner third), and type I (EAC and mucinous subtypes) endometrial adenocarcinoma. Besides these clinico-pathological factors, numerous biomarkers such as estrogen/progesterone receptors, bcl-2, catenin, Her2/neu and p53 were shown to predict prognosis in women with endometrial carcinoma [8,9].

Synuclein-γ (*SNCG*) (also known as breast cancer-specific protein 1) was initially cloned from infiltrating breast carcinoma cells [10]. This gene is located at the 10q23.2 locus and highly expressed in several cancer types such as advanced stage of ovarian, breast, liver, prostate and colon cancer [11–15]. It has been shown to promote cell growth, tumor invasiveness and metastasis and to interfere with drug-induced apoptosis [15,16]. In a recent study, we demonstrated the overexpression of SNCG mRNA in a number of USC tissue samples in comparison to EAC [17]. These findings suggested that SNCG merits further investigation both as a prognostic factor and as a therapeutic target. Thus, we examined SNCG protein expression using immunohistochemistry in paraffin-embedded tissues from 279 patients with endometrial cancers, all diagnosed and treated in

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one institution. We also analyzed the relationship between SNCG protein expression and clinicopathologic factors.

Materials and methods

Patients population

After obtaining the IRB approval, the Pathology archive at Roswell Park Cancer Institute, Buffalo-NY was searched for endometrial adenocarcinoma cases from January 2000 to December 2010. A chart review was conducted with extraction of clinical information including the patients' age at the time of diagnosis, the surgical stage, the post-operative therapy, the disease free survival (DFS), the site of recurrence, the cause and the time of death. All patients underwent a surgical staging procedure including an abdominal hysterectomy with bilateral salpingo-oophorectomy, with or without pelvic and para-aortic lymph nodes dissection and pelvic washing, depending on the tumor grade and the tumor stage. Patients were treated according to the National Comprehensive Cancer Network (NCCN) guidelines (www.cancer.gov).

Histological evaluation

Tumor grade was assessed using the International Federation of Gynecology and Obstetrics (FIGO) system and tumor stage was assigned based on 1992 FIGO surgical staging guidelines [18]. All slides were examined by an expert gynecologic pathologist for confirmation of the tumor type, tumor size, tumor grade, depth of myometrial invasion (MI) and presence of lymphovascular invasion (LVI).

Immunohistochemistry

Four micrometer thick sections were deparaffinized with xylene, and washed with ethanol. Sections were cooled 20 min and 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, steamed for 20 min and then sections were incubated with synuclein-γ antibody (monoclonal; 1:20 dilution; R&D systems MN, USA) for 1 h at room temperature. The diaminobenzidine complex was used as a chromogen. We chose invasive ductal carcinoma as positive control and negative control slides omitting the primary antibody were included in all assays. The stain intensity was diffuse and homogenous throughout the tumor. The extent of immunochemical reactivity was graded based on intensity as follows: 0 (negative), 1+ (weak), 2+ (moderate), 3+ (strong). For the sake of statistical analysis, negative and weak stains were grouped as group I (negative) and moderate and strong as group II (positive). 10 cases of normal endometrium were included to evaluate synuclein expression. Examples of normal endometrium, positive and negative cases are illustrated in Fig. 1A–D.

Statistical analyses

Statistical analyses were performed by R (http://www.r-project.org/). The clinical parameters used for modeling are age, tumor size, histologic subtypes, myometrial depth of invasion, LVI, FIGO grade, recurrence, status, and survival time. To test the association between the biomarker and the clinical parameters, Fisher's exact test was performed for categorical parameters and Welch t- test was used for the continuous ones. For survival analysis, Kaplan–Meier method with log-rank test was used to calculate the cumulative survival time, and check both the overall survival (OS) and disease free survival (DFS) difference between the patients with the different biomarker status. Multivariate cox proportional hazard model was used to determine the hazard ratio that represents the relative risk of death among patients with $SNCG^+$ compared with those with $SNCG^-$. All reported p values are two sided.

Results

The clinical and pathologic features of 279 patients with endometrial adenocarcinoma are summarized in Table 1. All patients (n = 279) had surgery for endometrial cancer with no previous chemotherapy or

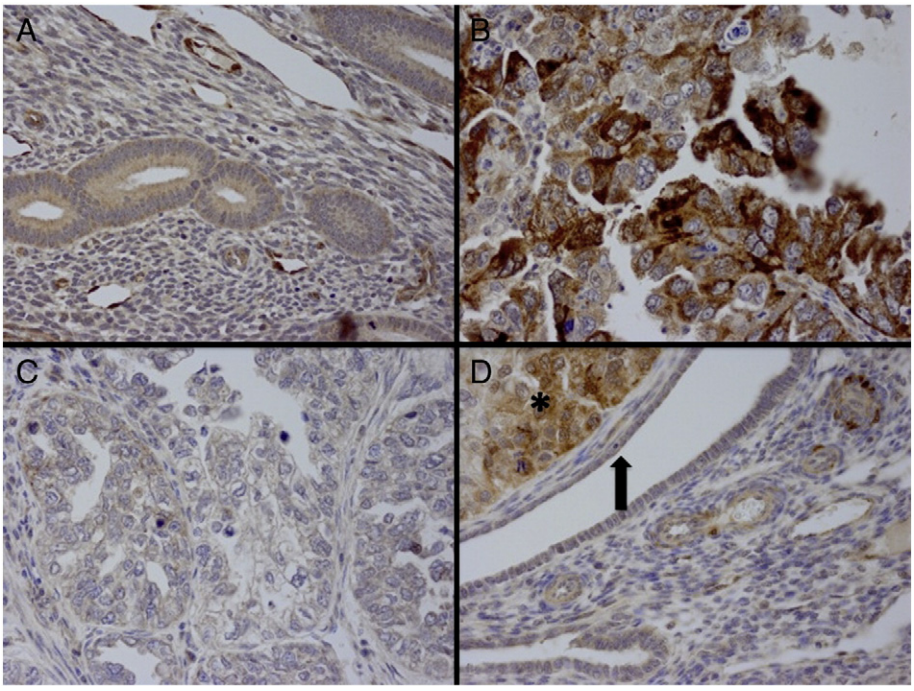

Fig. 1. A: Normal endometrium showed negative expression for synuclein (magnification ×40). B: Uterine serous carcinoma with strong positivity for SNCG (magnification ×40). C: Clear cell carcinoma with negative immunoexpression for SNCG (magnification ×40). D: Endometrioid adenocarcinoma FIGOIII (left upper corner marked by asterisk) strongly expressing SNCG immunostain, while the normal glands (marked in an arrow) are negative for SNCG expression (×40).