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Expression of Jun activation domain-binding protein 1 and p27 Kip1 in endometrial polyps and uterine leiomyomas

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Summary

Aim: The expression, localization, and density of Jab1 and p27 in endometrial polyps and uterine leiomyomas and the associations between these diseases and Jab1 and p27 based on clinical outcomes were explored. *Materials and Methods:* Tissues from 28 patients (14 with endometrial polyps and 14 with uterine leiomyomas) were used to investigate the expression of Jab1 and p27 by immunohistochemical analysis from November 2011 to April 2014. *Results:* Jab1 exhibited positive immunoreactivity: 78.5% in the endometrial polyp group in glandular epithelial cells and 71.4% in the uterine leiomyoma group in smooth muscle cells. In contrast to Jab1, p27 exhibited negative immunoreactivity: 76.9% in the endometrial polyp group and 78.6% in the uterine leiomyoma group. The lesions were larger in cases of expressed Jab1 only, rather than in cases with positive or negative expression of both proteins. Tissues that were recurrent did not express p27. *Conclusions:* Jab1 was expressed in the glandular epithelium of endometrial polyps and stromal layer of uterine leiomyomas, while p27 was suppressed in both diseases. Positive expression of Jab1 and negative expression of p27 were related to lesion growth.

Key words: Endometrium; Jab1; Leiomyoma; Polyps; Proteins; Uterus.

Introduction

Endometrial polyps and uterine leiomyomas are common benign diseases found in more than 25% of women [1, 2]. Both endometrial polyps and uterine leiomyomas are benign lesions involving localized overgrowth into the endometrial surface or uterine cavity. Endometrial polyps are related to localized gland overgrowth, and the progression of a polyp is related to gland density [3]. Uterine leiomyomas proliferate as smooth muscle cells in the uterus [4, 5]. The diseases occur in similar locations and produce similar symptoms, including abnormal uterine bleeding; their size gradually increases depending on the patient's body mass index, age, and hormone levels [6, 7]. These diseases are considered to be risk factors for endometrial cancer because a polyp or leiomyoma could develop into malignant endometrial cancer [8]. Therefore, it is important to understand the mechanisms of, and differences between, the two diseases. Histopathological examination after surgery enables the analysis and diagnosis of a polyp or leiomyoma [9]. Cyclin-dependent kinase inhibitor 1B (p27 Kip1 or p27) is a protein inhibitor that controls cell cycle progression at G1. The protein is ubiquitinated by Jun activation domain-binding protein 1 (Jab1) following specific phosphorylation, and the 26S proteasome disaggregates p27 [10]. The relationship between Jab1 and p27 in benign and borderline diseases has been extensively explored. The present authors previously showed that Jab1 was more highly expressed in malignant and borderline ovarian tumors than in benign ovarian tumors. Jab1 expression is increased in endometriosis compared to non-endometriosis tissues. Jab1 also tends to be more highly expressed in progressive and aggressive diseases. In contrast, p27 tends to be expressed less in malignant ovarian tumors than in benign ovarian tumors. The expression of p27 is similar between endometriosis and non-endometriosis tissues. Furthermore, the expression of p27 is diverse in epithelial ovarian tumors and endometriosis [11, 12]. Progressive and aggressive diseases tend to involve greater Jab1 expression and p27 suppression. Endometrial polyps, uterine leiomyomas, and endometriosis are benign diseases, though endometriosis is associated with chronic inflammation outside the uterus. However, researchers have not yet compared endometrial polyps and uterine leiomyomas: two different benign diseases of the uterus. Therefore, the present authors used immunohistochemistry to investigate the expression, localization, and density of Jab1 and p27 in endometrial polyps and uterine leiomyomas. They also explored the associations between these diseases and Jab1 and p27 based on clinical outcomes.

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Table 1. — *Clinical characteristics of the study subjects.*

	Endometrial polyp Mean ± [a]SD / No. of patients (%)	Uterine leiomyoma Mean ± SD / No. of patients (%)	[b]*p* value
Total no.	14	14	
Mean age (years)	46.2 ± 7.2	45.3 ± 7.1	0.73
Height (m)	1.6 ± 0.05	1.6 ± 0.04	0.67
Weight (kg)	57.0 ±10.1	58.4 ±7.7	0.68
[c]BMI	23.0 ± 4.3	23.7 ± 3.2	0.59
Menopause			0.62
Pre-	12 (85%)	11 (78%)	
Post-	2 (15%)	3 (22%)	
Term births (number of persons)	1.4 ± 0.9	1.5 ±1.2	0.73
Tissue size (mm)			
Width	14.1 ± 6.4	32.9 ± 15.6	<0.001
Length	8.1 ± 4.4	25.8 ± 11.9	<0.001
Multiple-occurrence			0.014
Single	14 (100%)	9 (64.3%)	
Multiple	0	5 (35.7%)	
Concomitant disease			0.32
Endometrial polyp	0	1 (7.1%)	
[e]HPV	0	2 (14.3%)	
Leiomyoma	3 (21.4%)	0	
Menorrhagia	1 (7.1%)	2 (14.3%)	
None	6 (42.9%)	4 (28.6%)	
Past history			0.32
Endometrial polyp	0	2 (14.3)	
Leiomyoma	0	1 (7.1%)	
Ovarian cyst	1 (7.1%)	1 (7.1%)	
None	13 (92.9%)	9 (64.3%)	
Recurrence			0.09
Yes	1 (7.1%)	5 (35.7%)	
No	13 (92.9%)	8 (57.1%)	

[a]SD: standard deviation. [b]p-values were measured by Student t-test, when necessary data were analyzed using the chi-square test.
[c]BMI (body mass index) = weight (kg)/height (m)^2. [d]DUB: dysfunctional uterine bleeding. [e]HPV: human papillomavirus.

Materials and Methods

This study included 28 patients (14 with endometrial polyps and 14 with submucosal uterine leiomyomas) who were admitted to the Department of Gynecological Surgery at a tertiary university hospital from November 2011 to April 2014. Tissues were collected during surgery, including hysteroscopy, hysteroscopic endometrial polypectomy, and hysteroscopic submucosal myomectomy. Clinical data were collected from electronic medical records. The study was approved by the hospital's institutional review board (#SCHCM 2011-11-15-02). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution with the 1964 Helsinki. Informed consent was obtained from all individual participants included in the study.

Each patient's endometrial tissue sample was cut into ten paraformaldehyde solution-fixed and paraffin-embedded 4-μm-thick sections. Each section was deparaffinized and rehydrated. Slides were heated at 90–100°C for ten minutes with 0.1 M sodium citrate buffer. After cooling for one hour, the slides were pre-incubated in a humidified chamber with 5% normal goat serum to prevent non-specific binding for one hour. The slides were then incubated at 4°C with primary antibodies (anti-p27 Kip 1 (1:250) or anti-Jab1 (1:250) in a moist chamber overnight. Horseradish peroxidase-conjugated goat anti-rabbit IgG or anti-mouse IgG (1:5,000) was used as the secondary antibody. The reaction products were visualized with diaminobenzidine. Negative controls were produced by excluding the primary antibody, and only brown-colored cells were considered positive. The immune reactivities of Jab1 and p27 were estimated using the ImageJ program (National Institutes of Health). H scores were ranked based on the positively stained tissue area, from grades 0–2. The classification criteria for grades 0–2 were as follows: < 5%, grade 0 (none), 5–30%, grade 1 (low), and > 30%, grade 2 (high). Grades 1 and 2 indicated positive (+) expression, while grade 0 indicated negative (-) expression. All endometrial polyp and leiomyoma sections were reviewed by two experimenters with experience in gynecologic pathology.

Data analyses were conducted using SPSS Version 14.0. Numerical or categorical variables were analyzed with a one-way ANOVA, chi-square test, and Student's *t*-test. A p-value < 0.05 was considered to indicate statistical significance.

Results

Table 1 summarizes the clinical characteristics of the patients with endometrial polyps or uterine leiomyomas. The groups did not differ significantly in terms of basic characteristics, including age, height, weight, and body mass

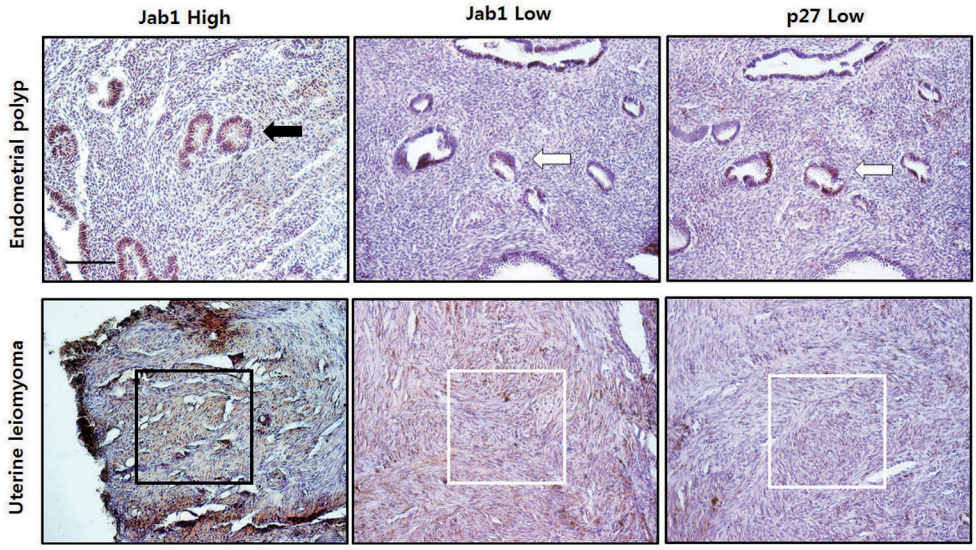

Figure 1. — Immunohistochemistry stains show expression of Jab1 and p27 in endometrial polyp and uterine leiomyoma. Jab1 is overexpressed at glandular of endometrial polyp (black arrow) and at stromal layer (black box) in uterine leiomyoma. Twenty percent of tissues low expressed Jab1 and p27 in the identical area (white arrow for endometrial polyp and white box for uterine leiomyoma). (×200, scale bar = 100 μm

index (BMI). Table 1 also lists variables including menopause, number of term births, tissue size, multiple occurrence, concomitant disease, past history, and recurrence. Overall, 85% of the endometrial polyp and 78% of the uterine leiomyoma cases occurred among premenopausal women. The mean tissue widths and lengths of the uterine leiomyomas were 32.9 ± 15.6 and 25.8 ± 11.9 mm, respectively. The mean tissue widths and lengths of the endometrial polyps were 14.1 ± 6.4 and 8.1 ± 4.4 mm, respectively. Multiple myoma was observed in five cases. The patients had various concomitant diseases and past histories, but these did not differ significantly between the two groups. The recurrence rates were 35.7% for the uterine leiomyoma group and 7.1% for the endometrial polyp group, but the *p*-value was not significant.

Figure 1 presents the immunohistochemical analysis results, comparing Jab1 and p27 expression between endometrial polyps and uterine leiomyomas. Jab1 and p27 expression was intensive in the glandular areas of endometrial polyp tissues and scattered in the stromal layers of uterine leiomyoma tissues. All brown-colored tissues were divided into three grades (high, low, and none) based on their H scores (Table 2). 'High' was defined as protein expressed in > 30% of the tissue area, 'low' as protein expressed in 5–30% of the tissue area, and 'none' as protein expressed in < 5% of the tissue area. Of the endometrial polyp samples, 57.1% were classified as having 'high' Jab1 expression, and 21.4% were classified as having 'low' Jab1 expression. Of the same samples, 7.7% were classified as having 'high' p27 expression and 15.4% were classified as having 'low' p27 expression. Of the endometrial polyp samples, 21.4% were classified as having no Jab1 expression ('none') and 76.9% were classified as no p27 expression ('none'). Of the uterine leiomyoma samples, 35.7% and 35.7% were classified as having 'high' and 'low' Jab1 expression, respectively, while 28.6% were classified as 'none.' For the uterine leiomyoma samples, 0, 21.4, and 78.6% were classified as having 'high,' 'low,' and no ('none') expression of p27, respectively.

Lesion length was investigated based on positive (+) or negative (-) protein expression to explore associations between the expression of the two proteins and the two diseases (Tables 3 and 4). The maximum length of each tissue sample was measured by ultrasonography to evaluate how the expression levels of Jab1 and p27 affected the sizes of the lesions. In the endometrial polyp group, 21.4% of the samples were positive for both Jab1 and p27, and the mean lesion size was 13.7 ± 3.8 mm. In the same group, 57.1% of the lesion samples expressed Jab1 only; their mean size was 14.3 ± 8.3 mm. Additionally, 21.4% of the lesion samples were negative for both proteins; their mean size was 14.0 ± 3.6 mm. Among the 14 cases of uterine leiomyoma, 21.4% had positive expression of both proteins, 50.0% had positive expression of Jab1 only, and 28.6% had negative expression of both proteins. The uterine leiomyoma samples had a mean lesion size of 32.0 ± 14.7 mm when they were positive for the expression of both proteins, a mean legion size of 33.7 ± 20.3 mm when they were positive for the expression of Jab1 only, and a mean size of 32.0 ± 9.2 mm when they were negative for the expression of both proteins. Lesions that expressed Jab1 only were the largest in both groups, but the *p*-values revealed no significant differences between the endometrial polyp and uterine leiomyoma groups. With regards to recurrence, endometrial polyps were recurrent in one case and uterine leiomyomas were recurrent in five cases. Of the five cases of

Table 2. — *Expression of Jab1 and p27 compared by expression grade of tissues.*

	Jab1							p27						
	High		Low		None			High		Low		None		
	n	%	n	%	n	%	*p* value	n	%	n	%	n	%	*p* value
Endometrial polyp	8	57.1	3	21.4	3	21.4	0.513	1	7.7	2	15.4	10	76.9	0.549
Uterine leiomyoma	5	35.7	5	35.7	4	28.6		0	0	3	21.4	11	78.6	

p values by chi-square test.

Table 3. — *Comparison of expression of Jab1 and p27 in endometrial polyp and uterine leiomyoma.*

	Endometrial polyp		Uterine leiomyoma		
Jab1/p27	n	%	n	%	*p* value
+/+	3	21.4	3	21.4	0.901
+/-	8	57.1	7	50.0	
-/+	0	0.0	0	0.0	
-/-	3	21.4	4	28.6	

p value by chi-square test.

Table 4. — *The average of lengthiest lesion size by level of Jab1 and p27 expression.*

	Endometrial polyp		Uterine leiomyoma	
Jab1/p27	Mean ± SD (mm)	*p* value	Mean ± SD (mm)	*p* value
+/+	13.7±3.8	0.990	32.0±14.7	0.982
+/-	14.3±8.3		33.7±20.3	
-/+	0		0	
-/-	14.0±3.6		32.0±9.2	

p value by one-way ANOVA.

recurrent uterine leiomyoma, three were positive for the expression of Jab1 only, and two were negative for the expression of both proteins (data not shown).

Discussion

The present authors found that Jab1 was expressed in more than 70% of glands with endometrial polyps and p27 was expressed in about 20% of the stromal layers in uterine leiomyomas. About 75% of the endometrial polyp and uterine leiomyoma lesions were negative for the expression of p27, but they did express Jab1. More than 50% of the tissues expressed Jab1 only, and the largest mean lesion sizes did not differ by disease. Lesion size and recurrence were not related to the expression of Jab1 or p27. Several previous studies investigated the proliferation of endometrial polyps and uterine leiomyomas. The development of endometrial polyps involves the overgrowth of glands with blood vessels and irregular proliferation of the epithelial lining. [13] Kim *et al.* [14] reported that uterine leiomyoma was linked with increased smooth muscle cells as well as reduced vessel density and glandular epithelia. The present authors previously demonstrated that Jab1 and p27 are related to endometriosis and epithelial ovarian tumors. [11, 12]. These proteins may be used to discriminate between endometriosis and borderline ovarian tumors. In endometriosis, Jab1 expression is known to increase, while p27 expression is similar in subjects with and without endometriosis. Jab1 expression is greater in borderline tumors than in benign tumors, and p27 expression is greater in benign tumors than in malignant tumors. Other studies have reported decreased p27 levels in tumors, and that its expression is linked with a poor prognosis [15]. Furthermore, p27 is expressed at lower levels in endometrial carcinoma than in normal endometrial tissues, and upregulated p27 can restrain the growth of human endometrial cells [16, 17]. Non-expression of p27 has been observed in many types of human cancer, including prostate, breast, cervical, and ovarian [18-20]. Therefore, the positive expression of Jab1 and negative expression of p27 might encourage the overgrowth of benign endometrial polyps and uterine leiomyomas. Additionally, endometrial polyps and uterine leiomyomas proliferate in the glandular epithelium and stromal layer, respectively, through expressed Jab1 and suppressed p27. The mean lesion size was larger in samples that expressed Jab1 only than in groups that expressed both Jab1 and p27 or groups that expressed neither protein. Jab1 overexpression and low levels of p27 are known to stimulate the progression and cause the rapid proliferation of some tumors, including bladder, thyroid, endometrial, and breast tumors [21, 22]. In endometrial cancer, tumor size is increased by dissemination and invasion [23]. This finding suggests that Jab1 expression is linked with lesion size. Regardless of Jab1 expression, p27 is expressed in many diseases, and its expression is similar between endometriosis and non-endometriosis tissues. In epithelial ovarian tumors, p27 is known to be more highly expressed in benign tumors than in borderline or malignant tumors [11]. Additionally, the expression of p27 was found to be downregulated in leiomyoma [15]. Together, these results

suggest that p27 expression is associated with many diseases, but its relationship with lesion size is not yet clear. Recurrence was observed in 35.7% of uterine leiomyoma cases. These tissues did not express p27, but negative expression of p27 was not statistically correlated with uterine leiomyoma. In atypical leiomyoma, the cell cycle markers p16, p21, p27, and p53 were not effective in predicting recurrence [24]. These results suggest that p27 expression is correlated with uterine leiomyoma. The expression levels of Jab1 and p27, and the sizes of the lesions, did not differ significantly between the endometrial polyp and uterine leiomyoma groups. Larger sample sizes are needed for future studies.

In conclusion, Jab1 was expressed at the glandular epithelium in endometrial polyps and at the stromal layer in uterine leiomyomas. Regardless of Jab1 expression, p27 expression was low or non-existent. The expression of Jab1 and non-expression of p27 affected lesion growth. The present results suggest that Jab1 and p27 play pivotal roles in the development of endometrial polyps and uterine leiomyomas.

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