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Distinguishing the progression of an endometrioma: Benign or malignant?

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ABSTRACT

Objective: To elucidate the immunohistochemical (IHC) differences of endometrioma tissues that may have the potential to progress to ovarian clear cell carcinoma (OCCC) by using KRAS, HNF1β, PIK3CA, PPP2R1A, and ARID1A as biomarkers.
Study design: This is a retrospective clinical study, which was conducted in an university hospital. The groups comprised 14 patients with endometrioma resection who later developed OCCC (non-healthy endometrioma-case group) and 66 patients with endometrioma resection who did not develop ovarian cancer in subsequent follow-ups (healthy endometrium-control group). IHC staining with KRAS, HNF1β, PIK3CA, PPP2R1A, and ARID1A antibodies was performed in paraffin blocks of endometriomas obtained in both groups. For KRAS, PIK3CA, PPP2R1A, and ARID1A, cell staining intensity on a scale from 0 (negative) to 3 (strongly positive), and for HNF1β, the percentage of stained cells (0–5) and the intensity of staining (0–3) were scored.
Results: KRAS, HNF1β, PIK3CA, PPP2R1A, and ARID1A were overexpressed in the case group samples compared with the endometrioma samples in the epithelial cells, and ARID1A and KRAS in the stroma were overexpressed in the case group samples compared with the matched control samples.
Conclusions: KRAS, HNF1β, PIK3CA, PPP2R1A, and ARID1A immunostaining scores in endometriomas previous to OCCC were significantly different than in endometriomas with no malignancy occurring in subsequent follow-ups, and were single predictors of OCCC. Hence, immunostaining with these biomarkers may be a method of identifying patients with endometrioma who have the potential to develop OCCC.

Introduction

Endometriosis is an enigmatic condition that is still not fully understood. There have been many studies on endometriosis, yet in spite of profound research, the pathogenesis is still unclear. The most broadly accepted theory was suggested by Sampson [1] who hypothesized that endometriosis originated from endometrial cells regurgitated through the fallopian tubes during menstruation. Sampson also defined the relationship between endometriosis and ovarian cancer for the first time with the theory of malignant transformation of endometriosis.

Endometriosis is a benign lesion but has the potential to become malignant and has similar characteristics to invasive cancer [2]. Patients with endometriosis are three times more likely to develop ovarian cancer [3,4]. Tumor suppressor genes and oncogenes have pivotal roles. Tumor suppressor genes code proteins involved in apoptosis and cell cycle regulation. If both copies of the genes are mutated, abnormal cells replicate and lead to cancer. Oncogenes are mutant genes. When they are activated, uncontrolled cellular growth and division contribute to the development of cancer. The carcinogenesis model categorizes epithelial ovarian cancer into two types. Endometriosis-associated ovarian cancers are included in Type I ovarian tumors. Type I ovarian tumors (low-grade serous, low-grade endometrioid, clear cell, and mucinous) are dormant, relatively genetically stable, and characterized by specific mutations of genes including KRAS, HNF1β, PIK3CA, PPP2R1A, and ARID1A

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[2,5–8]. Their expression can be found in the epithelium and stroma. In contrast, Type II ovarian tumors (high-grade serous and undifferentiated carcinomas, and malignant mixed mesodermal tumors) are highly aggressive, rapidly growing, and chromosomally unstable.

Malignancies associated with endometriosis most commonly evolve from an endometrioma [9]. It is known that two cancers may arise from endometrioma: ovarian endometrioid carcinoma and ovarian clear cell carcinoma (OCCC). Activation of oncogenic genes such as KRAS and PIK3CA, and inactivation of the tumor suppressor gene, ARID1A, seems to be the responsible mechanism for transformation into endometriosis-associated ovarian cancers [10,11]. However, the exact carcinogenic mechanisms and genetic issues linked to malignant transformation of an endometrioma remain unclear. OCCC is a common type of malignant transformation of endometriomas. The risk of direct malignant transformation has been estimated as 0.7–1.6% over an average of eight years [3,4]. The diagnosis of OCCC is based on morphologic and histologic features and is sometimes challenging. Common molecular genetic changes in OCCC are an activating PIK3CA mutation and a somatic inactivating ARID1A mutation.

In this study, we intended to elucidate the immunohistochemical (IHC) differences of endometrioma tissues that may have potential to progress to OCCC by using KRAS, HNF1β, PIK3CA, PPP2R1A, and ARID1A as biomarkers.

Materials and methods

Specimens

For this case-control study, we searched the medical records of the Pathology Department and identified 14 patients with OCCC who had a history of endometrioma resection because of benign reasons. We investigated the paraffin blocks of usual endometrioma samples of these 14 patients who later developed OCCC (non-healthy endometrioma). The patients with OCCC were matched with 66 controls who had a history of endometrioma resection but did not develop carcinoma in subsequent follow-ups (healthy endometrioma). The characteristics of patients are presented in Table 1.

The inclusion criteria for specimens were as follows: 1) presence of endometrial glands on the cyst wall with no signs of malignancy; 2) surgery indications were ultrasound findings (endometriomas >5 cm) and pain (dysmenorrhea, dyspareunia, dyschezia, chronic pelvic pain); 3) no signs of malignancy in ultrasound. The study Ethics Committee of Istanbul University School of Medicine approved the study (Istanbul, Turkey).

A collection of healthy endometriomas (n = 66) and non-healthy endometrioma tissue samples (n = 14) were subjected to IHC staining for KRAS, HNF1β, PIK3CA, PPP2R1A, and ARID1A to evaluate the timing of gain or loss of marker expression during the progression from endometrioma to OCCC.

Table 1
Characteristics of the patients.

Characteristics	Endometrioma (n = 66)	OCCC (n = 14)
Age, years (range)	23–38	24–41
Clinical indications for surgery and symptoms, number of patients (%)		
USG findings	45 (68.2)	8 (57.1)
Pain (dyspareunia, dysmenorrhea, dyschezia, chronic pelvic pain)	21(31.8)	6 (42.8)
Suggested treatment after surgery, number of patients (%)		
Oral contraceptive pills (OCP)	32 (48.4)	0
GnRH analog	10 (15.1)	0
Chemotherapy	0	14 (100)
No treatment	24 (36.3)	0

Immunohistochemistry analysis

IHC was performed on a selected representative paraffin block using a Ventana BenchMark XT Automated IHC/ISH staining module (Ventana Medical Systems Inc., Tucson, AZ, USA) in accordance with the manufacturer's protocol. All magnifications are at x40 and the antibodies have been validated for IHC. The antibodies used in this study are listed in Table 2. Two independent expert gynecologic pathologists evaluated the IHC staining using a quantitative scoring system.

Cell staining intensity was scored on a scale ranging from 0 (negative) to 3 (strongly positive) for KRAS, PIK3CA, PPP2R1A, and ARID1A. A positive expression pattern of KRAS and PIK3CA was accepted as membranous, whereas the staining pattern was cytoplasmic for PPP2R1A and nuclear for ARID1A. For HNF1β, the scoring method described by Allred et al. was used in line with the existing literature [12]. This is a semiquantitative grading system that indicates both the percentage of stained cells (0–5) and the intensity of staining (0–3).

Statistical analysis

All calculations were performed using SPSS 20.0.0 software (SPSS, Chicago, IL, USA) unless otherwise indicated. The Mann-Whitney U test was used to compare the mean IHC scores between the endometrioma and OCCC paraffin sections. Binary logistic regression analysis was used to quantify the effect of the studied biomarkers separately on OCCC outcomes. A P-value of less than 0.05 was considered statistically significant for all tests.

Results

Representative staining of the five biomarkers is presented in Fig. 1. For HNF1β and PIK3CA, we observed epithelial cell staining, but immunostaining was absent in the stroma (not shown) in both groups. For KRAS, PPP2R1A, and ARID1A, epithelial cell and stroma staining was observed in both groups.

Expression of KRAS and ARID1A both in epithelial cells and stroma, HNF1β in epithelial cells, PIK3CA in epithelial cells, and PPP2R1A in epithelial cells were significantly different (overexpressed) between the case group and control samples (all $p < 0.001$) (Table 3). Stromal expression of PPP2R1A was not significantly different (all $p > 0.05$).

We performed binary logistic regression analyses for biomarkers that presented statistically significant expression differences, namely KRAS, HNF1β, PIK3CA, and PPP2R1A, ARID1A (Table 4). The analyses revealed that KRAS expression in epithelial cells and stroma (epithelial cell odds ratio (OR): 6.47; 95% CI: [2.18–19.21];

Table 2
Antibodies used in the Study.

Antibody	Producer	Dilution
Anti-ARID1A Polyclonal antibody	Atlas Antibodies AB, Stockholm, Sweden	1/400
Anti-PPP2R1A Polyclonal antibody	ThermoFisher Scientific. Rockford, IL, USA	1/200
Anti-HNF1β Polyclonal antibody	Atlas Antibodies AB, Stockholm, Sweden	1/400
Anti-PI3KCA Monoclonal antibody	Bioscience, Concord, CA, USA	1/200
Anti-KRAS Polyclonal antibody	ThermoFisher Scientific, Rockford, IL, USA	1/200