Histol Histopathol (2012) 27: 593-599
DOI: 10.14670/HH-27.593

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Prognostic significance of BAF57 expression in patients with endometrial carcinoma

S. Kagami[1], T. Kurita[1], T. Kawagoe[1], N. Toki[1], Y. Matsuura[1], T. Hachisuga[1], A. Matsuyama[2], H. Hashimoto[2], H. Izumi[3] and K. Kohno[3]

[1]Departments of Obstetrics and Gynecology, [2]Pathology I and [3]Molecular Biology, University of Occupational and Environmental Health School of Medicine, Yahatanishi-ku, Kitakyushu, Japan

Summary. This study was conducted to elucidate the prognostic significance of BAF57 in patients with endometrial carcinoma. We investigated the relationship between the immunohistochemical expression of BAF57 and various clinicopathological variables in 111 endometrial carcinomas. Both univariate and multivariate regression analyses were performed. The correlations between the BAF57 expression and the other variables including estrogen receptor (ER) and p53 were examined. The high nuclear BAF57 expression was detected in 42 (37.8%) endometrial carcinomas, and 69 (62.2%) endometrial carcinomas were defined as having low nuclear BAF57 expression. The BAF57 expression was significantly associated with the surgical stage, grade of the tumor, myometrial invasion, lympho-vascular space invasion (LVSI) and lymph node metastasis. The 10-year overall survival rates of patients with low and high BAF57 expression were 96.9% and 58.2%, respectively ($p<0.001$). A multivariate analysis identified BAF57 expression as an independent prognostic factor. The BAF57 expression was significantly correlated with p53 expression ($r=0.312$, $P=0.001$), but was not correlated with ER expression ($r=-0.141$, $P=0.14$). The high BAF57 expression is an independent marker of poor prognosis of the patients in endometrial carcinomas. The inhibition of BAF57 activity may be one of the candidates for endometrial cancer therapy, especially therapy for aggressive tumors showing overexpression of p53.

Key words: Endometrial carcinoma, BAF57, Estrogen receptor, p53

Offprint requests to: Toru Hachisuga, M.D., Ph.D., Department of Obstetrics and Gynecology, University of Occupational and Environmental Health, 1-1, Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan. email: thachisu@med.uoeh-u.ac.jp

Introduction

The SWI/SNF complex is an ATP-dependent chromatin remodeling complex that alters the location or conformation of nucleosomes by using the energy of ATP hydrolysis (Muchardt and Yaniv, 2001). These complexes always contain a single catalytic subunit, BRM/SNFα or BRG1/SNFß, and several other variable BRG1-associated-factors (BAFs). BAF subunits largely contribute to SWI/SNF specificity and include BAF155, BAF170, INI1/SNF5, BAF250, BAF60, and BAF57. The chromatin-remodeling mechanism of the SWI/SNF complex has been best described with the transcriptional activity of nuclear receptors (Trotter and Archer, 2007).

BAF57 contains DNA-binding capability through its high-mobility-group (HMG) motif and kinesin-like coiled-coil domain. An interaction between the ER and BAF57 was identified in breast cancer cells (Garcia-Pedrero et al., 2006). The authors reported that BAF57 serves as a targeting subunit responsible for the recruitment of the complex to the ligand-bound ER during target gene activation. BAF57 is also reported to facilitate direct interaction with the androgen receptor (AR) in prostate cancer cells (Link et al., 2008). These recent studies suggest BAF57 plays an important role in the control of hormone-dependent proliferation in hormone sensitive cancer cells, but little is known regarding the function and role of BAF57 in endometrial carcinoma tissues, and there is no information regarding the relationship between BAF57 expression and clinicopathological factors, including the prognosis of patients with endometrial carcinoma.

In this study, we investigated the correlation between the expression of BAF57 and the clinicopathological profile of patients with endometrial carcinoma, and found BAF57 expression to be an independent prognostic indicator of patients with endometrial

carcinoma.

Materials and methods

Case selection

The study included 111 Japanese patients with endometrial carcinoma who had undergone surgery at the University of Occupational and Environmental Health hospital from 1990 and 2000. The surgical treatments of these 111 patients are summarized in Table 1.

Immunohistochemistry

Four-μm sections were cut from formalin-fixed paraffin-embedded tissue blocks, deparaffinized in xylene, and rehydrated through sequential changes of alcohol and distilled water. A polyclonal antibody was raised against BAF57 by multiple immunizations of a New Zealand white rabbit using a synthetic peptide. The sequence of the synthethic peptide for BAF57 was KEPPTDPIPEDEKKE (K plus amino acids 398-411). ER was detected using an ER· monoclonal antibody (clone 6F11, Novocastra, UK; dilution 1:50). P53 was detected using the ready-to-use p53 monoclonal antibody (clone DO-7, DAKO, Kyoto, Japan). The slides for ER and p53 were heated in an autoclave at 120°C for 5 min in 0.01 M citrate buffer (pH=6.0) before immunostaining. The slides for BAF57, ER and p53 were incubated with these antibodies for 2 hr at room temperature. Antibody binding was visualized using the EnVision+ Dual link system and diaminobenzidine as chromogen (DAKO, Kyoto, Japan). The slides were counterstained with methyl green and mounted.

Interpretation of immunohistochemical preparations

The cytoplasmic immunostaining for BAF57 was scored as low and high expression. There was no cytoplasmic immunostaining for ER and p53. The labeling index for BAF57, ER and p53 was defined as the percentage of neoplastic cells with clear nuclear immunoreactivity from the total number of neoplastic cells and was determined by counting 500 cells in the most active area of the specimen. The sections were evaluated by 2 independent observers with no knowledge of clinical data. Conflicting results were reviewed until final agreement was achieved. To evaluate the correlation between nuclear BAF57 expression and prognosis of the patients, three semiquantitative classes were used to describe the labeling index for nuclear BAF57: between none and less than 10% positive, between 10% and 50% positive, and more than 50% positive. Finally, the cases were classified into two groups of low BAF57 expression (between none and 50% positive) and high BAF57 expression (more than 50% positive).

Table 1. Summary of surgical treatment.

Treatment	
STH	1
STH+BSO	12
STH+BSO+pelvic lymphadenectomy	29
STH+BSO+pelvic lymphadenectomy+PALNBx	15
mRTH+BSO+pelvic lymphadenectomy	31
mRTH+BSO+pelvic lymphadenectomy+PALNBx	16
RTH+BSO+pelvic lymphadenectomy	2
RTH+BSO+pelvic lymphadenectomy+PALNBx	5
Total	111

STH: simple total hysterectomy; mRTH: modified radical hysterectomy; RTH: radical hysterectomy; BSO: bilateral salpingo-oophorectomy; PALNBx: para-aortic lymph node biopsy

Statistical analysis

Statistical analyses were carried out using the SPSS for Windows statistical software package, version 17.0.0 (SPSS, Chicago, IL, USA). The Mann-Whitney U-test was used for comparison of clinicopathological variables with low and high BAF57 expression. The Pearson correlation coefficient test was used to determine the correlations between continuous criteria. The survival time was calculated from date of initial surgery. The cumulative 10-year survival rate was determined using the Kaplan-Meier product-limited method. The log-rank test was used to test differences in survival within variables. The Cox proportional hazards model was used to identify and simultaneously evaluate any independent prognostic factors associated with relative survival. Statistical significance was considered to exist at a value of $P<0.05$.

Results

Immunohistochemical staining for BAF57

The cytoplasmic and nuclear expression of BAF57 was observed by immunohistochemistry (Fig. 1). The specificity of this BAF57 polyclonal antibody was tested by immunohistochemisry. After incubation of this antibody with the excess of synthesized peptides of BAF57, the positive immunostaining was almost eliminated (Fig. 2).

Low cytoplasmic expression for BAF57 was observed in 46 tumors and the high cytoplasmic expression for BAF57 was observed in 65 tumors. The nuclear expression for BAF57 was divided into three groups: between none and less than 10% positive in 46 (41.5%) tumors, between 10% and 50% positive in 23 (20.7%) tumors, and more than 50% positive in 42 (37.8%) tumors.

Relationship between nuclear BAF57 expression and clinicopathological variables

The age range of the 111 patients with endometrial

carcinoma was 30 to 81 years (mean, 55.4 years). All tumors were staged surgically according to the 1988 FIGO system. They included 28 patients with stage Ia disease, 38 patients with stage Ib disease, 12 patients with stage Ic disease, 4 patients with stage IIa disease, 4 patients with stage IIb disease, 7 patients with stage IIIa disease, 1 patient with stage IIIb disease, 12 patients with stage IIIc disease and 5 patients with stage IVb disease. The nuclear BAF57 expression correlated significantly with surgical stage, grade of the tumor, myometrial invasion, LVSI and lymph node metastasis but did not correlate with the age of the patients, cervical invasion and ovarian metastasis (Table 2). The high nuclear BAF57 expression was found in 14 (24.1%) of 58 grade 1 tumors, 16 (50.0%) of 32 grade 2 tumors, 8 (57.1%) of 14 grade 3 tumors, 3 (50.0%) of 6 uterine serous adenocarcinomas and 1 clear cell adenocarcinoma.

Correlation between BAF57 and the expression of ER and p53

The nuclear expression of ER and p53 was observed by immunohistochemistry (Fig. 3). The mean (±standard deviation; SD) labeling index for BAF57, p53, and ER was 37.8±36.6% (range; 0% to 97%), 16.5±23.3% (range; 0% to 98%), and 36.2±33.4% (range; 0% to 86%), respectively. The tumors showing more than a 50% labeling index for p53 involved 5 endometrioid adenocarcinomas and 6 non-endometrioid adenocarcinomas, involving 5 serous adenocarcinomas and 1 clear cell adenocarcinoma. The tumors showing more than a 50% labeling index for ER involved 46 endometrioid adenocarcinomas involving 31 grade 1 tumors, 12 grade 2 tumors and 3 grade 3 tumors. The coefficient of correlation between the labeling index of p53 and BAF57 was 0.312 (Fig. 4; P=0.001). The coefficient of correlation between labeling indices of ER and BAF57 was -0.141 (Fig. 5; P=0.14).

Prognosis

There was no correlation between the cytoplasmic expression of BAF57 and the prognosis of the patients. The 10-year overall survival rates of patients with nuclear BAF57 expression between none and less than

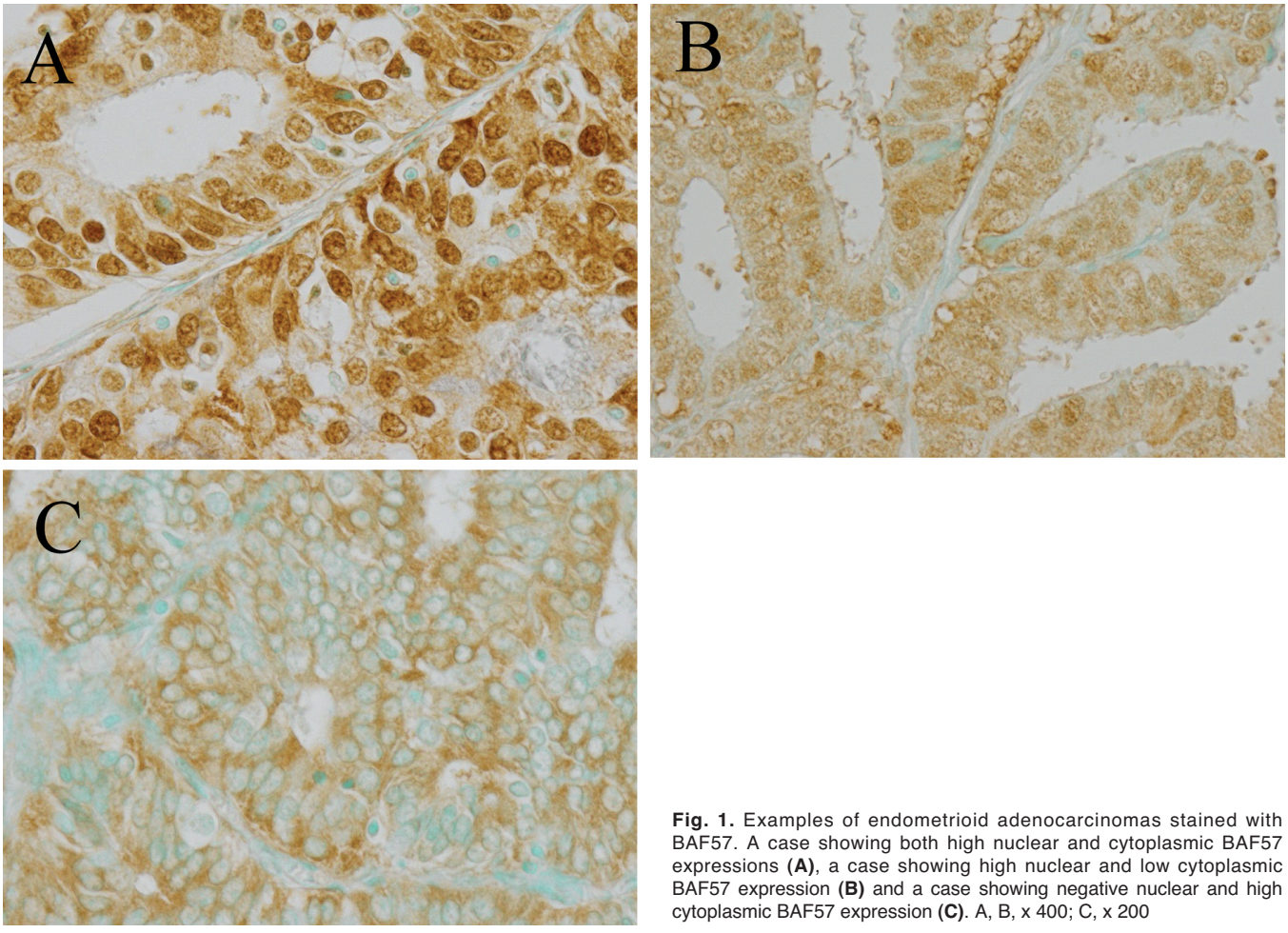

Fig. 1. Examples of endometrioid adenocarcinomas stained with BAF57. A case showing both high nuclear and cytoplasmic BAF57 expressions **(A)**, a case showing high nuclear and low cytoplasmic BAF57 expression **(B)** and a case showing negative nuclear and high cytoplasmic BAF57 expression **(C)**. A, B, x 400; C, x 200

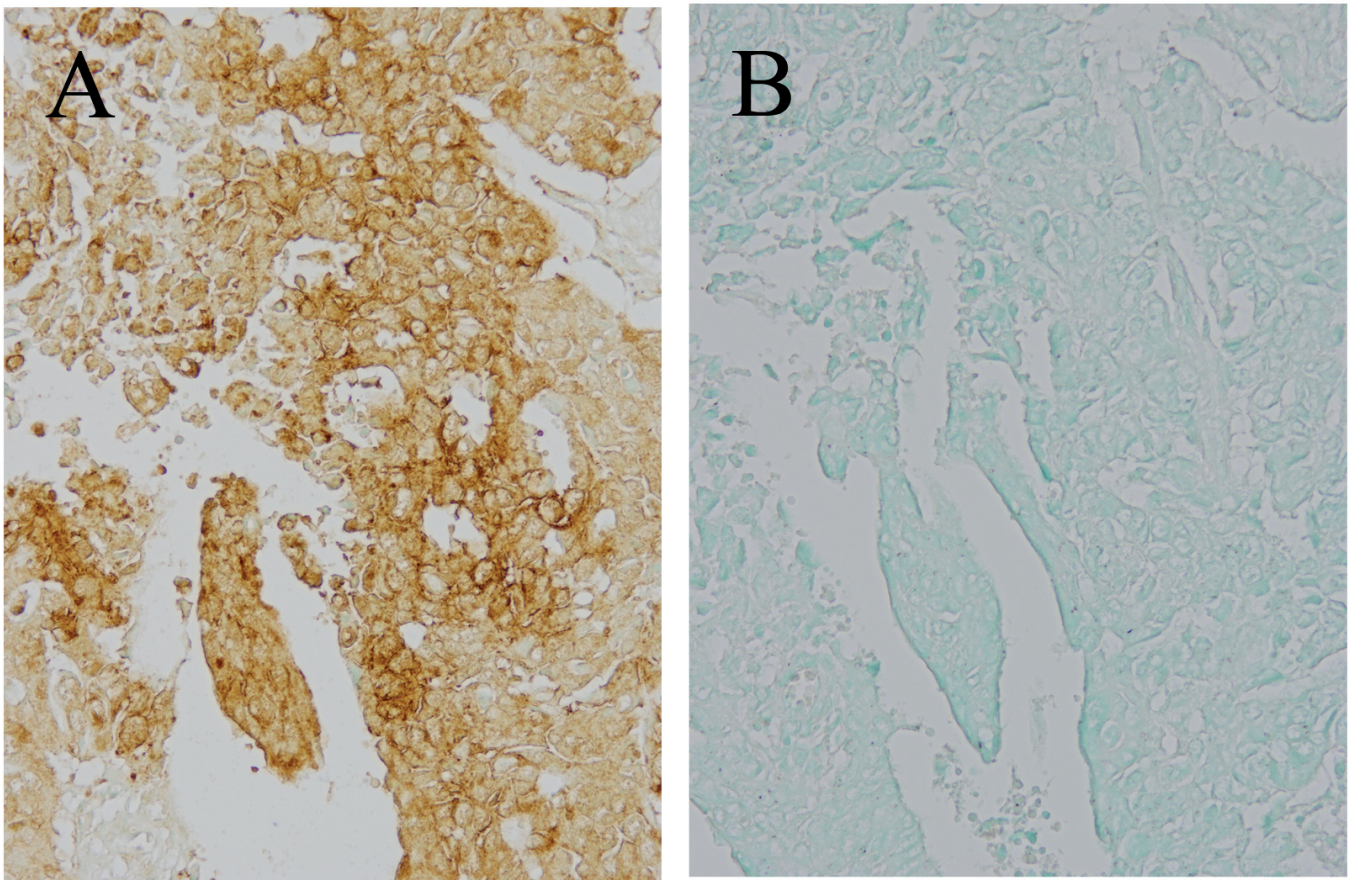

Fig. 2. Immunohistochemical BAF57 expression in endometrioid adenocarcinoma after incubation of an antiBAF57 polyclonal antibody **(A)** or an anti-BAF57 polyclonal antibody+synthesized peptides of BAF57 **(B)**. x 100

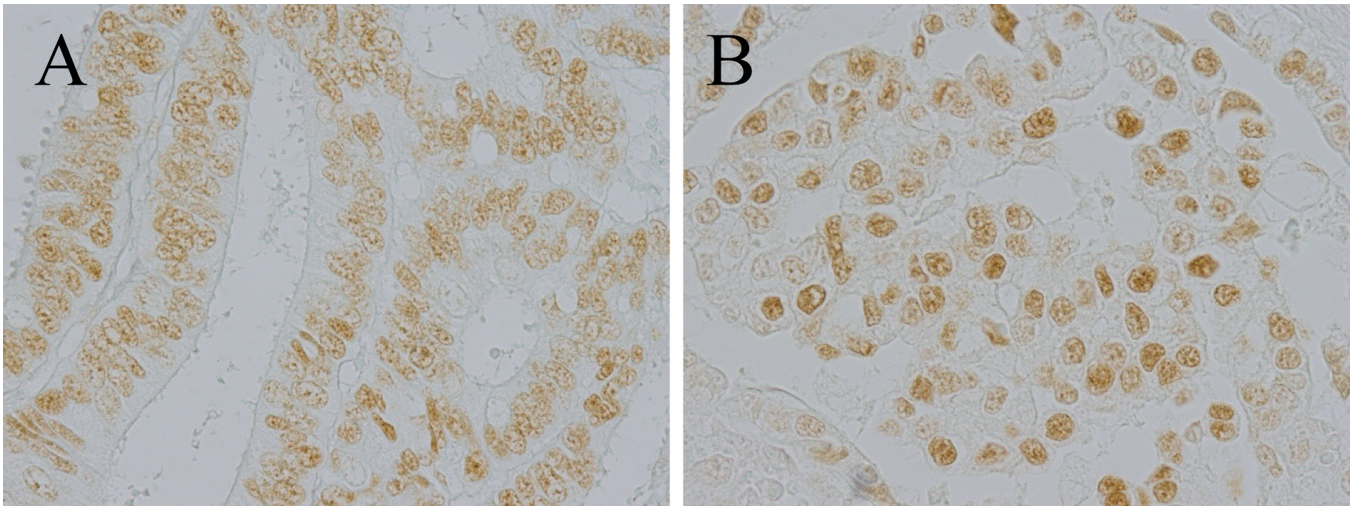

Fig. 3. A high nuclear expression for estrogen receptor in the endometrioid adenocarcinoma **(A)**. A high nuclear expression for p53 in the serous adenocarcinoma **(B)**. x 400

Table 2. Clinicopathological variables and BAF57 expression.

Variable	BAF57 expression		P value
	low n=69	high n=42	
Age			0.075
<50 years	18	5	
≥50 years	51	37	
Stage			0.007
I	54	24	
II	6	2	
III	8	12	
IV	1	4	
Grade			0.025
1 and 2	61	30	
3, serous and clear	8	12	
Myometrial invasion			<0.001
≤1/2	59	20	
>1/2	10	22	
LVSI			0.007
Negative	52	21	
Positive	17	21	
Cervical invasion			0.450
Negative	61	35	
Positive	8	7	
Ovarian metastasis			0.064
Negative	65	35	
Positive	4	7	
Lymph node metastasis			0.002
Negative	65	31	
Positive	4	11	

LVSI: lymphovascular space invasion.

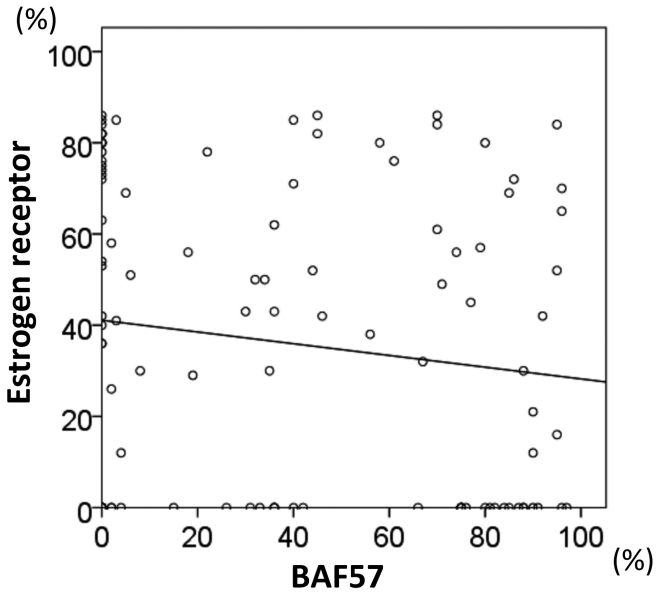

Fig. 5. Correlation between the labeling index of BAF57 and estrogen receptor.

10% positive, between 10% and 50% positive, and more than 50% positive were 97.4%, 95.8% and 58.2%, respectively. Finally, the cases were classified into two groups of 69 (62.2%) tumors with low BAF57

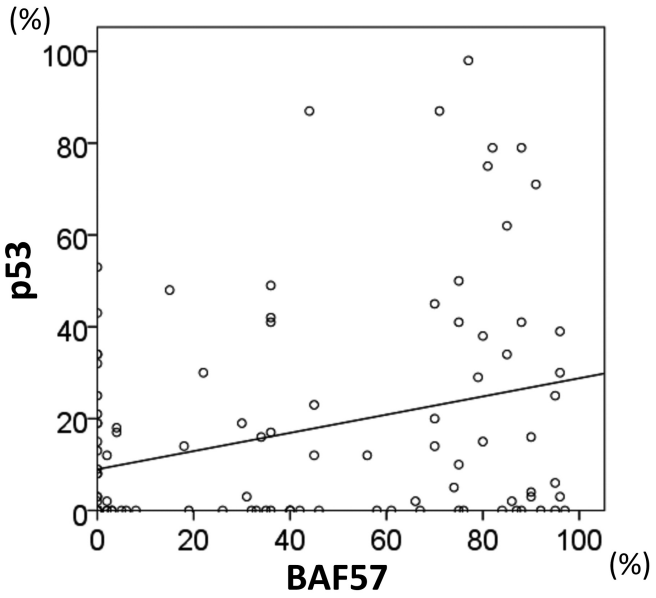

Fig. 4. Correlation between the labeling index of BAF57 and p53.

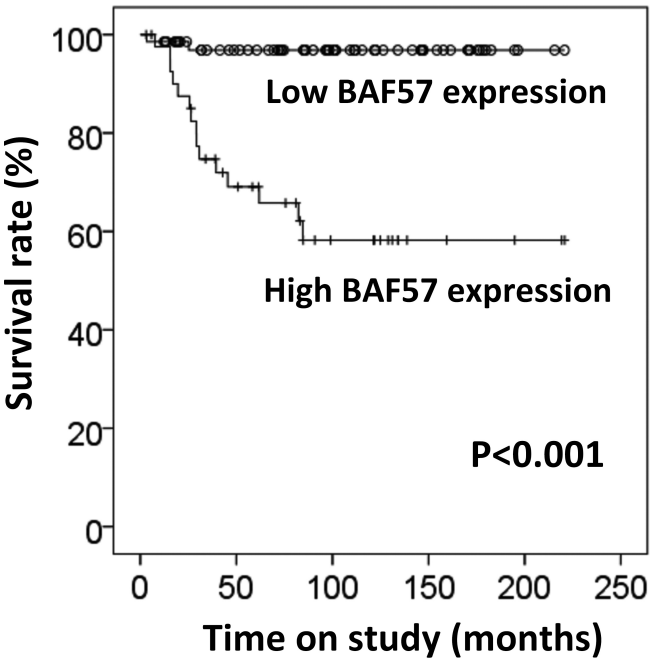

Fig. 6. The Kaplan-Meier curve for overall survival in 111 patients with endometrial carcinoma by BAF57 expression.

Table 3. Univariate and multivariate analyses of prognostic variables for overall survival by Cox proportional hazards model.

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	P value	HR	95%CI	P value
BAF57 expression; High vs, Low	14.045	3.207-61.510	<0.001	6.859	1.415-33.258	0.017
Grade; 1 and 2 vs.3, serous and clear	4.464	1.721-11.579	0.002	1.792	0.471- 6.826	0.392
Myometrial invasion; ≤1/2 vs. >1/2	9.186	2.992-28.209	<0.001	1.368	0.248- 7.551	0.719
LVSI; Negative vs. Positive	7.887	2.563-24.266	<0.001	2.103	0.422-10.469	0.364
Cervical invasion; Negative vs. Positive	6.367	2.409-16.826	<0.001	2.079	0.519- 8.327	0.301
Ovarian metastasis; Negative vs. Positive	6.198	2.288-16.789	<0.001	1.303	0.318- 5.335	0.714
Lymph node metastasis; Negative vs. Positive	14.06	5.288-37.384	<0.001	2.431	0.626- 9.447	0.200

LVSI:lymphovascular space invasion, HR:hazard ratio, 95%CI: 95% confidence interval.

expression (between none and 50% positive) and 42 (37.8%) tumors with high BAF57 expression (more than 50% positive). The 10-year overall survival rates of patients with low and high BAF57 expression were 96.9% and 58.2%, respectively (Fig. 6; P<0.001, log-rank test).

A univariate analysis showed the grade of the tumor, myometrial invasion, LVSI, cervical invasion, ovarian metastasis, lymph node metastasis and BAF57 expression to all be significantly associated with survival (Table 3). The multivariate analysis using the Cox proportional hazards model showed BAF57 expression as being an independent prognostic factor among the seven variables. Furthermore, multivariate analysis including p53 expression showed no independent prognostic factor among the eight variables.

Discussion

BAF57 is a subunit present in all mammalian SWI/SNF complexes that facilitates gene expression by remodeling nucleosomes and plays many important roles in epigenetic regulation during tumorigenesis, differentiation, and development (Muchardt and Yaniv, 2001). In the present study, the cytoplasmic and nuclear expression of BAF57 was observed by immunohistochemistry in endometrial carcinomas. The cytoplasmic expression of BAF57 gave no prognostic information, but a univariate survival analysis showed that the survival rate for patients with tumors showing low nuclear BAF57 expression was significantly better than that for patients with tumors showing a high nuclear BAF57 expression, and a multivariate analysis showed the nuclear BAF57 expression to be an independent prognostic factor. These results suggest that the nuclear expression of BAF57 may be associated with tumor activity. The nuclear BAF57 expression was high in the prostate cancer sample, especially the metastatic sample; therefore these data suggest that BAF57 expression is maintained or enhanced in prostate cancer (Link et al., 2008).

The SWI/SNF complex is reported to interact with various oncogenic and antioncogenic proteins, such as c-Myc (Cheng et al., 1999), Rb (Strober et al., 1996), BRCA1 (Bochar et al., 2000) and p53 (Lee et al., 2002). The p53 protein binds to several subunits of the SWI/SNF complex with p53-driven transcriptional activation (Lee et al., 2002). A study using an invasive human breast carcinoma cell line (BT549) lacking expression of BAF57 demonstrated the importance of BAF57 in cell growth regulation and suggested a novel link between the SWI/SNF complex and apoptosis (Wang et al., 2005). The human TP53 gene encodes a nuclear protein that induces growth arrest or apoptosis in response to endogenous and exogenous stress (Vogelstein et al., 2000). Most mutations of TP53 alter the conformation of p53, indirectly leading to a more stable protein that accumulates in tumor nuclei. p53 immunohistochemical staining is reported to correlate with p53 overexpression and gene mutation status (Jia et al., 2008). p53 is a significant prognostic factor for endometrial carcinoma (Uharcek, 2008) and is often observed in serous carcinomas of the endometrium (Kounelis et al., 2000). A labeling of more than 50% for p53 was found in 5 of 6 serous adenocarcinomas. A labeling of more than 50% for both p53 and BAF57 was found in 4 endometrioid adenocarcinomas, 3 serous adenocarcinomas and a clear cell adenocarcinoma. The correlation between p53 and BAF57 expression could not be explained by the histological subtypes. BAF57 overexpression may or may not be induced in compensation for loss of p53 function. On the other hand, a mutation in the BAF57 is reported in a breast cancer cell line (Kiskinis et al., 2006). The absence of a properly formed SWI/SNF including BAF57 may contribute to human cancer development and prognosis of the patients.

Furthermore, recent studies showed that the BAF57 SWI/SNF subunit facilitates direct interaction with the hormone receptors. The inhibition of the BAF57 function using dominant negative BAF57 mutants severely decreased the ER-dependent transcription from endogenous estrogen target genes (Garcia-Pedrero et al., 2006). SWI/SNF complex is critical for AR transcriptional activity, and the BAF57 SWI/SNF subunit facilitates direct interaction with the receptor (Link et al., 2008). The inhibitor derived from BAF57 (BIPep) is sufficient to inhibit androgen-dependent

prostate cancer cell proliferation (Link et al., 2008). Based on these findings, the correlation between BAF57 and ER expression was investigated. However, there was no correlation between expression of BAF57 and ER. This may be due to the small number of cases. Whether high nuclear BAF57 expression is activated by ER in endometrial carcinomas like breast carcinomas will be decided by experiments in the future using endometrial carcinoma cell lines. On the other hand, other factors may have influenced the BAF57 expression in endometrial carcinomas.

In conclusion, nuclear BAF57 expression was an independent prognostic factor for patients with endometrial carcinoma. The correlation between expression for BAF57 and p53 thus indicates that the inhibition of the BAF57 activity may be one of the candidates for endometrial cancer therapy, especially therapy for aggressive endometrial cancers showing an overexpression of p53.

References

Bochar D.A., Wang L., Beniya H., Kinev A., Xue Y., Lane W.S., Wang W., Kashanchi F. and Shiekhattar R. (2000). BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. Cell 102, 257-265.

Cheng S.-W.G., Davies K.P., Yung E., Beltran R.J., Yu J., and Kalpana G.V. (1999). C-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. Nat. Genet. 22, 102-105.

Garcia-Pedrero J.M., Kiskinis E., Parker M.G., and Belandia B. (2006). The SWI/SNF chromatin remodeling subunit BAF57 is a critical regulator of estrogen receptor function in breast cancer cells. J. Biol. Chem. 281, 22656-22664.

Jia L., Liu Y., Yi X., Miron A., Crum CP., Kong B. and Zheng W. (2008). Endometrial glandular dysplasia with frequent p53 gene mutation: A genetic evidence supporting its precancer nature for endometrial serous carcinoma. Clin. Cancer Res. 14, 2263-2269.

Kiskinis E., Garcia-Pedrero J.M., Villaronga M.A., Parker M.G., and Belandia B. (2006). Identification of BAF57 mutations in human breast cancer cell lines. Breast Cancer Res. Treat. 98, 191-198.

Kounelis S., Kapranos N., Kouri E., Coppola D., Papadaki H., and Jones M. (2000). Immunohistochemical profile of endometrial adenocarcinoma: A study of 61 cases and review of the literature. Mod. Pathol. 13, 379-388.

Lee D., Kim J.W., Seo T., Hwang S.G., Choi E.J., and Choe J. (2002). SWI/SNF complex interacts with tumor suppressor p53 and is necessary for the activation of p53-mediated transcription. J. Biol. Chem. 277, 22330-22337.

Link K.A., Balasubramaniam S., Sharma A., Comstock C.E.S., Godoy-Tundidor S., Powers N., Cao K.H., Haelens A., Claessens F., Revelo M.P. and Knudsen K.E. (2008). Targeting the BAF57 SWI/SNF subunit in prostate cancer: A novel platform to control androgen receptor activity. Cancer Res. 68, 4551-4558.

Muchardt C. and Yaniv M. (2001). When the SWI/SNF complex remodels…the cell cycle. Oncogene 20, 3067-3075.

Strober B., Dunaief J.L., Guha S. and Goff S. (1996). Functional interactions between the hBRM/hBRG1 transcriptional activators and the pRB family of proteins. Mol. Cell Biol. 16, 1576-1583.

Trotter K.W. and Archer T.K. (2007). Nuclear receptors and chromatin remodeling machinery. Mol. Cell Endocrinol. 265-266, 162-167.

Uharcek P. (2008). Prognostic factors in endometrial carcinoma. J. Obstet. Gynaecol. Res. 34, 776-783.

Vogeistein B., Lane D. and Levine A.J. (2000). Surfing the p53 network. Nature 408, 307-310.

Wang L., Baiocchi R.A., Pal S., Mosialos G., Caligiuri M. and Sif S. (2005). The BRG1- and hBRM-associated factor BAF57 induces apoptosis by stimulating expression of the cylindromatosis tumor suppressor gene. Mol. Cell Biol. 25, 7953-7965.

Accepted December 12, 2011