의학 석사 학위 논문

Expression of aurora kinases (aurora kinase A, aurora kinase B, phospho-aurora kinase A, and phospho-aurora kinase B) in uterine carcinosarcoma

자궁 암육종에서 aurora kinases 발현에 관한 연구

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Abstracts

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Introduction: Uterine carcinosarcoma (UCS) is an uncommon, biphasic uterine cancer which comprised of both a sarcomatous component (SC) and a carcinomatous component (CC). Despite some controversies on prognosis, CC is accepted as having potential for tumor progression and metastasis. As a driving force, CC induces sarcomatous metaplasia to form SC. Because CC has usually high mitotic count, expression patterns of mitotic kinase would be different between SC and CC. Aurora kinases are involved in separation and arrangement of chromosome, attachment of microtubule, and cytokinesis during mitosis. They regulate the G2/M phase, and contribute to carcinogenesis via abnormal cell proliferation and ceased apoptosis. The aim of this study is to investigate the expression pattern of aurora kinases according to the histologic components of UCS.

Methods: Typical areas for SC and CC were used to make tissue microarray cores. Immunohistochemistry was performed with antibodies for aurora kinase A (AURKA), aurora kinase B (AURKB), phospho-aurora kinase A (p-AURKA), and phospho-aurora kinase B (p-AURKB). The expression score was assessed by multiplying the intensity and cell proportion in nucleus and cytoplasm. For AURKA and AURKB, high expression was defined as ≥ 30% positive tumor

cells in the selected area. In p-AURKA and p-AURKB, samples with total score ≥ 6 were regarded as high expression. Medical records were retrospectively reviewed for clinical correlation.

Results: Histologic subtypes of SC were evenly divided into homologous and heterologous type. Most of the histology of CC was endometrioid (8/24, 33.3%), and others were serous (5/24, 20.8%), mucinous (1/24, 4.2%), adenosquamous type (5/24, 20.8%). Nuclear expression score of AURKB was much higher in CC than in SC (p=0.041). Polo-like kinase 1 expression was interrelated -with aurora kinases expression in cytoplasm and correlated with phosphorylated aurora kinases expression in nucleus. Nuclear expression of p-AURKA in CC was involved in tumor stage, lymph node metastasis, and omental involvement (p=0.027, 0.012, 0.037, respectively). Cytoplasmic expression of AURKB in CC and nuclear expression of p-AURKB in SC were correlated with positive vascular invasion (p=0.011 and 0.006).

Conclusions: The expression patterns of aurora kinases in UCS are involved in disease progression and vascular invasion. In addition, CC was the main part to express aurora kinases. This result supports the sarcomatous metaplasia, similar to the process of epithelial to mesenchymal transition. Inhibitors of aurora kinases are perspective chemotherapeutics for UCS.

Keywords: uterine carcinosarcoma, aurora kinase, immunohistochemistry

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I. Introduction

Uterine carcinosarcoma (UCS) is a biphasic tumor composed of a sarcomatous component (SC) from mesenchymal cells and a carcinomatous component (CC) from epithelial cells. It is rare, affecting 2 per 100,000 women annually (1-3). Five-year overall survival rate is about 25% to 39%, and disease-free survival rate is 11% (3). Some controversies have been existed about which component induces carcinogenesis and drives tumor progression. SC had been acknowledged as producing a poor prognosis of UCS since pure endometrial adenocarinoma showed favorable prognosis (4, 5). At present, CC is accepted as the main factor of tumor aggressiveness because of its high mitotic index (6). Revised 2009 FIGO staging was established based on this concept even if the new system is not likely to predict the survival rate of UCS (2).

UCS had been assumed to be either a collision tumor of two independent neoplasms or a combination tumor of two components originating from a single stem cell (5). Most experts support the conversion theory that SC originated from CC. Identical expression of p53 suggests that UCS has a monoclonal origin (7). CC, as a driving force of UCS, leads SC via sarcomatous metaplasia (4). In a sense, this process is analogous to the epithelial to mesenchymal transition (EMT) of the cancer stem cell (8, 9). The pathogenesis of UCS is regarded as a process of EMT (10). EMT also shares similarities with the underlying molecular alterations of metaplastic carcinoma of the breast, which is composed of ductal carcinomatous and trans-differentiated sarcomatous components (11).

There are two points to distinguish SC from CC: mitotic count and DNA ploidy. Mitotic count is usually higher in CC than in SC (6). Although epithelial neoplasm shows DNA diploidy, carcinosarcoma had aneuploidy subpopulation (12). In esophageal carcinosarcoma, sarcomatous area represented aneuploidy pattern, but carcinomatous area had diploidy pattern (13). Because both DNA ploidy and mitotic counts result from mitosis, expression patterns of mitosis-associated factors would be different in SC and CC.

Aurora kinases are kinds of serine-threonine kinases that are deeply involved in mitosis. There are three types of aurora kinases in human. At the centrosome, aurora kinase A (AURKA) initiates cells to enter mitosis if DNA damage is repaired (14). AURKA is associated with maturation and separation of centrosome, and arrangement of chromosome in metaphase (15). Aurora kinase B (AURKB) makes the cohesive form of sister chromatid in prophase, and attaches the kinetochore to the microtubule in metaphase. AURKB induces cytokinesis after telophase (14). Aurora kinase C (AURKC) is mainly involved in meiosis. There is little evidence that AURKC is connected to cancer. Polo-like kinase 1 (PLK1) is a spindle-assembly checkpoint and enables the functions of aurora kinases during mitosis (14).

Many previous reports demonstrated that aurora kinases were overexpressed in tumor cells. The aim of this study is to investigate the expression pattern of aurora kinases in the two histologic elements of UCS and to identify the clinical significance. We performed immunohistochemistry using the antibodies for AURKA, AURKB, and phosphorylated form of aurora kinases; phospho-aurora kinase A (p-AURKA) and phospho-aurora kinase B (p-AURKB).

II. Materials and Methods

II-1. Number of subjects

Samples were obtained from the patients with UCS who were diagnosed and underwent surgical procedure between 1997 and 2012 in Seoul National University Hospital. Level of significance was set to 0.05 (α=0.05), and power of the test was 80% (β=0.20). According to average and standard deviation of immunohistochemical expression of aurora kinases in previous literature (16), total 22 patients were needed (17, 18). Considering the drop-out rate, the number of patients was 25 according to PASS program. Though 25 samples of UCS were collected at first, one case was excluded from our study because it contained no proper component of CC. Consequently, the total number of subjects was 24. This study was approved by Seoul National University IRB: No. H-1208-097-422.

II-2. Production of tissue microarray

All tissue sections were stained with H&E method, and components of UCS were evaluated by two independent pathologists. Tissue microarray (TMA) was a validated tool to analyze the diagnosis, prognosis, and therapy for aurora kinases in uterine cancer (14, 19-21). To make TMA cores, two essential areas which were typically representative for CC and SC in one UCS sample were selected. Four-micrometer-thick sections of key slides were cut from paraffin-embedded samples. TMA was then created.

II-3. Immunohistochemistry

Sections were deparaffinized in xylene, then incubated in a dry oven at 60℃ for 1 hour. After deparaffinization, samples were dewaxed and hydrated at 72℃ for 3 minutes using alcohol for 3 times. Heat pretreatment was performed with epitomic retrieval solution at 100℃ for 20 minutes. Solution of pH 9.0 for AURKA and PLK1, and solution of pH 6.0 for AURKB were applied. With incubating for 5 minutes, endogenous peroxidase was blocked. After immunostaining using specific antibodies against AURKA (1:70, Santa-Cruz, SC-25425), AURKB (1:100, Santa-Cruz, SC-25426), and PLK1 (1:50, Thermo, PA5-15129) are incubated for 15 minutes, post-primary incubation was done for 8 minutes. The polymer was quenched with incubation for 8 minutes. DAB substrate was incubated for 10 minutes then counter hematoxylin method staining was done for 10 seconds. The bound antibody was detected using Bond polymer detection kit (Leica, Wetzlar, Germany). Sections were counterstained in hematoxylin for 10 seconds, then deparaffinized in xylene and hydrated with phosphate buffered saline (PBS). Antigen such as p-AURKA and p-AURKB was retrieved with pH 9.0 of cell conditioning1 solution at 100℃ for 60 minutes. Immunostaining was performed using specific antibodies against p-AURKA (1:200, Sigma, SAB4300270) and p-AURKB (1:100, Gentex, GTX85607) at 37℃ for 32 minutes. After primary antibody staining, the samples were ultrawashed with PBS. The bound antibody was detected using Ventana BenchMark XT Staining systems (Ventana, Basel, Switzerland). Samples were incubated for 4 minutes, followed by counterstaining with hematoxylin. Postcounterstain was performed with Bluing reagent in 4 minutes of incubation.

II-4. Analysis of expression pattern

Immunohistochemical intensity and cell proportion were analyzed in both nuclear and cytoplasmic area. All TMA cores were evaluated according to relative staining intensity (0, 1+, 2+, 3+) and proportion of stained cells (1, 0 to 5%; 2, 6 to 25%; 3, 26 to 50%; 4, 51 to 75%; 5, 76 to 100%) (21-23). A final score was calculated by multiplying the staining intensity and cell proportion in nuclear and cytoplasmic area (23). On the other hand, all samples for AURKA and AURKB were divided into two groups based on the proportion of positive staining cells. The samples which have more than 2+ of staining intensity and over 30% of stained cell proportion are indicated to high expression groups for AURKA and AURKB (24). The samples for p-AURKA and p-AURKB were classified into two groups based on the cut-off value of 6 (23). High expression groups of p-AURKA and p-AURKB were determined by expression scores ≥ 6. The comparison of immunohistochemical expression between SC and CC was analyzed with paired t-test for total score and McNemar test for expression groups.

Medical records of all patients with UCS were reviewed retrospectively. Analysis with clinical information for immunoexpression of aurora kinases was performed with t-test or Fisher's exact test. Statistical analyses were accomplished with SPSS software (version 18.0; SPSS, Inc., an IBM Company, Chicago, Illinois, USA). Null hypotheses of no difference were rejected if p-values were less than 0.05.

III. Results

III-1. Patients' characteristics

Twenty four patients were included in this study. They were diagnosed with UCS and underwent surgical management including total hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymphadenectomy, and omentectomy. Moreover, they were treated with adjuvant chemotherapy or radiation according to the surgical stage. Table 1 shows clinical characteristics of patients with UCS. Each UCS sample was analyzed in both CC and SC. In UCS samples, median proportion of SC was 6.5 times higher than that of CC (85%; range, 3-90 vs. 15%; range, 1-50). Mitotic index for 10 HPF were counted according to the elements of UCS. Median mitotic count for 10 HPF was 18 in SC and 12 in CC (p=0.253). Histology of SC was classified as either homologous or heterologous type. Pure homologous sarcoma is defined as sarcoma of uterine origin, whereas heterologous form is indicated to have distinct features of other organs. The types of heterologous sarcomas of our specimens are rhabdomyosarcoma, chondrosarcoma, and osteosarcoma in frequent order. The histology of CC was endometrioid (8/24, 33.3%), serous (5/24, 20.8%), mucinous (1/24, 4.2%), and adenosquamous type (5/24, 20.8%). This frequency is too common because the usual prevalence of adenosquamous pattern in endometrial carcinoma is 5% (25). However, a few CC cases were not clearly categorized in distinctive histology of endometrial cancer because of ambiguous features (5/24, 20.8%). Tumor size was measured by largest diameter, and median value of mass size was 5.5cm (range, 0-15).

Table 1. Patients' characteristics of uterine carcinosarcoma (UCS)

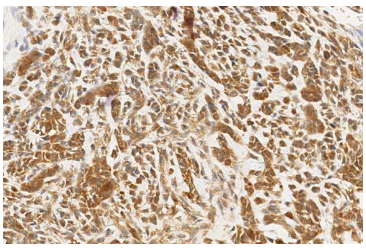
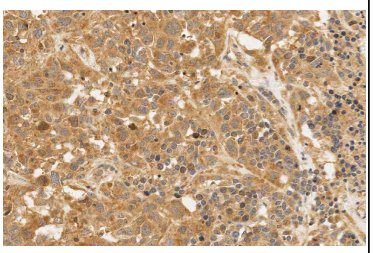
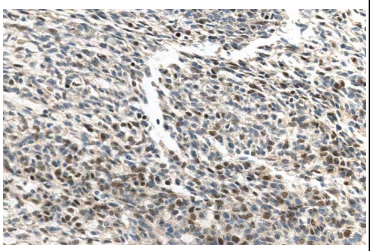
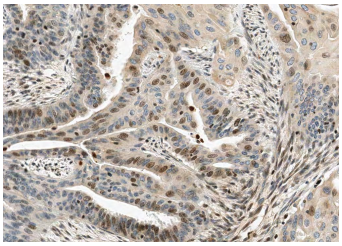
		Median (min - max)
Age (years)		65 (46 - 81)
BMI (kg/m^2)		24.9 (18.5 - 31.0)
		No. (%)
Histology of carcinomatous component (CC)	Endometrioid	8 (33.3)
	Serous	5 (20.8)
	Mucinous	1 (4.2)
	Adenosquamous	5 (20.8)
Histology of sarcomatous component (SC)	Heterologous	8 (33.3)
	Homologous	7 (29.2)
Myometrial invasion	Less than 1/2	10 (41.6)
	More than 1/2	14 (58.3)
Lymphatic invasion	Negative	13 (54.2)
	Positive	11 (45.8)
Vascular invasion	Negative	19 (79.2)
	Positive	5 (20.8)
Cervix involvement	None	19 (79.2)
	Stromal involvement	4 (16.7)
Adnexal involvement	Negative	18 (75.0)
	Positive	5 (20.8)
Lymph node metastasis	Negative	16 (66.7)
	Positive	4 (16.7)
Omentum involvement	Negative	19 (79.2)
	Positive	5 (20.8)
Peritoneal cytology	Negative	20 (83.3)
	Positive	4 (16.7)

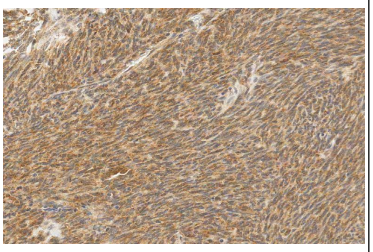
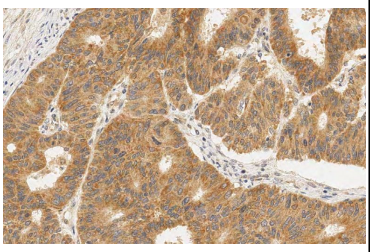
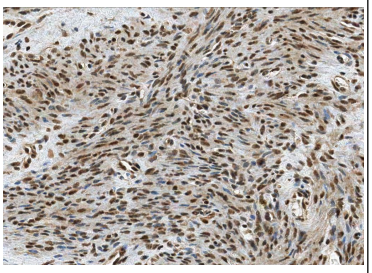
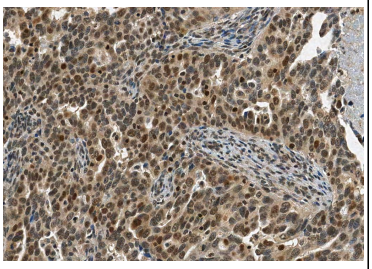
Abbreviations: UCS, uterine carcinosarcoma; CC, carcinomatous component; SC, sarcomatous component

III-2. Expression of aurora kinases and PLK1

Figure 1 illustrates the results of immunohistochemistry for the antibodies of aurora kinases in each histologic element. Table 2 represents both nuclear and cytoplasmic immnoexpression of aurora kinases in UCS samples. Many studies dealt with the immununohistochemical expression scores of AURKA and AURKB using semiquantitative system. Some found the nuclear expression pattern of aurora kinases (3, 23, 24, 26-29), but others discovered cytoplasmic expression of aurora kinases (1, 3, 22, 28-32). In this study, expression of AURKA and AURKB appeared at both nuclear and cytoplasmic area. However, p-AURKA and p-AURKB were definitely expressed in nucleus, not in cytoplasm. In table 2, nuclear expression score of AURKB was different in SC and CC (p=0.041). This tendency is equal to the group analysis for AURKB in table 3 (p=0.008). Nuclear expression of AURKB in CC was related with histology group of SC (p=0.033). The histology of SC of AURKB high expression group was heterologous type (9/10; 90.0%). In addition, homologous group of SC histology represents low expression of AURKB (8/9; 88.9%). PLK1 is the main molecule to work with aurora kinases during cellular mitosis. Table 4 shows the interaction between aurora kinases and PLK1 based on expression score. Expression of PLK1 is associated with cytoplasmic expression of AURKA and AURKB, whereas it is interrelated with nuclear expression of p-AURKA and p-AURKB.

Figure 1. The scores of immunohistochemical expression of aurora kinases are determined the rankings into high and low groups dependent on most appropriate cut-off value. High expression groups for AURKA and AURKB are regulated the samples which had over 30% of positive staining cells. For p-AURKA and p-AURKB, the group with more than 6 of immunoexpression score is set up to high expression group. This figures illustrates high cytoplasmic expression of AURKA and AURKB, and high nuclear expression of p-AURKA and p-AURKB (original magnification, 400X)

	Sarcomatous component (SC)	Carcinomatous component (CC)
AURKA		
p-AURKA		

AURKB		
p-AURKB		

Abbreviations: SC, sarcomatous component; CC, carcinomatous component; AURKA, aurora kinase A; p-AURKA, phospho-aurora kinase A; AURKB, aurora kinase B; p-AURKB, phospho-aurora kinase B

Table 2. Comparison of immunoexpression score of aurora kinases and PLK1 in both SC and CC

		SC	CC	95% CI	*p*
		Mean±SD	Mean±SD		
Nuclear expression score	AURKA	2.6±2.3	2.8±2.2	-1.17~0.76	0.659
	AURKB	1.1±2.6	2.5±3.3	-2.69~-0.06	0.041
	p-AURKA	5.5±3.5	5.1±2.5	-1.52~2.27	0.686
	p-AURKB	6.8±4.5	5.7±3.5	-0.38~2.55	0.139
	PLK1	2.5±3.9	2.2±4.0	-1.27~1.85	0.702
Cytoplasmic expression score	AURKA	4.3±4.0	6.1±5.1	-3.89~0.30	0.090
	AURKB	3.9±4.0	4.5±4.1	-2.65~1.31	0.493
	p-AURKA	0.5±1.4	0	-0.06~1.14	0.073
	p-AURKB	0	0	NA	NA
	PLK1	0.4±0.9	0.9±3.1	-1.95~0.87	0.433

Abbreviations: AURKA, aurora kinase A; AURKB, aurora kinase B; p-AURKA, phospho-aurora kinase A; p-AURKB, phospho-aurora kinase B; PLK1, polo-like kinase 1

Table 3. Expression analysis between immunoexpression group of aurora kinases in both SC and CC.

		SC		CC		*p*
		Low	High	Low	High	
		n (%)	n (%)	n (%)	n (%)	
Nuclear Expression group	AURKA	23 (95.8)	1 (4.2)	22 (91.7)	2 (8.3)	1.000
	AURKB	22 (91.7)	2 (8.3)	14 (58.3)	10 (41.7)	0.008
	p-AURKA	10 (41.7)	14 (58.3)	12 (50.0)	12 (50.0)	0.774
	p-AURKB	9 (37.5)	15 (62.5)	12 (50.0)	12 (50.0)	0.508
Cytoplasmic Expression group	AURKA	19 (79.2)	5 (20.8)	12 (50.0)	12 (50.0)	0.065
	AURKB	18 (75.0)	6 (25.0)	13 (54.2)	11 (45.8)	0.180
	p-AURKA	24 (100.0)	0	24 (100)	0	NA
	p-AURKB	24 (100.0)	0	24 (100)	0	NA

Table 4. Interrelation for immunohistochemical expression between aurora kinases and PLK1.

PLK1		SC		CC	
		95% CI	*p*	95% CI	*p*
Nuclear expression	AURKA	-1.42~1.58	0.909	-0.69~1.85	0.352
	AURKB	-2.84~-0.09	0.065	-1.68~2.26	0.762
	p-AURKA	1.44~4.56	0.001	0.89~4.95	0.007
	p-AURKB	2.35~6.23	<0.001	1.57~5.43	0.001
Cytoplasmic expression	AURKA	2.25~5.58	<0.001	3.08~7.25	<0.001
	AURKB	1.71~5.29	<0.001	1.55~5.70	0.001
	p-AURKA	-0.55~0.88	0.633	-2.23~0.40	0.163
	p-AURKB	-0.75~-0.01	0.047	-2.23~0.40	0.163

III-3. Clinical correlations with aurora kinases

In this study, neither nuclear nor cytoplasmic expression of AURKA were clinically significant. However, nuclear expression of p-AURKA in CC was correlated with tumor stage, lymph node metastasis, and omental involvement (p=0.027, 0.012, 0.037, respectively). All patients with positive lymph node metastasis and omental involvement belonged to the high expression group of p-AURKA in Table 5. Though the majority of patients with early stage were connected to the low expression group of p-AURKA, patients with advanced stage were corresponded to high expression group. Table 6 shows the clinical significance of expression pattern of AURKB. In CC, cytoplasmic overexpression of AURKB was significantly correlated with positive vascular invasion (p=0.011). Cytoplasmic expression score of AURKB was also related with vascular invasion (p=0.022). In SC, positive vascular invasion was closely connected with nuclear expression group and score of p-AURKB (p=0.006 and 0.004).

Table 5. Clinicopathologic correlations for p-AURKA expression according to the histologic components of UCS

	p-AURKA in SC			p-AURKA in CC		
	Low n (%)	High n (%)	*p*	Low n (%)	High n (%)	*p*
FIGO stage			0.673			0.027
I and II	6 (60.0)	10 (71.4)		11 (91.7)	5 (41.7)	
III and IV	4 (40.0)	4 (28.6)		1 (8.3)	7(58.3)	
Myometrial invasion			0.421			0.680
Less than 1/2	3 (30.0)	7 (50.0)		6 (50.00	4 (33.3)	
More than 1/2	7 (70.0)	7 (50.0)		6 (50.0)	8 (66.7)	
Lymphatic invasion			0.408			1.000
Negative	4 (40.0)	9 (64.3)		7 (58.3)	6 (50.0)	
Positive	6 (60.0)	5 (35.70		5 (41.7)	6 (50.0)	
Vascular invasion			0.615			0.317
Negative	7 (70.0)	12 (85.7)		11 (91.7)	8 (66.7)	
Positive	3 (30.0)	2 (14.3)		1 (8.3)	4 (33.3)	
Adnexal involvement			0.618			0.155
Negative	7 (70.0)	11 (84.6)		11 (91.7)	7 (63.6)	
Positive	3 (30.0)	2 (15.4)		1 (8.3)	4 (36.1)	
Lymph node metastasis			1.000			0.012
Negative	6 (75.0)	10 (76.9)		11 (100.0)	5 (50.0)	
Positive	2 (25.0)	3 (23.1)		0	5 (50.0)	
Omentum involvement			0.122			0.037
Negative	6 (60.0)	13 (92.9)		12 (100.0)	7 (58.3)	
Positive	4 (40.0)	1 97.1)		0	5 (41.7)	

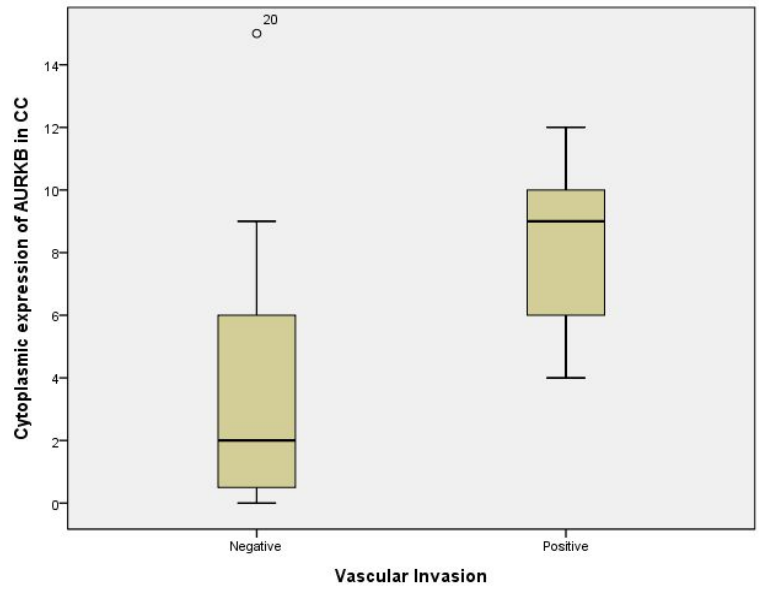
Abbreviation: FIGO, International Federation of Gynecology and Obstetrics

Table 6. Clinicopathologic correlations for AURKB and p-AURKB expression

	Cytoplasmic expression AURKB in CC			Nuclear expression p-AURKB in SC		
	Low n (%)	High n (%)	*p*	Low n (%)	High n (%)	*p*
FIGO stage			0.390			0.204
I and II	10 (79.6)	6 (54.5)		5 (50.0)	11 (78.6)	
III and IV	3 (23.1)	5 (45.5)		5 (50.0)	3 (21.4)	
Myometrial invasion			1.000			0.578
Less than 1/2	4 (30.8)	4 (36.4)		2 (20.0)	6 (42.9)	
More than 1/2	8 (61.5)	6 (54.5)		7 (70.0)	7 (50.0)	
Lymphatic invasion			1.000			1.000
Negative	7 (53.8)	6 (54.5)		5 (50.0)	8 (57.1)	
Positive	6 (46.2)	5 (45.5)		5 (50.0)	6 (42.9)	
Vascular invasion			0.011			0.006
Negative	13 (100.0)	6 (54.5)		5 (50.0)	14 (100.0)	
Positive	0	5 (45.5)		5 (50.0)	0	
Adnexal involvement			0.127			0.056
Negative	12 (92.3)	6 (60.0)		5 (55.6)	13 (92.9)	
Positive	1 (7.7)	4 (40.0)		4 (44.4)	1 (7.1)	
Lymph node metastasis			0.582			0.285
Negative	9 (90.0)	7 (70.0)		6 (66.7)	10 (90.9)	
Positive	1 (10.0)	3 (30.0)		3 (33.3)	1 (9.1)	
Omentum involvement			0.630			0.615
Negative	11 (84.6)	8 (72.7)		7 (70.0)	12 (85.7)	
Positive	2 (15.4)	3 (27.3)		3 (30.0)	2 (14.3)	

Figure 2 represents the box-plots for expression score of AURKB and p-AURKB based on vascular invasion. Positive vascular invasion group had higher cytoplasmic expression score of AURKB in CC and lower nuclear expression score of p-AURKB in SC. Median expression score of AURKB was 2.00 (range, 0-15) in negative vascular invasion and 9.00 (range, 4-12) in positive vascular invasion (p=0.022). In addition, median value of p-AURKB was 6.00 (range, 1-15) in negative vascular invasion and 4.00 (range, 3-5) in positive vascular invasion (p=0.004). There was negative correlation between nuclear expression score of p-AURKB in SC and cytoplasmic expression score of AURKB in CC. As shown in figure 3, the coefficient of linear regression between nuclear p-AURKB expression in SC and cytoplasmic AURKB expression in CC was -0.544 (p=0.013, R^2=0.248).

Figure 2. The correlation between vascular invasion and expression pattern of aurora kinases. Box plots for vascular invasion according to the cytoplasmic expression of AURKB in CC and nuclear expression of p-AURKB in SC (p=0.022 and 0.004).



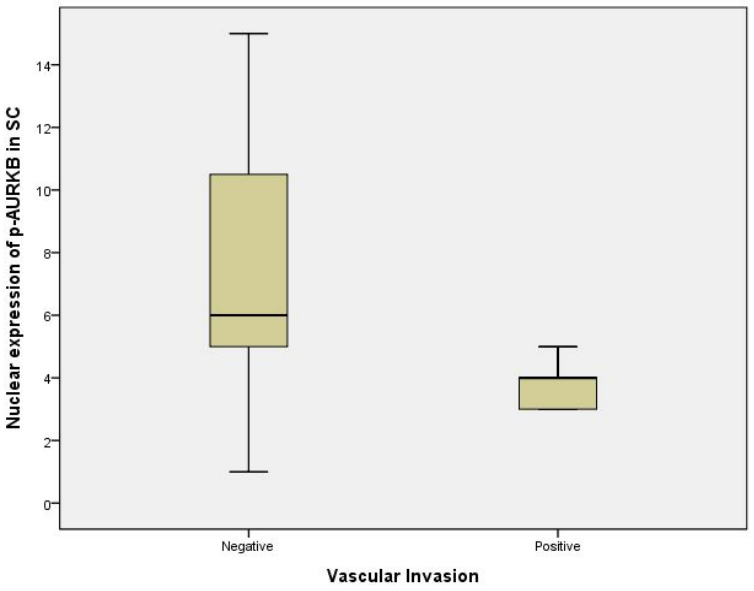
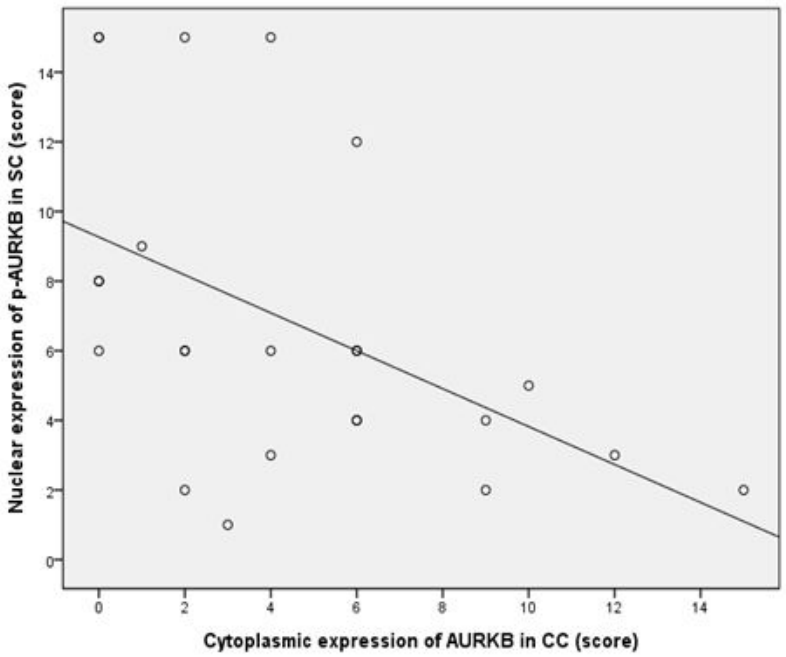


Figure 3. Simple linear regression analysis between two variables involved in vascular invasion. The coefficient of linear regression between p-AURKB nuclear expression and AURKB cytoplasmic expression was -0.544 (p=0.013, R^2=0.248). (Nuclear expression score of p-AURKB in SC) = [(-0.544) x (Cytoplasmic expression score of AURKB in CC)] + 9.264.



IV. Discussion

UCS is a biphasic tumor composed of SC and CC. CC, as a driving force, induces SC by sarcomatous metaplasia, which resembles EMT (7-10). The marker of EMT such as p-SMAD2/3 was more highly expressed in CC of UCS than in usual endometrial carcinoma (9). The unique characteristics of UCS such as increased mitotic count and aneuploidy result from an abnormal cell cycle. Aurora kinases belong to the mitotic kinases, which are involved in cell cycle. Previous studies found the overexpression of aurora kinases in uterine cancer. AURKA was highly expressed in non-endometrioid endometrial carcinoma (33), and AURKB overexpression was known as a poor prognostic factor in uterine endometrial carcinoma (31). AURKA and p-AURKA were overexpressed even in leiomyosarcoma (21, 23). An inhibitor of AURKA suppresses EMT in carcinoma cell lines (34). Therefore, expression patterns of aurora kinases are expected to be different in two histologic elements of UCS. The aim of this current study is to explore the expression pattern of aurora kinase in UCS and to find clinical significance.

Immunohistochemistry for antibodies of AURKA, AURKB, p-AURKA, and p-AURKB was performed using TMA in each component of UCS. To verify interrelation of aurora kinase and spindle assembly checkpoint, immunohistochemistry for PLK1 was also conducted. Various analytical methods were accomplished in previous studies for aurora kinases expression. While some researchers used expression score by semi-quantitative method or H-score (23, 31, 35, 36), others analyzed high and low expression groups by Allred proportion or appropriate manner in each study (22, 37). In this study,

immunohistochemical expression score was calculated from the multiplication of staining intensity and cell proportion. Furthermore, high expression group for AURKA and AURKB was determined by ≥ 2+ of staining intensity and over 30% of stained cell proportion (24). For p-AURKA and p-AURKB, samples with total score ≥6 were classified as high expression group (23).

AURKA is observed in cancer cells as well as normal cells especially in G2/M phase of mitosis. However, AURKA is much highly expressed in cancer cells than in normal proliferative cells, and known as poor prognostic factor and malignant marker in cancer. Gene of AURKA is located in chromosome 20q13.2, and it is amplified in cancers of breast, colon, bladder, ovary, and pancreas. In addition, mRNA and protein of AURKA was also elevated in cancer cells. Overexpressed AURKA induced chromosomal instability and carcinogenesis in colon cancer cell line (38). AURKA interacted with GSL-3β, and contributed to carcinogenesis in gastric cancer cell through β-catenin pathway (39). In vitro assay with ovarian cancer cell line, MK-0457 as an aurora kinase inhibitor reduced cell proliferation and increased cell apoptosis (40, 41). Overexpression of AURKA induced multinucleation and polyploidy by amplification of centrosome in vitro study using $p53^{+/-}$cells (42). Overexpression of AURKA is involved in genomic instability, DNA ploidy, and cellular senescence (43, 44). In other words, the main function of AURKA is to maintain genomic integrity (45).

AURKA acts in the cell cycle through phosphorylation. Immunohistochemical expression of p-AURKA was elevated in high grade tumor and advanced stage in bladder cancer (28). Thr-288 is the

phosphorylation site of AURKA within kinase activation loop of catalytic domain. Enzymatic activity increases as much as 7-fold in p-AURKA (46). Because Thr-288 is a target for proteosomal degradation in G1 phase during mitosis, p-AURKA regulates the activity and stability of AURKA (42, 43).

In this study, AURKA expression didn't have any specific correlations with clinical findings of UCS. However, nuclear expression of p-AURKA of CC was interrelated with well-known prognostic factors of uterine cancer. Although low expression group of p-AURKA was relevant to early stage, high expression group was to advanced stage (p=0.027). Furthermore, high expression cases of p-AURKA in CC were mainly metastatic groups for lymph node and omentum (p=0.012 and 0.037). Lymph node metastasis means over stage III disease. Moreover, omentum involvement is an indicator of abdominal metastasis and suggests stage IV disease. Expression of p-AURKA is expected to demonstrate tumor aggressiveness and advanced disease. In bladder cancer, high expression of AURKA was correlated with tumor stage and lymph node metastasis (30). AURKA functioned as a poor prognostic marker of esophageal cancer (24). Carcinogenic potential of AURKA was studied for the new generation of chemotherapeutics (47).

AURKB is located in chromosome 17p13.1 and it is a chromosomal passenger protein. AURKB is situated at chromatin in late G2. Then AURKB moves on the chromosome arms and inner centromere in the beginning of prometaphase, and it remains on this position within all metaphase. It then forms a central spindle in anaphase and contractile ring in telophase by interaction with PLK1. AURKB is an attractive molecule to bind microtubules to kinetochore during

prometaphase-metaphase transition after phosphorylation at serine or threonine residues (48-50). At last process of cell proliferation, AURKB is involved in cytokinesis. These mechanisms occur by association with other passenger proteins such as, inner centromere protein (INCENP) and survivin (14). Auto-phosphorylaion of Thr-232 is activated process of AURKB (51).

Metastatic melanoma has an elevated expression pattern of AURKB on immunohistochemistry (52). There were amplified mRNAs and proteins of AURKB in colon cancer (53). AURKB overexpression was associated with increased cell proliferation represented by Ki-67 in breast carcinoma (54). Furthermore, AURKB overexpression in malignant peritoneal and pleural effusions was known for the predictive factor of chemo-resistance feature in advanced ovarian cancer (55). Positive-AURKB cancer had poor prognosis than negative-AURKB (31). In colorectal cancer, nuclear AURKB expression was involved in lymph node metastasis (56).

In this study, CC had higher nuclear expression pattern of AURKB than SC (p=0.041). In addition, cytoplasmic overexpression of AURKB in CC is closely correlated with vascular invasion. These results were supported by the analysis for immunoexpression score and expression group (p=0.011 and 0.022). Almost of the histologic type of metastatic part of UCS was known as epithelial portion (57). Moreover, CC of UCS was known as a determining factor of tumor prognosis by metastasis and vascular invasion (58). In this study, nuclear expression of p-AURKB in SC was also connected with vascular invasion (p=0.006). The relationship between cytoplasmic expression of AURKB

in CC and nuclear expression of p-AURKB in SC can be expressed as a negative linear equation (p=0.013, R^2=0.248).

The interaction between angioinvasion and AURKB expression was introduced in chondrosarcoma samples (22). The interrelation between vascular invasion and AURKB was suggested through vascular endothelial growth factor (VEGF) (59). AURKB inhibitor bound VEGF receptor families, then it blocked vascular permeability and attenuates angiogenesis in vivo (28). Even more, there were abundant amount of localized VEGF and angiopoietin genes in UCS than in endometrial carcinoma (60). The results of this study corresponded to the relationship between angioinvasion and AURKB. Expression of p-AURKB was also correlated with vascular invasion in this study. Furthermore, AURKB inhibitor was considered as a promising chemotherapeutic drug for paclitaxel-resistance tumor because AURKB stabilize microtubule during mitosis (61).

This study revealed interaction of immunohistochemical expression between PLK1 and various aurora kinases. PLK1 expression was connected with non-phosphorylated aurora kinases in cytoplasm but phosphorylated aurora kinases in nucleus. It is expected that activated aurora kinases are primarily located in the nucleus although plain aurora kinases are mainly in the cytoplasm. PLK1 acts as a checkpoint of cell cycle. AURKA activates PLK1 by phosphorylation of residue Thr-210 on T-loop of PLK1 (62, 63). If DNA damage occurs, PLK1/Dcd25B makes cell to start proliferation after DNA repair. Cells with DNA damage proliferated into mitotic catastrophe (64). High levels of PLK1 were observed in tumors with metastatic potential and poor prognosis (65). PLK1-encoding siRNA induced G2/M arrest, apoptosis,

and mitotic spindle aberrations of osteosarcoma cell line by knockdown of PLK1 (66). PLK1 influenced tumor growth in dose-dependent manner in human sarcoma cell line. Furthermore, PLK1 depletion resulted in mitotic arrest and aneuploidy in p53-defective cell line (67). Both AURKA and PLK1 were more overexpressed in uterine leiomosarcoma than leiomyoma and normal endometrium. MK-5108 as an inhibitor of AURKA weakened the carcinogenetic potential of leiomyosarcoma cell line in xenograft model (23). Furthermore, AURKB interacted with PLK1 during mitosis and cytokinesis. Inhibition of PLK1 disturbed the proper function of AURKB (14).

The aim of this study is to investigate the expression pattern of aurora kinases in UCS. In this study, AURKB showed distinct expression pattern between CC and SC. Cytoplasmic expression of AURKB in CC and nuclear expression of p-AURKB in SC was correlated with vascular invasion. Moreover, nuclear expression of p-AURKA in CC was involved in tumor stage, lymph node metastasis, and omental involvement. Expression pattern of aurora kinases supports the conversion theory with sarcomatous metaplasia from CC, which is similar to EMT. Expressions of aurora kinases are expected to be involved in tumor metastasis and disease progression. Inhibitors of aurora kinases are perspective chemotherapeutics for aggressive carcinosarcoma with angioinvasive potential and high mitotic figures.

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초 록

서론: 자궁 암육종은 암종 부위와 육종 부위가 섞인 드문 형태의 자궁암으로, 예후가 매우 불량하다. 자궁 암육종의 구성 요소 중 공격성을 반영하는 인자에 대해서는 논란의 여지가 있다. 자궁 암육종의 구성 요소별로 분열 지수와 염색체 배수성이 다르기 때문에, 체세포 분열에 관련하는 인자를 중심으로 자궁 암육종을 살펴보고자 한다. Aurora kinase는 체세포 분열 과정에서 중심체 분리와 방추체 형성, 염색체 분리, 세포질 분리에 관여하며, 종양에서 높게 분포한다고 알려져 있다. 이 연구에서는 자궁 암육종의 구성 요소에 따른 aurora kinase의 발현 양상을 확인하고 그 임상적 의의를 파악하고자 한다.

방법: 1997년부터 2012년까지 서울대학교병원에서 진단받고 치료받은 자궁 암육종 환자 총 24명을 대상으로 조직 마이크로어레이를 제작하여 aurora kinase 항체에 대해 면역조직화학염색을 시행하였다. 핵과 세포질 각각에서 염색된 양상을 관찰하였으며, 염색된 세포의 강도와 비율을 곱하여 산출한 점수를 바탕으로 분석하였고, 발현이 높은 집단과 낮은 집단으로도 분류하여 비교하였다.

결과: 육종 부위는 homologous와 heterologous 간에 비슷하게 분포하였으나, 암종 부위는 endometrioid, serous, mucinous, adenosquamous 순서로 다양하게 분포하였다. 면역조직화학염색 결과 육종 부위보다 암종 부위에서 aurora kinase B (AURKB) 발현이 높게 나타났다 (p=0.041). Polo-like kinase 1과 aurora kinase 발현과의 상관관계를 보면, aurora kinase A (AURKA), AURKB는 세포질에서, phospho-aurora kinase A (p-AURKA), phospho-aurora kinase B (p-AURKB)는 핵에서 유의하게 나타났다 (p<0.001, 0.001, 0.007, 0.001). 암종 부위에서 발현된 p-AURKA는 종양의 병기, 림프절 전이, 장간막 침범과 상관관

계를 보였다 (p=0.027, 0.012, 0.037). 암종 부위에서 발현된 AURKB와 육종 부위에서 발현된 p-AURKB는 혈관 침범과 관련성을 보였다 (p=0.011, 0.006). 또한 암종 부위 AURKB 발현과 육종 부위 p-AURKB 발현은 음성 선형 관계를 나타냈다 (p=0.013).

결론: AURKB는 암종 부위와 육종 부위에서 유의한 발현 차이를 보였으며, AURKB와 p-AURKB 발현은 혈관 침범 여부와 상관성을 보였다. 암종에서 발현된 p-AURKA는 자궁 암육종의 병기, 림프절 전이, 장간막 침범 여부와 관련 있었다. Aurora kinase는 자궁 암육종의 조직학적 구성 요소 중 암종 부위에서 높게 발현하며, 종양의 전이 혹은 침범 양상과 관련을 보였다. 예후가 불량한 자궁 암육종의 차기 치료제로 aurora kinase 억제제 사용이 유망하다고 사료된다.

주요어: 자궁 암육종, aurora kinase, 면역조직화학염색

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