

Original Article

Loss of BAP1 expression is a very rare event in gastrointestinal stromal tumors

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Abstract: Gastrointestinal stromal tumors (GISTs), the most common mesenchymal tumors affecting the gastrointestinal tract, are primarily driven by activating *KIT* and *platelet-derived growth factor receptor alpha (PDGFRA)* mutations and respond to targeted tyrosine kinase therapy. However, GISTs exhibit various clinical behaviors, regardless of the proposed risk classification. Here, we investigated the expression of BRCA1-associated protein-1 (BAP1) in GIST and analyzed its prognostic significance and utility as a marker in the context of differential diagnosis. Among a total of 226 GIST, only one (0.44%, 1/226 cases) GIST exhibited a loss of BAP1 expression. In the univariate analysis, small intestinal and colorectal GISTs, GISTs with necrosis, recurrence/metastasis, or a higher risk of malignancy were associated with poor overall survival. In the multivariate analysis, GISTs with a higher risk of malignancy or recurrence/metastasis were identified as independent prognostic factors. We conclude that a loss of BAP1 expression is a very rare event, then *BAP1* would not play a major role in pathogenesis of GIST.

Keywords: Gastrointestinal stromal tumor, BAP1, BRCA1-associated protein, immunohistochemistry

Introduction

Gastrointestinal stromal tumors (GISTs) comprise the most common type of mesenchymal neoplasms of the gastrointestinal tract. These tumors are resistant to conventional chemotherapy and radiotherapy [1, 2]. In adults, the majority of GISTs harbor characteristic oncogenic mutations in *KIT* (80-85%) and *PDGFRA* (5-7%) and respond to targeted tyrosine kinase (TK) therapy [2, 3]. However, approximately 10-15% of GISTs are *KIT/PDGFRA* wild-type (WT) and are less sensitive to TK inhibitors (TKI).

BRCA1-associated protein-1 (BAP1) is a deubiquitinating enzyme that plays important roles in chromatin modulation, DNA transcription and cell cycle regulation, cellular growth, and DNA repair [3-5]. *BAP1* is now recognized as a tumor suppressor gene, and germline *BAP1* mutations have been associated with autosomal dominant cancer syndromes that include cutaneous and uveal melanoma, renal cell carcinoma, cutaneous basal cell carcinoma, and malignant mesothelioma [6]. Somatic *BAP1* mutations have also been investigated in the context of mesothelioma, intrahepatic cholangiocarci-

noma (ICC), clear cell renal cell carcinoma, and atypical cutaneous spitzoid tumors [3, 6-9]. Immunohistochemistry (IHC) for BAP1 appears to serve as a reliable and highly sensitive/specific marker of *BAP1* mutation or inactivation, irrespective of underlying genetic alterations [4, 7, 10]. Accordingly, the BAP1 expression status has been described as clinically significant in a variety of human tumors [6, 9, 11-13].

The present study aimed to investigate the BAP1 expression statuses of GISTs, to assess the clinical and pathological significance, and to identify the utility in differential diagnosis from other tumors. To the best of our knowledge, BAP1 expression status has not previously been assessed in GIST.

Materials and methods

Patient characteristics

Between 1997 and 2016, a total of 226 GISTs from the stomach (154 cases), small intestine (67 cases), colon and rectum (3 cases), and extra gastrointestinal locations (pelvic cavity and abdominal cavity) were included. Medical

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Table 1. Clinicopathologic features of GISTs

Clinicopathologic factors	Number (%)	Death* (%)
M:F	120 (53.1):106 (46.9)	20 (10.8):13 (7.0)
Location		
Stomach	154 (68.1)	15 (12.3)
Small intestine	67 (29.6)	16 (11.6)
Colon and rectum	3 (1.3)	2 (1.1)
Others	2 (0.9)	0 (0)
Cell type		
Spindle	206 (91.1)	30 (16.2)
Epithelioid	9 (4.0)	2 (1.1)
Mixed	11 (4.9)	1 (0.5)
Risk of malignancy		
None	26 (11.5)	0 (0)
Very low	69 (30.5)	2 (1.1)
Low	45 (19.9)	2 (1.1)
Moderate	40 (17.7)	7 (3.8)
High	46 (20.4)	22 (11.9)
Mucosal invasion	25 (11.1)	6 (3.2)
Necrosis	37 (16.4)	13 (7.0)
Recurrence or metastasis	26 (11.5)	18 (9.7)
CD117 positive	222 (98.2)	33 (17.8)
DOG1 positive	222 (98.2)	32 (17.3)
BAP1 loss	1 (0.44)	0 (0)

*Available follow-up: 185 cases.

records were reviewed to determine each patient's age, sex, most recent follow-up visit, survival status, and the presence or absence of GIST-related disease. The following clinicopathologic characteristics were also assessed: tumor location, tumor size, mitotic count, tumor cell type, necrosis, mucosal ulceration, and recurrence or metastasis. The risk of malignant behavior was classified according to the system proposed by the National Comprehensive Cancer Network (NCCN) Guideline [14], and further classified as low, moderate, or high risk (Table 1). Overall survival (OS) was defined as the time from surgical resection to death or the last follow-up. The follow-up period ended in October 2016 (OS range: 0-215 months) and we could get follow-up data from 185 patients. Patients with a higher risk malignant behavior or metastasis received imatinib therapy. This study was approved by our institutional Human Ethics Review Board.

Tissue microarray block construction

We obtained two to five 2-mm cores from the most representative tumor area of each case,

and arrayed in a tissue microarray (TMA) block. One core from breast carcinoma, thyroid papillary carcinoma, normal gastric mucosa, palatine tonsil, or uterine leiomyoma was obtained and arrayed in a TMA block, and used as control tissues. We made total 11 TMA blocks with 226 cases.

Immunohistochemistry

Immunohistochemistry for BAP1 was performed on an automated Benchmark[®] platform (Ventana Medical Systems, Tucson, AZ, USA). For BAP1, a mouse monoclonal antibody (clone C-4, Santa Cruz Biotechnology, Dallas, TX, USA) was used at a 1:100 dilution after onboard heat-induced epitope retrieval in a high-pH CC1 buffer (99°C, 1 h). Staining was visualized using the UltraView[™] universal DAB detection kit (Automated BenchMark[®], Ventana), which included a hydrogen peroxide substrate and a 3,3'-diaminobenzidine chromogen solution. The slides

were subsequently counterstained with hematoxylin.

Interpretation of IHC

The slides were assessed by an investigator who was blinded to the patients' clinicopathologic information. We defined a loss of BAP1 expression as a complete absence of nuclear staining in tumor cells. Lymphocytes and background stromal cells served as the positive controls.

Statistical analyses

The chi-square test and Fisher's exact test were used to examine correlations between categorical variables. Overall survival was defined as described above. Disease-free survival (DFS) was defined as the postoperative interval without a known recurrence or metastasis. Survival rates were calculated using the Kaplan-Meier method. Associations between survival rates and various clinicopathologic factors were evaluated using the log-rank test. A Cox proportional hazard regression model was used to evalu-

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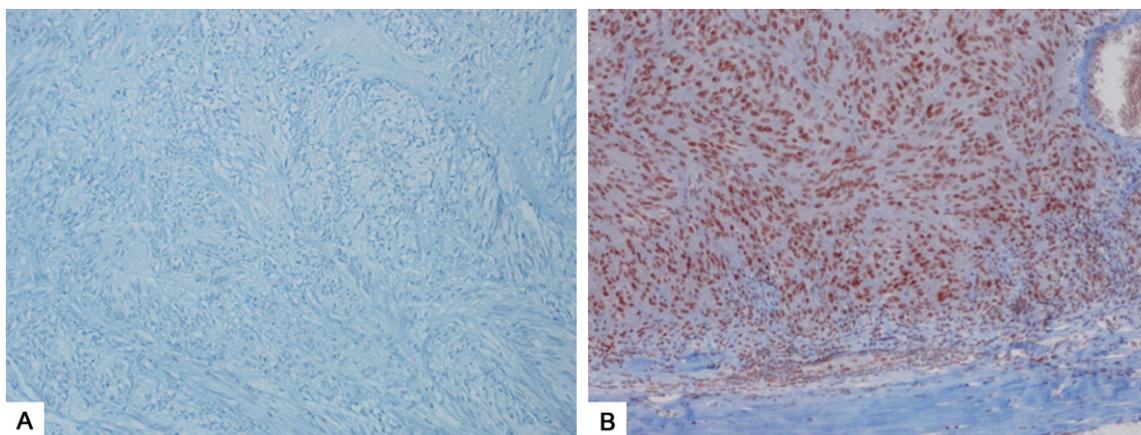


Figure 1. BRCA1-associated protein-1 (BAP1) expression in GIST. (A) loss of BAP1 expression (B) retained BAP1 expression ($\times 10$).

Table 2. Univariate and multivariate analyses of clinicopathologic factors affecting the overall survival of patients with gastrointestinal stromal tumors

Variables	Univariate analysis			Multivariate analysis		
	β	HR (95% CI)	<i>p</i>	β	HR (95% CI)	<i>p</i>
Small intestine vs stomach	.715	2.044 (0.998-4.183)	.050	-.719	0.487 (0.204-1.160)	.104
Colorectum vs stomach	1.874	6.517 (1.471-28.860)	.014	-.079	0.924 (0.159-5.356)	.924
Other location vs stomach	-10.245	0.000	.978	-12.946	0.000	.979
Moderate risk vs low risk	2.330	10.281 (2.975-35.531)	<0.0001	2.340	8.199 (2.308-29.123)	.001
High risk vs low risk	3.043	20.974 (7.187-61.209)	<0.0001	2.640	12.231 (3.692-40.525)	<0.0001
Necrosis	1.549	4.705 (2.288-9.679)	<0.0001	.146	1.157 (0.484-2.768)	.743
Recurrence or metastasis	2.246	9.448 (4.700-18.995)	<0.0001	.945	2.573 (1.163-5.692)	.020

HR: hazard ratio; CI: confidence interval.

Table 3. Univariate and multivariate analyses of clinicopathologic factors affecting the disease-free survival of patients with gastrointestinal stromal tumors

Variables	Univariate analysis			Multivariate analysis		
	β	HR (95% CI)	<i>p</i>	β	HR (95% CI)	<i>p</i>
Small intestine vs stomach	.903	2.467 (1.218-4.997)	.012	-.484	0.616 (0.269-1.414)	.254
Colorectum vs stomach	1.930	6.888 (1.559-30.433)	.011	-.493	0.611 (0.106-3.522)	.581
Other location vs stomach	-10.167	0.000	.982	-12.320	0.000	.979
Moderate risk vs low risk	2.244	9.432 (2.715-32.761)	<0.0001	2.021	7.550 (2.095-27.202)	.002
High risk vs low risk	3.323	27.751 (9.433-81.636)	<0.0001	2.568	13.038 (3.886-43.743)	<0.0001
Necrosis	1.507	4.513 (2.199-9.264)	<0.0001	-.126	1.157 (0.484-2.768)	.743
Recurrence or metastasis	2.973	19.549 (9.278-41.193)	<0.0001	1.693	5.434 (2.393-12.338)	<0.0001

ate the significance of the prognostic factors. Variables with significant result in the univariate analysis were analyzed in the multivariate Cox proportional hazard regression analysis. Hazard ratios (HR) and associated 95% confidence intervals (CI) were calculated for each variable. All comparisons were performed using SPSS, version 23.0 (SPSS Inc., Chicago, IL, USA). A $p < .05$ was considered to indicate statistical significance.

Results

Clinicopathologic characteristics

One hundred twenty-six male and 120 female patients with a median age of 58.5 years (range: 22-88 years) were included in this study. The median tumor size was 4.79 cm (range: 1-23 cm). CD117 and DOG1 expression were detected in 222 cases (98.2%), respectively.

SDHB negativity was observed only in two wild-type gastric GISTs in a 56-year-old female and 15-year-old male patient. These SDHB-negative GISTs exhibited diffuse strong positive CD117 and DOG1 staining.

Immunohistochemistry of BAP1 and its clinical significance

Of the 226 GISTs, only one case (0.44%) exhibited a loss of BAP1 expression (**Figure 1A**). All other cases exhibited diffuse homogeneous BAP1 positivity (**Figure 1B**). We next stained a whole section from a representative tumor block to confirm the loss of BAP1 expression, and observed that the tumor cells were completely negative for BAP1. Statistically, we observed no significance associations between loss of BAP1 expression and clinicopathologic factors. In univariate analysis, small intestinal and colorectal GISTs, GISTs with necrosis, recurrence/metastasis, or a higher risk of malignancy were significantly associated with a poor OS and DFS. In a multivariate analysis, GISTs with a higher risk of malignancy and recurrence/metastasis were confirmed as independent prognostic factors (**Tables 2, 3**).

Discussion

In a study of 226 GISTs cases, a loss of BAP1 expression was observed in only one small intestinal GIST. The indicated patient was a 33-years-old man without a family or personal history of BAP1-associated malignancy. The tumor was a 9 cm × 5.5 cm-sized mass with mucosal ulceration, 1/50 high-powered field mitosis rate, spindle cell histology, diffuse strong CD117 and DOG1 positivity, and *KIT* mutation. Irrespective of imatinib therapy, the tumor metastasized to abdominal wall (12 months later) and brain (90 months later), but no other malignancy was not detected for follow-up period. Recently, germline mutation in BAP1 have been reported in causing a hereditary tumor syndrome that gives an increased risk of cancers [15]. Although we did not perform BAP1 germline mutation test for this case, he was unlikely BAP1-associated hereditary syndrome because he did not have BAP1-related family history or BAP1-associated malignancy.

Studies of BAP1 expression have increased since the first report of the diagnostic utility of this marker for mesothelial lesions. A loss of

BAP1 expression was found to be 100% specific for malignant mesothelioma and could be used to distinguish malignant mesothelioma from benign mesothelial proliferation [9, 16]. However, studies to determine the clinical significance of BAP1 expression in several human tumors has yielded varied results. A meta-analysis of BAP1 expression in cancers revealed that 1) BAP1 is generally a poor prognostic marker for cancers, except mesothelioma; 2) *BAP1* mutations are associated with high-grade colorectal and renal cell carcinomas; and 3) *BAP1*-mutated cancers more commonly occur in women than in men [6]. In contrast, mesotheliomas with loss of BAP1 expression showed a better outcome compared to those with BAP1 expression, and this association was notable among epithelioid cases [13]. In lung cancer, Fan observed a 52.5% rate of BAP1 loss among advanced cases, and this loss was associated with lymph node metastasis and poor OS [17]. However, two other studies identified a loss of BAP1 expression in only one case of primary non-small cell lung cancer (1/101 and 1/257 cases), and suggested that BAP1 could be used to distinguish primary lung carcinoma from malignant mesothelioma [13, 18, 19]. In gastric carcinoma, decreased BAP1 expression was associated with a higher histologic grade, TNM stage, metastasis, and reduced OS [20]. Similarly, in clear cell renal cell carcinoma, a loss of BAP1 was associated with a larger tumor size, higher TNM stage, higher nuclear grade, metastasis, and reduced OS [12, 21]. In two studies of intrahepatic cholangiocarcinoma (ICC), Misumi observed that a loss of BAP1, an independent prognostic factor, was associated with mass-forming small duct type ICC, reduced perineural invasion and mucin production [22]. In cholangiocarcinoma, *BAP1* mutation was found to be correlate with aggressive disease and poor responses to standard therapies [23]. However, recent studies have revealed rare losses of BAP1 in lung and other human cancers. A loss of BAP1 expression was observed in only one of 306 cases of pancreatic ductal adenocarcinoma, despite the close anatomical proximity and similarities of this type of cancer (e.g., aspects of embryogenesis) with the bile duct [24]. These findings suggest that a loss of BAP1 expression could be used to distinguish a diagnosis of pancreatic ductal adenocarcinoma from ICC. Furthermore, a loss of BAP1 expression was very rare among peritoneal and

gynecological serous adenocarcinomas and could facilitate a differential diagnosis of abdominal mesothelioma [13].

A loss of BAP1 expression has shown to be 100% specific for malignancy. However, Hwang observed that losses of BAP1 were observed in 15% of sarcomatous/desmoplastic mesotheliomas. In contrast, no BAP1 loss was observed in sarcomatoid carcinoma [25]. These findings and our study results suggest that BAP1 loss might not assist a differential diagnosis in a small biopsy that includes KIT (-) GIST, sarcomatoid carcinoma, and sarcomatoid/desmoplastic mesothelioma. The germline and somatic mutation of *BAP1* is considered uncommon event in GIST, since most *BAP1* mutation is highly associated with loss of protein expression.

In summary, we conducted the first study of BAP1 expression status in a large cohort of GISTs. The loss of BAP1 expression was observed in only one case, and it suggests that BAP1 expression loss is a very rare event in GIST and *BAP1* would not play a major role in pathogenesis of GIST. The risk of malignancy and recurrence/metastasis were confirmed as independent prognostic factors for GIST.

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Disclosure of conflict of interest

None.

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References

- [1] Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH and Weiss SW. Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Hum Pathol* 2002; 33: 459-465.
- [2] Hornick JL and Fletcher CD. The role of KIT in the management of patients with gastrointestinal stromal tumors. *Hum Pathol* 2007; 38: 679-687.
- [3] Ventii KH, Devi NS, Friedrich KL, Chernova TA, Tighiouart M, Van Meir EG and Wilkinson KD. BRCA1-associated protein-1 is a tumor suppressor that requires deubiquitinating activity and nuclear localization. *Cancer Res* 2008; 68: 6953-6962.
- [4] Murali R, Wilmott JS, Jakrot V, Al-Ahmadie H, Wiesner T, McCarthy SW, Thompson JF and Scolyer RA. BAP1 expression in cutaneous melanoma: a pilot study. *Pathology* 2013; 45: 606-609.
- [5] Wadt KA, Aoude LG, Johansson P, Solinas A, Pritchard A, Crainic O, Andersen MT, Kiilgaard JF, Heegaard S, Sunde L, Federspiel B, Madore J, Thompson JF, McCarthy SW, Goodwin A, Tsao H, Jonsson G, Busam K, Gupta R, Trent JM, Gerdes AM, Brown KM, Scolyer RA and Hayward NK. A recurrent germline BAP1 mutation and extension of the BAP1 tumor predisposition spectrum to include basal cell carcinoma. *Clin Genet* 2015; 88: 267-272.
- [6] Luchini C, Veronese N, Yachida S, Cheng L, Nottegar A, Stubbs B, Solmi M, Capelli P, Pea A, Barbareschi M, Fassan M, Wood LD and Scarpa A. Different prognostic roles of tumor suppressor gene BAP1 in cancer: a systematic review with meta-analysis. *Genes Chromosomes Cancer* 2016; 55: 741-749.
- [7] Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, Creaney J, Lake RA, Zakowski MF, Reva B, Sander C, Delsite R, Powell S, Zhou Q, Shen R, Olshen A, Rusch V and Ladanyi M. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat Genet* 2011; 43: 668-672.
- [8] Gossage L, Murtaza M, Slatter AF, Lichtenstein CP, Warren A, Haynes B, Marass F, Roberts I, Shanahan SJ, Claas A, Dunham A, May AP, Rosenfeld N, Forshew T and Eisen T. Clinical and pathological impact of VHL, PBRM1, BAP1, SETD2, KDM6A, and JARID1c in clear cell renal cell carcinoma. *Genes Chromosomes Cancer* 2014; 53: 38-51.
- [9] Farzin M, Toon CW, Clarkson A, Sioson L, Watson N, Andrici J and Gill AJ. Loss of expression of BAP1 predicts longer survival in mesothelioma. *Pathology* 2015; 47: 302-307.
- [10] Yoshikawa Y, Sato A, Tsujimura T, Emi M, Morinaga T, Fukuoka K, Yamada S, Murakami A, Kondo N, Matsumoto S, Okumura Y, Tanaka F, Hasegawa S, Nakano T and Hashimoto-Tamaoki T. Frequent inactivation of the BAP1 gene in epithelioid-type malignant mesothelioma. *Cancer Sci* 2012; 103: 868-874.
- [11] Shen C, Wang Y, Wei P and Du X. BRCA1-associated protein 1 deficiency in lung adenocarcinoma predicts poor outcome and increased tumor invasion. *BMC Cancer* 2016; 16: 670.

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- [12] Minardi D, Lucarini G, Milanese G, Di Primio R, Montironi R and Muzzonigro G. Loss of nuclear BAP1 protein expression is a marker of poor prognosis in patients with clear cell renal cell carcinoma. *Urol Oncol* 2016; 34: 338.e18.
- [13] Andrici J, Jung J, Sheen A, D'Urso L, Sioson L, Pickett J, Parkhill TR, Verdonk B, Wardell KL, Singh A, Clarkson A, Watson N, Toon CW and Gill AJ. Loss of BAP1 expression is very rare in peritoneal and gynecologic serous adenocarcinomas and can be useful in the differential diagnosis with abdominal mesothelioma. *Hum Pathol* 2016; 51: 9-15.
- [14] Demetri GD, von Mehren M, Antonescu CR, DeMatteo RP, Ganjoo KN, Maki RG, Pisters PW, Raut CP, Riedel RF, Schuetze S, Sundar HM, Trent JC and Wayne JD. NCCN task force report: update on the management of patients with gastrointestinal stromal tumors. *J Natl Compr Canc Netw* 2010; 8 Suppl 2: 4.
- [15] McDonnell KJ, Gallanis GT, Heller KA, Melas M, Idos GE, Culver JO, Martin SE, Peng DH and Gruber SB. A novel BAP1 mutation is associated with melanocytic neoplasms and thyroid cancer. *Cancer Genet* 2016; 209: 75-81.
- [16] M McGregor S, McElherne J, Minor A, Keller-Ramey J, Dunning R, Husain AN, Vigneswaran W, Fitzpatrick C and Krausz T. BAP1 immunohistochemistry has limited prognostic utility as a complement of CDKN2A (p16) fluorescence in situ hybridization in malignant pleural mesothelioma. *Hum Pathol* 2017; 60: 86-94.
- [17] Fan LH, Tang LN, Yue L, Yang Y, Gao ZL and Shen Z. BAP1 is a good prognostic factor in advanced non-small cell lung cancer. *Clin Invest Med* 2012; 35: E182-9.
- [18] Owen D, Sheffield BS, Ionescu D and Churg A. Loss of BRCA1-associated protein 1 (BAP1) expression is rare in non-small cell lung cancer. *Hum Pathol* 2017; 60: 82-85.
- [19] Andrici J, Jung J, Sheen A, D'Urso L, Sioson L, Pickett J, Parkhill TR, Verdonk B, Wardell KL, Singh A, Clarkson A, Watson N, Toon CW and Gill AJ. Loss of BAP1 expression is very rare in peritoneal and gynecologic serous adenocarcinomas and can be useful in the differential diagnosis with abdominal mesothelioma. *Hum Pathol* 2016; 51: 9-15.
- [20] Yan S, He F, Luo R, Wu H, Huang M, Huang C, Li Y and Zhou Z. Decreased expression of BRCA1-associated protein 1 predicts unfavorable survival in gastric adenocarcinoma. *Tumour Biol* 2016; 37: 6125-6133.
- [21] Joseph RW, Kapur P, Serie DJ, Parasramka M, Ho TH, Cheville JC, Frenkel E, Parker AS and Brugarolas J. Clear cell renal cell carcinoma subtypes identified by BAP1 and PBRM1 expression. *J Urol* 2016; 195: 180-187.
- [22] Misumi K, Hayashi A, Shibahara J, Arita J, Sakamoto Y, Hasegawa K, Kokudo N and Fukayama M. Intrahepatic cholangiocarcinoma frequently shows loss of BAP1 and PBRM1 expression, and demonstrates specific clinicopathological and genetic characteristics with BAP1 loss. *Histopathology* 2017; 70: 766-774.
- [23] Al-Shamsi H, Anand D, Shroff RT, Jain A, Zuo M, Conrad C, Vauthey JN and Javle MM. BRCA-associated protein 1 mutant cholangiocarcinoma: an aggressive disease subtype. *J Gastrointest Oncol* 2016; 7: 556-561.
- [24] Tayao M, Andrici J, Farzin M, Clarkson A, Sioson L, Watson N, Chua TC, Sztynka T, Samra JS and Gill AJ. Loss of BAP1 expression is very rare in pancreatic ductal adenocarcinoma. *PLoS One* 2016; 11: e0150338.
- [25] Hwang HC, Pyott S, Rodriguez S, Cindric A, Carr A, Michelsen C, Thompson K, Tse CH, Gown AM and Churg A. BAP1 immunohistochemistry and p16 FISH in the diagnosis of sarcomatous and desmoplastic mesotheliomas. *Am J Surg Pathol* 2016; 40: 714-718.