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Decreased Expression of 14-3-3σ Is Predictive of Poor Prognosis for Patients with Human Uterine Papillary Serous Carcinoma

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Uterine papillary serous carcinoma (UPSC) morphologically resembles ovarian serous carcinoma and is categorized as a type II endometrial cancer. UPSC comprises about 10% of all types of endometrial cancer and has an aggressive clinical course and a poor prognosis. The 14-3-3σ gene was originally discovered as a p53-inducible gene; its expression is induced by DNA damage in a p53-dependent manner, which leads to G2 arrest and repair of damaged DNA. Moreover, it has been reported that expression of 14-3-3σ is frequently lost in various types of human cancer, including ovarian cancer. We therefore examined the association between 14-3-3σ expression determined by immunohistochemistry and clinical outcomes of 51 patients with UPSC. UPSC was considered positive for 14-3-3σ when > 30% of tumor cells were stained with a specific antibody. Of these patients, 29 (58.7%) showed positive immunoreactivity for 14-3-3σ and 22 (41.3%) had decreased 14-3-3σ staining. Decreased immunoreactivity for 14-3-3σ was associated with stage (P = 0.001) and lymphovascular space involvement (P = 0.005). Moreover, decreased 14-3-3σ expression was an independent risk factor for reduced overall survival (P = 0.0416) in multivariate analysis. Direct bisulfite sequencing was performed to evaluate the methylation status of the 27 CpG islands in the promoter region and first exon of the 14-3-3σ gene. These CpG islands were hypermethylated in 30% of 14-3-3σ-positive UPSC and 80% of 14-3-3σ-negative UPSC, although the difference was not statistically significant. These findings suggest that decreased expression of immunoreactive 14-3-3σ may be a predictor of poor prognosis in patients with UPSC.

Keywords: 14-3-3σ; immunohistochemistry; prognostic factor; uterine cancer; uterine papillary serous carcinoma
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Introduction

Human uterine papillary serous carcinoma (UPSC) is categorized as a type II endometrial cancer and was first identified by Hendrickson's group as a clinically progressive and distinct neoplasm that morphologically resembles ovarian papillary serous carcinoma. UPSC accounts for 10% of all endometrial cancers and generally occurs in postmenopausal women (Hendrickson et al. 1982; Bancher-Todesca et al. 1998). Unlike patients with other subtypes of type I endometrial cancer, patients with UPSC are unlikely to be obese, hypertensive or diabetic, or to have a history of hormone replacement therapy, which are typical risk factors for type I endometrial cancer (Hendrickson et al. 1982; del Carmen et al. 2012). UPSC is an aggressive neoplasm with a high recurrence rate, rapid and deep myometrial invasion, and frequent lymphovascular space involvement (LVSI) (Hendrickson et al. 1982; Bancher-Todesca et al. 1998). UPSC patients without myometrial invasion also have extrauterine disease, similar to patients with a deeply invasive tumor (Grice et al. 1998). The 5-year survival rate varies from 15 to 51% in stage I UPSC (Lauchlan 1981). Thus, the poor prognosis of UPSC is similar to that of other high-grade uterine endometrial carcinomas.

The 14-3-3σ gene was originally discovered as a p53-inducible gene that was responsive to gamma irradiation and other DNA-damaging agents (Hermeking et al. 1997). Expression of 14-3-3σ is induced by DNA damage

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in a p53-dependent manner and prevents the cdc2-cyclin B1 complex from entering the nucleus, with subsequent induction of G2 arrest that allows repair of damaged DNA (Cheng et al. 2004). Expression of 14-3-3σ is frequently lost in human breast, gastric, liver, prostate, and lung cancers (Suzuki et al. 2000; Umbricht et al. 2001; Cheng et al. 2004; Kuroda et al. 2007), and these findings indicate that loss of 14-3-3σ is involved in failure to control the cell cycle and results in G2-type chromosomal aberrations. We have shown that decreased expression of 14-3-3σ is significantly associated with a poor prognosis in patients with epithelial ovarian cancer (Akahira et al. 2004). However, the relationship between 14-3-3σ expression and the prognosis of UPSC, which morphologically resembles ovarian papillary serous carcinoma, has yet to be examined.

In this study, we investigated the expression of 14-3-3σ in human UPSC tissues and evaluated the correlation between 14-3-3σ expression and clinicopathological parameters in patients with UPSC. We also examined the methylation status of the 14-3-3σ gene to determine if the 14-3-3σ expression mechanism is modified in human UPSC tissues.

Materials and Methods

Patients and Tissues

UPSC tissues were obtained from 51 patients after surgery performed at Tohoku University Hospital between January 2001 and September 2009. The median age of the patients at the time of surgery was 64.6 years old (range: 37-86 years old). The tissues were immediately fixed in 10% formalin or embedded in OCT compound, and then frozen in liquid N_2 and stored at −80ºC until use. No patient had received preoperative irradiation or chemotherapy. Information on age, performance status on admission, histology including the percentage of the tumor comprised of UPSC, stage, myometrial invasion, LVSI, lymph node invasion and overall survival were retrieved from a review of patient charts. The median follow-up time was 49 months (range: 3-116 months). Disease-free survival and overall survival were calculated from the time of initial surgery to recurrence and/or death or the date of last contact. The standard primary surgical treatment for endometrial carcinoma at Tohoku University Hospital in the period of the study was total abdominal hysterectomy, salpingo-oophorectomy, pelvic and/or para-aortic lymphadenectomy, followed by cytology of the peritoneal washings. If the patient was diagnosed with UPSC in a specimen obtained by curettage before surgery, omentectomy was also performed. Of the 51 patients, 45 received platinum-based chemotherapy and 6 did not receive any chemotherapy. Four out of the six patients received postoperative radiotherapy. Histological grading of UPSC was performed using FIGO (International Federation of Gynecology and Obstetrics) criteria. All patients in the study had endometrial carcinoma comprised of > 10% UPSC. Tumors with ≥ 50% UPSC were defined as “pure”, and those with 10-50% UPSC and ≥ 10% of other histological components as “mixed”. All archival specimens were retrieved from surgical pathology files at Tohoku University Hospital. The specimens were processed in 10% formalin, fixed for 24-48 h, paraffin embedded, and sectioned (thickness of 3 μm). This clinical research protocol was approved by the ethics committee of Tohoku University Graduate School of Medicine.

Immunohistochemistry

Immunohistochemical analysis was performed with the streptavidin-biotin amplification method using a Histofine kit (Nichirei, Tokyo, Japan). Polyclonal antibody for 14-3-3σ (N-14) and monoclonal antibody for p53 (B20.1) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and Biomeda (Foster City, CA, USA), respectively. For immunostaining of 14-3-3σ and p53, slides were heated in an autoclave at 121°C for 5 or 15 min in 0.01 M citric acid buffer [2 mmol/L citric acid and 9 mmol/L trisodium citrate dehydrate (pH 6.0)] following deparaffinization for antigen retrieval. Dilutions of the primary antibodies were 1:300 for 14-3-3σ and 1:50 for p53. The antigen-antibody complex was visualized with 1 mmol/L 3,3′-diaminobenzidine solution [in 50 mmol/L Tris-HCl buffer (pH 7.6) and 0.006% H_2O_2] and counterstained with hematoxylin. Tissue sections of non-neoplastic breast cancer were used as a positive control for 14-3-3σ, and colon cancer tissue was used as positive control for p53. In the immunohistochemical analysis, 14-3-3σ was detected by cytoplasmic staining and p53 by nuclear staining (Kuroda et al. 2007). Tumor cells were considered positive for 14-3-3σ when > 30% of cells were stained, and positive for p53 when > 10% of cells showed nuclear staining (Kuroda et al. 2007). Immunohistochemical expression of 14-3-3σ and p53 was independently reviewed by two of the authors (E.H. and Y.M.). A slide with a difference of > 5% between the two readings was reviewed jointly until a consensus was reached.

DNA preparation and bisulfite direct Sequencing

Genomic DNA from frozen tissues was extracted using an AquaPure Genomic DNA Kit (Bio-Rad, Hercules, CA, USA) and 1 μg was treated with sodium bisulfite using an EpiTect Bisulfite Kit (Qiagen, Valencia, CA, USA). Sequence analysis of genomic DNA templates treated with bisulfite (Ferguson et al. 2000) was performed using the following primers: 5′-GAG AGA GTT AGT TTG ATT TAG AAG G-3′ (sense primer) and 5′-CTT ACT AAT ATC CAT AAC CTC C-3′ (antisense primer), which generated a 473-bp PCR product. Hot-start PCR was performed at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 1 min. A band of the correct size was isolated and DNA was extracted using a QIAquick Gel Extraction Kit (Qiagen). Purified DNAs were sequenced with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Statistical analysis was performed using SAS ver. 5.0 (Statview, Cary, NC, USA). Relationships between 14-3-3σ expression and patient characteristics were evaluated in a cross-table using a χ^2 test. Correlations between 14-3-3σ and p53 immunoreactivity were assessed by Mann-Whitney *U* test. Overall survival curves were generated using the Kaplan-Meier method and significance was evaluated by log-rank test. Univariate and multivariate analyses were performed with a Cox proportional hazards model. $P < 0.05$ was considered to be significant in all tests.

Results

Immunohistochemistry in patients with UPSC

Initially, 14-3-3σ and p53 were examined immunohistochemically in normal uterine tissues. The immunoreactive 14-3-3σ was found in the cytoplasm of glandular cells in normal uterine tissue, whereas p53 immunoreactivity in

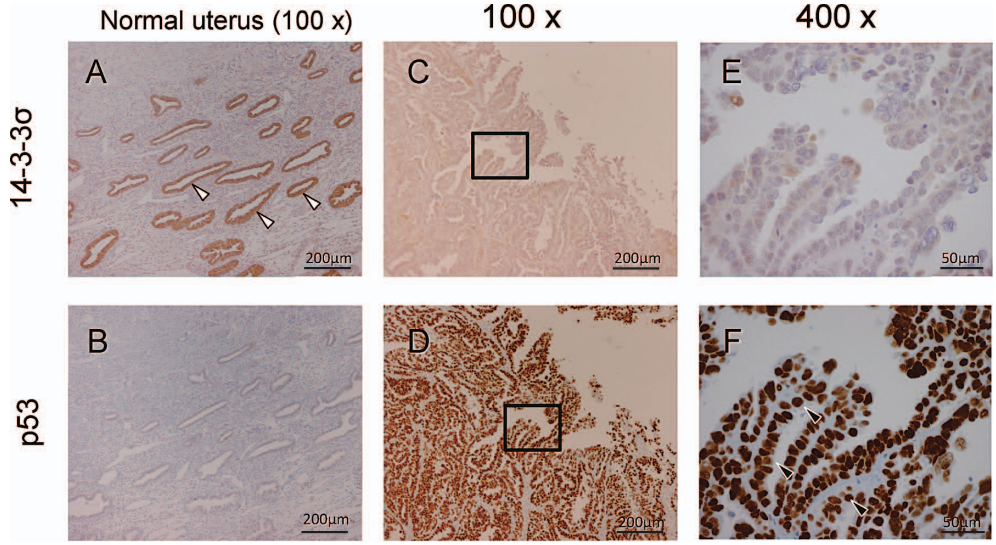

Fig. 1. Immunohistochemistry of 14-3-3σ and p53 in UPSC and normal uterine tissue.
A, B; 14-3-3σ was strongly expressed in the cytoplasm (white arrowheads) in normal uterine tissue (A), but p53 was negative (B). C, E; the immunoreactivity of 14-3-3σ was decreased or faintly detected in the cytoplasm in UPSC tissue. D, F; p53 immunoreactivity was detected in nuclei (black arrowheads) of UPSC cells. E and F are higher magnifications (400 ×) of the boxed areas in C and D (100 ×), respectively.

Table 1. Relationships between 14-3-3σ Immunoreactivity and Clinicopathological Parameters in 51 patients with UPSC.

	Total (n = 51)	%	14-3-3σ immunoreactivity		P value
			+ (n = 29)	− (n = 22)	
Stage					
I/ II	25	49.0%	20	5	0.001
III/ IV	26	51.0%	9	17	
Histology					
Pure	37	72.5%	19	18	0.196
Mixed	14	27.5%	10	4	
LVSI					
Positive	17	33.3%	5	12	0.005
Negative	34	66.7%	24	10	
p53 Immunoreactivity					
Positive	41	80.4%	23	18	0.823
Negative	10	19.6%	6	4	

LVSI, lymphovascular space involvement.

normal tissue was faint (Fig. 1A and B). We then examined 14-3-3σ and p53 immunoreactivities in UPSC tissues from 51 patients. The 14-3-3σ immunoreactivity was detected in the cytoplasm of cancer cells (Fig. 1C, E), whereas p53 was confined to nuclei (Fig. 1D, F). The 14-3-3σ immunoreactivity was detected in 29 of the 51 patients (56.9%), whereas no staining or < 30% staining was observed in 22 patients (43.1%) (Table 1). The p53 immunoreactivity was detected in nuclei of UPSC cells (Fig. 1D, F) in 41 patients (80.4%).

Relationships between clinicopathological parameters and immunohistochemistry

An examination of relationships of 14-3-3σ expression with clinicopathologic variables showed a significant association of the 14-3-3σ immunoreactivity with Stage (I/II vs. III/IV) (P = 0.001) and LVSI (Positive vs. Negative) (P = 0.005) (Table 1). Positive immunoreactivity of 14-3-3σ tended to be inversely correlated with that of p53, but the relationship was not significant (P = 0.823). Univariate analysis of the prognostic significance of each variable (Table 2) showed that 14-3-3σ immunoreactivity (P = 0.0001), Stage (P = 0.0001), and LVSI (P = 0.0158) were significantly associated with overall survival. The

Table 2. Univariate Analyses of Predictors of Disease Free and Overall Survival in 51 Patients with UPSC.

Variable	Overall Survival	Disease free Survival
	P value	*P* value
14-3-3σ (Positive vs. Negative)	0.0001	0.0003
Stage (I/II vs. III/IV)	0.0001	0.0002
Histological type (Pure vs. Mixed)	0.0870	0.1273
LVSI (Positive vs. Negative)	0.0158	0.0026
p53 (Positive vs. Negative)	0.8078	0.3411

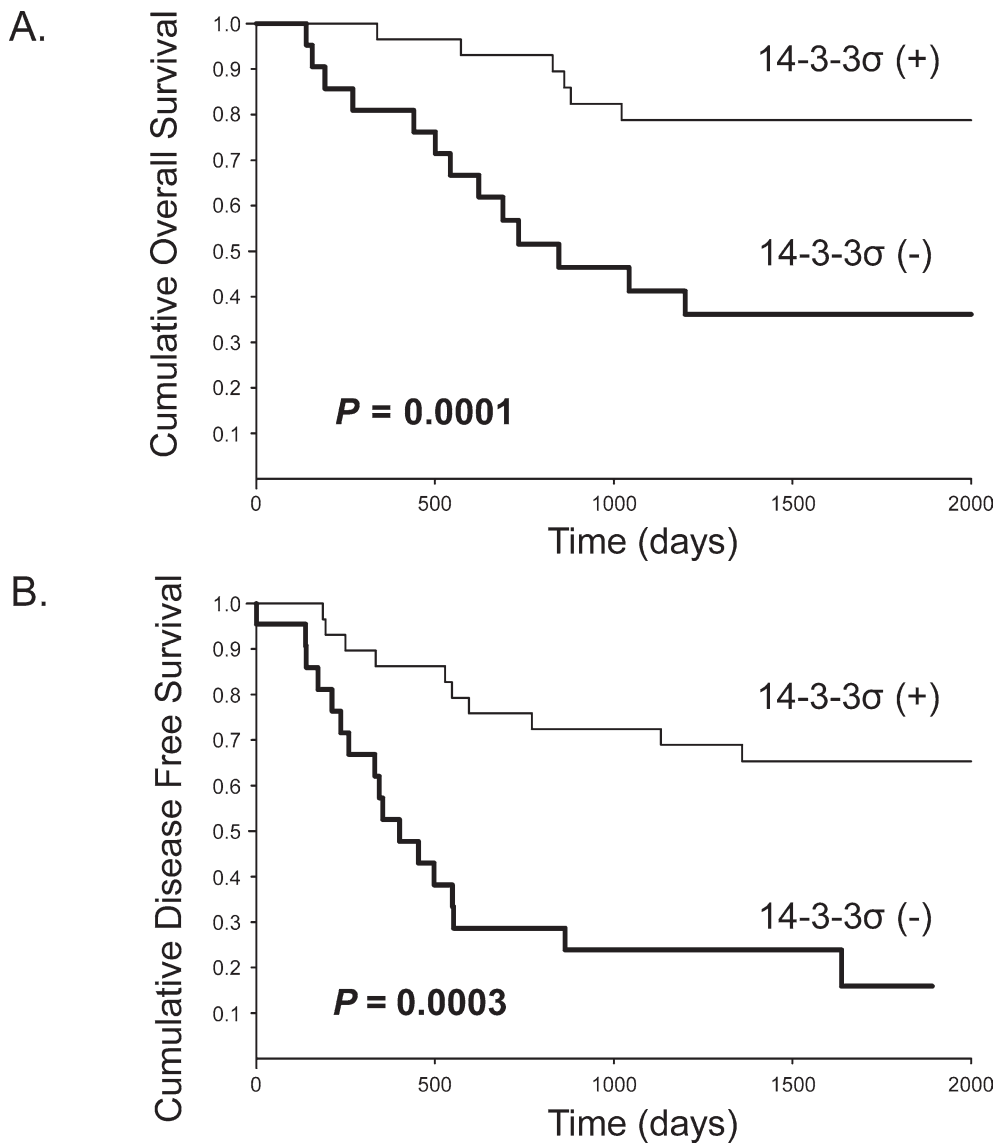

Fig. 2. Kaplan-Meier overall survival and recurrence curves for patients with UPSC.
A. Relationship of 14-3-3*σ* immunoreactivity and survival in patients with UPSC. B. Relationship of 14-3-3*σ* immunoreactivity and recurrence in patients with UPSC. In A and B, the numbers of patients were 29 for 14-3-3*σ* (+) and 22 for 14-3-3*σ* (+).

14-3-3*σ*-negative patients showed significantly worse overall and disease-free survival compared to 14-3-3*σ*-positive patients ($P = 0.0001$ in Fig. 2A and $P = 0.0003$ in Fig. 2B). Multivariate analysis (Table 3) showed that loss of expression of 14-3-3*σ* ($P = 0.0416$) and stage ($P = 0.0315$) were significant independent prognostic factors for overall survival, but LVSI did not show this relationship ($P = 0.4506$).

Table 3. Multivariate Analyses of Predictors for Disease Free and Overall Survival in 51 Patients with UPSC.

Variable	Overall Survival	Disease free Survival
	P value	*P* value
14-3-3σ (Positive vs. Negative)	0.0416	0.0830
Stage (I/II vs. III/IV)	0.0315	0.0459
LVSI (Positive vs. Negative)	0.4506	0.1640

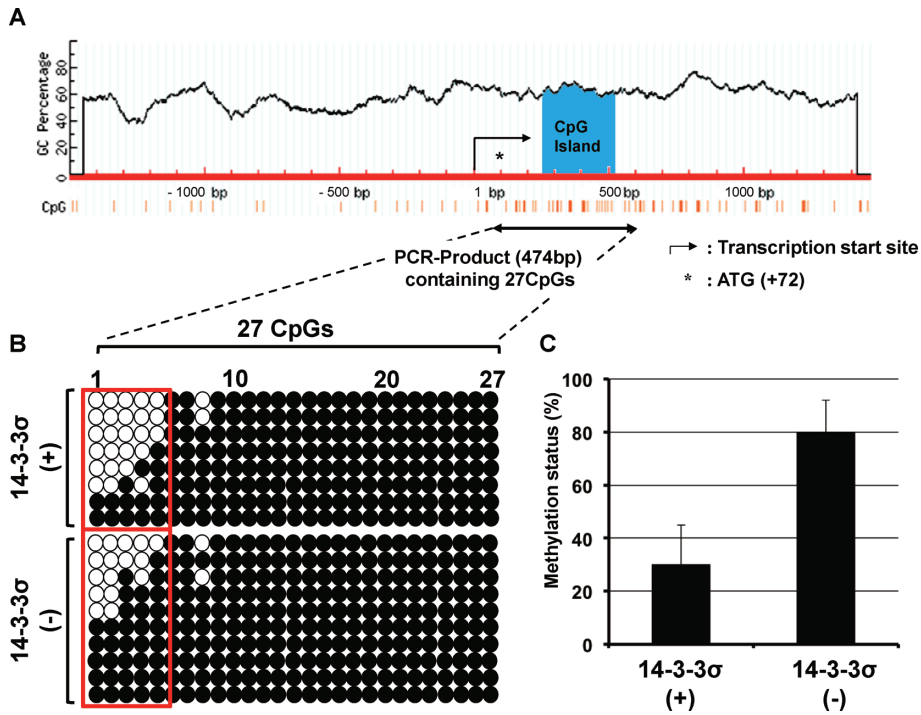

Fig. 3. Analysis of 14-3-3σ CpG island DNA methylation in patients with UPSC.
A. Location of PCR primers and CpG islands used for bisulfite direct sequence analysis of the 14-3-3σ gene. B. Methylation status of 27 CpG sites in the 14-3-3σ gene in UPSC samples: (●) methylated CpG sites, (○) unmethylated sites. The box indicates the region used for statistical analysis. C. Comparison of methylation status (%) in 14-3-3σ positive (+) patients (n = 29) and negative (−) patients (n = 22). Each value is shown as the mean ± SE.

Methylation status of the 14-3-3σ gene in UPSC tissues

To clarify the relationship between the 14-3-3σ immunoreactivity and gene expression in UPSC, we analyzed the methylation status of the 14-3-3σ gene by bisulfite sequencing. The primer sequences are depicted schematically in Fig. 3A. A total of 27 CpG islands were selected since these CpGs in the 14-3-3σ gene are frequently hypermethylated in breast cancer (Ferguson et al. 2000). In our samples, the median methylation frequencies were 30 ± 14.8% and 80 ± 12% in 14-3-3σ-negative and -positive UPSC, respectively (Fig. 3B and C). There was a tendency for a difference in methylation frequency in the vicinity of the transcription start site (Fig. 3A, arrow), but this was not significant.

Discussion

In this study, 14-3-3σ immunoreactivity was absent in 43.1% of patients with UPSC. This rate of negative cases was higher than that found in our studies of other gynecological cancers (i.e., ovarian serous adenocarcinoma, 26.5%; endometrial endometrioid adenocarcinoma, 24.7%) (Akahira et al. 2004; Ito et al. 2005). We designated tissues with > 50% UPSC as "pure" (37/51 patients, 72.5%) and those with 10-50% UPSC and ≥ 10% of other components as "mixed" (14/51 patients, 27.5%). Halperin et al. (2002) found no significant difference in overall survival between patients with 100% UPSC (n = 10) and those with mixed tumors containing at least 25% UPSC (n = 24). Thus, the presence of as little as 10% UPSC is sufficient to influence disease behavior and outcome based on the effect on disease-free survival (Halperin et al. 2002). However, in our patients, the histological type (pure vs. mixed) was not a significant risk factor for disease-free survival.

Results from previous reports support an association between inactivation of the 14-3-3σ gene and tumor progression. The 14-3-3σ gene is frequently inactivated in

intrahepatic cholangiocarcinoma (Kuroda et al. 2007) and its expression is decreased in poorly differentiated bladder squamous cell carcinoma (Ostergaard et al. 1997). In breast carcinomas, loss of 14-3-3σ expression becomes marked in progression from atypical hyperplastic lesions to ductal carcinoma *in situ* (Simooka et al. 2004). Loss of 14-3-3σ protein is also evident in prostate carcinoma and its precursors (Cheng et al. 2004; Urano et al. 2004). Akahira et al. (2004) showed that loss of 14-3-3σ expression correlated with advanced disease and/or high grade histology and was significantly associated with a poor prognosis in epithelial ovarian carcinoma. Finally, decreased immunoreactive 14-3-3σ is significantly associated with poor prognosis and/or recurrence in endometrial endometrioid adenocarcinoma (Ito et al. 2005). These results, along with those in the current study, suggest that the loss or decrease of 14-3-3σ expression may be an early event in carcinogenesis in various cancers. In this study, there was no significant difference in expression of 14-3-3σ between cases of early-stage and advanced-stage cancers. Thus, loss of 14-3-3σ expression in UPSC may be associated with an aggressive biological phenotype and may play an important role in prognosis and/or recurrence, in addition to being a relatively early event in carcinogenesis.

Hypermethylation of CpG islands is a well-known epigenetic mechanism of inactivation of tumor suppressor genes. Silencing of gene expression by CpG hypermethylation is an early event in cancer development and may precede the neoplastic process in some cases (Esteller et al. 2001). Mhawech et al. (2005) found that downregulation of 14-3-3σ expression in prostate, endometrial and ovarian carcinomas is associated with 14-3-3σ CpG methylation. Osada et al. (2002) showed histological type-specific inactivation of 14-3-3σ, which suggests that a DNA methylation-independent mechanism may also be involved in loss of 14-3-3σ expression in primary tumors. Our results suggested a higher rate of aberrant DNA methylation in UPSC negative for 14-3-3σ compared to the 14-3-3σ-positive cases, although the difference was not significant (Fig. 3B and C).

Expression of 14-3-3 is regulated in a p53-dependent manner (Hermeking et al. 1997). Mutation of p53 is common in UPSC, and thus there is a possibility that other factors are regulating expression of 14-3-3σ in UPSC (Mhawech et al. 2005). microRNAs are a novel class of gene regulators involved in endocrine function and cancer (Ricarte Filho and Kimura 2006). We have found that microRNA expression correlates with prognosis in UPSC patients (Hiroki et al. 2010) and that microRNA-34b has an important role in the molecular pathogenesis of UPSC (Hiroki et al. 2012). Therefore, downregulation of 14-3-3σ gene expression by a microRNA may occur in patients with UPSC.

In conclusion, our findings demonstrate that the absence of 14-3-3σ protein determined immunohistochemically is a potential prognostic marker in UPSC. Further studies are needed to examine the relationship between 14-3-3σ gene expression and methylation status. The results may provide molecular markers for evaluation of prognosis and selection of optimal therapy for women with UPSC.

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Conflict of Interest

The authors declare no conflict of interest.

References

Akahira, J., Sugihashi, Y., Suzuki, T., Ito, K., Niikura, H., Moriya, T., Nitta, M., Okamura, H., Inoue, S., Sasano, H., Okamura, K. & Yaegashi, N. (2004) Decreased expression of 14-3-3 sigma is associated with advanced disease in human epithelial ovarian cancer: its correlation with aberrant DNA methylation. *Clin. Cancer Res*., **10**, 2687-2693.

Bancher-Todesca, D., Neunteufel, W., Williams, K.E., Prainsack, D., Breitenecker, G., Friedlander, M.L. & Hacker, N.F. (1998) Influence of postoperative treatment on survival in patients with uterine papillary serous carcinoma. *Gynecol. Oncol*., **71**, 344-347.

Cheng, L., Pan, C.X., Zhang, J.T., Zhang, S., Kinch, M.S., Li, L., Baldridge, L.A., Wade, C., Hu, Z., Koch, M.O., Ulbright, T.M. & Eble, J.N. (2004) Loss of 14-3-3sigma in prostate cancer and its precursors. *Clin. Cancer Res*., **10**, 3064-3068.

del Carmen, M.G., Birrer, M. & Schorge, J.O. (2012) Uterine papillary serous cancer: a review of the literature. *Gynecol. Oncol*., **127**, 651-661.

Esteller, M., Corn, P.G., Baylin, S.B. & Herman, J.G. (2001) A gene hypermethylation profile of human cancer. *Cancer Res*., **61**, 3225-3229.

Ferguson, A.T., Evron, E., Umbricht, C.B., Pandita, T.K., Chan, T.A., Hermeking, H., Marks, J.R., Lambers, A.R., Futreal, P.A., Stampfer, M.R. & Sukumar, S. (2000) High frequency of hypermethylation at the 14-3-3 sigma locus leads to gene silencing in breast cancer. *Proc. Natl. Acad. Sci. USA*, **97**, 6049-6054.

Grice, J., Ek, M., Greer, B., Koh, W.J., Muntz, H.G., Cain, J., Tamimi, H., Stelzer, K., Figge, D. & Goff, B.A. (1998) Uterine papillary serous carcinoma: evaluation of long-term survival in surgically staged patients. *Gynecol. Oncol*., **69**, 69-73.

Halperin, R., Zehavi, S., Langer, R., Hadas, E., Bukovsky, I. & Schneider, D. (2002) Uterine papillary serous carcinoma (pure and mixed type) compared with moderately and poorly differentiated endometrioid carcinoma. A clinicopathologic study. *Eur. J. Gynaecol. Oncol*., **23**, 300-304.

Hendrickson, M., Ross, J., Eifel, P., Martinez, A. & Kempson, R. (1982) Uterine papillary serous carcinoma: a highly malignant form of endometrial adenocarcinoma. *Am. J. Surg. Pathol*., **6**, 93-108.

Hermeking, H., Lengauer, C., Polyak, K., He, T.C., Zhang, L., Thiagalingam, S., Kinzler, K.W. & Vogelstein, B. (1997) 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. *Mol. Cell*, **1**, 3-11.

Hiroki, E., Akahira, J., Suzuki, F., Nagase, S., Ito, K., Suzuki, T., Sasano, H. & Yaegashi, N. (2010) Changes in microRNA expression levels correlate with clinicopathological features and prognoses in endometrial serous adenocarcinomas. *Cancer Sci*., **101**, 241-249.

Hiroki, E., Suzuki, F., Akahira, J., Nagase, S., Ito, K., Sugawara, J., Miki, Y., Suzuki, T., Sasano, H. & Yaegashi, N. (2012) MicroRNA-34b functions as a potential tumor suppressor in

endometrial serous adenocarcinoma. *Int. J. Cancer*, **131**, E395-404.

Ito, K., Suzuki, T., Akahira, J., Sakuma, M., Saitou, S., Okamoto, S., Niikura, H., Okamura, K., Yaegashi, N., Sasano, H. & Inoue, S. (2005) 14-3-3sigma in endometrial cancer: a possible prognostic marker in early-stage cancer. *Clin. Cancer Res*., **11**, 7384-7391.

Kuroda, Y., Aishima, S., Taketomi, A., Nishihara, Y., Iguchi, T., Taguchi, K., Maehara, Y. & Tsuneyoshi, M. (2007) 14-3-3sigma negatively regulates the cell cycle, and its down-regulation is associated with poor outcome in intrahepatic cholangiocarcinoma. *Hum. Pathol*., **38**, 1014-1022.

Lauchlan, S.C. (1981) Tubal (serous) carcinoma of the endometrium. *Arch. Pathol. Lab. Med*., **105**, 615-618.

Mhawech, P., Benz, A., Cerato, C., Greloz, V., Assaly, M., Desmond, J.C., Koeffler, H.P., Lodygin, D., Hermeking, H., Herrmann, F. & Schwaller, J. (2005) Downregulation of 14-3-3sigma in ovary, prostate and endometrial carcinomas is associated with CpG island methylation. *Mod. Pathol*., **18**, 340-348.

Osada, H., Tatematsu, Y., Yatabe, Y., Nakagawa, T., Konishi, H., Harano, T., Tezal, E., Takada, M. & Takahashi, T. (2002) Frequent and histological type-specific inactivation of 14-3-3 sigma in human lung cancers. *Oncogene*, **21**, 2418-2424.

Ostergaard, M., Rasmussen, H.H., Nielsen, H.V., Vorum, H., Orntoft, T.F., Wolf, H. & Celis, J.E. (1997) Proteome profiling of bladder squamous cell carcinomas: identification of markers that define their degree of differentiation. *Cancer Res*., **57**, 4111-4117.

Ricarte Filho, J.C. & Kimura, E.T. (2006) MicroRNAs: novel class of gene regulators involved in endocrine function and cancer. *Arq. Bras. Endocrinol. Metabol*., **50**, 1102-1107.

Simooka, H., Oyama, T., Sano, T., Horiguchi, J. & Nakajima, T. (2004) Immunohistochemical analysis of 14-3-3 sigma and related proteins in hyperplastic and neoplastic breast lesions, with particular reference to early carcinogenesis. *Pathol. Int*., **54**, 595-602.

Suzuki, H., Itoh, F., Toyota, M., Kikuchi, T., Kakiuchi, H. & Imai, K. (2000) Inactivation of the 14-3-3 sigma gene is associated with 5' CpG island hypermethylation in human cancers. *Cancer Res*., **60**, 4353-4357.

Umbricht, C.B., Evron, E., Gabrielson, E., Ferguson, A., Marks, J. & Sukumar, S. (2001) Hypermethylation of 14-3-3 sigma (stratifin) is an early event in breast cancer. *Oncogene*, **20**, 3348-3353.

Urano, T., Takahashi, S., Suzuki, T., Fujimura, T., Fujita, M., Kumagai, J., Horie-Inoue, K., Sasano, H., Kitamura, T., Ouchi, Y. & Inoue, S. (2004) 14-3-3sigma is down-regulated in human prostate cancer. *Biochem. Biophys. Res. Commun*., **319**, 795-800.