ARID1A expression in ovarian clear cell carcinoma with an adenofibromatous component

（腺線維腫を有する卵巣明細胞腺癌における Adenine-thymine–rich interactive domain 1A の発現について）

千葉大学大学院医学薬学府

先端医学薬学専攻

（主任：生水真紀夫教授）

錦見　恭子

Abstract

Aims: The carcinogenesis of ovarian clear cell carcinoma (CCC) has been hypothesized to comprise two different pathways: an adenofibroma-carcinoma sequence and an endometriosis-carcinoma sequence. However, the difference in the genetic basis of these two pathways remains unclear. Recent studies have suggested that an *ARID1A* mutation and the loss of the corresponding protein, BAF250a, are frequent events in CCC. Herein, we investigated the difference in the loss of BAF250a expression in adenofibroma-related CCC and endometriosis-related CCC.

Methods and Results: In total, 93 cases of surgically treated CCC were evaluated. The presence of adenofibroma and endometriosis associated with carcinoma was determined by reviewing hematoxylin and eosin-stained slides for each case. BAF250a expression in carcinoma was examined immunohistochemically. The loss of BAF250a expression was detected in carcinomas in 50 of 93 (54%) cases, including 5/18 (28%) with adenofibroma alone, 30/45 (67%) with endometriosis alone, 8/18 (44%) with both conditions, and 7/12 (58%) with neither condition. The loss of BAF250a expression was significantly less frequent in CCC cases with adenofibroma than in cases with endometriosis ($p = 0.01$, Fisher's exact test).

Conclusions: The action of *ARID1A* in carcinogenesis differs between adenofibroma-related CCC and endometriosis-related CCC.

Introduction

Adenine-thymine–rich interactive domain 1A (*ARID1A*) has recently been identified as a tumour suppressor gene, which is associated with various human neoplastic lesions, including gynaecological cancers.[1-5] Genome-wide sequencing analysis has shown approximately 50% of ovarian clear cell carcinomas (CCC) and 30% of ovarian endometrioid carcinomas, but none in high-grade serous carcinoma, to harbour somatic mutations in *ARID1A*,[2, 3] *ARID1A* encodes BAF250a, a component of the ATP-dependent chromatin remodelling complex SWI/SNF, containing BRG1 or BRM,[2] which plays a crucial role in cell proliferation and differentiation.[4-8] Recent studies in mice have shown that *ARID1A* (BAF250a) promotes the growth of ovarian carcinomas by interacting with p53[4], PTEN[9], or PIK3CA.[10] The loss of BAF250a expression—detected by immunostaining—has recently been shown to be significantly correlated with an *ARID1A* mutation,[2,11] and it can therefore be used as a surrogate marker.

Ovarian CCC is frequently associated with endometriosis and less often with adenofibroma. The carcinogenesis pathways of endometriosis-related CCC and adenofibroma-related CCC are hypothesized to differ from each other[12-14] despite the fact that both endometriosis and adenofibroma are occasionally present in association with the same CCC concurrently.[15-17] The molecular abnormality underlying endometriosis-related CCC has been ascribed to the highly inflammatory, stressful environment of an endometriotic lesion,

leading to the activation of the PTEN-PIK3CA-mTOR pathway.[18] However, the molecular abnormality underlying adenofibroma-related CCC remains unclear. The present study was conducted to investigate differences in the loss of BAF250a expression in adenofibroma-related CCC and endometriosis-related CCC to clarify the difference in the genetic background and carcinogenesis between these two lesions.

Materials and Methods

Patients and tissue samples

The Institutional Review Board of Chiba University Graduate School of Medicine approved this research protocol (Approval Number 1903, 21 August 2014). Ninety-three patients with CCC who were surgically treated between 2000 and 2012 at Chiba University Hospital and affiliated hospitals in Chiba, Japan, were included in the study. All available hematoxylin and eosin-stained slides of the ovarian tumours were reviewed by two gynaecologic pathologists to confirm the histological type of the tumours on the basis of the WHO classification[19] and for the presence of adenofibroma and/or endometriosis associated with carcinoma. In the present study, adenofibroma was defined by the presence of proliferation foci of tubules lined by a single layer of tumour cells, with minimal nuclear atypia and a marked stromal fibromatous component.[15-17] We classified endometriosis into two categories: one with endometriotic cyst formation contiguous with or adjacent to CCC, and the other being distant

from CCC, without endometriotic cyst formation.

Immunohistochemical staining

Formalin-fixed paraffin-embedded tissue sections (4-μm-thick) were deparaffinized, and immunohistochemistry was performed using the antibody against ARID1A (BAF250a) (HPA005456, Sigma-Aldrich, Tokyo, Japan) at a dilution of 1:400. Auto-stainer Link48 (Dako, Tokyo Japan) was used for immunostaining. Antigen retrieval was performed by incubating sections in low pH Target Retrieval Solution (Dako) at 98°C for 20 min.

Immunohistochemical staining was scored using a previously described method.[1, 20] The percentage of positively stained epithelial cells was scored as 0 (0–9%), 1 (10–25%), 2 (26–50%), or 3 (51–100%), and the intensity was scored as 0 (undetectable), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). The total immunostaining score was defined as percentage positivity score × staining intensity score and ranged from 0 to 9. We considered total immunostaining scores of 0–4 as loss of BAF250a expression and scores of 6 or 9 as retained BAF250a expression.

Statistical analysis

Loss of BAF250a expression in adenofibroma-related CCC was compared with that in endometriosis-related CCC using Fisher's exact test. Logistic regression analysis was used to

clarify the correlation between adenofibroma and loss of BAF250a expression after adjusting for the presence of endometriosis. A survival curve for patients with pT1 was calculated using the Kaplan–Meier method and compared by the log-rank test. Statistical analyses were performed using the Statistical Package for Social Sciences, version II for Windows (SPSS Inc., Chicago, IL, USA); $p < 0.05$ was considered statistically significant.

Results

Patient characteristics and associated lesions

The patients' characteristics and BAF250a expression status are summarised in Table 1. All patients were surgically treated, with no residual tumour. The follow-up period ranged from seven months to 150 months, with a median of 60 months. Overall, 86 of 93 patients presented with stage pT1, and the remaining 7 presented with pT2 or pT3. Of 93 CCCs, 18 (19%) were associated with adenofibroma alone, 45 (48%) with endometriosis alone, 18 (19%) with both components, and 12 (13%) with neither of them. No patient with atypical endometriosis was identified.

Loss of BAF250a expression in adenofibroma, endometriosis, and carcinoma

None of the 80 cases with adenofibroma and/or endometriosis showed a loss of BAF250a expression in these components, and all had a total immunostaining score of 9. In contrast,

the extent of BAF250a immunoreactivity in the carcinomatous component varied with regard to staining intensity and the percentage of positive cells (Figure 1). Loss of BAF250a expression (score 0–4) was detected in the carcinomatous component in 50 (54%) of 93 patients, and the breakup is as follows: 45 (52%) of 86 patients with pT1 disease, and 5 (71%) of 7 patients with pT2/pT3 disease. The difference between the two groups was not statistically significant ($p = 0.445$, Fisher's exact test). Patients with loss of BAF250a expression in CCC included 5 (28%) of 18 with adenofibroma alone, 30 (67%) of 45 with endometriosis alone, 8 (44%) of 18 with both adenofibroma and endometriosis, and 7 of 12 (58%) with neither condition (Table 2). Loss of BAF250a expression was found to be significantly less frequent in CCC associated with adenofibroma alone than in CCC with endometriosis alone when analysed by Fisher's exact test ($p = 0.01$). Because 18 patients had both endometriosis and adenofibroma, data were adjusted for endometriosis to clarify the correlation between adenofibroma and loss of BAF250a expression (Table 3). Univariate and multivariate analyses showed that the loss of BAF250a expression was less frequent in CCC with an adenofibromatous component. In 63 CCC patients with endometriosis, loss of BAF250a expression in the carcinomatous component occurred in 26 (55%) of 47 patients with endometriotic cysts contiguous with or adjacent to CCC and in 12 (75%) of 16 patients with endometriosis distant from CCC without cyst formation. Loss of BAF250a expression in CCC did not differ between the two types of endometriosis ($p = 0.24$, Fisher's exact test).

Prognostic impact of BAF250a expression

The 5-year progression-free survival rates in the 86 patients with pT1 CCC with and without loss of BAF250a expression were 82% and 79%, respectively. Kaplan–Meier analysis revealed no significant relationship between BAF250a immunoreactivity and progression-free survival in pT1 CCC patients ($p = 0.89$, log-rank test) (Figure 2).

Discussion

In this study, we have shown that loss of BAF250a expression was significantly less frequent in adenofibroma-associated CCC than in endometriosis-associated CCC. This is the first report showing a genetic difference between adenofibroma-associated CCC and endometriosis-associated CCC. Our results suggest that the mutation in *ARID1A*, which encodes BAF250a, may not be as strongly associated with the development of adenofibroma-related CCC as it is with endometriosis-related CCC, and that the role of *ARID1A* in carcinogenesis may differ between the two groups. We observed a loss of BAF250a expression in only 28% in adenofibroma-related CCC cases, indicating that another non-*ARID1A* genetic aberration might underlie this condition. In contrast, loss of BAF250a expression in CCC with endometriosis was observed in 67% of the patients, consistent with previous studies.[2, 21, 23] This indicates that the *ARID1A* mutation plays an important role in the

development of CCC derived from endometriosis. Furthermore, the high frequency of *ARID1A* mutations in our study, as well as in earlier reports, and in ovarian endometrioid adenocarcinoma but not in high-grade serous carcinoma[2, 3] suggests that *ARID1A* mutations might be associated with carcinomas related to endometriosis. In evaluating BAF250a immunostaining, we use the total score defined as percentage positivity score × staining intensity score ranging from 0 to 9.[1, 20] Cells without *ARID1A* mutations, including normal cells, are expected to show positive staining for BAF250a. We considered 'loss of BAF250a expression' as significant decreases in staining intensity and in percentage of stained cells, and used the cut off index as a score of 4 or less to avoid overestimating the loss of BAF250a expression. We believe our evaluation method is justifiable, given that our result of BAF250a expression in CCC with endometriosis was consistent with that of published studies.[2, 21- 23]

In our study, loss of BAF250a expression was not observed in the adenofibromatous component in any of the cases studied. This implies that *ARID1A* mutations do not occur in these putative precursor lesions and are not early events in adenofibroma-related CCC. This is in conflict with the findings of Yamamoto et al., who reported the loss of BAF250a expression in six of 14 cases of CCC associated with adenofibroma; benign (three of three), and borderline (six of six) clear cell adenofibroma components adjacent to carcinoma were found to lack BAF250a expression.[23] A possible reason for the discrepancy between our results and those of Yamamoto et al. is the difference in the criteria used for adenofibroma

diagnosis. Diagnostic criteria for the distinction between clear cell adenofibroma and borderline adenofibroma, and between borderline adenofibroma and CCC, have not been firmly established.[15-17] Although we only designated cases as adenofibroma when tubules with minimal nuclear atypia were present, we occasionally observed a few tubules with moderate atypia mixed with tubules of carcinoma in the abundant fibrous stroma of adenofibroma-related CCC. In these cases, we considered the lesion to be part of the carcinoma and thus did not include it as an adenofibroma component.

There have been several reports of loss of BAF250a expression not only in carcinoma but also in endometriosis and atypical endometriosis.[2, 21-23] According to these reports, an *ARID1A* mutation occurred in atypical endometriosis before the development of carcinoma, and it was an early event in the malignant transformation of ovarian endometriosis. In the present study, we were not able to identify foci of atypical endometriosis in any of the cases included. Moreover, in contrast to some previous reports,[21-23] the endometriotic component did not show loss of BAF250a expression in our study, perhaps owing to differences in the histological features of endometriotic lesions and experimental protocols. In our study, we included endometriosis associated with carcinoma, regardless of location and size. In most of our endometriosis patients, endometriotic lesions were subtle, often with a limited number of epithelial cells present. In studies by Ayhan et al. and Yamamoto et al., loss of BAF250a expression was observed in the epithelial cells of endometriotic lesions but not in endometrial

stromal cells.[21, 23] Thus, it is possible that in our patients, the epithelial cells of some endometriotic lesions had indeed lost BAF250a expression, but this could not be detected because of epithelial exfoliation. Alternatively, methodological attributes for these discrepancies might include differences in the antibodies, antibody dilutions, and immunohistochemical assessment methods used. To conclude whether *ARID1A* mutation occurs in conventional endometriosis and is an early event during malignant transformation of ovarian endometriosis, larger studies and additional methods such as DNA or RNA sequencing analysis[2, 11] are needed.

There are conflicting reports concerning the prognostic value of BAF250a expression in CCC. No prognostic impact of the loss of BAF250a expression was detected for pT1 CCC cases in our study. Maeda et al. reported no significant difference in overall survival in 121 cases of CCC with or without loss of BAF250a expression.[11] In contrast, Katagiri et al. reported that CCC patients showing loss of BAF250a expression had a shorter progression-free interval than those with normal levels of BAF250a.[24] Both these studies included CCC cases of all stages (FIGO stages III/IV in 31 of 121 cases and 15 of 60 cases in the former and latter studies, respectively), and that of Katagiri et al. included 12 of 60 cases with residual tumours of ≥2 cm, whereas in the present study, we included pT1 CCC cases only. It is possible that loss of BAF250a expression may be a prognostic factor in advanced-stage CCC; however, further in-depth studies are required to validate this concept.

In conclusion, we identified a genetic difference in the carcinogenesis pathways of adenofibroma-related and endometriosis-related CCC by revealing that loss of BAF250a expression was significantly less frequent in adenofibroma-related CCC than in endometriosis-related CCC. Our results suggest that other non-*ARID1A* genetic aberrations might underlie adenofibroma-related CCC. We expect that our results might aid in the discovery of such mutations. Patients with CCC are resistant to currently available cytotoxic drugs. Therefore, another implication of our present findings is the possibility of individualization of CCC treatment once drugs targeting *ARID1A* are developed.

Acknowledgments

We thank Tsutomu Matsui, Kikuyo Kawashima, and Takashi Onodera for their technical assistance. We also thank Kouichiro Hirashiki, Youichi Unno, Yoshihiko Izawa, Naotake Tanaka, Michio Sanada, Hideaki Iwasaki, Noriko Yamamoto, Hideo Matsui, Reiko Matsumoto, Yuka Sakuma, Nobuo Endo, Yoshimasa Kawarai, Masaaki Kamiyama, Takako Kato, Makoto Kawada, Kaori Kuroda and Yuko Okazima for sample collections.

Author contributions: Kyoko Nishikimi and Takako Kiyokawa conceived and carried out experiments; Kyoko Nishikimi and Shinichi Tate analysed data; Takako Kiyokawa and Masami Iwamoto confirmed the histology and evaluated the immunohistochemistry; and Shinichi Tate and Makio Shozu collected clinical data and specimens. All authors were involved in writing the paper and approve of the submitted and published versions.

References

[1] Wang DD, Chen YB, Pan K, et al. Decreased expression of the ARID1A gene is associated with poor prognosis in primary gastric cancer. PLOS ONE 2012; 7(7): e40364.

[2] Wiegand KC, Shah SP, Al-Agha OM, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med 2010; 363: 1532-1543.

[3] Jones S, Wang TL, Shih IeM, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science 2010; 330: 228-231.

[4] Wu RC, Wang TL, Shih IeM. The emerging roles of ARID1A in tumor suppression. Cancer Biol Ther 2014; 15(6): 655-664.

[5] Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic tumor suppressor? Cancer Discov 2013; 3(1): 35-43.

[6] Ho L, Crabtree GR. Chromatin remodelling during development. Nature 2010; 463: 474-484.

[7] Wang X, Nagl NG, Wilsker D, et al. Two related ARID family proteins are alternative subunits of human SWI/SNF complexes. Biochem J 2004; 383: 319-325.

[8] Van Rechem C, Boulay G, Leprince D. HIC1 interacts with a specific subunit of SWI/SNF complexes, ARID1A/BAF250A. Biochem Biophys Res Commun 2009; 385: 586-590.

[9] Guan B, Rahmanto YS, Wu RC et al. Roles of deletion of Arid1a, a tumor suppressor, in

mouse ovarian tumorigenesis. J Natl Cancer Inst 2014; 106: (7) pii: dju146.

[10] Chandler RL, Damrauer JS, Raab JR et al. Coexistent ARID1A-PIK3CA mutations promote ovarian clear-cell tumorigenesis through pro-tumorigenic inflammatory cytokine signalling. Nat Commun 2015; 6: 6118.

[11] Maeda D, Mao TL, Fukayama M, et al. Clinicopathological significance of loss of ARID1A immunoreactivity in ovarian clear cell carcinoma. Int J Mol Sci 2010; 11(12): 5120-5128.

[12] Yamamoto S, Tsuda H, Takano M, Hase K, Tamai S, Matsubara O. Clear cell adenofibroma can be a clonal precursor for clear cell carcinoma of the ovary: a possible alternative ovarian clear cell carcinogenic pathway. J Pathol 2008; 216: 103-110.

[13] Yamamoto S, Tsuda H, Suzuki K, Takano M, Tamai S, Matsubara O. An allelotype analysis indicating the presence of two distinct ovarian clear cell carcinogenic pathways: endometriosis-associated pathway vs. clear cell adenofibroma-associated pathway. Virchows Arch 2009; 455(3): 261-270.

[14] Veras E, Mao TL, Ayhan A, et al. Cystic and adenofibromatous clear cell carcinomas of the ovary: distinctive tumors that differ in their pathogenesis and behavior: a clinicopathologic analysis of 122 cases. Am J Surg Pathol 2009; 33: 844-853.

[15] Roth LM, Langley FA, Fox H, Wheeler JE, Czernobilsky B. Ovarian clear cell adenofibromatous tumors. Benign, of low malignant potential, and associated with invasive

clear cell carcinoma. Cancer 1984; 53(5): 1156-1163.

[16] Bell DA, Scully RE. Benign and borderline clear cell adenofibromas of the ovary. Cancer 1985; 56: 2922-2931.

[17] Zhao C, Wu LS, Barner R. Pathogenesis of ovarian clear cell adenofibroma, atypical proliferative (borderline) tumor, and carcinoma: clinicopathologic features of tumors with endometriosis or adenofibromatous components support two related pathways of tumor development. J Cancer 2011; 2: 94-106.

[18] Gounaris I, Charnock-Jones DS, Brenton JD. Ovarian clear cell carcinoma—bad endometriosis or bad endometrium? J Pathol 2011; 225: 157-160.

[19] Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO Classification of Tumours of Female Reproductive Organs, 4th edn. Lyon: International Agency for Research on Cancer, 2014; 34.

[20] Fadare O, Gwin K, Desouki MM, et al. The clinicopathologic significance of p53 and BAF-250a (ARID1A) expression in clear cell carcinoma of the endometrium. Mod Pathol 2013; 26(8): 1101-1110.

[21] Ayhan A, Mao TL, Seckin T, et al. Loss of ARID1A expression is an early molecular event in tumor progression from ovarian endometriotic cyst to clear cell and endometrioid carcinoma. Int J Gynecol Cancer 2012; 22(8): 1310-1315.

[22] Xiao W, Awadallah A, Xin W. Loss of ARID1A/BAF250a expression in ovarian endometriosis and clear cell carcinoma. Int J Clin Exp Pathol 2012; 5(7): 642-650.

[23] Yamamoto S, Tsuda H, Takano M, Tamai S, Matsubara O. Loss of ARID1A protein expression occurs as an early event in ovarian clear cell carcinoma development and frequently coexists with PIK3CA mutations. Mod Pathol 2012; 25(4): 615-624.

[24] Katagiri A, Nakayama K, Rahman MT, et al. Loss of ARID1A expression is related to shorter progression-free survival and chemoresistance in ovarian clear cell carcinoma. Mod Pathol 2012; 25(2): 282-288.

Table 1. Clinicopathological features of 93 ovarian clear cell carcinomas and loss of BAF250a expression (#1NX, lymphadenectomy not performed)

Parameters	n	Loss of BAF250a expression (%)
Total	93	50 (54)
Mean age [years (range)]	56 (32–84)	
Tumour infiltration		
T1a	27	11 (41)
T1b	1	1 (100)
T1c	58	33 (57)
T2a	0	0 (0)
T2b	0	0 (0)
T2c	5	3 (60)
T3a	1	1 (100)
T3b	0	0 (0)
T3c	1	1 (100)
Lymph node metastasis		
N0	51	31 (61)
N1	1	0 (0)
NX#1	41	19 (46)
Received adjuvant chemotherapy		
Yes	80	48 (60)
No	13	2 (15)
Recurrence		
Yes	20	12 (60)
No	73	38 (52)

Table 2. Loss of BAF250a expression in carcinoma, adenofibroma, and endometriosis

Associated lesion	No. of cases	Loss of BAF250a expression		
		Adenofibroma	Endometriosis	Carcinoma
Adenofibroma+/Endometriosis+	**18**	**0 (0%)**	**0 (0%)**	**8 (44%)**
Adenofibroma+/Endometriosis-	**18**	**0 (0%)**	**-**	**5 (28%)***
Adenofibroma-/Endometriosis+	**45**	**-**	**0 (0%)**	**30 (67%)***
Adenofibroma-/Endometriosis-	**12**	**-**	**-**	**7 (58%)**

*$p = 0.01$, Fisher's exact test

Table 3. Univariate and multivariate analysis of loss of BAF250a expression in carcinoma associated with adenofibroma and endometriosis

Associated lesion	No. of cases	No. of cases with loss of BAF250a expression in carcinoma	Univariate analysis			Multivariate analysis		
			Odds ratio	95% CI	*p*-value	Odds ratio	95% CI	*p*-value
Adenofibroma+	**36**	**13 (36%)**	**0.3**	**0.1–0.8**	**0.01**	**0.3**	**0.1–0.9**	**0.03**
Endometriosis+	**63**	**38 (60%)**	**0.6**	**0.2–1.1**	**0.10**	**1.8**	**0.7–4.6**	**0.25**

Figure 1. Representative histological features (A–D; hematoxylin and eosin staining) and BAF250a immunostaining (E–H) of clear cell carcinoma (CCC) and associated adenofibroma or endometriosis (×100). (A,B) CCC associated with adenofibroma. (C,D) CCC associated with endometriosis. (E) BAF250a expression is retained in both CCC and adenofibroma. (F) BAF250a expression is lost in CCC but retained in adenofibroma. (G) BAF250a expression is retained in both CCC and endometriosis. (H) BAF250a expression is lost in CCC but retained in endometriosis.

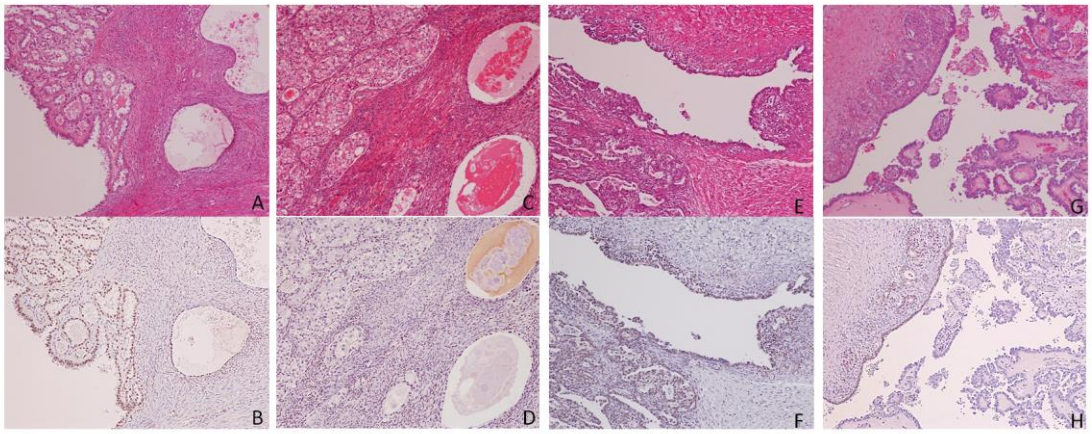
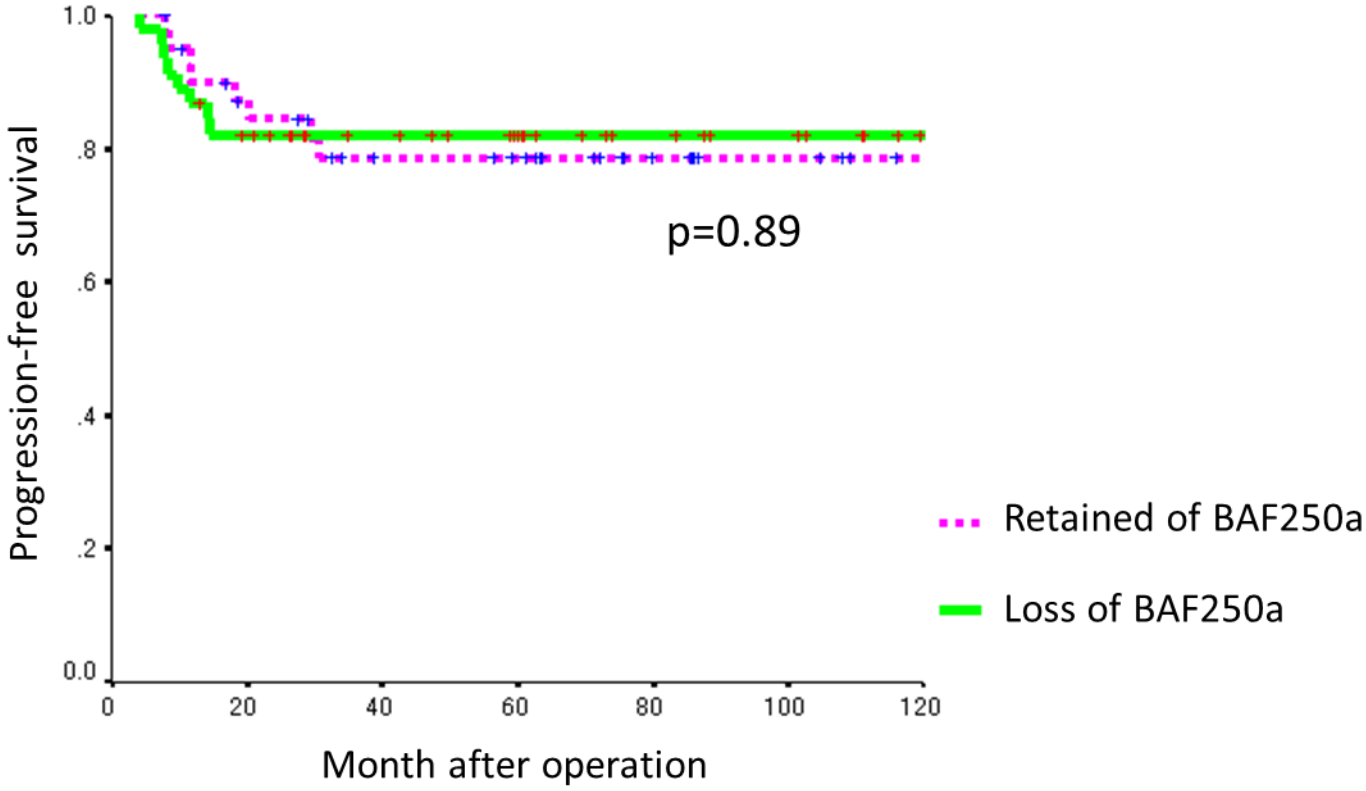


Figure 2. Kaplan–Meier curves of progression-free survival and BAF250a expression in patients with pT1 cancer.



Histopathology Vol.67 Issue 6 p.866-871

平成27年6月7日　公表済