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ORIGINAL ARTICLE

Function and clinical significance of SUMOylation in type I endometrial carcinoma

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Abstract

Objective This study elucidated the function and role of SUMOylation in type I endometrial carcinoma.
Methods Fifty type I endometrial carcinoma cases and para-cancer tissue samples were collected. The expression levels of ubiquitin-conjugating enzyme E2 I (Ube2i, Ubc9) and small ubiquitin-like modifier 1 (SUMO1)/sentrin-specific peptidase 1 (SENP1) proteins were examined using immunohistochemistry and the correlation with clinicopathological parameters was analyzed.
Results Ubc9 expression in type I endometrial carcinoma tissues was significantly higher than that in the para-cancer tissues; in contrast, the expression of the SENP1 protein was markedly lower than that in the para-cancer tissues. Ubc9 and SENP1 expression levels were negatively correlated and were associated with tumor differentiation, but not age, depth of invasion, tumor stage, and lymph node metastasis.
Conclusion SUMOylation modification plays a major role in the pathogenesis and development of type I endometrial carcinoma. Thus, it could be a potential target for the treatment of endometrial cancer.
Keywords SUMOylation; Ubc9; sentrin-specific peptidase 1 (SENP1); immunohistochemistry

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Endometrial carcinoma, a malignant epithelial tumor that primarily occurs in perimenopausal and postmenopausal women, is becoming one of the most common tumors of the female reproductive system. Furthermore, it is the third most common gynecological malignancy leading to death (underlying ovarian and cervical cancers). Histologically, endometrial carcinoma is classified as type I and II. Accounting for 90% of endometrial carcinomas, type I is a low-grade tumor associated with estrogen. Recent studies have shown the involvement of gene alterations including in phosphatase and tensin homolog (PTEN) and KRAS proto-oncogene, GTPase (K-RAS) as well as microsatellite instability in the pathogenesis of carcinoma. However, the underlying molecular mechanisms are unknown.

Small ubiquitin-like modifier (SUMO), which is a highly conserved protein is expressed as a small ubiquitin-related modification in eukaryotes [1] and regulates the function of target proteins by SUMOylation. This modification is mainly mediated by covalent binding with substrate proteins. The SUMOylation process requires the actions of the E1-activating and E2-conjugating enzymes and E3 ligases [2]. Previous studies have demonstrated that the only ubiquitin-conjugating enzyme, E2 I (Ube2i, Ubc9), plays a key role in the development of tumors [3]. SUMOylation is a dynamic and reversible process, and the SUMO-substrate combination can be separated by specific enzymes. Previous studies identified the expression of SUMO-specific proteases (SENPs), which are responsible for separating SUMO and its target proteins, in various tumor tissues and showed their participation in the pathophysiology of tumor development [4-6]. Studies found that SENP1 expression was significantly elevated in prostate, gastric, and breast cancer tissues

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[7-9]; however, no related reports on its expression and function in endometrial carcinoma are available. Thus, our prospective study used immunohistochemical staining to detect the differential expression of Ubc9 and SENP1 proteins in type I endometrial cancer tissues to evaluate the role of SUMO modification in ontogenesis and development of type I endometrial carcinoma.

Data and methods

Clinical data

Specimens from 50 patients with type I endometrial carcinoma and adjacent normal tissues were collected randomly after surgical resection in the patients at the Qingdao Municipal Hospital. The samples were confirmed by postoperative pathological diagnosis. These included 9, 16, and 25 cases with high, moderate, and low differentiation, respectively. The range and median age of the patients was 42–70 and 56 years, respectively, consisting of 14 and 36 patients who were < 50 and ≥ 50 years old, respectively. According to the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging method, 21, 20, 9, and 0, patients were in stages I, II, III, and IV, respectively, whereas 23 and 27 patients showed the presence and absence of lymph node metastasis, respectively.

Reagents

Rabbit anti-human SENP1 and anti-human Ubc9 antibodies (Abcam, UK), enzyme-labeled sheep anti-mouse/rabbit IgG polymer (Zhongshan Company, Beijing), animal serum (sheep, Maixin Biotechnology Co., Ltd.), and 3% hydrogen peroxide (Maixin Biotechnology Co., Ltd.) were used in this study

Test methods

The specimens were fixed for 24 h in 10% formalin, embedded in paraffin, and sliced into 6 μm thick sections, followed by xylene dewaxing, gradient alcohol dehydration, high-pressure repair, 3% hydrogen peroxide-based removal of endogenous enzymes, blocking with animal serum (sheep), and incubation with primary antibodies: rabbit anti-human SENP1 polyclonal antibody (1:400) and rabbit anti-human Ubc9 antibody (1:1000). Subsequently, the secondary antibody, enzyme-labeled goat anti-mouse anti-rabbit IgG/polymer was added, followed by 3, 3'-diaminobenzidine (DAB) staining, hematoxylin counterstaining, acid alcohol differentiation, dehydration, transparency treatment, sealing with a neutral gum piece, and microscopic examination. The testicular tissue was used as a positive control (according to the instructions from the SENP1 antibody manufacturer). Phosphate-buffered saline (PBS) was used as a negative control instead of the primary antibody.

Determination standard

The determination standard used was that proposed in the method of Liang *et al* [10], which specifies to observe the specimen 200 times under an optical microscope. Cells with distinct brown granules were considered positive. The Ubc9 protein was positively expressed primarily in the cytoplasm of the tumor and stromal cells and brown granules with different thicknesses and depths were also observed. The SENP1 protein was mainly positively expressed in the nucleus. Each tissue slice was randomly examined in 10 high-power visual fields and evaluated based on the depth of the cell color using a point scale that was divided into four grades: no color, 0 points; weak, 1 point; medium, 2 points; and strong, 3 points. The following percentages of positive tumor cells were assigned to the four grades; ≤10%, >10–30%, >30–60%, >60% to 0, 1, 2, and 3 points, respectively. The final result was the sum of two phases: 0-1 point, "-"; 2-3 points, "+"; 4-5 points, "++"; 6 points, "+++." The double-blind method was used to determine the final result, and each slice was assessed by two pathologists and re-enumerated when the difference was >10%.

Statistical analysis

The statistical package for the social sciences (SPSS) version 16 software was used to analyze the data using the Chi-squared (χ^2) test. Fisher's probability and Spearman's methods were used for the correlation analysis and a $P < 0.05$ was considered statistically significant.

Results

Expression of SENP1 protein in endometrial carcinoma and adjacent normal tissues

The Ubc9 protein was primarily expressed in the tumor and adjacent tissues. However, the positive expression level was significantly lower in the adjacent normal tissues [18% (9/50)] than it was in the tumor tissues [84% (42/50)]. In contrast, the positive Ubc9 expression level in the endometrial carcinoma tissue was significantly higher than that in the adjacent normal tissue was ($P < 0.001$, Table 1 and Fig 1).

Expression of SENP1 protein in endometrial

carcinoma and adjacent normal tissues

The SENP1 protein was primarily positively expressed in the adjacent normal and tumor tissues. However, the positive expression level was significantly lower in tumor tissues at 54% (27/50) than it was in the adjacent normal tissues at 100% (50/50). Thus, the positive SENP1 expression level in endometrial carcinoma was significantly lower than that in the adjacent normal tissue ($P < 0.001$, Table 2 and Fig 2).

Relationship between Ubc9 protein expression and clinicopathological features

The analysis of the relationship between Ubc9 protein expression and clinicopathological features showed that the expression was related to the degree of tumor differentiation ($P < 0.05$). However, no correlation was established with the patients' age, depth of invasion, FIGO stage, and lymph node metastasis ($P > 0.05$, Table 3 and Fig 1).

Table 1 Expression of Ubc9 protein in endometrial carcinoma and adjacent normal tissues (*n*)

Group	Case	Ubc9				χ^2 value	*P*-value
		-	+	++	+++		
Carcinoma	50	8	9	11	22		
Adjacent tissues	50	41	6	2	1	48.22	< 0.001

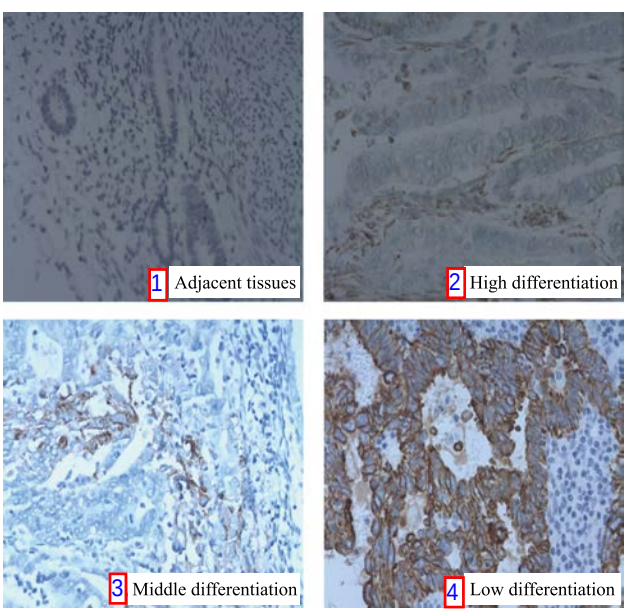

Fig. 1 Expression of Ubc9 in type I endometrial carcinoma and its adjacent tissues (HE, ×200)

Table 2 Expression of SENP1 protein in endometrial carcinoma and adjacent normal tissues (*n*)

Group	Case	SENP1				χ^2 value	*P*-value
		-	+	++	+++		
Carcinoma	50	23	3	6	18		
Adjacent tissues	50	0	5	7	38	30.72	< 0.001

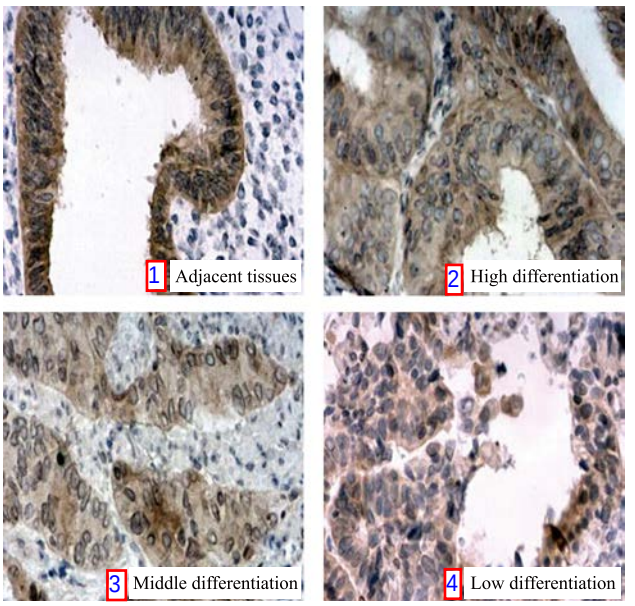

Fig. 2 Expression of SENP1 in type I endometrial carcinoma and its adjacent tissues (HE, × 200)

Table 3 Relationship between Ubc9 protein expression and clinical pathological features (*n*)

Group	Case	Ubc9				*P*-value
		-	+	++	+++	
Age (years)						
< 50	14	1	2	5	6	
≥ 50	36	7	7	6	16	0.425
Depth of invasion						
< 1/2 muscle layer	19	3	4	6	6	
≥ 1/2 muscle layer	31	5	5	5	16	0.472
Lymph node metastasis						
yes	27	4	4	7	12	
no	23	4	5	4	10	0.849
Tumor degree						
low	25	1	4	8	12	
middle	16	2	3	2	9	
high	9	5	2	1	1	0.021
FIGO Stage						
I	21	4	5	5	7	
II	20	3	3	4	10	
III	9	1	1	2	5	0.912

Table 4 Relationship between SENP1 protein expression and clinical pathological features

Group	Case	SENP1				*P*-value
		-	+	++	+++	
Age (years)						
< 50	14	7	2	3	2	
≥ 50	36	16	1	3	16	0.096
Depth of invasion						
< 1/2 muscle layer	19	8	1	2	8	
≥ 1/2 muscle layer	31	15	2	4	10	0.919
Lymph node metastasis						
yes	27	12	2	4	9	
no	23	11	1	2	9	0.863
Tumor degree						
low	25	18	1	3	3	
middle	16	4	2	2	8	
high	9	1	0	1	7	0.003
FIGO Stage						
I	21	9	0	2	10	
II	20	8	2	3	7	
III	9	6	1	1	0	0.292

Table 5 Correlation between Ubc9 and SENP1

	Ubc9	SENP1	r value	*P*-value
-	8	23		
+	9	3		
++	11	6		
+++	22	18		
Total	50	50	-0.20	0.006

Relationship between SENP1 protein expression and clinicopathological characteristics

The analysis of the relationship between SENP1 protein expression and clinicopathological characteristics revealed that the expression of SENP1 was related to the degree of tumor differentiation ($P < 0.05$); however, no association was noted with patient's age, depth of tumor invasion, FIGO stage, and lymph node metastasis ($P > 0.05$, Table 4 and Fig 2).

Correlation between Ubc9 and SENP1

The Spearman's analysis showed that the expression of Ubc9 was negatively correlated with that of SENP1 ($P < 0.05$, Table 5).

Discussion

SUMOylation modification is a dynamic and reversible process mediated primarily by activation, binding, and connection, which leads to modification [11]. Although SUMOylation and ubiquitination are similar, SUMOylation is more stable and cannot be degraded easily by a protease compared to ubiquitination [12]. The covalent link between SUMO and its target proteins requires a cascade of enzymes including; Ubc9, which is the only E2 conjugating enzyme and was first discovered in the lower animals [13]. E2 is correlated with cell meiosis and cell cycle regulation [14]. Ubc9 plays a major role in cell cycle and DNA damage repair, and these pathways are directly or indirectly related to tumorigenesis [2]. In the early stage of malignant cell transformation, the expression levels of Ubc9 and SUMO proteins were increased significantly [12]. Recent studies have also shown that Ubc9 is highly expressed in several tumors such as breast, ovarian, and lung cancers [15–16]; however, only a few reports are available about endometrial carcinoma.

The reverse reaction of SUMOylation modification, which separates SUMO and the target proteins, is termed deSUMOylation and this process is primarily mediated by SENPS [17]. SENP1 belongs to the SENP family of proteins and catalyzes the separation of numerous SUMO-target protein combinations. Although the specific mechanism of the action of SENPs in the pathogenesis and development of tumors is unclear, studies have shown that the disruption of SUMOylation balance promotes tumor occurrence and development. SENPs, as critical enzymes in the deSUMOylation process, play a vital role in maintaining the dynamics of SUMOylation [18]. Therefore, further studies on the function of SENPs and the mechanism underlying deSUMOylation would provide insights into the processes that balance SUMOylation modifications and contribute to elucidating the mechanism of tumor occurrence and progression.

Tumor growth requires nutrients and hypoxia, which is one of the factors affecting the supply of nutrients, is mainly mediated by the coordination of the hypoxia-inducible factor (HIF)-1α [19]. Cheng *et al* [20] reported that knockout of the SENP1 gene could enhance the SUMOylation of HIF-1α, leading to its αdegradation. Therefore, SENP1 gene knockout mice do not survive, which might be attributed to the lack of new blood vessels formation, thereby illustrating the vital role of SENP1 in tumor occurrence and development.

In this study, we used Ubc9 and SENP1 antibodies to examine SUMOylation in type I endometrial carcinoma and adjacent normal tissues. The results showed that the level of positive Ubc9 expression was significantly higher in the tumor tissue than that in the adjacent normal tissues ($P < 0.05$). The positive SENP1 expression level was

significantly lower in tumor tissues than that in the adjacent normal tissues ($P < 0.05$), indicating that SUMOylation considerably affected type I endometrial carcinoma occurrence and development. The present study also found that the expression levels of the two proteins were related to the degree of tumor differentiation ($P < 0.05$). Moreover, Ubc9 and SENP1 were negatively correlated and played a crucial role in the observed SUMOylation and deSUMOylation, respectively. However, whether this function is antagonistic or the proteins interactive with each to maintain the SUMOylation dynamics is yet to be studied. Furthermore, in advanced melanoma, inhibition of Ubc9 expression increases the sensitivity of melanoma to chemotherapy [21]. Therefore, whether SENP1 can be used to antagonize Ubc9 to improve tissue sensitivity to chemotherapy in endometrial carcinoma should be addressed. An intensive study of the mechanism underlying SUMOylation might identify a target for endometrial carcinoma treatment.

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