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Immunohistochemical detection of MTAP and BAP1 protein loss for mesothelioma diagnosis: Comparison with 9p21 FISH and BAP1 immunohistochemistry

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ABSTRACT

Objectives: Differentiating malignant pleural mesothelioma (MPM) from reactive mesothelial hyperplasia (RMH) is still challenging. Detection of homozygous deletion (HD) of 9p21 region including $p16^{INK4A}$ (*p16*) by fluorescence *in situ* hybridization (FISH) and immunohistochemical detection of loss of BRCA1 associated protein 1 (BAP1), are reliable markers for MPM diagnosis. However, not all laboratories are equipped to perform 9p21 FISH; immunohistochemistry (IHC) is a more common and feasible technique. Thus, we sought to develop a IHC-based method that could predict the deletion of *p16* in MPM in concordance with 9p21 FISH.
Materials and methods: We examined the expression of the 9p21.3-related proteins (p14, p15, p16, and methylthioadenosine phosphorylase (MTAP)) and BAP1 using IHC in 51 MPM and 25 RMH cases, and assessed their correlation with HD of *p16* detected by FISH. The diagnostic usefulness of IHC of the 9p21.3-related proteins and BAP1 and their combinations was assessed using the cut-off values set by receiver operating characteristic (ROC) analysis.
Results: Among the 9p21.3-related proteins, MTAP IHC findings showed best concordance with 9p21 FISH results (kappa coefficient of 0.69) and a specificity of 100%. We also examined the combinations of MTAP IHC with the other products. The loss of p16 and MTAP had better concordance (kappa coefficient of 0.71), although lower specificity (85%). For differentiating MPM from RMH, only MTAP showed 100% specificity among the 9p21.3-related proteins, as did BAP1 IHC and 9p21 FISH. Among BAP1 combinations, only that of BAP1 with MTAP showed 100% specificity. Its sensitivity was 76.5%, which was lower than BAP1 IHC and 9p21 FISH combination (84.3%), but higher than BAP1 IHC alone (60.8%) or 9p21 FISH alone (60.8%).
Conclusions: A combination of MTAP or BAP1 loss detected by IHC can likely detect MPM with good sensitivity and 100% specificity, and serve as useful ancillary IHC for discriminating MPM from RMH.
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Abbreviations: BAP1, BRCA1 associated protein 1; CEA, carcinoembryonic antigen; DAPI, 4′,6-diamidino-2-phenylindole; EPP, extrapleural pneumonectomy; FISH, fluorescence *in situ* hybridization; HD, homozygous deletion; IHC, immunohistochemistry; L/D, loss or decrease; MPM, malignant pleural mesothelioma; MTAP, methylthioadenosine phosphorylase; PD, pleurectomy and decortication; RMH, reactive mesothelial hyperplasia; ROC, receiver-operating characteristic; RT, room temperature; SSC, saline-sodium citrate; TTF-1, thyroid transcription factor-1; VATS, video-assisted thoracic surgery; WHO, World Health Organization; WT-1, Wilms' tumor-1.

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1. Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive malignancy associated with asbestos exposure. With heavy use of asbestos in industries in the past, the disease is expected to rise in incidence in Japan for the next one or two decades, though it has peaked in the United States [1]. Despite the poor prognosis and the median survival time of less than 20 months [2–6], early detection and treatment could lead to prolonged survival [5,6]. Thus, precise diagnosis in the early stage of the disease is critical.

One of the challenges in early MPM diagnosis is the difficulty in differentiating MPM from reactive mesothelial hyperplasia (RMH). The histological and cytological findings of RMH can often mimic those of MPM [7–9]. Identifying tumor cell invasion of the surrounding adipose tissue is the gold standard for MPM diagnosis [7], but sometimes it cannot be assessed in small biopsy specimens.

Recently, loss of nuclear expression of BRCA1 associated protein 1 (BAP1), detected using immunohistochemistry (IHC), has been reported to be useful for distinguishing MPM from RMH [10–14]. *BAP1* gene encodes a nuclear ubiquitin carboxy-terminal hydrolase located in 21.1 region of the short arm of chromosome 3 (3p21.1) [15]. Loss of BAP1 expression has been reported in 56–81% of epithelioid MPM cases, with a specificity of 100% for differentiating MPM from RMH thus far [11–14].

The other useful method for discriminating MPM versus RMH is the detection of homozygous deletion (HD) of $p16^{INK4A}$ (*p16*, also known as *CDKN2A*) using fluorescence *in situ* hybridization (FISH) [10,13,14,16–18]. *p16* is located in 21.3 region of the short arm of chromosome 9 (9p21.3). *p16* HD has been found in 45–86% of epithelioid MPM cases, while so far RMH cases have never been reported to harbor *p16* HD [13,14,16,17]. In MPM and RMH cases, we previously demonstrated the concordance of the deletion status of *p16* detected by FISH between histologic specimens and cytologic smears, and proposed the possibility of early MPM diagnosis by using 9p21 FISH for the smears of pleural effusions, which are one of the early symptoms of MPM [19]. However, FISH assay cannot be performed in all laboratories because of its complexities, skillful procedures involved, and higher costs. Previously, several studies examined the usefulness of p16 IHC to detect the deletion status of *p16* [18,20,21]. However, the results were variable.

In addition to *p16*, several genes such as $p14^{ARF}$, $p15^{INK4B}$ and *methylthioadenosine phosphorylase* (*MTAP*) are located in the 9p21.3 locus, and are co-deleted with *p16* in some MPM cases [17,22–27]. In pancreatic cancer, a combined loss of MTAP and p16 protein expression has been proposed as a surrogate marker for HD of *p16* [28]. Loss of expression of the 9p21.3-related proteins (p14, p15, p16, and MTAP) detected by IHC has been reported in MPM by several groups [18,20,21,29,30]. However, whether IHC detection of the loss of or decrease in the expression of these 9p21.3-related proteins could predict the deletion status of *p16* as detected by FISH in MPM, has not been elucidated.

The aim of this study was to investigate IHC detection of the 9p21.3-related proteins in MPM and their correlation with the deletion status of *p16* detected by FISH. Furthermore, we examined the usefulness of IHC based detection of 9p21.3-related proteins independently or in combination with BAP1 IHC for differentiating MPM versus RMH.

2. Materials and methods

2.1. Case selection

Fifty-one MPM and 25 RMH cases obtained from patients with bullae formation, were collected from the pleural lesion file of the Department of Pathology, Fukuoka University Hospital and Kyushu University Hospital, Fukuoka, Japan, between 2001 and 2015. The file included consultation cases. Anonymous use of redundant tissues is part of the standard patient treatment agreement. These materials are not used if the patient objects to this arrangement. The study protocol was approved by the Ethics Committee of the Fukuoka University and Kyushu University Hospitals. Mesothelioma diagnosis and classification were performed according to the World Health Organization (WHO) classification issued in 2015 [31]. The mesothelial nature of each tumor was confirmed using IHC (calretinin, D2-40, cytokeratin 5/6, and Wilms' tumor-1 (WT-1) as positive mesothelial markers; thyroid transcription factor-1 (TTF-1), Ber-EP4, and carcinoembryonic antigen (CEA) as negative markers). Sarcomatoid-type tumors were excluded from the analysis, because of their lower frequency of BAP1 loss [11,12] and our future plan to apply the results to smear cytology.

2.2. Immunohistochemistry

Immunohistochemical staining was performed on the formalin-fixed, paraffin-embedded, 4-μm-thick tissue sections using the following antibodies after epitope retrieval using pH 9.0 Tris-EDTA buffer at 95 °C for 40 min: anti-p14 mouse monoclonal (Sigma-Aldrich Corporation, St Louis, MO, USA; 1:500; 4 °C, overnight), anti-p15 mouse polyclonal (Abcam, Cambridge, UK; 1:500; room temperature (RT), 1 h), anti-p16 mouse monoclonal (BD Pharmingen, San Diego, CA, USA; 1:50; 4 °C, overnight), anti-MTAP rabbit monoclonal (Abcam; 1:500; RT, 1 h), and anti-BAP1 mouse monoclonal (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:100; RT, 1 h) antibodies. Immunoreacted cells were visualized with 3, 3′-diaminobenzidine, and the nuclei were counterstained with hematoxylin. Staining for p15, MTAP and BAP1 was carried out with the Dako Omnis (Agilent Technologies Company, Glostrup, Denmark).

The staining conditions for each antibody were established using mesothelioma cell lines that were known to harbor HD of 9p21 region and *BAP1* gene alterations (ACC-MESO-4; RIKEN BioResource Center Cell Bank, Tsukuba, Japan, and NCI-H2452; American Type Culture Collection, Manassas, VA, USA) as negative controls. Specimens from normal tonsil tissue (for BAP1), liver tissue (for MTAP), and phyllodes tumor (for p14, p15, and p16) served as positive controls. Non-mesothelial immunoreactive cells (e.g., inflammatory cells, fibroblasts, pneumocytes, and endothelial cells) were used as internal positive control in each staining protocol.

Nuclear staining in mesothelial cells with the same or higher intensity than internal positive control was regarded as positive in p14, p15, p16 or BAP1 IHC. Cytoplasmic positivity was interpreted as a non-specific reaction. In MTAP IHC, cytoplasmic expression with nuclear staining in mesothelial cells at same as or higher intensity than internal positive control was interpreted as positive. Representative IHC features are shown in Fig. 1. In each case, at least 500 cells in 5–10 representative high power fields were evaluated, and the proportion of positively stained cells was calculated.

2.3. FISH

9p21 FISH was performed on the formalin-fixed, paraffin-embedded, 4-μm-thick tissue sections using Vysis LSI CDKN2A SpectrumOrange/CEP9 SpectrumGreen Probes (Abbott Japan, Tokyo, Japan) as previously described [9,14,19]. Briefly, the sections were treated with 2 × saline-sodium citrate buffer (SSC) containing 0.3% Tween 20 (SSC-0.3T; Sigma-Aldrich), incubated in pretreatment solution (Histology FISH Accessory Kit; Dako; 1:20) at 95 °C for 10 min, and then digested using pepsin solution (Dako) at 37 °C for 5 min. After refixation in 10% buffered formalin at RT for 5 min, the preparations were treated in 2 × SSC-0.3T at 45 °C for 30 min, dehydrated in ethanol, and dried, and exposed to the probes. The

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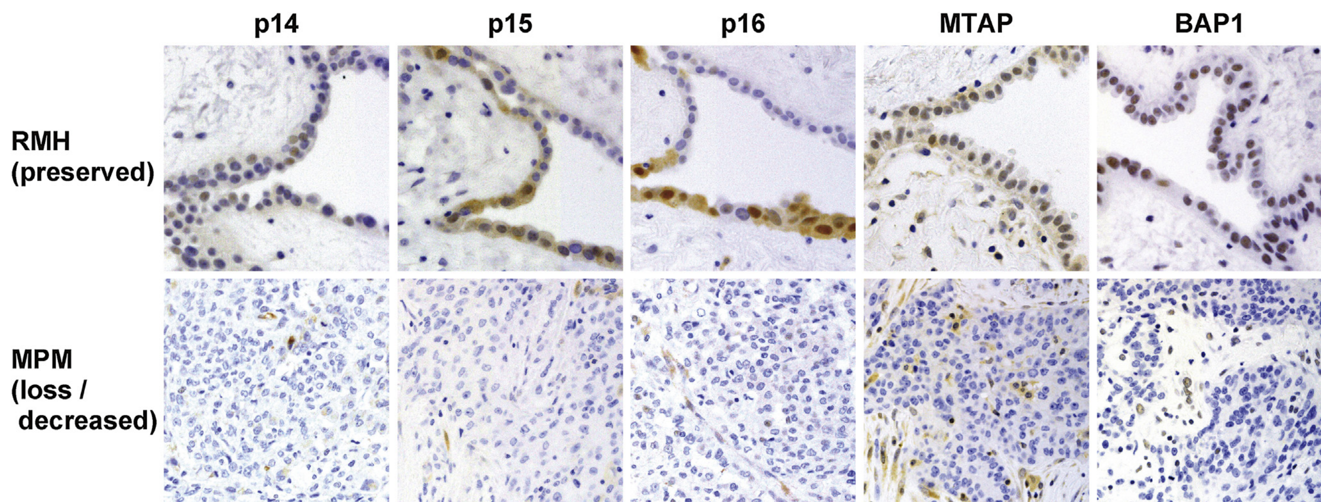

Fig. 1. Representative immunohistochemical (IHC) features of the 9p21.3-related proteins and BAP1. Preserved IHC detection of p14, p15, p16, methylthioadenosine phosphorylase (MTAP), and BAP1 in reactive mesothelial hyperplasia (RMH) cases are shown in the top row. Expression of p14, p15, p16, and BAP1 were observed in the nuclei, while that of MTAP was seen in the cytoplasm. The loss of expression of these proteins in malignant pleural mesothelioma (MPM) cases is shown in the bottom row. Original magnification × 200.

probes and preparations were denatured at 80 °C for 5 min in the probe solution provided (Abbott Japan), followed by hybridization at 37 °C for 48 h in the ThermoBrite unit (Abbott Japan). The preparations were washed in 2 × SSC-0.3T at 72 °C for 3–5 min and in 2 × SSC containing 0.1% Tween 20 at RT for 3–5 min. The nuclei were counterstained using 4′,6-diamidino-2-phenylindole (DAPI) in the antifade reagent (Vector Laboratories, Burlingame, CA, USA). The analyses were performed using a fluorescence microscope (Axio Imager Z1; Carl Zeiss Microimaging, Jena, Germany) and the Isis analysis system (Metasystems, Altlussheim, Germany) equipped with filter sets with single- and dual-band excitors for SpectrumGreen, SpectrumOrange, and DAPI (UV, 360 nm).

HD was defined as the lack of both 9p21 signals. At least 100 cells were scored for each case. Lymphocytes present in each preparation were used as internal negative controls and emitted two signals per FISH probe. This result indicated that the 9p21 FISH signal loss was not due to pre-analytical factors (e.g., fixation or processing).

2.4. Statistical analysis

Receiver operating characteristic (ROC) and logistic regression analyses were performed to evaluate the utility of IHCs of the 9p21.3-related proteins and BAP1 and that of 9p21 FISH for differentiation between MPM and RMH. Areas under the curves (AUCs) were compared using the chi square test. Kappa statistics were used to assess the correlation between the results of IHCs of the 9p21.3-related proteins and 9p21 FISH. Statistical analyses were performed using R version 3.2.4 software (R Foundation for Statistical Computing, Vienna, Austria). A P value <0.05 was considered to indicate a statistically significant result.

3. Results

3.1. Clinical features

Forty-two of the 51 MPM cases were male and nine were female (M:F ratio: 4.7:1). The mean age at diagnosis was 63.8 years (range: 33–81 years). Sixteen of the 51 MPM cases were obtained by biopsy, and the other cases were obtained by surgery including extrapleural pneumonectomy (EPP) and pleurectomy and decortication (PD). The histologic subtype was epithelioid in 44 of the tumors and biphasic in seven of them. All of the 25 RMH cases were male (the mean age: 34.4 years; range: 18–78 years).

3.2. ROC analysis and setting of the cut-off values

Based on the proportion of positively stained cells in each case of MPM and RMH in each IHC, the cut-off values for MPM differentiation from RMH were set by ROC analysis at 14.8%, 3.8%, 8.5%, 32.2%, and 19.4%, for p14, p15, p16, MTAP, and BAP1, respectively (Fig. 2A). The cases in which the proportion of positively stained cells were lower than the cutoff values were designated expression loss or decrease (L/D) positive. For 9p21 FISH, the cut-off value was set at 11.0%, and the cases in which the proportion of HD positive cells were higher than the cut-off value were designated HD positive.

3.3. IHCs and 9p21 FISH results

The distributions of the proportion of positively stained cells in each case are shown by the beeswarm plot in Fig. 2B. Those of the HD-positive cell proportion for 9p21 FISH are also shown. BAP1 and MTAP showed similar characteristics; stain-positive cases showed high proportions of positivity, and stain-negative cases showed low proportions of positivity. Furthermore, BAP1 and MTAP protein expressions were well preserved in all 25 RMH cases. On the other hand, L/D assessment was difficult for p14, p15, and p16. A wide range of positivity was seen for HD using 9p21 FISH, but HD-positive cases were all MPM cases.

All IHC and 9p21 FISH assay results for each case, assessed by using the cut-off values, are shown in Fig. 2C. Twenty-eight (54.9%), 10 (19.6%), 21 (41.2%), 23 (45.1%), and 31 (60.8%) of the MPM cases were L/D-positive by IHC for p14, p15, p16, MTAP, and BAP1, respectively. Thirty-one (60.8%) of the 51 MPM cases were *p16* HD-positive by FISH. In RMH cases, p14, p15, and p16 showed L/D positivity in 9, 1, and 5 cases, respectively, whereas none of the 25 cases was L/D-positive for MTAP and BAP1 by IHC and *p16* HD-positive by FISH.

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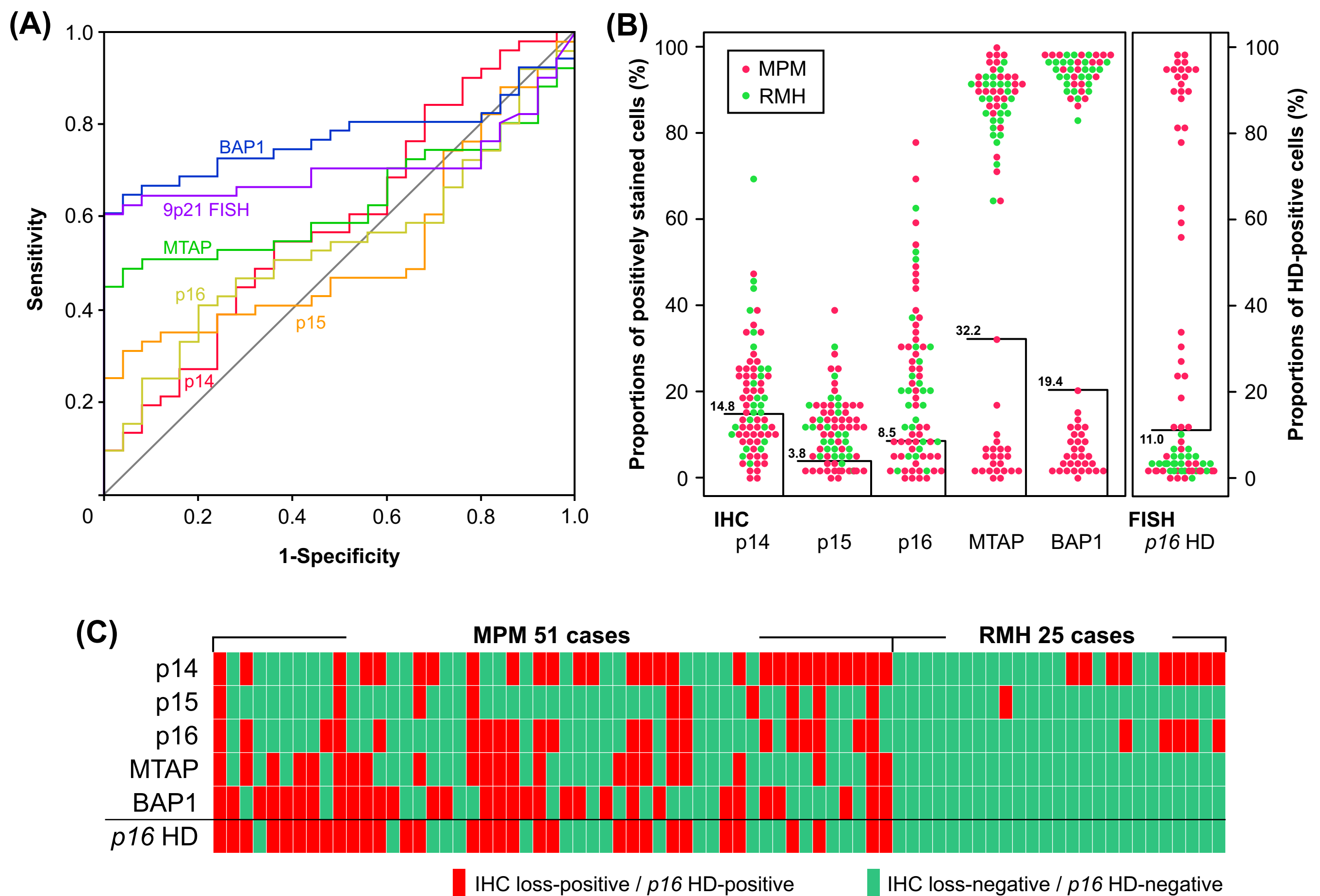

Fig. 2. (A), Receiver operating characteristic (ROC) analysis of immunohistochemistry (IHC) of the 9p21.3-related proteins and BAP1, and fluorescence *in situ* hybridization (FISH) of *p16*. (B), Beeswarm plots of the proportion of positively stained cells for the 9p21.3-related proteins and BAP1, and homozygous deletion (HD) of *p16* detected by FISH. The lines and values in this plot indicate the cut-off points determined for each assay. (C), The results of IHC of the 9p21.3-related proteins and BAP1, and 9p21 FISH in the 51 cases of malignant pleural mesothelioma (MPM) and the 25 cases of reactive mesothelial hyperplasia (RMH). The colors of the cells represent IHC loss-positive or *p16* HD-positive (in red), or IHC loss-negative or *p16* HD-negative (in green).

Table 1
Correlation between immunohistochemical expression of the 9p21.3-related proteins and 9p21 FISH results in mesothelioma cases.

9p21 FISH	HD positive (n = 31)		HD negative (n = 20)		Sensitivity (%)	Specificity (%)	Kappa statistic
IHC	L/D	P	L/D	P			
p14	18	13	10	10	58.1	50.0	0.08
p15	9	22	1	19	29.0	95.0	0.20
p16	18	13	3	17	58.1	85.0	0.40
MTAP	23	8	0	20	74.2	100	0.69
p14/p15	19	12	11	9	61.3	45.0	0.06
p14/p16	22	9	10	10	71.0	50.0	0.21
p14/MTAP	26	5	10	10	83.9	50.0	0.35
p15/p16	19	12	4	16	61.3	80.0	0.39
p15/MTAP	24	7	1	19	77.4	95.0	0.69
p16/MTAP	27	4	3	17	87.1	85.0	0.71
p14/p15/p16	22	9	11	9	71.0	45.0	0.16
p14/p15/MTAP	26	5	11	9	83.9	45.0	0.30
p14/p16/MTAP	27	4	10	10	87.1	50.0	0.39
p15/p16/MTAP	27	4	4	16	87.1	80.0	0.67
p14/p15/p16/MTAP	27	4	11	9	87.1	45.0	0.34

FISH, fluorescence *in situ* hybridization; HD, homozygous deletion; IHC, immunohistochemistry; L/D, loss or decreased; P, preserved.

3.4. Correlation between IHCs of the 9p21.3-related proteins and 9p21 FISH

Next, we investigated the correlation between IHC results of the 9p21.3-related proteins and *p16* HD detected by FISH. Fig. 3 shows a representative case, in which *p16* HD was positive by FISH and accordingly, p14, p15, p16, and MTAP were also L/D-positive by IHC. The results are summarized in Table 1. MTAP IHC had the best concordance with the 9p21 FISH results, with a kappa coefficient of 0.69. For predicting *p16* HD detected by FISH, L/D of MTAP had 100%

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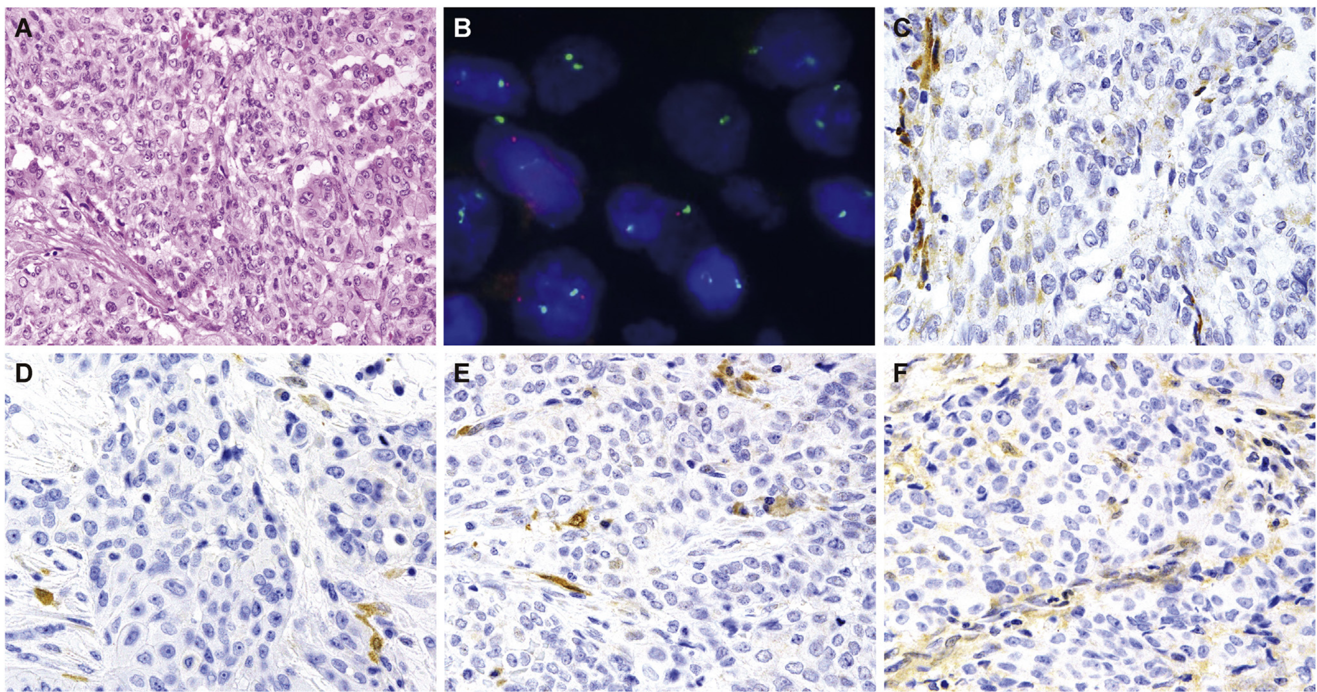

Fig. 3. An example of positive loss/decreased immunohistochemical expression of the 9p21.3-related proteins. This mesothelioma case showed homozygous deletion of *p16* detected using fluorescence *in situ* hybridization (FISH), and loss/decreased immunostaining of p14, p15, p16, and methylthioadenosine phosphorylase (MTAP). (A) H&E staining of the specimen, (B) 9p21 FISH, (C) p14 immunohistochemistry (IHC), (D) p15 IHC, (E) p16 IHC, and (F) MTAP IHC. Original magnifications × 400 (A, C, D, E and F) and × 1000 (B).

specificity, while that of p14, p15, and p16 had lower specificity. We also examined the combinations of MTAP with other gene products. The L/D of p16 or MTAP in combination had better concordance, with a kappa coefficient of 0.71. However, the specificity was 85%. The other combinations, including IHCs of three protein products, had poorer concordance because of their lower specificities.

3.5. Diagnostic utilities of IHCs of the 9p21.3-related proteins and their combinations with BAP1

Finally, we examined the usefulness of 9p21.3-related protein IHCs and their combinations with BAP1 in the differentiation between MPM and RMH (Table 2). Only MTAP IHC had 100% specificity, although its sensitivity was as low as 45.1%. The combination of p14, p15, p16, and MTAP, improved the sensitivity to 74.5%, but the specificity was decreased to 60.0%. Among IHC combinations with BAP1, only that of BAP1 with MTAP exhibited a specificity of 100%. Its sensitivity was 76.5%, which was lower than that of a combination of BAP1 and 9p21 FISH (84.3%). However, it was higher than that of BAP1 IHC alone (60.8%) or 9p21 FISH alone (60.8%) significantly ($P = 0.0386$ and 0.0229, respectively). Two example cases with different combinations of MTAP and BAP1 loss are shown in Fig. 4 (AB, L/D of BAP1; CD, L/D of MTAP).

4. Discussion

This is the first report to demonstrate the usefulness of a combination of MTAP and BAP1 IHC in differentiation of MPM versus RMH. This combination had a 100% specificity and 76.5% sensitivity in the differentiation of MPM from RMH in our study. The sensitivity was lower by approximately 10% than that of a combination of BAP1 IHC and 9p21 FISH, but was significantly higher than those of BAP1 IHC alone (60.8%) and 9p21 FISH alone (60.8%). Moreover, the results show that MTAP IHC may contribute to fill up the gap of BAP1 preserved MPM, recruiting 8 cases of BAP1 preserved MPM, because loss of MTAP IHC expression was shown in 8 out of 20 cases that showed preserved BAP1 expression (8/20, 40%, Fig. 2C). Additionally, these results may lead to a possible reduction of 15.7% (8/51) of FISH analysis. Considering the fact that IHC is a more common and feasible technique compared with FISH, this combination of MTAP and BAP1 IHCs may be useful for the differentiation of early stage MPM from RMH in any institution or laboratory.

In this study, we first examined the correlation between the combinations of IHCs of 9p21.3-related gene products and *p16* HD detected by FISH, and found that MTAP IHC was the most reliable in predicting *p16* HD status. The correlation of p16 IHC and *p16* HD was also previously examined, with variable results [18,20,21]. The differences in IHC conditions such as clones of antibodies used or fixation and/or staining procedures may be the factors that caused the observed discrepancies. There is also a possibility that p16 protein may not be expressed constantly; it plays an important role in cell proliferation and its expression is strictly regulated. Hypermethylation of the promotor regions is also a mechanism implicated in the decreased expression of *p16* gene in some tumors [32,33]. Also in our study, some RMH cases showed p16 IHC signal lower than the cut-off value determined in ROC analysis. The same was true for p14 and p15 IHC.

The variation in the deletion length of the 9p21.3 region in MPM may also influence the IHC results. The commercially available FISH probe we used is of ~222 kb length, which fully covers the region from *MTAP* to $p15^{INK4B}$. Therefore, the results of 9p21 FISH might also depend on the deletion status of not only *p16* but also that of *MTAP* and $p15^{INK4B}$ gene regions, and if so, it could explain the fact that some MPM cases showed preserved IHC detection of p14, p15, or p16 in spite of the presence of *p16* HD detected by FISH. However, we did not investigate the status of $p14^{ARF}$, $p15^{INK4B}$, *p16,* and *MTAP* genes in MPM directly in this study. There may be a further complexity in the expression of these proteins and their correlation with gene deletion.

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Table 2
Diagnostic utility of immunohistochemistry of the 9p21.3-related proteins and BAP1 and 9p21 FISH for distinguishing MPM from RMH.

	MPM (n = 51)		RMH (n = 25)		Sensitivity (%)	Specificity (%)
	positive[†]	negative[†]	positive[†]	negative[†]		
p14	28	23	9	16	54.9	64.0
p15	10	41	1	24	19.6	96.0
p16	21	30	5	20	41.2	80.0
MTAP	23	28	0	25	45.1	100
p14/p15	30	21	10	15	58.8	60.0
p14/p16	32	19	9	16	62.7	64.0
p14/MTAP	36	15	9	16	70.6	64.0
p15/p16	23	28	6	19	45.1	76.0
p15/MTAP	25	26	1	24	49.0	96.0
p16/MTAP	30	21	5	20	58.8	80.0
p14/p15/p16	33	18	10	15	64.7	60.0
p14/p15/MTAP	37	14	10	15	72.5	60.0
p14/p16/MTAP	37	14	9	16	72.5	64.0
p15/p16/MTAP	31	20	6	19	60.8	76.0
p14/p15/p16/MTAP	38	13	10	15	74.5	60.0
BAP1	31	20	0	25	60.8	100
BAP1/p14	43	8	9	16	84.3	64.0
BAP1/p15	38	13	1	24	74.5	96.0
BAP1/p16	42	9	5	20	82.4	80.0
BAP1/MTAP	39	12	0	25	76.5	100
9p21 FISH	31	20	0	25	60.8	100
BAP1/9p21 FISH	43	8	0	25	84.3	100

MPM, malignant pleural mesothelioma; RMH, reactive mesothelial hyperplasia.

[†] For immunohistochemistry (IHC), positive indicates expression loss or decreased-positive, while, for fluorescence *in situ* hybridization (FISH), positive indicates homozygous deletion-positive. Negative indicates expression loss or decrease-negative for IHC and homozygous deletion-negative for FISH.

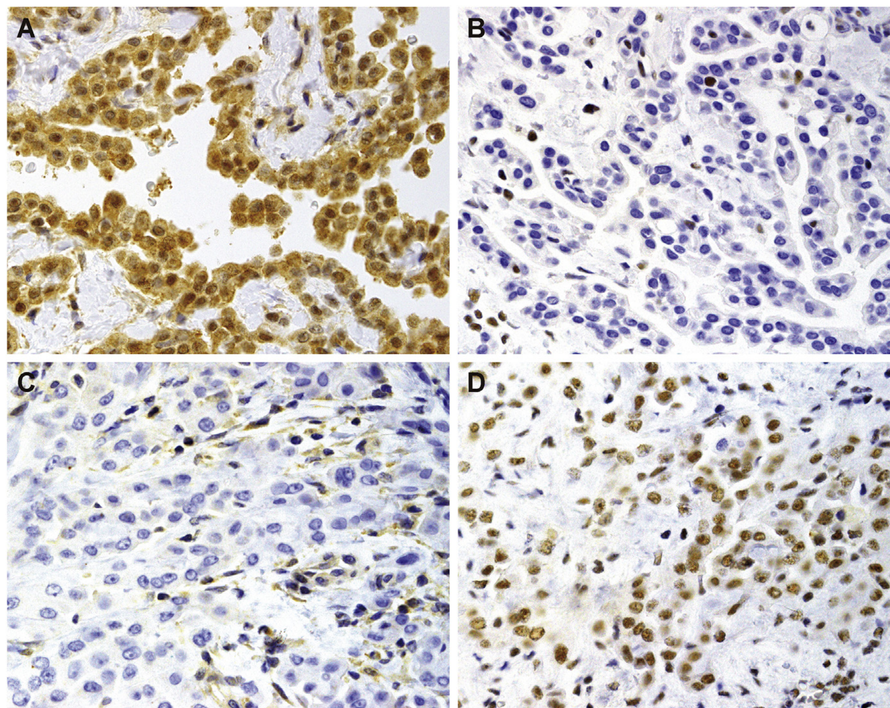

Fig. 4. Examples of immunohistochemistry (IHC) of methylthioadenosine phosphorylase (MTAP) and BAP1 in malignant pleural mesothelioma (MPM) cases. The top row shows a MPM case with preserved staining of MTAP (A) and loss/decreased staining of BAP1 (B). In contrast, the case in the bottom row exhibits loss/decreased staining of MTAP (C) but preserved staining of BAP1 (D). Original magnifications × 400.

MTAP is an enzyme that plays an important role in polyamine metabolism and salvage of adenine and methionine, expressed in normal human tissues ubiquitously, and has been reported to be deficient in a variety of tumor types [17,34–39]. *MTAP* gene is often co-deleted with $p16^{INK4A}$ gene in tumors including mesothelioma [17,27,33–35,40]. Krasinskas et al. reported that *MTAP* was co-deleted in every case of peritoneal mesothelioma with *p16* HD detected by FISH [40]. Nobori et al. reported the primary mechanism leading to MTAP IHC loss was partial or complete HD of *MTAP* gene [38]. Hypermethylation of the promoter region is also considered a possible cause of MTAP deficiency [41].

MTAP IHC in MPM was previously described by Zimling et al. [42]. Using tissue sections, they showed decreased MTAP expression in 65% of MPM cases and 23% of cases with reactive mesothelial proliferations. In our study, 45% of the MPM and none of the RMH cases showed loss of or decreased MTAP expression. This

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discrepancy may be explained by the difference in not only the experimental conditions for IHC but also in the methods for data interpretation and evaluation. The use of ROC-based cut-off value might have resulted in lower sensitivity of MTAP IHC in our study than theirs, although 100% specificity was achieved using our methodology. Furthermore, in our study, the proportion of cells positive for MTAP IHC in MPM cases showed a bimodal distribution like BAP1 IHC, similar to the previous report in non-small cell lung cancer [43]. These features make it easy to interpret the IHC results for distinguishing MPM from RMH. A major limitation of our study is the relatively small sample size. Further studies are required to assess the usefulness of MTAP and BAP1 IHCs, independently or in combination, in the medical diagnosis and management of MPM. For clinical use, setting the proper and easily evaluable cut-off value, for example 50% [43], may be also needed. Analysis of sarcomatoid subtype is also favorable. Our study included 7 cases of biphasic MPM, all of which showed positive HD by 9p21 FISH. Homogeneous MTAP expression loss by IHC was observed in both epithelioid and sarcomatoid components in 6 of them, and the other showed homogeneously preserved expression of MTAP in both components. However, further studies with more cases of sarcomatoid MPM are needed in order to analyze the usefulness of MTAP IHC in diagnosis of sarcomatoid MPM.

In conclusion, a combination of MTAP and/or BAP1 loss detected by IHC can likely detect MPM with good sensitivity that is higher than that of BAP1 IHC alone or 9p21 FISH alone. This combination could therefore serve as a useful ancillary IHC method for the discrimination of MPM from RMH, although a combination of BAP1 IHC and 9p21 FISH remains the most accurate ancillary tool. However, it is important to confirm the mesothelial origin of the tumor before applying combinations of MTAP and BAP1 IHCs or BAP1 IHC and 9p21 FISH for MPM diagnosis.

Conflict of interest

The authors declare no conflict of interest.

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