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Recurrent tumor suppressor alterations in primary pericardial mesothelioma

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Abstract

Primary pericardial mesotheliomas are extremely rare, accounting for <1% of all mesotheliomas, and their molecular genetic features and predisposing factors remain to be determined. Here, we report the clinicopathologic, immunohistochemical, and molecular genetic findings of three pericardial mesotheliomas without pleural involvement. Three cases diagnosed between 2004 and 2022 were included in the study and analyzed by immunohistochemistry and targeted next-generation sequencing (NGS); corresponding nonneoplastic tissue was sequenced in all cases. Two patients were female and one male, aged between 66 and 75 years. Two patients each had prior asbestos exposure and were smokers. Histologic subtypes were epithelioid in two cases and biphasic in one case. Immunohistochemical staining identified expression of cytokeratin AE1/AE3 and calretinin in all cases, D2–40 in two cases, and WT1 in one case. Staining for tumor suppressors revealed loss of p16, MTAP, and Merlin (NF2) expression in two cases, and loss of BAP1 and p53 in one case. Abnormal cytoplasmic BAP1 expression was observed in an additional case. Protein expression abnormalities correlated with NGS results, which showed concurrent complete genomic inactivation of *CDKN2A/p16*, *CDKN2B*, *MTAP*, and *NF2* in two mesotheliomas and of *BAP1* and *TP53* in one mesothelioma each, respectively. In addition, one patient harbored a pathogenic *BRCA1* germline mutation which resulted in biallelic inactivation in the mesothelioma. All mesotheliomas were mismatch repair proficient and showed several chromosomal gains and losses. All patients died from disease. In summary, our studies demonstrate that pericardial mesotheliomas share common morphologic, immunohistochemical, and molecular genetic features with pleural mesothelioma including

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Author Contributions

I.-M.S. and L.M.S. performed study concept and design; I.-M.S. and L.M.S. performed development of methodology and writing of the paper; All authors provided acquisition, analysis, and interpretation of data; A.M.-E., M.M.H. and R.F.P. provided technical and material support; All authors read and approved the final paper.

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Ethics Approval

Patient sample collection and analysis were conducted following protocols approved by the Dana-Farber/Brigham and Women's Hospital Institutional Review Board. The study was performed in accordance with the Declaration of Helsinki.

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recurrent genomic inactivation of canonical tumor suppressors. Our study adds new insights into the genetic landscape of primary pericardial mesothelioma and highlights *BRCA1* loss as a potential contributing factor in a subset of cases, thereby contributing to refined precision diagnostics for this rare cancer.

Keywords

Mesothelioma; pericardium; asbestos; next-generation sequencing; *BRCA1*; germline; tumor suppressor; cell cycle regulator

Introduction

Mesothelioma most commonly arises in the pleura, accounting for ~90% of cases, followed by the peritoneum in ~10% of cases.¹ Primary pericardial mesotheliomas are extremely rare and comprise <1% of all mesotheliomas, with an estimated annual incidence of 10–15 new cases in the United States.^{1,2} To date, only few cases of pericardial mesothelioma have been described, mostly as case reports^{3,4}, with the largest single-site series including 12 cases.⁵ They most commonly affect adults ~60 years of age and patients usually present with dyspnea, chest pain, cough, signs of constrictive pericarditis, and heart failure.^{1,2,5–7} Given its nonspecific clinical presentation, pericardial mesothelioma is diagnosed a median of three months after clinical presentation and often post mortem.^{3,7} Pericardial mesothelioma spreads locally around the large vessels and into the myocardium and follows a very aggressive clinical course with a mean survival of 2.5–6 months.^{6,7} While asbestos exposure has long been established as a predisposing factor for pleural mesothelioma⁸, its role in the development of primary pericardial mesothelioma remains controversial. While some studies observed a significant association between occupational asbestos exposure and pericardial mesothelioma^{6,7} with detection of asbestos fibers in the lungs of affected patients^{9,10}, other groups did not identify any such association.^{1,5}

Most reported cases of pericardial mesothelioma exhibited epithelioid morphology^{5–7} and showed expression of immunohistochemical markers typically observed in pleural mesothelioma, such as keratins, calretinin, D2–40 (podoplanin), and WT1, and negativity for TTF1 and claudin-4.³ To date, only three cases of pericardial mesothelioma included in two different studies have been analyzed by next generation sequencing (NGS) and exhibited genomic aberrations that inactivated *CDKN2A/B*, *BAP1*, *TP53*, and *NF2*,¹¹ representing aberrations typically observed in pleural mesothelioma.^{11,12} However, a possible role of tumor suppressor germline mutations, which have been identified as predisposing factor for the development of pleural mesothelioma (e.g., *BAP1* mutations)^{13,14}, remains to be determined in pericardial mesothelioma.

Here, we report the clinicopathologic, immunohistochemical, and molecular genetic findings in a series of three primary pericardial mesotheliomas.

Materials and Methods

Case Selection

This study was performed with the approval of the Institutional Review Board at Brigham and Women's Hospital. Cases were retrieved from the surgical pathology files of Brigham and Women's Hospital. In total, three epithelioid primary pericardial mesotheliomas without pleural involvement diagnosed between 2004 and 2022 were identified and examined postmortem (cases #1 and #3) or on surgical resection (case #2).

Immunohistochemistry

Immunohistochemistry was performed for all mesotheliomas on 4- μ m-thick formalin-fixed paraffin-embedded whole tissue sections using the antibodies and staining condition listed in Table 1. Appropriate positive and negative controls were used throughout. All stains were independently evaluated by two anatomic pathologists (I.-M.S. and L.M.S.). For the tumor suppressors p16, MTAP, BAP1, Merlin (NF2), and p53, expression was considered lost (i.e., negative) when there was loss of nuclear (p16, BAP1, p53), cytoplasmic (MTAP), or membranous (Merlin) expression. In addition, loss of nuclear BAP1 expression and concurrent cytoplasmic BAP1 expression was considered abnormal. Strong nuclear expression of p53 in >95% of tumor cells was considered abnormal/overexpressed. Nonneoplastic cells (vascular endothelial cells and inflammatory cells) were used as internal positive control.

Targeted Next-Generation Sequencing

Next-generation sequencing was performed using our institutional targeted sequencing platform, OncoPanel, which interrogates the exonic sequences of 447 cancer-associated genes for mutations and copy number variations, and 191 introns across 60 genes for gene rearrangements.^{15,16} DNA extraction from formalin-fixed paraffin-embedded tissue (QIAamp DNA mini kit, Qiagen, Valencia, CA, USA), construction of hybrid-capture libraries, sequencing using the Illumina HiSeq 2500 (Illumina, San Diego, CA, USA), and sequence data analysis was performed as previously described.¹⁶ All detected alterations (including single nucleotide variants, copy number alterations, and translocation calls) were reviewed and annotated manually.

Results

Clinicopathologic Findings

Table 2 summarizes the clinicopathologic findings of the three primary pericardial mesotheliomas. The cases included one male and two female patients aged 75, 66, and 68 years, respectively. The tumors were located in the pericardium without pleural involvement by imaging studies and/or examination at autopsy.

Patient #1 was a 75-year-old male with a history of cardiac arrhythmias (right and left bundle branch block, atrial flutter, and atrial fibrillation) who presented with hypoxia and recurrent pericardial effusion with evidence of pericardial thickening leading to biventricular dysfunction. He had a history of asbestos exposure and was a non-smoker. The patient

underwent an open mediastinotomy with attempted pericardiectomy which demonstrated an irregular, thickened, nodular pericardium plastered to and encasing the heart and ascending aorta. Intraoperative frozen section examination was highly suggestive of mesothelioma. The tumor was partially removed but was ultimately deemed unresectable. The patient expired the same day in cardiogenic shock and underwent autopsy examination limited to the chest and abdomen which revealed a tan to white solid mass which diffusely replaced the pericardium extending from the base of the heart to the apex (Figure 1A). Locoregional lymph nodes were involved by metastatic spread. Given the absence of pleural involvement, a diagnosis of primary pericardial mesothelioma was made.

Patient #2 was a 66-year-old female with a history of systemic hypertension, hypothyroidism, and gastroesophageal reflux disease who was hospitalized for dyspnea and intermittent epigastric pain. She had a history of asbestos exposure and a smoking history of 40 pack-years. During her hospitalization, she was found to have a large pericardial effusion and nodularity of the pericardium. Imaging studies demonstrated an ill-defined soft tissue mass along the anterior and the inferior pericardium immediately adjacent to the diaphragm and along the ascending aorta with the most prominent nodular area of pericardial enhancement noted near the cardiac apex. The pleura was not involved. Histopathologic examination of a pericardial biopsy revealed mesothelioma. She subsequently underwent radical pericardiectomy with radical left lymphadenectomy and heated intraoperative chemotherapy. Histopathologic examination of the resected specimens revealed mesothelioma extensively involving the pericardium and abutting the diaphragmatic skeletal muscle. Given the absence of pleural involvement, a diagnosis of primary pericardial mesothelioma was made. She was subsequently discharged and died 15 months later from disease recurrence.

Patient #3 was a 68-year-old female with a history of gastric carcinoma (status post partial gastrectomy 7 years earlier), diffuse atherosclerosis, and peripheral vascular disease with remote myocardial infarction and stroke who presented with chest pain, abdominal pain, and complete heart block. The patient was a smoker with no known asbestos exposure. Physical exam revealed decompensated congestive heart failure and pericardial and pleural effusions. Cardiac catheterization identified severe three-vessel disease, and the patient was admitted to undergo cardiac surgery for coronary bypass grafting which was ultimately cancelled after an angiogram showed complete atherosclerotic occlusion of the distal aorta at the level of the renal arteries. The patient developed ventricular tachycardia and subsequently expired. She underwent unrestricted autopsy examination which revealed a tan to white solid mass and diffusely replacing the pericardium extending from the base to the apex (Figure 1B). Given the absence of pleural involvement, a diagnosis of primary pericardial mesothelioma was made.

Radiologic Findings

Figure 1C–E summarizes the radiologic findings for the three primary pericardial mesotheliomas. In patient #1, contrast-enhanced chest CT images showed a loculated pericardial effusion with soft tissue in the superior pericardial recess and anterior pericardial space as well as circumferential thickening and enhancement of the visceral and parietal

pericardium (Figure 1C). In patient #2, contrast-enhanced chest CT images displayed nodular pericardial thickening with nodules invading the epicardial fat (Figure 1D). In patient #3, CT imaging was not available. Three-chamber echocardiography imaging revealed a pericardial effusion with nodular soft tissue along the visceral pericardium (Figure 1E).

Histopathologic Features

Histopathologic examination revealed high-grade epithelioid mesothelioma in case #1, lowgrade epithelioid mesothelioma in case #2 with atypical tumor cells growing in sheets and strands and forming glandular structures (Figure 2A–D). Case #3 represented a biphasic mesothelioma with sarcomatoid components accounting for 60% of the tumor, consisting of moderately to severely atypical tumor cells growing in solid sheets with scattered glandular structures (Figure 2E, F). The tumor cells showed a higher degree of nuclear pleomorphism than cases #1 and #2. Geographic necrosis was present.

Immunohistochemistry Results

All three primary pericardial mesotheliomas expressed cytokeratin AE1/AE3 and calretinin by immunohistochemistry (Table 2, Figure 3A–C). Staining for D2–40 was positive in case #1, whereas WT1 was negative in cases #1 and #3 (cytoplasmic staining) and positive in case #2 (nuclear staining) (Figure 3A–C). TTF1 and claudin-4 were negative in all three cases (Table 2, Figure 3A–C). Staining for tumor suppressors frequently dysregulated in mesothelioma revealed concurrent loss of p16 and MTAP expression in cases #1 and #2, loss of BAP1 expression in case #2, loss of Merlin expression in cases #2 and #3, and loss of p53 expression in case #3 (Figure 4A–C). In addition, case #3 showed loss of nuclear BAP1 expression and abnormal cytoplasmic expression (Figure 4A–C).

Targeted Next-Generation Sequencing Results

All three mesotheliomas and corresponding non-neoplastic tissues (liver in cases #1 and #3, lymph node in case #2) underwent successful targeted next-generation sequencing. The overall mean target coverage (MTC) was 208 (range, 27–484); one case (the mesothelioma of patient #3) had a low MTC of 27 which is likely attributable to post-mortem DNA degradation. Figure 5A demonstrates that the protein expression abnormalities of p16, MTAP, BAP1, Merlin, and p53 correlated with genomic aberrations involving *CDKN2A*, *CDKN2B*, *MTAP*, *BAP1*, *NF2*, and *TP53*, respectively. The frequency and mutational pattern of NGS genomic results for the three pericardial mesotheliomas are summarized in Figure 5B and largely corresponded to the genomic aberrations reported by the cBioPortal for Cancer Genomics (project GENIE v13.0-public, N=167,423 samples total; N=963 mesotheliomas, including N=800 pleural, N=154 peritoneal, and N=9 testicular mesotheliomas)^{17,18}, shown in Figure 5C, with *CDKN2A*, *CDKN2B*, and *MTAP* generally being inactivated through homozygous deletions, and *BAP1*, *NF2*, *TP53*, and *BRCA1* usually targeted by inactivating mutations.

The mesothelioma of patient #1 showed a homozygous deletion involving *CDKN2A*, *CDKN2B*, and *MTAP*, a heterozygous deletion of *BAP1*, and heterozygous deletion of *NF2*, gain of *WT1*, and a pathogenic frameshift mutation of *BRCA1* (p.E1210Rfs*)

(allele fraction [AF] 0.8; PolyPhen-2 score: 0.995) (Figure 6A). The same mutation was detected as a heterozygous mutation in the patient's corresponding non-neoplastic tissue (AF 0.5) (Figure 6B) thereby credentialing it as a germline variant. The mesothelioma of patient #2 displayed a homozygous deletion involving *CDKN2A*, *CDKN2B*, and *MTAP*, a homozygous deletion involving *BAP1*, a nonsense mutation of *NF2* (p.Y144*, AF 0.11) with loss of one copy of *NF2*, and gain of *WT1*. No germline mutations were identified in the non-neoplastic tissue of patient #2. The mesothelioma of patient #3 displayed a two-copy loss of *NF2*, homozygous *TP53* nonsense mutation (p.Q331*; AF 0.74), and gain of *WT1* (Figure 6C). No genomic abnormality involving *BAP1* was identified upon manual review of the sequencing results. No germline mutations were identified in the non-neoplastic tissue of patient #3. All three mesotheliomas were MMR proficient. The mean tumor mutational burden per Megabase was 4.31 (range, 3–6) which is comparable to an average of 4.56 (range, 1–56) mutations per Megabase for all mesotheliomas included in project GENIE (N=831).

Overall, copy number analysis revealed several chromosomal gains and losses in all three mesotheliomas, whereas no copy number alterations were identified in the corresponding nonneoplastic tissues (Figure 7).

Discussion

Primary pericardial mesotheliomas are extremely rare and unlike pleural mesotheliomas, their etiologic factors remain elusive. The three cases of pericardial mesothelioma reported herein largely reflect the clinicopathologic features described for patients with pleural mesotheliomas, including a patient age of ~70 years as well as prior asbestos exposure and smoking history reported in two patients each. While it is commonly accepted that tobacco smoking and asbestos exposure interact synergistically for the causation of lung cancer, and asbestos exposure has been long been established as risk factor for pleural mesothelioma¹⁹, their role for the development of pericardial mesothelioma has not been definitively determined. Some studies observed a significant association between occupational asbestos exposure and pericardial mesothelioma^{6,7} whereas others have not.^{1,5} While two of the patients reported in our study each had a history of smoking and asbestos exposure, our study is limited by small case numbers and our observations do not allow for conclusions about causation.

Histopathologic examination revealed epithelioid or biphasic histomorphology as reported previously for pericardial mesotheliomas.^{5,20} In addition, we observed immunohistochemical expression of markers commonly expressed in mesothelioma, such as cytokeratin AE1/AE3, calretinin, D2–40, and WT1 as well as negativity for TTF1 and claudin-4.

The results of our immunohistochemical analyses point towards frequent tumor suppressor inactivation in pericardial mesotheliomas, including expression abnormalities of p16, MTAP, BAP1, Merlin, and p53 resulting from genomic inactivation of *CDKN2A/p16*, *CDKN2B*, *MTAP*, *BAP1*, *NF2*, and *TP53*, respectively, which are also frequently dysregulated in pleural mesothelioma.²¹ Our findings are in line with those reported by Offin *et al.* and Kato

et al. who previously described genomic aberrations inactivating *CDKN2A/B*, *BAP1*, *TP53*, and *NF2*, in altogether three patients' pericardial mesotheliomas.^{5,11}

Cytoplasmic expression of BAP1 with concurrent loss of nuclear expression observed in case #3 has been reported in pleural mesothelioma by De Rienzo and colleagues.²² This staining pattern is considered abnormal since BAP1 is normally localized in the nucleus. Aberrant cytoplasmic expression suggests an underlying mutation affecting the nuclear localization signal of BAP1.²² However, we were not able to identify a *BAP1* genomic abnormality in case #3, which might be explained by technical limitations of our NGS assay or the low MTC in this case.

The results of our analyses indicate that pericardial mesotheliomas share genomic aberrations with pleural mesotheliomas and might therefore be driven by the same biologic pathways. Similarly, mesothelioma of the tunica vaginalis, another rare subtype of mesothelioma, was found to exhibit a similar mutational profile comparable to those of the pleura/peritoneum; however, *CDKN2A* and *BAP1* alterations appeared to be less common.²³ A subset of cases had potentially targetable alterations in driver genes (*PTCH1* and *TSC1*) that are unusual in mesotheliomas at other anatomical sites²³ and were not identified in the three pericardial mesotheliomas reported here.

Comparison with the genomic data from our institution included revealed that the three pericardial mesotheliomas had a mutational burden comparable to all mesotheliomas included in project GENIE. In addition, one patient with pericardial mesothelioma harbored a pathogenic *BRCA1* germline mutation (p.E1210Rfs*) that has previously been reported 17 times in ClinVar as a germline variant in patients with hereditary breast and ovarian carcinoma (VCV000037534.35).²⁴ Mesothelioma patients have been reported to harbor germline mutations in *CDKN2A* and *BAP1* (encoding BAP1 or BRCA1 associated protein-1)^{13,14,25}, as well as other cancer susceptibility genes, such as *ATM*, *CHEK2*, *TP53*, *BRCA1*, and *BRCA2*.²⁶ As discussed by Panou *et al.*, these findings suggest that predominantly aberrations in genes involved in DNA damage response pathways play important roles in the development of pleural mesothelioma. The identification of a *BRCA1* germline mutation in one of our patients indicates that deficient DNA damage response pathways might give rise to pericardial mesothelioma as well. While *BRCA1* germline mutations have initially been reported in patients with hereditary breast and ovarian cancer syndrome, additional tumor types have been added to the list of neoplasms associated with *BRCA1* germline variants, including gastric cancer, pancreatic cancer, and biliary tract cancer.²⁷ In breast, pancreatic, and ovarian cancers, *BRCA1* germline mutations are associated with failure of DNA double-strand break-repair by homologous recombination.²⁸ Importantly, homologous recombination pathway alterations can occur concurrently with canonical driver mutations, as reported in lung cancer²⁹ and pancreatic cancer.³⁰ Our studies provide evidence of biallelic *BRCA1* inactivation in the mesothelioma of patient #1 which occurred concurrently with mutations in mesothelioma driver genes (e.g., biallelic inactivation of *CDKN2A*, *CDKN2B*, and *MTAP*) indicating that *BRCA1* inactivation is being selected for in the tumor cells and likely contributed to tumor development and/or progression.

BRCA1 mutations have been reported to render patients with ovarian or breast cancer particularly sensitive to cisplatin chemotherapy³¹ and poly-ADP ribose polymerase (PARP) inhibitors^{32,33}, which induce synthetic lethality of alternate DNA repair pathways and thereby lead to cell death. Based on its interaction with *BRCA1*, *BAP1* has been shown to increase sensitivity to PARP inhibitors in patients with mesothelioma. The three cases of pericardial mesothelioma reported herein behaved aggressively and the patients died between 0 and 15 months after diagnosis. Offin *et al.*, reported that trimodality therapy (i.e., surgical resection, adjuvant chemotherapy, and radiation) may improve outcomes in selected patients with primary pericardial mesothelioma.⁵ Ongoing studies are exploring the efficacy of targeting DNA damage repair pathway deficiencies in patients with mesothelioma³⁴ and it remains to be determined whether such approaches might also benefit patients with pericardial mesothelioma in the future.

In summary, we report three pericardial mesotheliomas which showed morphologic, immunohistochemical, and molecular genetic features typical of pleural mesothelioma including recurrent genomic inactivation of *CDKN2A/p16*, *CDKN2B*, *MTAP*, *BAP1*, *NF2*, and *TP53*. Our study adds new insights into the genetic landscape of primary pericardial mesothelioma and highlights *BRCA1* loss as a potential contributing factor in a subset of cases, thereby contributing to refined precision diagnostics for this rare cancer.

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Data Availability

As per genomic data sharing guidelines, somatic sequence and assay data are deposited in a publicly available database sponsored by the American Association for Cancer Research (AACR) Project GENIE. Variant calls and a limited clinical dataset from patients are sent to the Synapse platform, developed by Sage Bionetworks, where the data are harmonized and protected health information (PHI) removed in a secure Health Insurance Portability and Accountability Act (HIPAA)-compliant environment that provides data governance.

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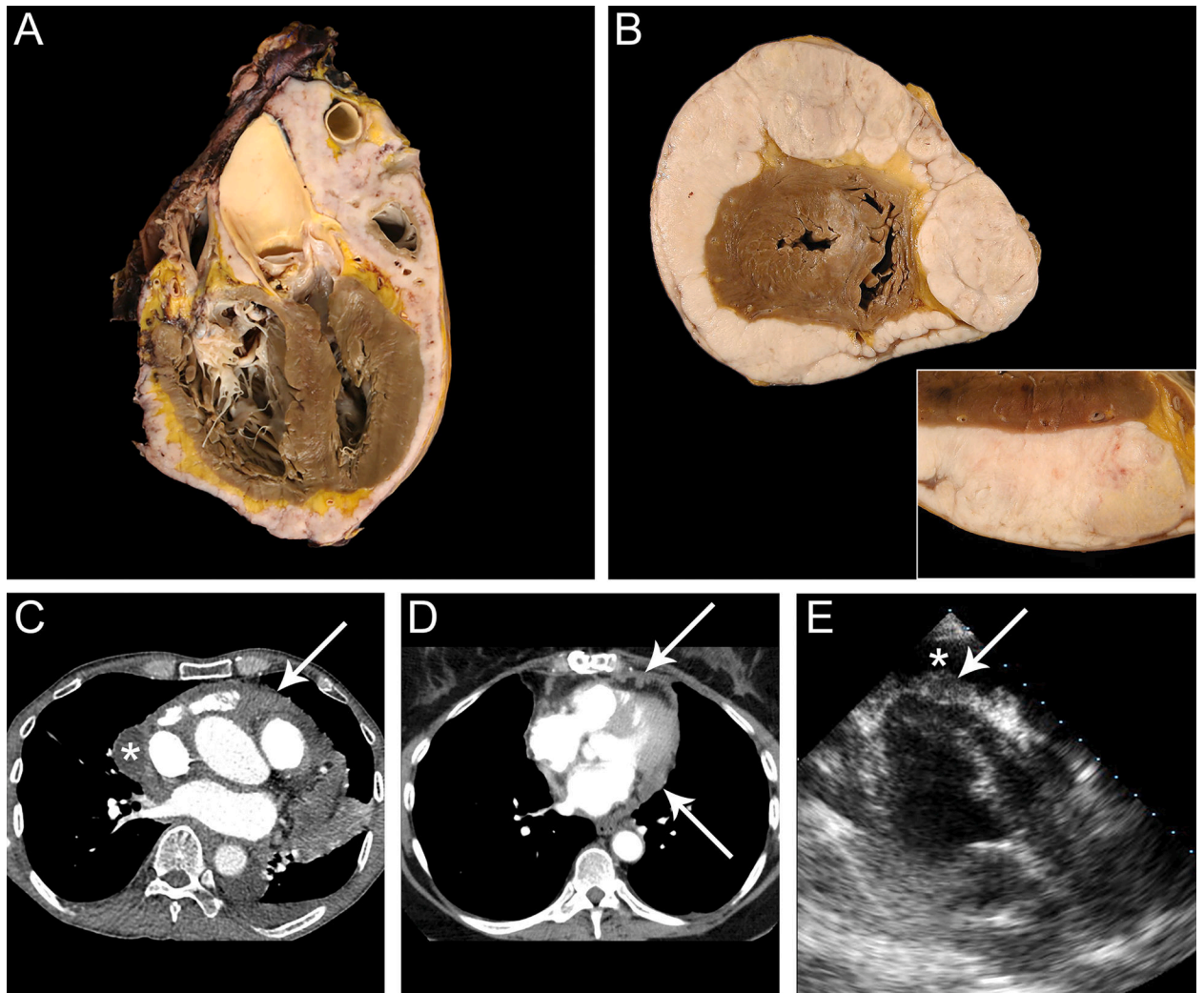


Figure 1. Gross and radiologic findings of primary pericardial mesothelioma.

Gross examination at autopsy in cases #1 (**A**) and #3 (**B**; **B**, inset) revealed a solid tan to white mass replacing the pericardium and completely encasing the heart from the base to the apex. **C** Contrast-enhanced chest CT imaging in case #1 showed a loculated pericardial effusion (*) with soft tissue in the superior pericardial recess and anterior pericardial space as well as circumferential thickening and enhancement of the visceral and parietal pericardium (arrow). **D** In case #2, contrast-enhanced chest CT imaging displayed nodular pericardial thickening with nodules invading the epicardial fat (arrows). **E** Three-chamber echocardiography imaging revealed a pericardial effusion (*) with nodular soft tissue along the visceral pericardium (arrow).

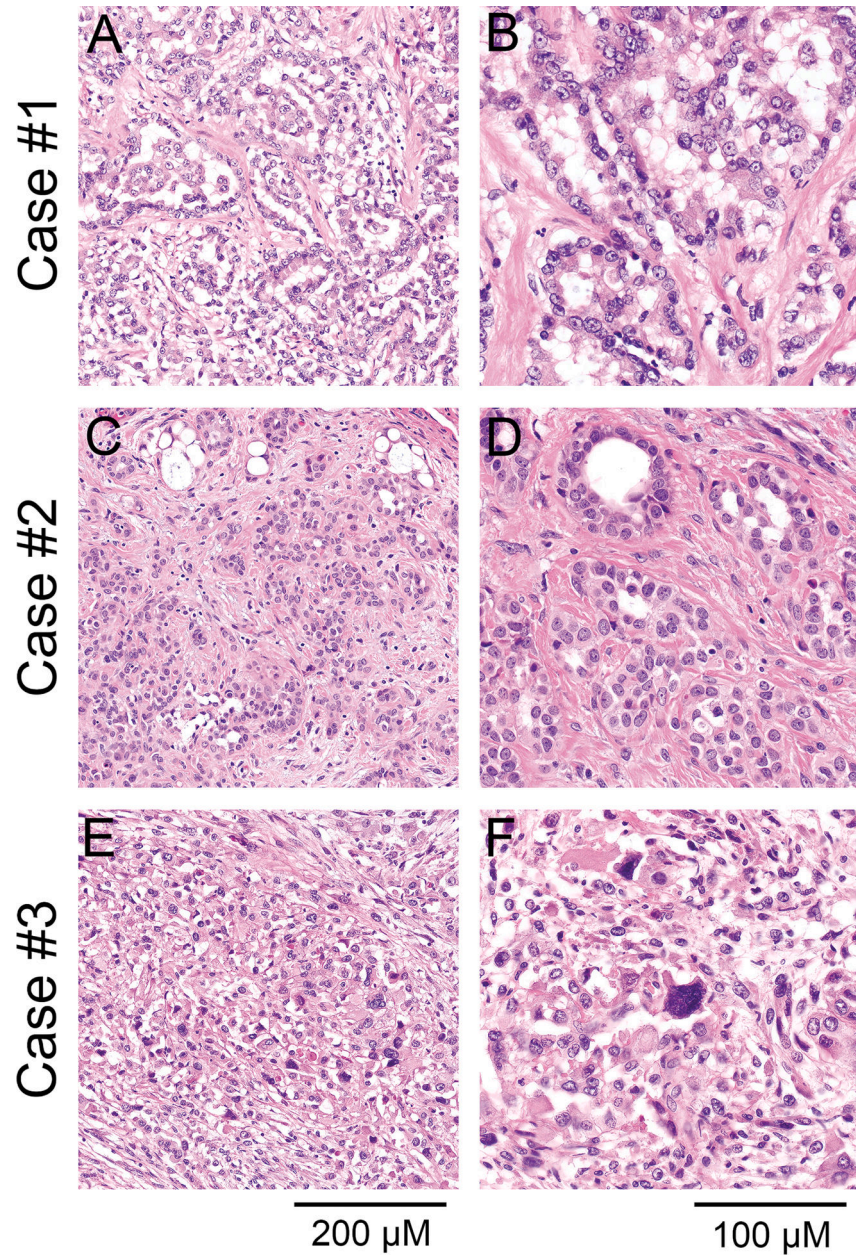


Figure 2. Histopathologic features of primary pericardial mesothelioma. Histopathologic examination revealed epithelioid mesothelioma in cases #1 (**A**, x200; **B**, x400) and #2 (**C**, x200; **D**, x400) with moderately atypical tumor cells growing in sheets and strands and forming glandular structures. **E** (x200), **F** (x400) Case #3 represented a biphasic mesothelioma consisting of moderately to severely atypical tumor cells growing in solid sheets with scattered glandular structures; hematoxylin-eosin stain.

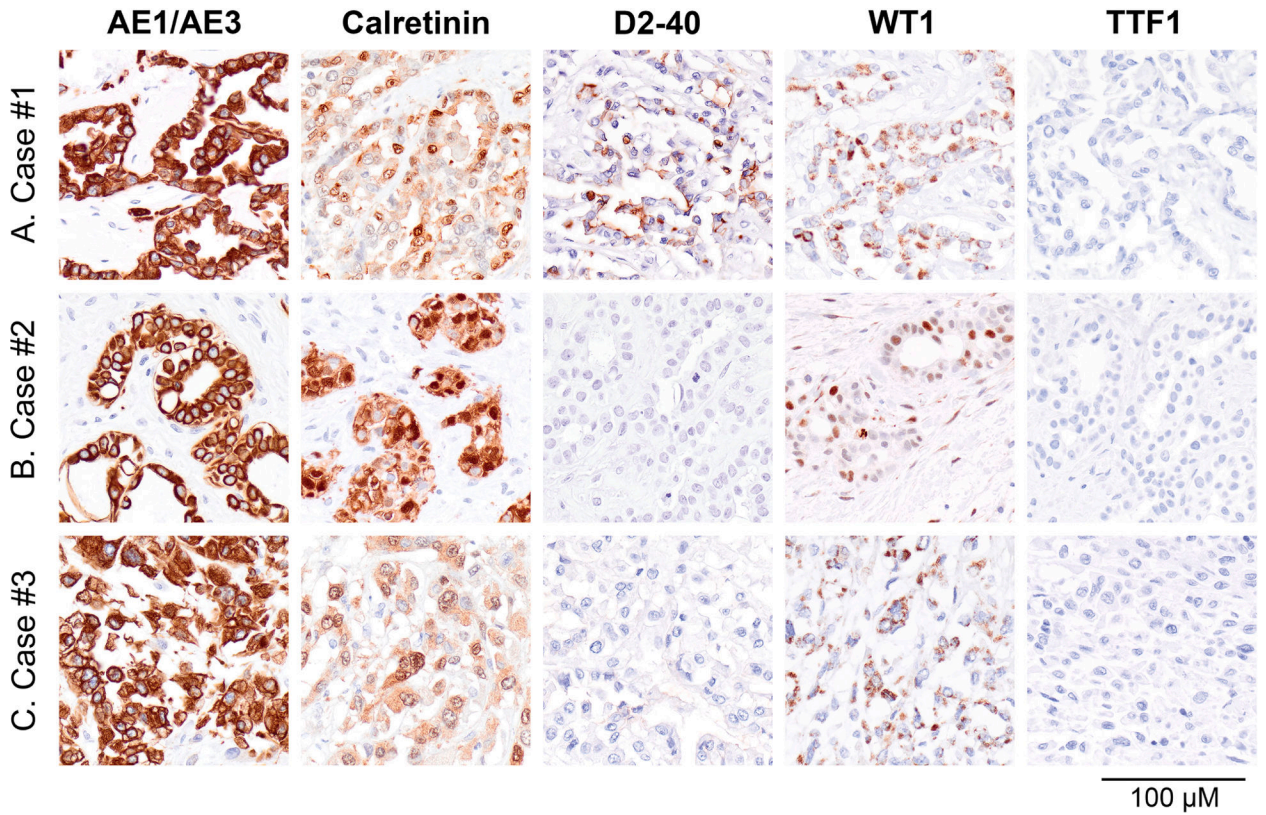


Figure 3. Results of immunohistochemical staining for mesothelioma markers in primary pericardial mesothelioma.

A-C All three cases expressed cytokeratin AE1/AE3 and calretinin by immunohistochemistry. A-C Staining for D2-40 was positive in case #1, whereas WT1 was negative in cases #1 and #3 (cytoplasmic staining) and positive in case #2 (nuclear staining). A-C TTF1 and claudin-4 stains were negative in all three cases.

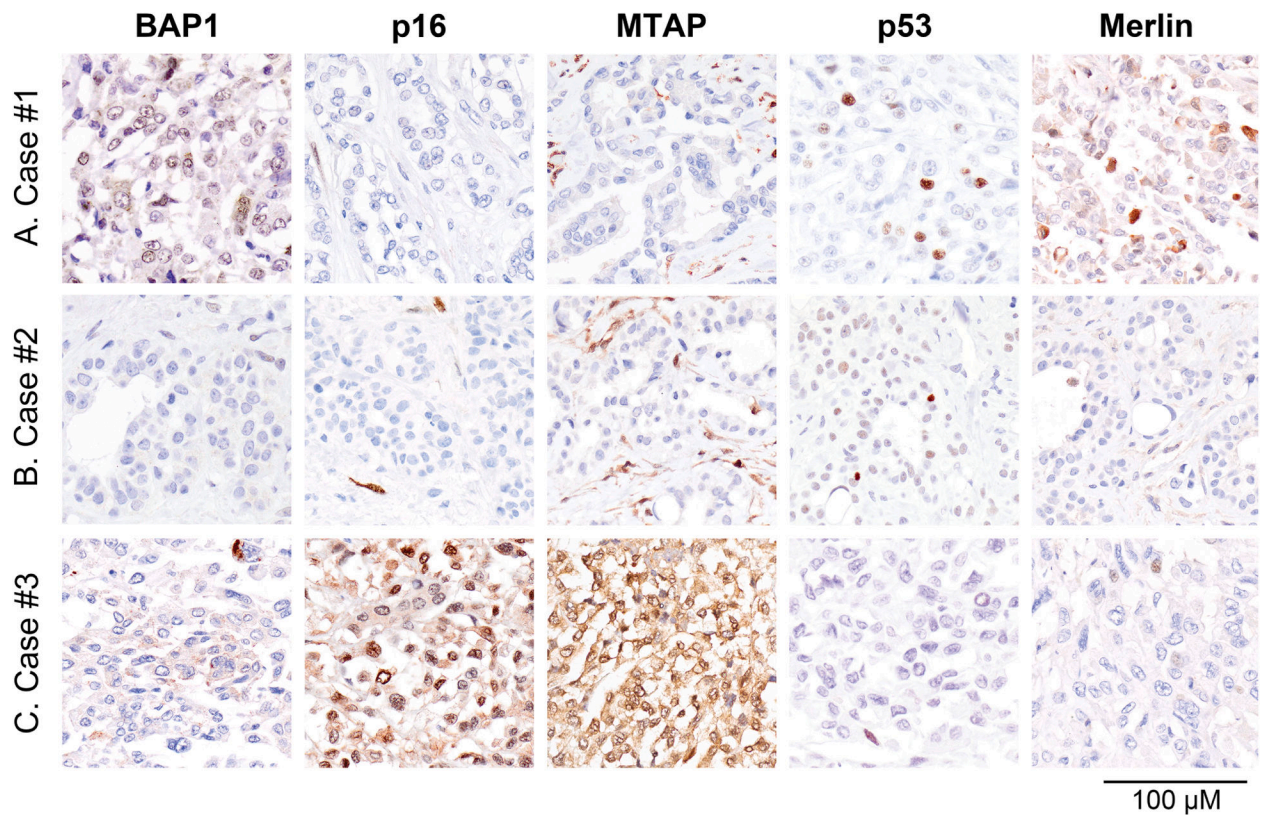


Figure 4. Results of immunohistochemical staining for tumor suppressor abnormalities in primary pericardial mesothelioma.

A-C Immunohistochemical staining revealed loss of BAP1 expression in case #2, concurrent loss of p16 and MTAP expression in cases #1 and #2, loss of p53 expression in case #3, and loss of Merlin expression in cases #2 and #3. For each marker, vascular endothelial cells and inflammatory cells served as internal control.

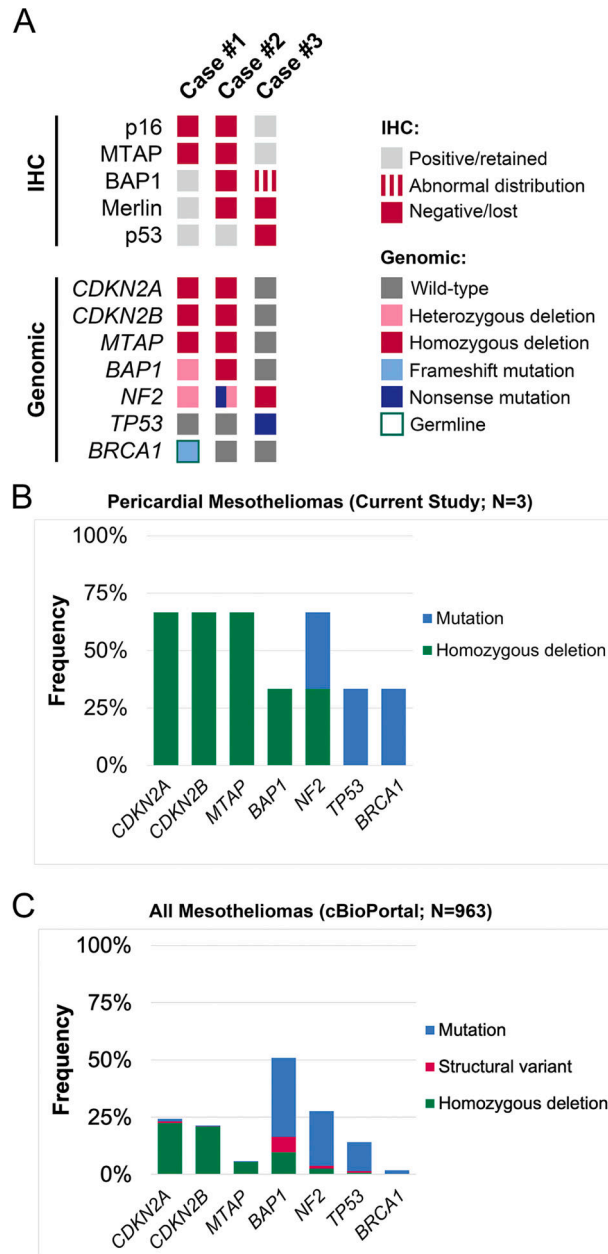


Figure 5. Results of targeted next-generation sequencing for tumor suppressor aberrations in primary pericardial mesothelioma.

A Heat map displaying results of protein expression analysis by immunohistochemistry and targeted next-generation sequencing in primary pericardial mesotheliomas. Frequency of recurrent genomic aberrations and inactivating mechanism for *CDKN2A*, *CDKN2B*, *MTAP*, *BAP1*, *NF2*, *TP53*, and *BRCA1* in three pericardial mesotheliomas (**B**) and in all mesotheliomas included in the cBioPortal for Cancer Genomics (project GENIE v13.0-public, N=167,423 samples total; N=963 mesotheliomas, including N=800 pleural, N=154 peritoneal, and N=9 testicular mesotheliomas) (**C**).

Table 1.

Immunohistochemical antibodies and staining conditions.

Antibody	Clone	Dilution	Source	Pretreatment	Detection system
AE1/AE3	AE1/AE3	1:200	Agilent, Santa Clara, CA, USA	10 mins protease	Envision Plus
Calretinin	Polyclonal	1:200	Invitrogen, Carlsbad, CA, USA	pH 6 citrate buffer, pressure cooker	Envision Plus
D2-40	D2-40	1:100	Biologend, San Diego, CA, USA	None	Envision Plus
WT1	6F-H2	1:75	Agilent, Santa Clara, CA, USA	pH 6 citrate buffer, pressure cooker	Envision Plus
TTF1	8G7G3/1	1:800	Agilent, Santa Clara, CA, USA	pH 6 citrate buffer, pressure cooker	Envision Plus
Claudin-4	3E2C1	1:300	Invitrogen, Carlsbad, CA, USA	pH 6 citrate buffer, pressure cooker	Envision Plus
BAP1	C-4	1:100	Santa Cruz Biotechnology, Dallas, TX, USA	pH 6 citrate buffer, pressure cooker	Envision Plus
p16	BC42	1:50	Biocare, Pacheco, CA, USA	pH 6 citrate buffer, pressure cooker	Envision Plus
MTAP	42-T	1:200	Santa Cruz Biotechnology, Dallas, TX, USA	pH 6 citrate buffer, pressure cooker	Novolink
p53	DO-7	1:500	Agilent, Santa Clara, CA, USA	pH 6 citrate buffer, pressure cooker	Envision Plus
Merlin (NF2)	D1D8	1:250	Cell Signaling Technology, Danvers, MA, USA	pH 6 citrate buffer, pressure cooker	Novolink

Table 2.

Clinicopathologic features of three cases of primary pericardial mesothelioma.

	Case #1	Case #2	Case #3
Age/Sex	75/M	66/F	68/F
Clinical presentation	Hypoxia, pericardial effusion, and biventricular dysfunction	Dyspnea and intermittent epigastric pain	Chest pain, abdominal pain, and complete heart block
Pleural involvement	No	No	No
Prior asbestos exposure	Yes	Yes	N/A
Smoker	No	Yes (40 Py)	Yes (N/A)
Treatment	Partial pericardial resection	Extensive pericardial resection	None
Follow-up status	DOD (<1 week)	DOD (15 months)	DOD (<1 week)
Histologic subtype	Epithelioid	Epithelioid	Biphasic (sarcomatoid component: 60%)
Histologic grade	High	Low	-
Immunohistochemistry			
AE1/AE3	Positive	Positive	Positive
Calretinin	Positive	Positive	Positive
D2-40	Positive	Negative	Negative
WT1	Negative	Positive	Negative
TTF1	Negative	Negative	Negative
Claudin-4	Negative	Negative	Negative

DOD: Died of disease; F: Female; M: Male; N/A: Not available; Py: Pack years

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