The correlation between expression of synuclein-γ, glucose transporter-1, and survival outcomes in endometrioid endometrial carcinoma

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Summary

Purpose: To evaluate the correlation between immunohistochemical expression of synuclein-γ, glucose transporter-1, and survival outcomes in endometrioid endometrial carcinoma. *Materials and Methods:* A tissue microarray was constructed using formalin-fixed, paraffin-embedded tissue that included 23 early and 18 advanced cases. The intensity and area of the immunohistochemical reactions were evaluated using the semi-quantitative scoring system. *Results:* Synuclein-γ expression was higher in the advanced stage, although it was not statistically significant (p = 0.51). Glucose transporter-1 was overexpressed in the advanced stage (p = 0.01). Synuclein-γ (score = 0 vs > 0) and glucose transporter-1 (score ≤ 7 vs > 7) did not show any differences in overall survival (p = 0.54, *p* = 0.48) and disease-free survival (p = 0.61, p = 0.14). *Conclusion:* In this study the expression of synuclein-γ and glucose transporter-1 were not considered to be a prognostic factor and were not related with survival outcomes in endometrioid endometrial carcinoma.

Key words: Endometrial carcinoma; Synuclein-γ; Glucose transporter-1.

Introduction

Endometrial carcinoma is the most common malignancy of the female genital tract in the Western world and the fourth most common cancer in women after breast, lung, and colon cancer [1].

There are two different subtypes of endometrical cancer recognized: estrogen-related (type I, endometrioid) and non-estrogen related (type II, non-endometroid). Approximately 75% of cases are classified as endometrioid adenocarcinomas. Other histologies include uterine serous carcinoma (5% to 10%), clear cell carcinoma (5%), and a variety of relatively rare carcinomas [2].

Synucleins, a family of neuronal proteins consisting of synuclein-α, synuclein-β, and synuclein-γ, are implicated in various neurodegenerative disorders [3, 4].

A strong correlation between synuclein-γ expression and metastasis is observed regardless of the cancer type. Also, in breast cancer, synuclein-γ is causatively linked to antimicrotubule drug resistance [5, 6].

Glucose transporter-1 is a member of Na+-independent glucose transporters. This protein is largely undetectable in normal epithelium and benign tumors, but is expressed in a variety of tumors including cervix, lung, gastric, and colorectal carcinoma. It is also associated with lymph node metastasis and poor prognosis [7-10].

This study was designed to evaluate the differences in the immunohistochemical expression of synuclein-γ and glucose transporter-1 in early- and advanced-stage endometrioid endometrial carcinoma, in order to determine the correlation between the expression of synuclein-γ and glucose transporter-1 with survival outcomes. Furthermore, the relationship of the expression of synuclein-γ and glucose transporter-1 with the clinicopathological parameters of endometrioid endometrial carcinoma was investigated.

Materials and Methods

This study was performed on formalin-fixed, paraffin-embedded tissue samples obtained from 41 women who were diagnosed with endometrioid endometrial carcinoma. These slides were reviewed by two pathologists.

All patients had been treated surgically with: total hysterectomy, bilateral salpingo-oophorectomy, pelvic or/and para-aortic lymph node sampling or dissection, and omentectomy. Patient chart review was performed retrospectively. Surgical staging was restaged by revised International Federation of Gynecology and Obstetrics (FIGO), 2009 criteria. Microscopic grading was based on the FIGO grading system. The cases were divided into two groups. Group 1 was early-stage and group 2 was advanced-stage.

A microarray instrument was used. Conventional hematoxylin and eosin (H & E) slides were reviewed and representative area without necrosis or hemorrhage were marked. Cores of paraffin, two mm in diameter, were taken from the tissue blocks at sites corresponding to the previously selected areas on the H&E slides and these cores were placed in empty blocks in the

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Table 1. — *Patients' characteristics and clinicopathologic parameters.*

	Group 1 (n = 23)	Group 2 (n = 18)
Age, mean (± SD), years	47.4 ± 9.05	55.6 ± 10.3
BMI, mean (± SD)	24 ± 2.9	24 ± 1.9
Stage		
I	23	
III		15
IV		3
Grade		
1	23	5
2		6
3		7
Preop CA 125, mean (± SD)	32.3 ± 40.5	123.1 ± 189.1
Tumor size, mean (± SD), cm	1.7 ± 1.22	5.25 ± 2.8
Cervical stroma involvement	0	18
Invasion depth, mean (± SD), mm	4.17 ± 4.5	14.1 ± 11.6
% of myometrium, mean (± SD)	16.7 ± 15.9	62.4 ± 36.0
Lymphovasscular invasion, n	1	8
Lymph node metastasis, n	0	7
Follow up period, mean (± SD), months	34.9 ± 20.5	26.8 ± 17.8
Recurrence or persistant disease	1	10
Disease-related death	1	7

Table 2. — *Immunohistochemical scores of synuclein-γ and glucose transporter-1.*

		Total			Area			Intensity		
		s	n	%	s	n	%	s	n	%
Synuclein-γ	Group 1	5	1	4.3	4	2	8.7	1	3	13
		6	3	13.0	5	2	8.7	2	1	4.4
	Group 2	5	3	16.7	2	2	11.1	1	3	16.7
		6	2	11.1	3	1	5.6	2	1	5.6
					4	1	5.6	3	1	5.6
					5	2	11.1			
		Total			Area			Intensity		
		s	n	%	s	n	%	s	n	%
Glucose transport-1	Group 1	6	9	39.1	5	22	95.6	1	9	39.1
		7	1	4.3				2	1	4.3
		8	12	52.2				3	12	52.2
	Group 2	7	3	16.7	4	1	5.6	1	1	5.6
		8	15	83.3	5	17	94.4	2	4	22.2
								3	13	72.2

(s: scores, n: number of patients).

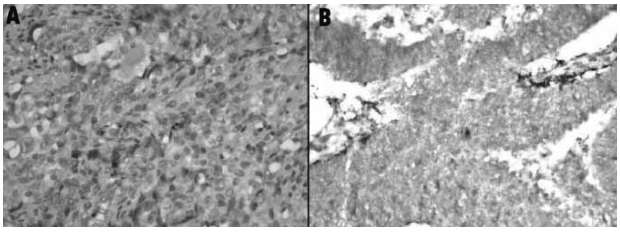

Figure 1. — Immunohistochemical expression of (A) synuclein-γ (B) glucose transporter-1. (A and B: brown cytoplasmic staining or membranous staining, X 400).

kit. The cores were punched at one mm intervals and arranged in a six by ten grid. The grid system in which each core had a coordinate reference (X-axis and Y-axis) was used to allow cross-referencing between core location and parent case. Once the microarrays were completed, all blocks were incubated at 60°C for 30 min. The slides were dewaxed and rehydrated prior to antigen retrieval.

Immunohistochemical staining was performed using automatic stainer. The tissue sections that were used measured four μm in thickness and were mounted on positively-charged slides.

Synuclein-γ and glucose transporter-1 were used as primary antibody. A section of normal appendix was used as the positive control and negative controls were obtained by omitting the primary antibody. An iView DAB Detection kit was used as secondary antibody and hematoxylin was used for counter staining. Synuclein-γ and glucose transporter-1 were interpreted as positive when adenocarcinoma cells showed cytoplasmic staining or membranous staining (Figure 1).

The immunohistochemical results were scored using a semiquantitative scoring system. This system assesses the percentage of positive cells (none = 0; < 1 % = 1; 1% to 10%, = 2; 10% to 33% = 3; 34% to 67% = 4; and > 67% = 5) and the intensity of staining (none = 0; weak = 1; intermediate = 2; and strong = 3). The intensity and percentage scores were added to give a final score of 0 to 8. These slides were reviewed by two pathologists.

The correlation between synuclein-γ and glucose transporter-1 with clinicopathological parameters such as FIGO Stage, grade, preoperative CA 125, endometrial tumor size, metastasis, and lymphovascular space invasion (LVSI) was analyzed using Spearman's correlation test and the differences in two groups were analyzed using a non-parametric and Mann-Whitney test. The Kaplan-Meier and log-rank test were used to analyze the relationship between the immunohistochemical expression of synuclein-γ and glucose transporter-1 and the survival outcomes. Statistical analysis was performed by the SPSS15 for Windows software. A p value < 0.05 was considered significant.

Results

Forty-one cases of endometrioid endometrial carcinoma, 2009 revised FIGO Stages I, III, and IV were retrieved from the Kyungpook National University Hospital cancer registry. All data is expressed as the mean ± the standard deviation. The mean age at the time of surgery was 47.4 ± 9.05 years in group 1 and 55.6 ± 10.3 years in group 2. In group 1, all 23 patients were in Stage 1, grade 1. In group 2, of 18 patients, 15 were in Stage III, three in Stage IV, and in histologic differentiation, five patients were grade 1, six grade 2, and seven grade 3. The preoperative levels of CA 125 were 32.3 ± 40.5 in group 1 and 123.1 ± 189.1 in group 2. The endometrial tumor sizes both groups were 1.7 ± 1.22 cm and 5.25 ± 2.8 cm, respectively. The depths of myometrial invasion were 4.17 ± 4.5 mm and 14.1 ± 11.6 mm, respectively. The percentages of myometrial invasion were 16.7 ± 15.9% and 62.4 ± 36.0%, respectively. In group 1, one patient showed LVSI and in group 2, eight patients showed the same. Postoperative pelvic or para-aortic lymph node metastasis was not observed in any of the patients in group 1 but was observed in seven patients in group 2. The follow-up periods of the two groups were 34.9 ± 20.5 months and 26.8 ± 17.8 months, respectively. Cases where the disease recurred or persisted after the operation

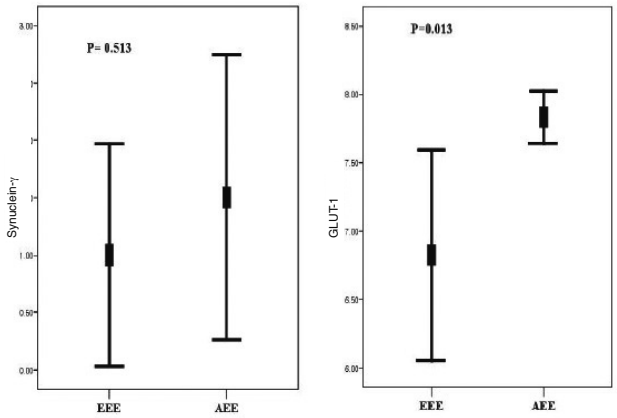

Figure 2. — Comparison of immunohistochemical expression of synuclein-γ and glucose transporter-1 in early and advanced endometrial endometrioid carcinoma (EEE: early-stage, AEE: advanced-stage).

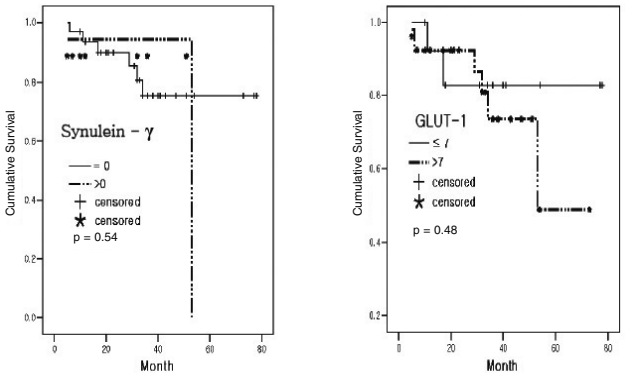

Figure 3. — Relation between disease-free survival and immunohistochemical expression of synuclein-γ, and glucose transporter-1 (GLUT-1).

included one patient in group 1 and ten in group 2. Disease-related deaths occurred in one patient in group 1 and seven in group 2 (Table 1). The immunohistochemical scores of synuclein-γ and glucose transporter-1 in two groups are shown in Table 2. The immunoreactivity values of synuclein-γ and glucose transporter-1 in the two groups are shown in Figure 2 as an error bar graph. Although synuclein-γ was not statistically significant, synuclein-γ (p = 0.513) and glucose transporter-1 (p = 0.013) showed higher immunoreactivity values in group 2. The interrelationships between synuclein-γ and glucose transporter-1 and six clinicopathological parameters: 1) histologic grades, 2) endometrial tumor sizes, 3) percentages of myometrial invasion, 4) invasion depths, 5) lymph node metastases, and 6) lymphovascular invasions were analyzed. Synuclein-γ and glucose transporter-1 showed positive relationships (p = 0.021). Glucose transporter-1 showed a positive relationship only with histological grade (p = 0.030).

Synuclein-γ did not show any differences in overall survival and disease-free survival between cases where it was expressed and cases where it was not expressed (p = 0.541, p = 0.061). Glucose transporter-1 also did not show any differences in overall survival and disease-free survival between cases where its total score was higher than 7 and cases where its total score was lower than 7 (p = 0.482, p = 0.141) (Figure 3).

Discussion

Synuclein-α, β, and γ expression were not detected by immunohistochemistry in normal ovarian epithelium. Eighty-seven percent of ovarian carcinomas were found to express at least one type of synuclein, and 42% expressed all three synucleins-α, β, and γ simultaneously. The expression was different in different histological types: 45.4% in undifferentiated types, 50% in endometrioid types, 66% in mucinous types and 85.4% in serous papillary types [11]. In uterine papillary serous carcinoma (UPSC) patients, synuclein-γ expression by immunohistochemistry (IHC), correlated with advanced-stage and decreased progression-free survival [12].

Recently, as the importance of antimicrotubule drugs in the treatment of gynecological adenocarcinomas has been emphasized, the importance of synuclein-γ has been considered. Antimicrotubule drugs have been considered to be the most important drugs for the treatment of advanced-stage endometrioid endometrial carcinoma, which accounts for the majority of endometrial cancers.

In this study, four of 23 cases (17%) showed expressions in group 1 and five of 18 cases (27%) showed expressions in group 2, thereby showing an entire expression rate of nine out of 41 cases (21.9%). There were no statistically significant differences between the two groups (p = 0.513). On reviewing this study and previous studies on ovary carcinoma and UPSC, it can be seen that papillary serous carcinomas are the most highly-related with the expression of synuclein-γ in two organs.

In the current study, the significant lower expression of synuclein-γ than the expression in ovarian carcinoma and insignificance as prognostic factor show the possibility that the lower drug resistance against antimicrotule drug will occur in endometrioid endometrial carcinoma, but further clinical study is needed.

The development of a malignant tumor is an energy-dependent process supported by increased glucose metabolism, which in turn produces a corresponding increase in glucose transporter proteins located in cellular membrane. Glucose transporter-1 mediates glucose uptake and thus facilitates anaerobic glycolysis. This protein is largely undetectable in normal epithelium and benign tumors but is expressed in a variety of tumors including cervix, lung, gastric, and colorectal carcinoma and is associated with poor prognosis [7, 8].

Haber *et al*. studied the association of glucose transporter-1 expression with the prediction of outcome for colon cancer, and they found that patients with carcinomas

with greater than 50% glucose transporter-1 positive malignant cells had a mortality rate two to three times higher than those whose tumors had less than 50% positivity.

They also showed that high glucose transporter-1 expression was also correlated with a greater frequency of lymph node metastases and concluded that the level of glucose transporter-1 expression might be an independent prognostic factor in colon cancer [10].

Similarly, in cervical carcinoma, a significant relationship between an absence of glucose transporter-1 expression and increasing the likelihood of metastasis-free survival was shown [7].

In this study, 40 of 41cases were strongly immunostained. Immunohistochemical scores were higher with a statistical significance in group 2 ($p = 0.013$). Glucose transporter-1 expression was directly correlated with tumor grades, although it did not show any significant correlation with other clinicopathological parameters. The expression was not related with lymph node metastasis and survival outcomes.

Conclusion

The expression of synuclein-γ was not correlated with clinicopathological parameters, could not be shown to have the role of a prognostic factor, and was not related to survival outcomes in endometrioid endometrial carcinoma. Glucose transporter-1 was strongly expressed in both early – and advanced-stages and showed significant difference in both groups. Furthermore it showed correlation with histologic grade; however, it did not show any difference in survival outcomes. In the future, further clinical studies evaluating the relationship between antimicrotubule drug resistance and survival outcomes according to expression of synuclein-γ and glucose transporter-1 in endometrial carcinoma are needed.

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