Malignant mesothelioma with a novel *BAP1* germline frameshift mutation treated with dual immune checkpoint inhibitors: A case report

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Abstract. Germline pathogenic mutation of the *BAP1* gene is a common molecular event in malignant mesothelioma (MM). A patient with a positive family history of tenacious peritoneal effusions presented with hydropneumothorax and suffered from recurrent pleural and peritoneal effusions since. Tuberculosis (TB) was assumed by preceding clinicians who prescribed futile anti-TB regimens. Finally, a diagnostic laparoscopy and omental biopsy revealed the histology of MM. Next-generation sequencing uncovered a novel *BAP1* germline frameshift mutation (c. 1077_1083delinsTG, pPhe360fs), which was rated as pathogenic due to its potential to introduce a termination codon, resulting in nonsense-mediated mRNA decay and due to the fact of *BAP1* protein nuclear loss in tumor tissue. Dual immunotherapy with nivolumab and ipilimumab was given for 3 cycles and only achieved stable disease. Steven-Johnson syndrome occurred afterward and was relieved after steroid treatment. The present study reported a case of MM with a new *BAP1* frameshift mutation, treated by dual immune checkpoint inhibitors, achieving a modest drug effect and serious skin-related adverse events.

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Abbreviations: BAP1, BRCA1-associated protein 1; TB, tuberculosis; NGS, next generation sequencing; MM, malignant mesothelioma; IHC, immunohistochemistry; ATM, Ataxia-Telangiectasia mutated; TMB, tumor mutation burden; SJS, Steven-Johnson syndrome; ICIs, immune checkpoint inhibitors

Key words: malignant mesothelioma, *BAP1* germline mutation, dual immune checkpoint inhibitors, Steven-Johnson syndrome, case report

Introduction

Malignant mesothelioma (MM) is a rare cancer, which poses a formidable challenge to clinicians. Individuals carrying germline mutations in the tumor suppressor gene BRCA1-associated protein 1 (*BAP1*) are predisposed to develop MM; however, familial cases are seldom reported (1). The prognostic significance of *BAP1* mutations in predicting response to systemic therapies remains elusive. In the systemic treatment of MM, conventional chemotherapy offers only a moderate survival benefit and is associated with poor outcomes (2). Of note, the dual immune checkpoint inhibitors (ICIs) regimen of nivolumab plus ipilimumab has become a standard first-line treatment for MM, significantly extending overall survival compared to chemotherapy in a phase 3 trial (median overall survival: 18.1 vs. 14.1 months; hazard ratio, 0.74; P=0.0020) (2). The current study presented a Chinese patient and their family whose hereditary MMs were associated with a novel *BAP1* frameshift mutation, underscoring the importance of genetic factors in MM pathogenesis, and reported on the efficacy of dual ICIs treatment in this context.

Case presentation

A 48-year-old male patient with no history of asbestos exposure was admitted to Peking Union Medical College Hospital (Beijing, China) for recurrent pleural and peritoneal effusion in December 2022. The patient had presented with bilateral hydropneumothorax at the age of 30 years. The disease was resistant to anti-tuberculosis (TB) treatment and lingered on into the patient's forties. The patient suffered from another

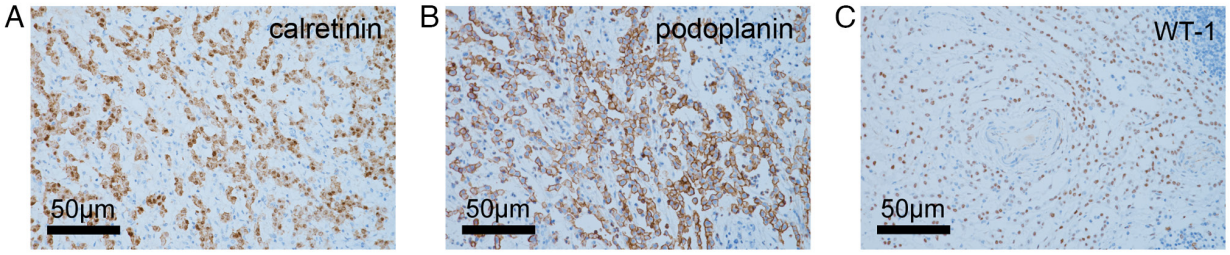

Figure 1. Malignant mesothelioma diagnosis confirmed by positive immunohistochemistry for (A) calretinin, (B) podoplanin and (C) WT-1 (scale bars, 50 μm). WT-1, Wilms' tumor 1.

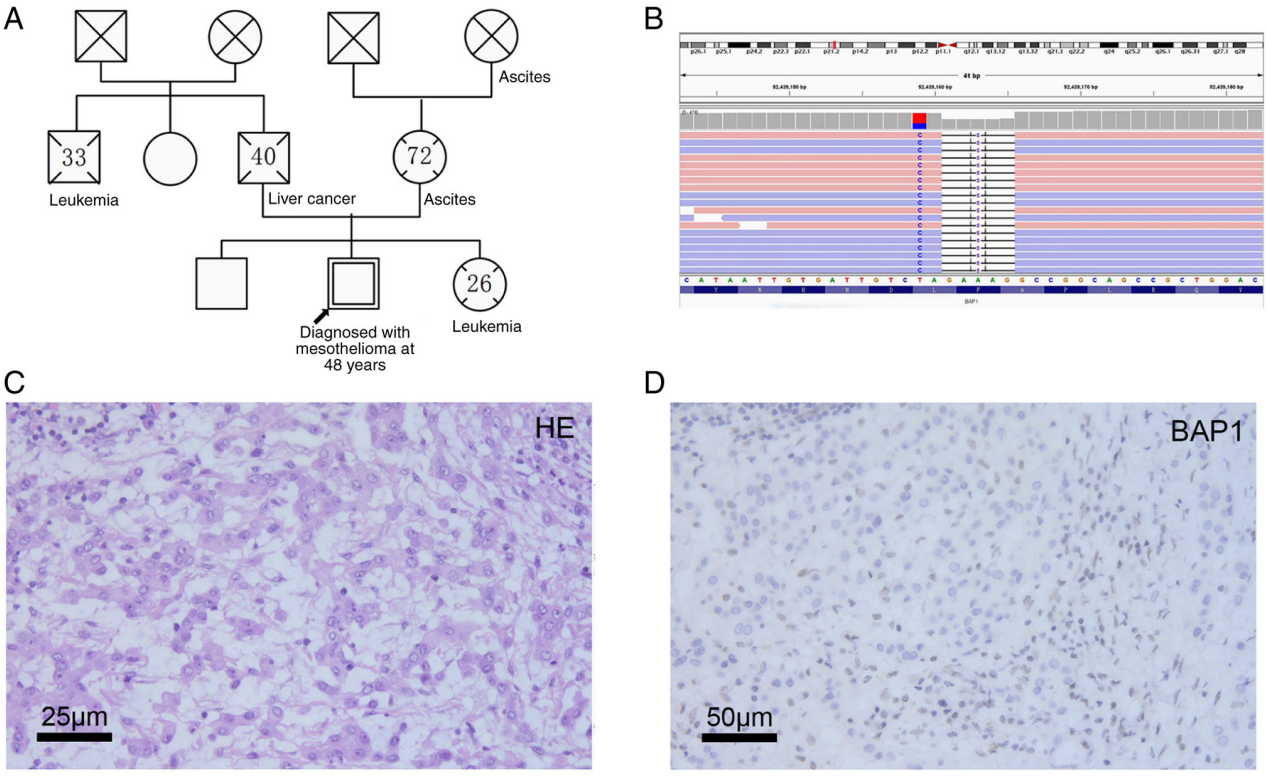

Figure 2 Diagnosis of malignant mesothelioma associated with germline *BAP1* mutation. (A) Pedigree of the patient. (B) Next-generation sequencing showed a novel *BAP1* germline frameshift mutation, compared to the GRCh37 human reference genome. (C) Histology of the tumor (HE stain; magnification, x400; scale bar, 25 μm) showed heterotypical cells infiltration in omentum. (D) Immunohistochemistry with *BAP1* stain showed nuclear protein loss of tumor cells, but not in immune cells (magnification, x200; scale bar, 50 μm). *BAP1*, BRCA1-associated protein 1; GRCh37, genome reference consortium human build 37; HE, hematoxylin-eosin.

episode of pleural and peritoneal effusion since January 2022. Symptoms including abdominal swelling and pain persisted after 9 months of the standard 4-drug anti-TB regimen. A positive blood T-SPOT.TB assay but negative findings of the Xpert MIB/RIF test, acid-fast staining and bacterial culture test using peritoneal effusion suggested no active TB infection. An abdominopelvic MRI showed a collapsed right rib cage, thickened right pleural membrane, omentum and pelvic peritoneum, as well as large effusion. A diagnostic laparoscopy was performed in late December 2022 and a biopsy to nodules throughout the omentum uncovered an MM with immunohistochemistry (IHC) positive for calretinin, podoplanin and Wilms' tumor 1 (WT-1) (Fig. 1A-C). Another IHC test indicated a programmed death 1 (PD-1) ligand 1 (PD-L1) (22C3) combined positive score (CPS) of 10. IHC staining was performed using an automated immunostainer (Ventana BenchMark ULTRA; Roche Diagnostics), following the manufacturer's recommended standard protocol. The antibodies utilized were anti-calretinin (cat. no. PA0346; Leica Biosystems), anti-podoplanin (cat. no. IR0720), anti-WT-1 (cat. no. IR055) and anti-PD-L1 (cat. no. M3653; all from DAKO; Agilent Technologies, Inc.). PD-L1 expression was assessed using the CPS, calculated as the number of PD-L1-stained cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100 (3). In line with clinical practice and prior investigations, the predefined cutoff was set at 1 (3).

The patient has a maternal history of suspected peritoneal mesotheliomas. The patient's mother and grandmother both died in their seventies due to tenacious peritoneal effusions after rounds of unsuccessful anti-TB treatment. Furthermore, the patient's younger sister had died of acute leukemia at age 26.

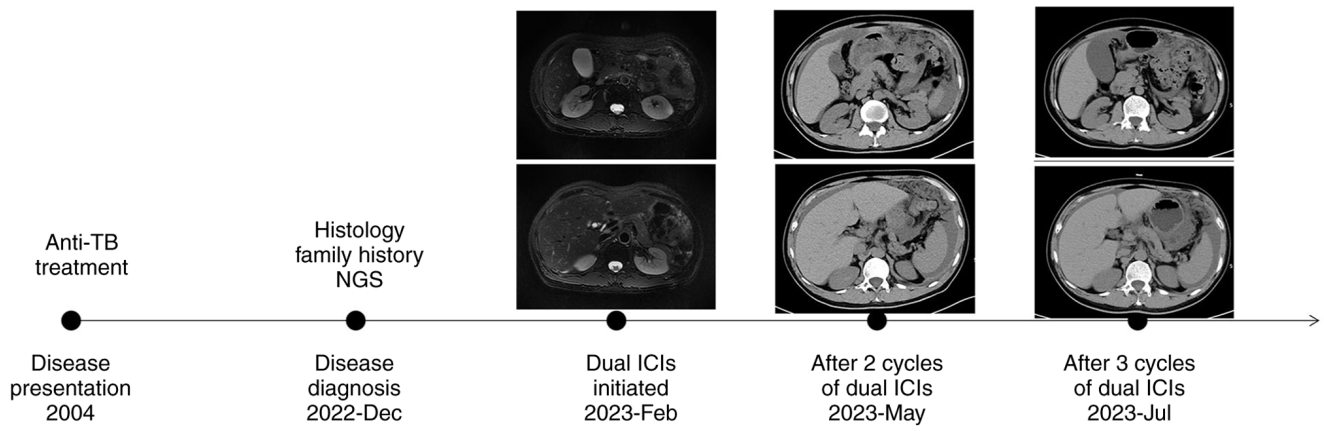

Figure 3. Time course of the disease and the transition of the two characteristic layers (upper and lower) of CT imaging during dual immune checkpoint inhibitors. TB, tuberculosis; NGS, next-generation sequencing; ICI, immune checkpoint inhibitor.

The patient's family cancer history is shown in a pedigree chart in Fig. 2A. Next-generation sequencing (NGS) was performed using a 520-gene panel for multiple solid tumors. Libraries were prepared using a target enrichment method, followed by sequencing on the NextSeq 550 Dx platform (Illumina, Inc.). NGS analysis of the tumor tissue and the patient's control blood sample revealed a novel, heterozygous, germline frameshift mutation of the *BAP1* gene (c.1077_1083delinsTG, pPhe360fs) (Fig. 2B), which was interpreted as likely pathogenic, since it may lead to protein loss through nonsense-mediated mRNA decay by introducing a termination codon (4). The Combined Annotation Dependent Depletion score of this mutation was 34 and the minor allele frequency was 51.88% (5). Copy number variation gain of the Ataxia-Telangiectasia mutated (ATM) gene was also observed, which was a 3.9-fold gain. The tumor mutation burden was 0 mutations/Mb. Tissue samples were fixed in 10% neutral-buffered formalin at room temperature for 24 h. Sections (4 μm) were stained with hematoxylin for 5 min and eosin for 2 min, then examined under a light microscope. The hematoxylin and eosin (H&E) staining showed heterotypical cells infiltration in omentum (Fig. 2C), and IHC showed nuclear total loss of the BAP-1 protein in tumor cells but not in immune cells (Fig. 2D), indicating loss of function in the tumor. The IHC protocols have been described previously. The anti-BAP1 antibody (used at 1:50 dilution) was obtained from Abcam (cat. no. EPR22826-65). Regrettably, the patient did not consent to any genetic analysis and consultation provided to their child and samples of the patient's living maternal relatives were unavailable.

According to positive PD-L1 expression and the patient's wishes, nivolumab (Opdivo; Bristol Myers Squibb) 3 mg/kg every 2 weeks and ipilimumab (Yervoy; Bristol Myers Squibb) 1 mg/kg every 6 weeks were prescribed since February 2023. After 2 cycles, the patient's symptoms were slightly alleviated but computed tomography (CT) imaging showed stable disease (Fig. 3). However, the patient developed Steven-Johnson syndrome (SJS) after the 3rd cycle of dual ICIs, resulting in a disruption of ICI treatment. SJS symptoms were relieved after 80 mg/day (1.5 mg/kg) methyprednisolone (Pfizer) for 7 days, followed by 60 mg/day (1.0 mg/kg) prednisone (Tianjin Lisheng Pharmaceutical) for 5 days, then tapered by 10 mg every 3 days until 10 mg/day, followed by a further taper of 5 mg every 7 days until completion. At two months after the cessation of steroids, the patient received 3 cycles of combination therapy with pemetrexed (Huiyu Pharmaceutical) 500 mg/m^2, cisplatin (Qilu Pharmaceutical) 75 mg/m^2 and bevacizumab (Avastin; Roche) 15 mg/kg, every 3 weeks. CT following the 3rd cycle of therapy showed a reduction of pleural and peritoneal effusion but unchanged thickening of pleura and omentum. Considering the favorable prognosis of mesothelioma carrying a germline BAP1 mutation (6), as well as the rather torpid clinical course, the patient decided not to continue chemotherapy and still had a good performance status at the last follow-up in October 2024. The patient was lost to follow-up thereafter. Regarding the patient's maternal history of suspected peritoneal mesothelioma, attempts to obtain accurate information about the patient's cousins were unsuccessful.

Discussion

The present study was the very first report of this original germline *BAP1* frameshift mutation (c.1077_1083delinsTG, pPhe360fs) in a newly-found Chinese family with hereditary MMs, to the best of our knowledge. *BAP1*, a deubiquitinase, exerts its functions of orchestrating transcription, DNA replication and repair in the nucleus, as well as promoting cell death and maintaining metabolism homeostasis in the cytoplasm, through deubiquitination of its miscellaneous substrates in these cellular activities (7). While various rare germline BAP1 mutations have been detected in breast cancer and renal cancer in the Chinese population (8-10), there have been no specific reports about any Chinese families affected by BAP1 hereditary mesothelioma.

Several questions need to be answered. Firstly, *BAP1* germline mutation usually involves one allele and a second hit is required to trigger total loss of the protein function and the development of mesotheliomas. It may be speculated that exposure to carcinogens other than asbestos or post-translational modification may be the second hit, although DNA methylation-mediated *BAP1* inactivation has not been detected (11). Besides, the development of mesotheliomas in Bap1$^{+/-}$ mice or in familial carriers of *BAP1* mutations independently of carcinogens has also been reported (7). Another notable molecular

event was the co-occurring ATM gain in the number of copies. The upregulation of the ATM gene and other genetic alterations in the DNA damage repair pathway in *BAP1* haploinsufficient mesotheliomas were also observed by a previous study (12). *BAP1* is known as a substrate of ATM phosphorylation in DNA replication stress (13), but the role of the *BAP1* germline mutation in carcinogenesis and mechanisms of secondary changes needs to be investigated in the future.

According to the role of *BAP1* in DNA double-strand break repair and the generally lower aggressiveness of mesothelioma with germline *BAP1* mutations (7), one may wonder what is the optimal initial systemic strategy for such patients with advanced diseases: Platinum-doublet chemotherapy, dual ICIs or the combination of chemotherapy with anti-PD-1 antibodies (2,14). Loss of *BAP1* was suggested to be associated with improved survival in patients with pleural mesothelioma but also with resistance to cisplatin-based chemotherapy through apoptosis inhibition (15,16). These contradictory phenomena were the reflections of its multiple biological activities and the uncertainty of the value of *BAP1* alterations as a predictive marker for current systemic therapies. The patient of the present study received dual ICIs according to their own will. However, the efficacy was modest, although findings have shown that *BAP1* deletion correlates with an inflammatory tumor microenvironment and is a potential target for checkpoint blockade (12). Additionally, the patient had positive PD-L1 expression (CPS=10). As confirmed by the CheckMate 743 (2), PD-L1 positivity did not correlate with the degree of benefit from nivolumab plus ipilimumab in this patient. The instigation of SJS associated with this regimen suggests an off-target effect by activated cytotoxic T cells. The optimal treatment modality for MM associated with germline *BAP1* mutation should be explored in future comparative studies.

In conclusion, a novel *BAP1* germline frameshift mutation (c. 1077_1083delinsTG, pPhe360fs) was found to be associated with a Chinese patient with MM. Dual ICIs achieved a modest effect in this patient and treatment was disrupted by a serious skin immune-related adverse event.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in the National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA009720) and are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human/browse/HRA009720.

Authors' contributions

NZ, SY and LZ was involved in the conception and design of the study. SY and LZ provided administrative support. HW, XG, SY, LZ and YC provided study materials or patients and were involved in the acquisition of data. NZ, CW, MW and MY performed data analysis and interpretation. All authors wrote the manuscript. NZ and MW edited the manuscript. All authors have read and approved the final manuscript. SY and LZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable. Written informed consent was obtained from the patient for genetic testing.

Patient consent for publication

Written informed consent was obtained from the patient for the publication of this case report, including accompanying images and genetic test results.

Competing interests

The authors declare that they have no competing interests.

References

1. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, Cox NJ, Dogan AU, Pass HI, Trusa S, *et al*: Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 43: 1022-1025, 2011.
2. Baas P, Scherpereel A, Nowak AK, Fujimoto N, Peters S, Tsao AS, Mansfield AS, Popat S, Jahan T, Antonia S, *et al*: First-line nivolumab plus ipilimumab in unresectable malignant pleural mesothelioma (CheckMate 743): A multicentre, randomised, open-label, phase 3 trial. Lancet 397: 375-386, 2021.
3. Kulangara K, Zhang N, Corigliano E, Guerrero L, Waldroup S, Jaiswal D, Ms MJ, Shah S, Hanks D, Wang J, *et al*: Clinical utility of the combined positive score for programmed death ligand-1 expression and the approval of pembrolizumab for treatment of gastric cancer. Arch Pathol Lab Med 143: 330-337, 2019.
4. Carbone M, Yang H, Pass HI, Krausz T, Testa JR and Gaudino G: BAP1 and Cancer. Nat Rev Cancer 13: 153-159, 2013.
5. Novelli F, Yoshikawa Y, Vitto VAM, Modesti L, Minaai M, Pastorino S, Emi M, Kim JH, Kricek F, Bai F, *et al*: Germline BARD1 variants predispose to mesothelioma by impairing DNA repair and calcium signaling. Proc Natl Acad Sci USA 121: e2405231121, 2024.
6. Carbone M, Pass HI, Ak G, Alexander HR Jr, Baas P, Baumann F, Blakely AM, Bueno R, Bzura A, Cardillo G, *et al*: Medical and surgical care of patients with mesothelioma and their relatives carrying germline BAP1 mutations. J Thorac Oncol 17: 873-889, 2022.
7. Carbone M, Harbour JW, Brugarolas J, Bononi A, Pagano I, Dey A, Krausz T, Pass HI, Yang H and Gaudino G: Biological mechanisms and clinical significance of BAP1 mutations in human cancer. Cancer Discov 10: 1103-1120, 2020.
8. Wu J, Wang H, Ricketts CJ, Yang Y, Merino MJ, Zhang H, Shi G, Gan H, Linehan WM, Zhu Y and Ye D: Germline mutations of renal cancer predisposition genes and clinical relevance in Chinese patients with sporadic, early-onset disease. Cancer 125: 1060-1069, 2019.
9. Dong L, Zhang H, Zhang H, Ye Y, Cheng Y, Li L, Wei L, Han L, Cao Y, Li S, *et al*: The mutation landscape of multiple cancer predisposition genes in Chinese familial/hereditary breast cancer families. Cancer Biol Med 19: 850-870, 2021.

10. Kong W, Yang T, Wen X, Mu Z, Zhao C, Han S, Tian J, Zhang X, Zhou T, Zhang Y, *et al*: Germline mutation landscape and associated clinical characteristics in Chinese patients with renal cell carcinoma. Front Oncol 11: 737547, 2021.
11. Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Luk H, Baumann F, Zhang YA, Gazdar A, Kanodia S, *et al*: High Incidence of Somatic BAP1 alterations in sporadic malignant mesothelioma. J Thorac Oncol 10: 565-576, 2015.
12. Shrestha R, Nabavi N, Lin YY, Mo F, Anderson S, Volik S, Adomat HH, Lin D, Xue H, Dong X, *et al*: BAP1 haploinsufficiency predicts a distinct immunogenic class of malignant peritoneal mesothelioma. Genome Med 11: 8, 2019.
13. Ismail IH, Davidson R, Gagné JP, Xu ZZ, Poirier GG and Hendzel MJ: Germline mutations in BAP1 impair its function in DNA double-strand break repair. Cancer Res 74: 4282-4294, 2014.
14. Chu Q, Perrone F, Greillier L, Tu W, Piccirillo MC, Grosso F, Lo Russo G, Florescu M, Mencoboni M, Morabito A, *et al*: Pembrolizumab plus chemotherapy versus chemotherapy in untreated advanced pleural mesothelioma in Canada, Italy, and France: A phase 3, open-label, randomised controlled trial. Lancet 402: 2295-2306, 2023.
15. Louw A, Panou V, Szejniuk WM, Meristoudis C, Chai SM, van Vliet C, Lee YCG, Dick IM, Firth T, Lynggaard LA, *et al*: BAP1 loss by immunohistochemistry predicts improved survival to first-line platinum and pemetrexed chemotherapy for patients with pleural mesothelioma: A validation study. J Thorac Oncol 17: 921-930, 2022.
16. Oehl K, Vrugt B, Wagner U, Kirschner MB, Meerang M, Weder W, Felley-Bosco E, Wollscheid B, Bankov K, Demes MC, *et al*: Alterations in BAP1 are associated with cisplatin resistance through inhibition of apoptosis in malignant pleural mesothelioma. Clin Cancer Res 27: 2277-2291, 2021.