Gastric metastasis from nodular malignant melanoma of the auricle with multigene aberrations: A rare case report and literature review

YA-CHEN YUAN[1*], YING LI[2*], YUN PAN[1] and BO GAO[1]

[1]Department of Pathology, The First Affiliated Hospital of Dali University, Dali, Yunnan 671000;
[2]Department of Pathology, People's Hospital of Xiangyun County, Xiangyun, Yunnan 672100, P.R. China

Abstract. Primary malignant melanoma (MM) of the external ear accounts for a low proportion of cases of cutaneous MM, and its incidence in non-white women is very low. The stomach is a rare metastatic site for MM. Gastric metastasis of MM of the external ear is extremely rare, and the associated gene alterations and mechanisms are poorly understood. The present report describes the case of a 58-year-old Asian woman who had a mass on the left auricle for 5 years and was diagnosed with nodular MM with the BRAF V600E mutation after surgical resection. Postoperative metastases to the stomach and descending duodenum appeared 1 year after resection. After 11 months of BRAF-targeted therapy and immunotherapy, the patient developed drug resistance and died from systemic metastases to the brain, lungs, liver, left adrenal gland and peritoneum. Genetic testing revealed additional aberrations in MYB, p16, MYC and PTEN. The clinical characteristics of MM of the external ear and gastric metastatic MM were also summarized through a retrospective literature review. Immunohistochemical staining is critical in the diagnosis of gastric metastasis from MM of the external ear. This disease often requires a multidisciplinary treatment approach, including surgery, targeted therapy and immunotherapy. The present study provides some genetic information about this rare disease and discusses appropriate treatment strategies. The findings of the present study suggests that the surgical margin size, tumor histological type and number of genetic aberrations may be closely associated with metastasis potential, therapeutic efficacy and patient outcome.

Correspondence to: Dr Bo Gao, Department of Pathology, The First Affiliated Hospital of Dali University, 32 Carlsberg Avenue, Dali, Yunnan 671000, P.R. China
E-mail: gaobo229@163.com

*Contributed equally

Introduction

Melanoma is a highly malignant tumor that originates from melanocytes and can occur in different sites or tissues, including the skin, ocular uvea, pia mater and mucosa, for example, that of the digestive, respiratory and genitourinary tracts. Although malignant melanoma (MM) accounts for only 1% of all diagnosed cutaneous cancers (1), it is responsible for the highest number of skin cancer-associated deaths. MM of the external ear is rare. Among head and neck MMs, the majority (70-90%) occur on the face, mainly on the cheek, while the external ear accounts for only 7% of cases (2). The incidence of primary melanoma of the external ear is increasing, as is the incidence of cutaneous melanoma (3); however, case reports of primary external ear MM are rare.

MM primarily metastasizes through lymphatic and hematologic routes, and distant metastases are most commonly found in the lungs, liver and brain. Gastrointestinal metastases of MM are rare, and as the main site of gastrointestinal metastasis is the small intestine, metastasis of MM to the stomach is even rarer (4). Due to its rarity, to the best of our knowledge there are no case reports or series evaluating the gastric metastasis of MM from the external ear that provide useful disease characterization to help clinicians choose appropriate treatments.

The present study reports the case of a patient with MM originating from the left auricle with initial metastasis to the stomach and descending duodenum, followed by multiple metastases to the brain, lungs, peritoneum, liver and left adrenal gland. The molecular genetic alterations of the tumor were investigated to help select the appropriate targeted therapeutic regimen, and the presence of multiple mutated genes was identified.

Case report

Materials and methods. BRAF V600E mutation was detected using a Human BRAF V600E Mutation Detection Kit (Production lot number 11221112601X; Amoy Diagnostics Co., Ltd.) for medical diagnosis. According to the manufacturer's instructions, amplification of the reference gene (internal control) and BRAF V600E mutation in samples were detected

by amplification-refractory mutation system polymerase chain reaction (ARMS-PCR) using HEX and FAM fluorescent channels, respectively. The primers used for PCR were provided as part of the kit. To achieve reliable results, the HEX signal of the sample to be tested should be a positive curve, with a Cq value of between 13 and 21. The cutoff value of Cq for BRAF V600E mutation is 30. Immunohistochemical staining was performed on 4 μm-thick paraffin sections. The ready-to-use primary antibodies (MAB-0098, MAB-0275, Kit-0007, MAB-0671, Kit-0024, RMA-0815, MAB-0743, MAB-0742, MAB-0707, MAB-0672, MAB-0735, MAB-0716 and MAB-0717; MXB Biotechnologies Co., Ltd.) and an EliVision plus Detection Kit (KIT-9901; MXB Biotechnologies Co., Ltd.) were used for immunohistochemical staining.

Case presentation. A 58-year-old female patient was hospitalized at the People's Hospital of Xiangyun County (Xiangyun, China) in March 2019 due to a black mass the approximate size of a fava bean behind the left ear that had been present for ~5 years with itching but no tinnitus, stuffiness, hearing loss or pus discharge from the external ear canal. Upon physical examination, it was observed that the black mass was soft with clear boundaries and an absence of pain and redness, and both auricles exhibited normal morphology. Five days after initial hospitalization, mass resection and arbitrary flap plasty were performed. The resected mass comprised hard grayish-black tissue, 1.5x1x0.8 cm^3 in size, with a black protrusion of diameter 0.8 cm in the center (Fig. 1A). The mass was diagnosed as nodular MM of the left auricle by intraoperative frozen section examination. When observed under a microscope, nestlike, nodular and diffuse melanocyte dysplasia was observed, with tumor cells displaying marked pleomorphism and high proliferative activity (Figs. 1B and C). Immunohistochemical staining showed that the tumor cells were positive for human melanoma black 45 (HMB45; Fig. 1D), melan-A (Fig. 1E) and S-100 (Fig. 1F) but negative for pan cytokeratin (CK-pan), leukocyte common antigen and p40. Formalin-fixed paraffin-embedded tissue sections were further analyzed to detect genetic variations. Fluorescence *in situ* hybridization (FISH) revealed MYB deletion (Fig. 2A), p16 deletion (Fig. 2B), MYC amplification (Fig. 2C), PTEN deletion (Fig. 2C) and no marked changes in the copy numbers of Ras responsive element binding protein 1 (RREB1; Fig. 2A) and cyclin D1 (CCND1; Fig. 2B). ARMS-PCR revealed the BRAF V600E mutation (Fig. 3), and no common NRAS (G12D, G12S, G13D, G13R, G12C, G12V, G12A, G13V, A59D, Q61R, Q61K, Q61L, Q61H, K117N, and A146T) mutations were found. Furthermore, DNA sequencing revealed no mutations in exons 9, 11, 13, or 17 of c-KIT. PCR followed by high-resolution melting analysis showed that the tumor was negative for seven microsatellite instability biomarkers, namely activin A receptor type 2A; BTB domain containing 7; death inducer-obliterator 1; MRE11 homolog, double strand break repair nuclease; ryanodine receptor 3; SEC31 homolog A, COPII coat complex component; and sulfatase 2. These findings indicated microsatellite stability.

The patient was hospitalized again at the People's Hospital of Xiangyun County in March 2020 due to epigastric pain, nausea, vomiting and abdominal distension. Protruding lesions with erosion and melanin deposition were observed in the gastric body, gastric antrum and descending part of the duodenum by gastroscopic examination (Fig. 4). Pathological biopsy revealed gastric mucosa hyperemia, nestlike cell clusters in the lamina propria, and lymphocyte and plasma cell infiltration (Fig. 5A-C). *Helicobacter pylori* was not detected. Immunohistochemical staining showed that the lesion cells were positive for HMB45 (Fig. 5D), melan-A (Fig. 5E) and S-100 (Fig. 5F), weakly positive for CD56 (Fig. 5G), and negative for CK-pan, Syn and CgA. The Ki-67 proliferation index was ~40% (Fig. 5H). Due to the medical history, histopathologic features and expression of protein markers in the biopsy specimen, the patient was diagnosed with metastatic external ear MM. Subsequently, the patient received targeted treatment with vemurafenib, which was administered during multiple immunotherapy sessions at a specialized oncology hospital.

The patient experienced abdominal swelling for ~2 weeks in February 2021. Computed tomography examination revealed multiple hyperdense shadows in the brain (Fig. 6A), bilateral small pulmonary nodules in the lungs (Fig. 6B), diffuse slightly hyperdense nodules of varying sizes in the liver (Fig. 6C), space-occupying lesions in the left adrenal gland (Fig. 6D) and peritoneal thickening (Fig. 6E), suggesting systemic metastases of the tumor. The patient had seroperitoneum in which malignant tumor cells were detected by cytological examination (Fig. 7A). Immunohistochemical staining showed that the tumor cells were positive for HMB45 (Fig. 7B), melan-A (Fig. 7C), S-100 (Fig. 7D), Ki-67 (50%) (Fig. 7E) and vimentin (Fig. 7F), and the peritoneal mesothelial cells were positive for CK-pan (Fig. 7G), calretinin (Fig. 7H) and mesothelial cell (Fig. 7I), indicating metastatic MM.

In March 2021, the patient developed secondary epilepsy, hypoproteinemia, electrolyte disturbance and cancerous cachexia. The patient abandoned treatment and died two days after discharge from the hospital (Fig. 8).

Literature review

A total of 6,577 cases of MM of the external ear reported in 11 articles were reviewed in the present study (Table I) (3,5-14). The cases in all articles had MM of the external ear, and the specific site was reported in some of the articles. The tumors primarily occurred in the helix. Analysis of these cases indicated that MM of the external ear frequently occurs in middle-aged and older adults and occasionally in adolescents. Men (5,603 cases) were more frequently affected than women (974 cases). Patients with MM of the external ear were mostly asymptomatic or had some local symptoms at diagnosis, including ear swelling, otorrhea, hearing loss and bleeding. Regional lymph node metastasis was common, and the other metastatic sites were primarily the liver, lung, brain, parotid gland and retromandibular tissue. Regarding prognosis, a long disease duration and high tumor stage were unfavorable factors for survival.

A detailed literature review of 22 cases of gastric metastatic melanoma was performed (Table II) (15-36). The sex of one patient was not reported, and of the remaining patients, 14 (66.7%) were male and 7 (33.3%) were female. The mean age at diagnosis was 63.1 years (range, 36-89 years). Excluding five patients with unclear metastatic site in the stomach, the majority of the gastric metastatic melanomas occurred in
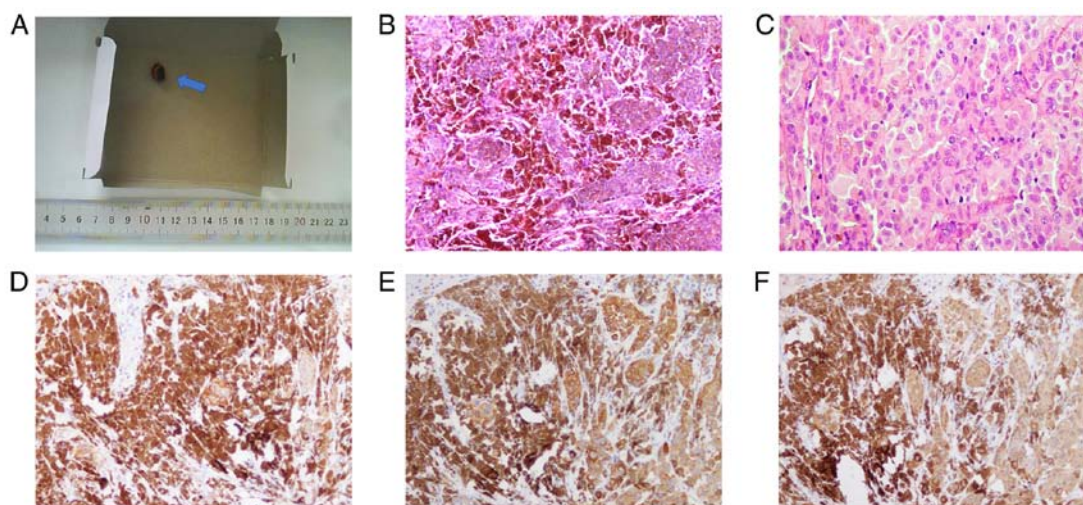
Figure 1. Pathological evaluation of MM of the external ear in the present case. (A) Resected specimen of the left auricle. Hematoxylin and eosin staining of the resected auricular MM specimen at (B) x200 and (C) x400 magnification. Immunohistochemical staining showed that tumor cells in the MM were positive for (D) human melanoma black 45, (E) melan-A and (F) S-100; magnification, x400. MM, malignant melanoma.
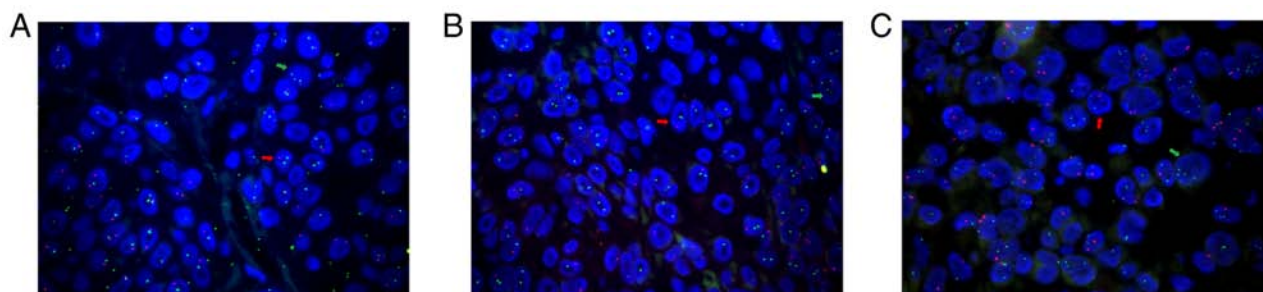

Figure 2. Copy number alterations of multiple genes in the auricular MM detected by fluorescence *in situ* hybridization. The MM was found to be (A) positive for 6q23 (MYB) deletion (44%) and negative for 6p25 (RREB1) amplification (4%), (B) positive for 9p21 (P16) deletion (80%) and negative for 11q23 (CCND1) amplification (2%) and (C) positive for 8q24 (MYC) amplification (average 2.8 signals) and 10q23 (PTEN) deletion (70%) (x1,000 magnification). The specific validated cutoff values for the probes used in the assay are as follows: 1 red signal (red arrow) in ≥40% of nuclei is considered an abnormal loss of MYB; >2 green signals (green arrow) in ≥29.0% of nuclei is considered an abnormal gain of RREB1; ≤1 red signal (red arrow) in ≥20% of nuclei is considered an abnormal loss of P16; >2 green signals (green arrow) in ≥38.0% of nuclei is considered an abnormal gain of CCND1; average >2.5 green signals (green arrow) in nuclei is considered an abnormal gain of MYC; 1 red signal (red arrow) in ≥20% of nuclei is considered an abnormal loss of PTEN. MM, malignant melanoma; RREB1, Ras responsive element binding protein 1; CCND1, cyclin D1.
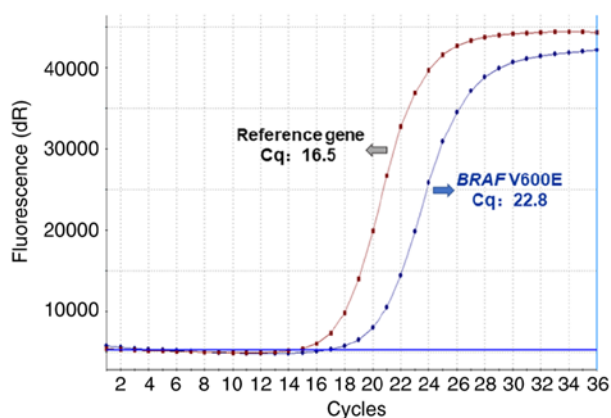

Figure 3. Amplification curve of BRAF V600E mutation in the auriclar MM detected by ARMS-PCR. Amplification of the reference gene (internal control) and BRAF V600E mutation were detected by ARMS-PCR using HEX and FAM fluorescent channels, respectively. The Cq values for the reference gene and BRAF V600E were 16.5 and 22.8, respectively, suggesting that the present case of MM was positive for the BRAF V600E mutation. MM, malignant melanoma; ARMS-PCR, amplification-refractory mutation system polymerase chain reaction.

the gastric body (41.2%) and fundus (29.4%), and the rest occurred in the gastric antrum, cardia and pylorus. The whole stomach was involved in one case. On the basis of the articles containing clear reports of 19 patients' symptoms, it was found that the main gastrointestinal symptoms were black stools or hematemesis (52.6%), abdominal pain (36.8%) and nausea or emesis (26.3%). Less common symptoms included anorexia and abdominal distention. Endoscopic or intraoperative findings were mostly masses, nodules, protruding lumps or polyps (77.3%), ulcerations (45.5%) and melanin pigmentation (31.8%). Multiple mucosal erosions were less common manifestations. The primary site was not reported for one patient, and the skin was the most common primary site in the remaining patients (16 cases, 72.7%), including eight with acral MM. Two cases of gastric metastatic MM originated in the eye, two in the mucosa and one in the sphenoid sinus. Following the exclusion of six patients with missing time-to-metastasis data, the mean and median time intervals to metastasis were 4.4 and 2 years, respectively, with a range from 0.5 to 15 years. Individual differences in tumor metastasis among the patients are evident.
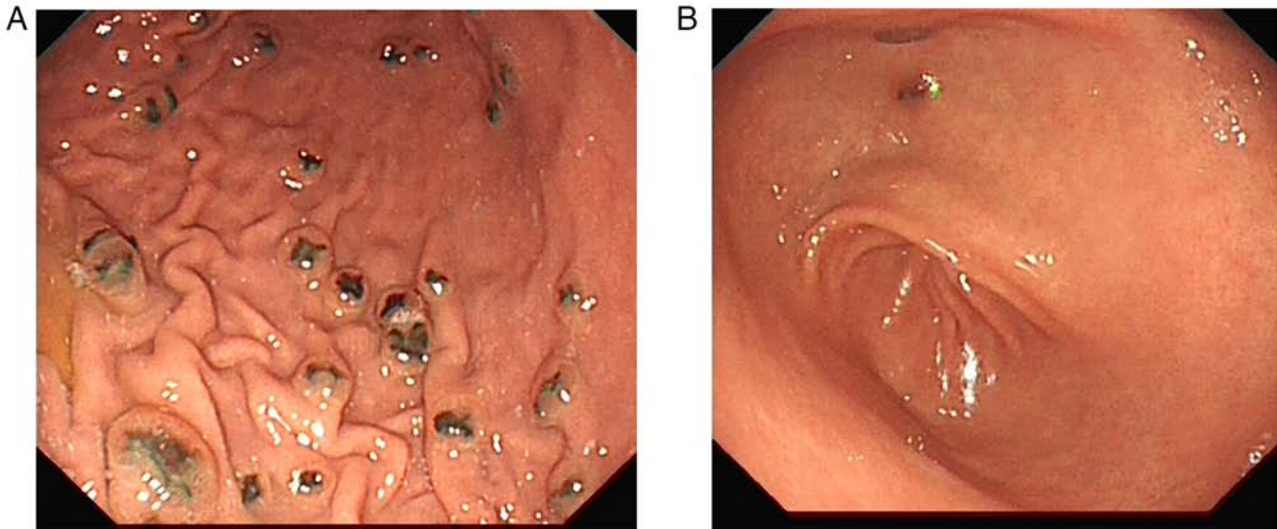
Figure 4. Gastroscopic findings. Black protruding lesions in the (A) gastric body and (B) antrum.

Discussion

Primary MM of the external ear is a rare type of melanoma, accounting for ~1% of cutaneous MM and 7% of MM of the head and neck (2). In the present review it was identified that tumor lesions mainly occurred in the helix and anthelix, followed by the earlobe (*lobules auriculae*) and auricle (*concha auriculae*). The external auditory canal was a less common site. The most common subtype of external ear MM was superficial spreading melanoma (40.1%), followed by malignant freckle-like nevus (33.7%) and nodular melanoma (16.4%) (8,37). MM of the external ear is primarily observed in the white population (99.3%), with a much lower incidence (<0.5%) in the non-white population (37). Melanin pigmentation is well known for protecting the skin from the harmful effects of UV radiation. A negative association exists between the degree of skin pigmentation and the incidence of external ear MM (37). The average incidence rates of MM are 1.015 cases per 100,000 men and 0.126 cases per 100,000 women (37). The patient described in the present case report is an Asian woman, and MM is extremely rare in individuals with these demographic characteristics.

Patel *et al* (37) reported that the 5- and 10-year disease-specific survival rates for MM of the external ear are 89.4 and 84.8%, respectively, and another study suggested that the 5-year overall and cancer-specific survival rates for MM of the outer ear are 78.8 and 90.0%, respectively (3). Patients with a malignant freckle-like nevus have been shown to have favorable survival outcomes, while those with nodular melanoma generally have generally poor outcomes (37). It has been reported that the time of cancer-specific death can be independently predicted by age, Breslow thickness, stage, ulceration and lymphatic and distant metastasis (3). Certain studies have implied that MM of the external ear may be more aggressive than other head and neck MMs (7,38), while others contend that anatomical location does not affect tumor recurrence rates and survival outcomes (3,37,39).

MM is a highly aggressive cancer that metastasizes relatively early, particularly to the lymph nodes, brain, liver and lungs. The preauricular and postauricular lymph nodes, anterior and posterior regions of the neck and parotid gland are common sites of local metastasis for melanoma of the external ear. Consistent with the systemic review of external ear MM published by Toia *et al* (8), the literature review in the present study observed that regional lymph nodes were the most frequent sites of metastasis, and distant metastatic sites were primarily liver, lung, brain and other head and neck tissues. However, gastrointestinal metastases of MM are uncommon (4,40,41). As reported by López *et al* (42), gastrointestinal metastases of MM involve the small intestine (51-71%), stomach (27%) and colon (22%). In the present case and literature review, it was found that gastric metastatic MM typically occurs in the gastric body and fundus, with various manifestations observed in endoscopy or surgery, including masses, nodules, bulges, ulcerations, polyps, mucosal pigmentation and erosions. A review of studies on small intestinal melanomas suggested that 60% of deceased patients with MM had gastrointestinal metastases, but only 1.5-4.0% were detected before death (43). The present retrospective literature study revealed that a total of 22 cases of gastric MM were reported between 1960 and 2022, including only one patient with a primary lesion in the ear, which was a 46-year-old white male. To the best of our knowledge, this is the first case report of gastric metastasis from auricular MM in a non-white female.

Prophylactic removal of nevus of the external ear is advocated in the literature to prevent the development of MM (44). Approximately 8% of cases of MM in the ear are non-pigmented, which can easily lead to misdiagnosis. As these tumors are insensitive to radiotherapy, wide local excision should be performed as soon as possible if malignancy is suspected. Surgery, with or without lymph node dissection, remains the preferred treatment strategy for MM (8). Numerous adjuvant therapies have been investigated for the treatment of cutaneous MM following complete surgical excision. The US Food and Drug Administration (FDA) has approved ipilimumab, nivolumab and pembrolizumab for the adjuvant treatment of MM at stage III and above (45). Immune
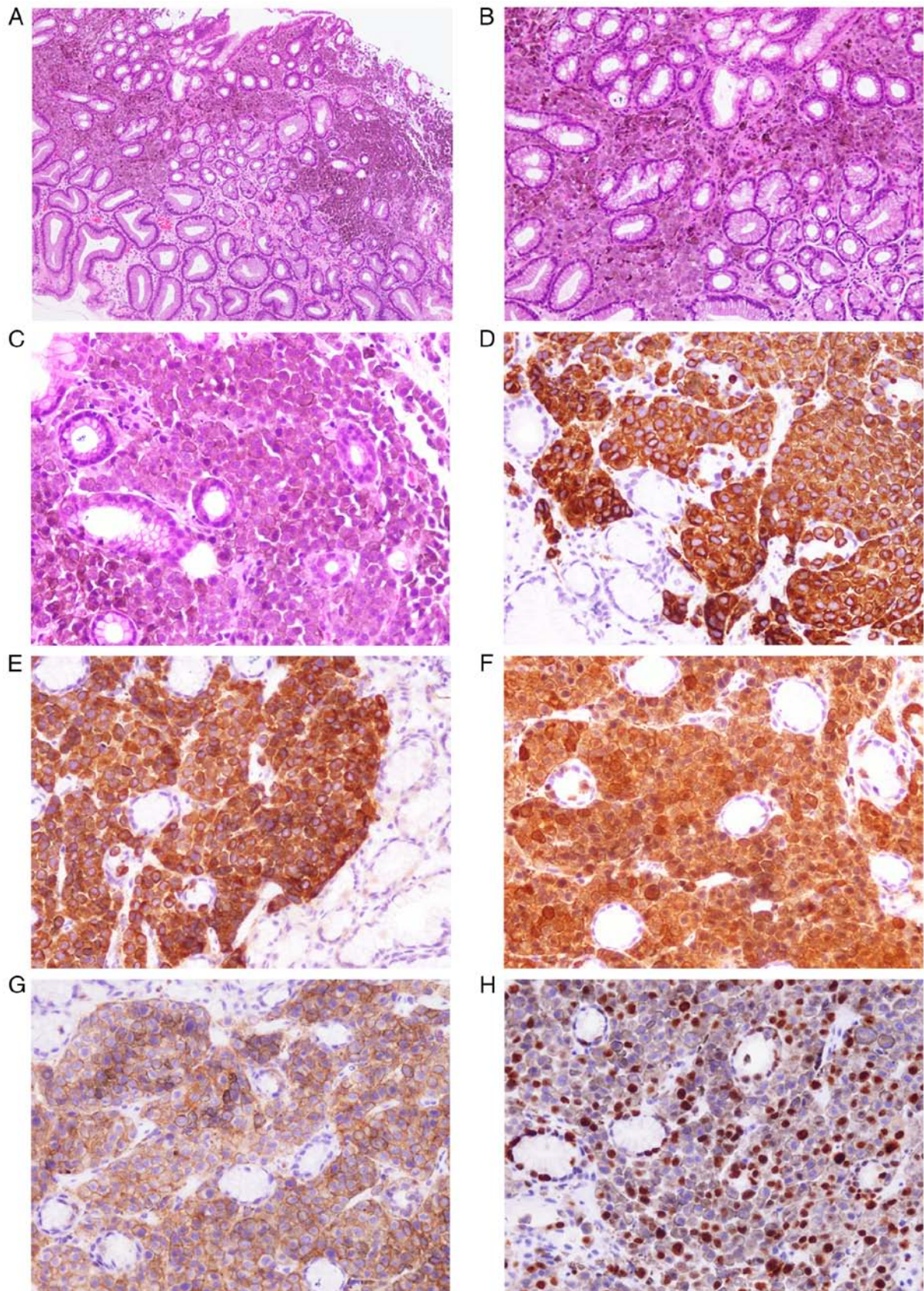
Figure 5. Pathological evaluation of the gastric metastatic malignant melanoma. Hematoxylin and eosin staining of the biopsy specimen of the gastric mucosa at (A) x100, (B) x200 and (C) x400 magnification. Immunohistochemical staining showed tumor cells positive for (D) human melanoma black 45, (E) melan-A, (F) S-100, (G) CD56 and (H) Ki-67; magnification, x400.

checkpoint inhibitors represent a promising new type of systemic therapy for the adjuvant treatment of MM. Regarding

the surgical treatment of MM from the external ear, the safe margin of resection remains unclear. A minimum resection
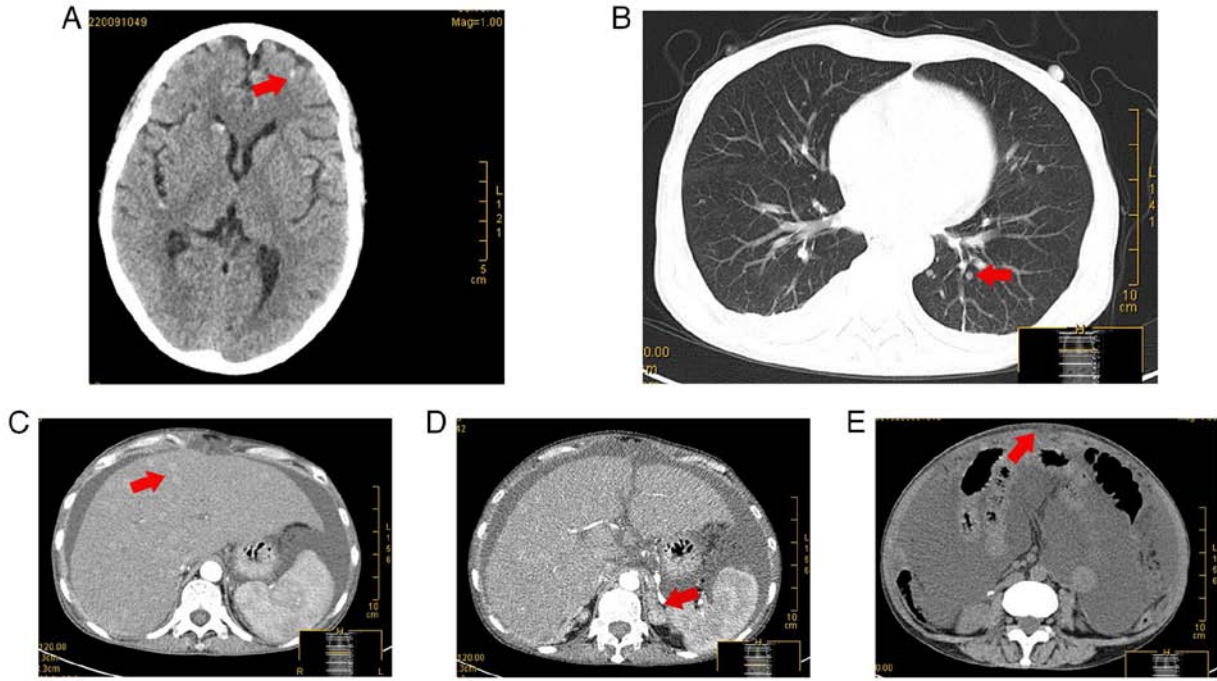
Figure 6. Computed tomography scans of the patient. Metastatic lesions in the (A) brain, (B) lungs, (C) liver, (D) left adrenal gland and (E) peritoneum were detected.
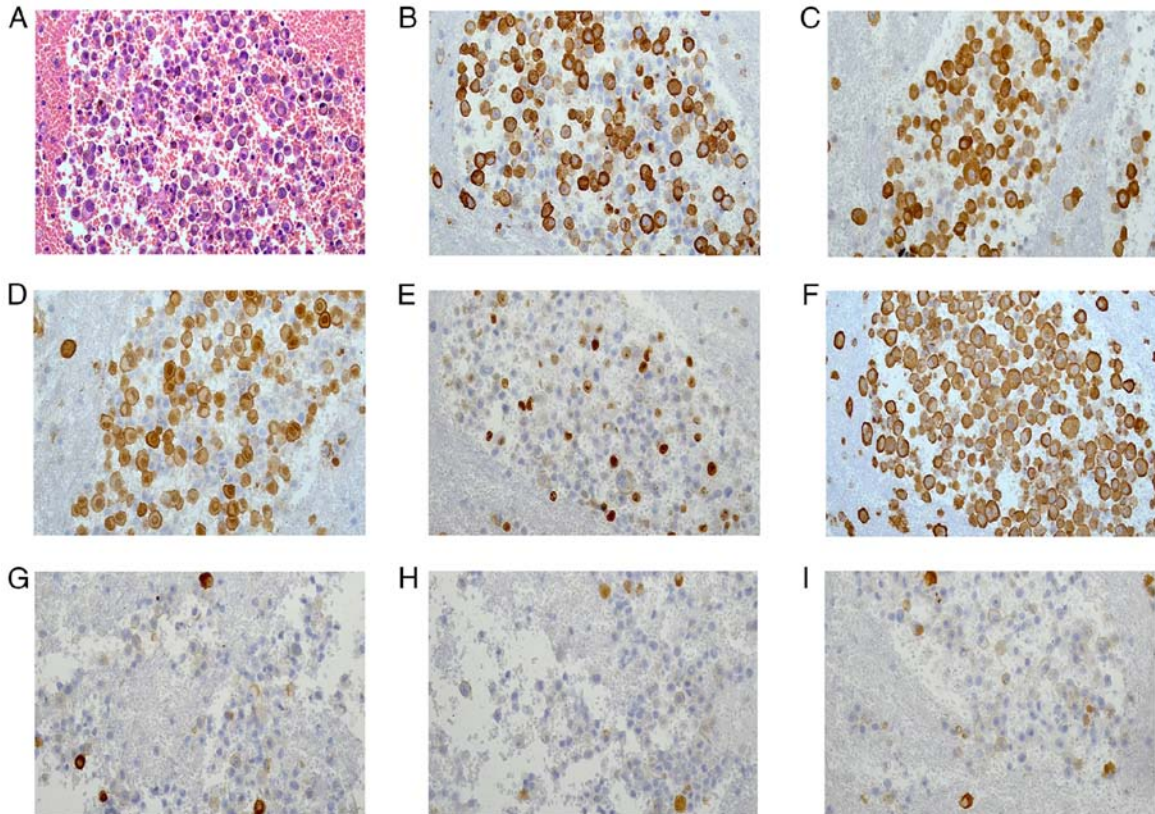

Figure 7. Pathological evaluation of the metastatic malignant melanoma cells in the seroperitoneum. (A) Hematoxylin and eosin staining of a paraffin-embedded cell block of the seroperitoneum (x400 magnification). Immunohistochemical staining showed tumor cells positive for (B) human melanoma black 45, (C) melan-A, (D) S-100, (E) Ki-67 and (F) vimentin and peritoneal mesothelial cells positive for (G) pan cytokeratin, (H) calretinin and (I) mesothelial cell (x400 magnification).

margin of 1 cm is commonly reported; however, some authors advocate a resection margin of 2 cm if the tumor thickness is >2.0 mm. There is a trend towards surgical approaches with reduced resection margins, with the advised minimum extent

Table I. Cases of malignant melanoma of the external ear reported in the literature (n=6,577).

First author/s, year	Cases, n	Age, years	Sex	Primary site	Clinical symptoms	Metastatic sites	Outcomes	(Refs.)
Amando García et al, 2003	1	59	Male	External ear canal	Earache, suppurative otorrhea, preauricular swelling	Parotid gland	Disease-free survival for 9 months	(5)
Hannan and Parikh, 2006	1	40	Male	External ear canal	Deafness	Retromandibular tissue	N/A	(6)
Gowthami et al, 2014	1	11	Female	Right post-auricular	Pain, swelling, intermittent bleeding	Right temporal bone	Died	(7)
Toia et al, 2015	845	59.4 (mean)	Male (78%), female (22%)	Helix (57%); earlobe (17%); post-auricular (9%); auricle (6%); antihelix and scapha (4%); external ear canal (0.8%); other (6.2%)	N/A	Regional lymph nodes (10%)	Five-year survival rate: stage 0, 100%; stage I, 71%; stage II, 53%; stage III, 0%	(8)
Frost et al, 2017	45	63 (median)	Male (80%), female (20%)	External ear	N/A	Sentinel lymph nodes (7%); distant metastasis (4.4%)	Survival rate: Overall, 80%; stage I/II, 84%; stage III/ IV, 56%	(9)
Deep et al, 2017	5,481	66.7 (mean)	Male (86.5%) female (13.5%)	External ear	N/A	N/A	Survival rate: Overall, 90%; stage I, 95.3%; stage II, 81.1%; stage III, 56.6%; stage IV, 20.5%	(3)
Harrison et al, 2017	41	61 (median)	Male (73%), female (27%)	External ear	N/A	Liver and brain (5%)	Survival rate: Overall, 90%; 5-year, 100%; 10-year, 71%	(10)
Straccia, et al 2020	1	43	Female	Right auricle	N/A	Pleura	N/A	(11)
Truong et al, 2020	156	62.5 (median)	Male (86%), female (14%)	Helix (61.5%); earlobe, (13.5%); other (25%)	N/A	Sentinel lymph nodes (14.1%)	Survival rate, 74.4%	(12)
Fiorio et al, 2021	4	69 (mean), 67.5 (median)	Male (25%), female (75%)	Right earlobe (50%); left helix (50%)	Asymptomatic	Regional lymph nodes (25%)	During the follow-up, with no signs of disease progression	(13)
Pająk et al, 2022	1	43	Female	Left helix	Asymptomatic	N/A	Lost to follow-up	(14)

N/A, no relevant information available.

being >0.5 cm (3,46). The resection margin in the present case was much smaller than the minimum resection standard advocated in the aforementioned studies, which may a primary reason for the postoperative metastasis of the tumor.

Table II. Cases of gastric metastatic malignant melanoma reported in the literature (n=22).

First author/s, year	Sex	Age, years	Metastatic site	Clinical symptoms	Endoscopic or intraoperative findings	Primary site	Time interval between primary and metastatic lesions	(Refs.)
Davis and Zollinger, 1960	Female	76	Greater gastric curvature	Anorexia, nausea and black stools	Multiple polypoid lesions	Upper arm	1 year	(15)
Morini *et al*, 1980	Female	77	Posterior wall of the gastric body	Abdominal pain, dyspepsia, lack of appetite, and black stools	Protruding lump	Left hip	6 years	(16)
Mimica and Tomić, 2002	Male	51	Gastric body and antrum	Abdominal pain	Multiple polypoid lesions	N/A	N/A	(17)
Malladi *et al*, 2005	Male	80	Fundus, upper part and pylorus of the stomach	Abdominal pain, dyspepsia and weight loss	Multiple black nodules with ulceration	Sole of the right foot	2 years	(18)
Matsubayashi *et al*, 2005	Male	53	Upper part of the stomach	N/A	Dark brown lesions with clear boundaries	Gums	Metastatic at diagnosis	(19)
Wu *et al*, 2011	Male	72	Stomach	Dysphagia	Superficial ulcers and ulcerative nodules	Esophagus	Metastatic at diagnosis	(20)
Law *et al*, 2011	Female	84	Stomach	Hematemesis	Multiple pigmented nodules and mucosal swelling	Right foot	1 year	(21)
Eivazi-Ziaei and Esmaili, 2014	Male	56	Lesser gastric curvature	Abdominal pain	Ulcers and lumps	Right heel	2 years	(22)
El-Sourani *et al*, 2014	Female	43	Lesser gastric curvature	Black stools and anemia	Lump	Right breast	2 years	(23)
Zhao *et al*, 2015	Male	57	Stomach	Diarrhea	Multiple erosion and lumps	Sphenoid sinus	Half a year	(24)
Ruiz-Cuesta *et al*, 2014	N/A	49	Gastric body	Epigastric pain, nausea, weight loss	Ulcerated lump	Right lower limb	13 years	(25)
Morita *et al*, 2019	Male	55	Whole stomach	N/A	Mucosa covered by tumor cells with pitting and melanin pigmentation	Uvea	14 years	(26)
Farshad *et al*, 2018	Male	89	Gastric cardia	Hematochezia	Bleeding mass and ulcerations	Chest	15 years	(27)
Santos-Seoane *et al*, 2019	Male	79	Gastric fundus	N/A	Polypoid lesion	Left forearm	3 years	(28)
Sachdeva *et al*, 2020	Male	59	Gastric body, fundus and antrum	Black stools and weight loss	Multiple black ulcerated nodules	Multiple skin areas throughout the body	10 months	(29)
Anandabaskaran *et al*, 2020	Male	68	Gastric incisura	Abdominal pain and hematochezia	Pitted ulcerations with melanin pigmentation around the periphery	Right foot	7 years	(30)

Table II. Continued.

First author/s, year	Sex	Age, years	Metastatic site	Clinical symptoms	Endoscopic or intraoperative findings	Primary site	Time interval between primary and metastatic lesions	(Refs.)
Groudan et al, 2020	Female	66	Gastric antrum	Nausea and hematemesis	Ulcerations and fungoid mass	Vulva	Metastatic at diagnosis	(31)
Yoshimoto et al, 2021	Female	82	Gastric body	Black stools	White semicircular ulcerated mass	Left toe	N/A	(32)
Monti et al, 2021	Male	56	Stomach	Severe dyspepsia	Ulcerations and plaques	Glans penis	Metastatic at diagnosis	(33)
Zhu et al, 2022	Male	36	Gastric body and fundus	Anorexia, nausea and emesis	Hemispherical protrusive lesion	Sole of the right foot	6 months	(34)
Maksimaityte et al, 2022	Male	46	Gastric body and fundus	Nausea and abdominal enlargement	Multiple red polyps	Right ear auricle	1 year	(35)
Bharwad et al, 2022	Female	55	Stomach	Abdominal pain, hematochezia, dizziness and fatigue	Multiple small pigmented lesions	Choroid of the right eye	2 years	(36)

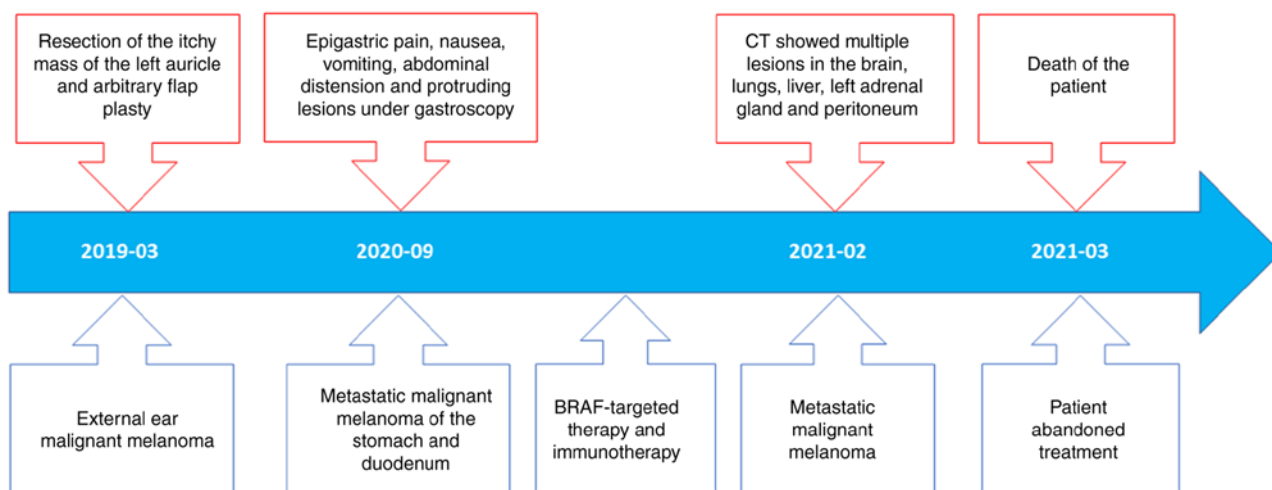N/A, no relevant information available.

Figure 8. Timeline of the history of the present case. CT, computed tomography.

Positive results for specific immunohistochemical markers, including HMB45, melan-A, S-100, SRY-box transcription factor 10, melanoma-associated antigen (PNL-2), tyrosinase and melanocyte inducing transcription factor, are critical for the diagnosis and differential diagnosis of MM (47). HMB45 is a glycoprotein expressed in melanosomes with extremely high specificity; melan-A, PNL-2 and tyrosinase also have high specificity; while S-100 is more sensitive but less specific for the detection of MM. The appropriate marker for detection and diagnosis can be selected on the basis of the marker characteristics. In the present study, HMB45, melan-A, and S-100 were found to be expressed in the tumor cells in the primary lesion, gastric metastatic lesion and seroperitoneum of the patient.

The initiation and development of MM involve multiple genes. BRAF gene mutation is the most prevalent genetic alteration in 40-60% of cutaneous MM cases, and this mutation results in constitutive activation of the MAPK pathway (48-50). Vemurafenib, dabrafenib and encorafenib are US FDA-approved inhibitors targeting the BRAF V600 mutation, and a combination of BRAF inhibitors and MEK inhibitors, including trametinib and cobimetinib, has become

the standard treatment for advanced MM with BRAF V600 mutation (51,52). Furthermore, atezolizumab plus vemurafenib plus cobimetinib was approved by the FDA for patients with BRAF V600 mutation-positive unresectable or metastatic MM in July 2020 (53). Therefore, molecular testing for BRAF mutations in MM is critical for selecting the most appropriate treatment. Genetic information is rarely mentioned in reported cases of MM of the external ear, and the present case was positive for the BRAF V600E gene mutation. BRAF mutations are associated with a higher tumor stage, lymph node metastasis and an increased depth of tumor infiltration (54). Brain metastases are more likely to occur in patients in which the MM has BRAF mutations than in those with wild-type BRAF (55). Consistent with this, 11 months following the gastric metastasis of external ear MM, the involvement of multiple organs, including the brain, had occurred in the present case.

Numerous molecular genetic studies have demonstrated that although BRAF mutations have been detected in both MM and melanocytic nevi, the genetic alterations of the two presentations differ substantially. MM frequently shows a large number and variety of chromosomal abnormalities, including 6q23 (MYB), 6q25 (RREB1), 9p21 (p16), 11q23 (CCND1), 8q24 (MYC) and 10q23 (PTEN), which can be amplified, deleted or translocated (56). By contrast, these genetic loci are rarely changed in melanocytic nevi. Multiple molecular testing by FISH confirmed the amplification of the MYC gene and deletion of the MYB, p16 and PTEN genes in the present case. Additionally, RREB1 and CCND1 copy numbers were not markedly changed.

The proto-oncogene c-MYC is strongly associated with various types of cancer. It has been demonstrated that the copy number of c-MYC is increased in the tumor region of nodular MM (57). In a study of 43 cases of uveal melanoma, extra copies of c-MYC were detected in 70% of cases (58). Of the 30 patients with extra copies of c-MYC, 13 (43%) had amplification, 14 (47%) had an intermediate relative increase in copy number, and 3 (10%) had a simple gain of chromosome 8 (58). In a study of 44 cases of primary acral melanoma, c-MYC was found to be amplified in 54.5% of cases (59). In addition, c-MYC gene expression may be positively associated with the metastatic potential of MM (57). An association has also been identified between larger tumor size and c-MYC amplification (58).

The MYB gene is localized in chromosome 6q22-q23. Deletions and translocations involving this region of the long arm of chromosome 6 occur frequently in MM, and rearrangements of the MYB gene have been reported in MM (60). Romano *et al* (61) observed deletion of the MYB gene in five of seven cases (71%) of melanoma of the nail apparatus, with the percentage of abnormal cells ranging from 52 to 94%. Two of the patients in the study had lymph node metastasis and died from widespread metastatic disease, and these two patients had amplification of RREB1, CCND1 and MYC as well as MYB deletion (61).

As a tumor suppressor, p16 has been shown to play a crucial role in tumor progression rather than initiation. In healthy cells, p16 blocks the phosphorylation of retinoblastoma protein by binding to CDK4/6, leading to cell cycle arrest. Dysfunction of the p16 protein diminishes its ability

to regulate cell proliferation, resulting in uncontrolled cell division and the growth and development of tumors (62). p16 alterations frequently occur in sporadic melanomas (62). In a study of vertical growth phase melanoma, absent or minimal nuclear p16 protein expression was found in 45% of primary melanomas, and further loss of p16 expression was observed in 77% of metastatic lesions; the absence of p16 expression independently predicted poor survival in the patients (62).

PTEN is a well-known tumor suppressor that is frequently mutated, inactivated or deleted in various malignancies. The MEK/ERK pathway contributes to the inhibition of PTEN by activating c-Jun, which activates the PI3K/AKT pathway, causing apoptosis resistance, tumor growth and metastasis in melanoma cells (63,64). Loss of PTEN function has been reported in 35% of melanomas and is often detected in advanced melanomas along with BRAF mutations (65). Dankort *et al* (66) induced the expression of BRAF V600E specifically in the melanocytes of mice; the mice developed benign melanocytic hyperplasias that did not progress to melanoma within 15-20 months. By contrast, when the expression of BRAF V600E was combined with PTEN gene silencing, melanoma with 100% penetrance, a short latency period and metastases to the lymph nodes and lungs developed, suggesting that the BRAF V600E mutation combined with PTEN deletion induces MM metastasis (66). It has been reported that PTEN deletion is a possible predicting factor of intrinsic resistance to BRAF inhibitor therapy (67). Notably, BRAF inhibitor treatment was ineffective in the present patient. In addition, PTEN deletion has been described as a driver of immune evasion, with PTEN deletion in MM being associated with the increased expression of programmed death-ligand 1 and immunosuppressive cytokines, decreased T-cell infiltration, and resistance to T-cell-mediated tumor cell lysis (64). PTEN deletion has also been suggested to mediate immune evasion by the attenuation of tumor antigen cross-presentation, resulting in T-cell exclusion and indirectly influencing B-cell function (67).

In conclusion, the present report describes a rare case of MM in an Asian female with postoperative metastasis to the stomach and descending duodenum from the auricle and secondary metastases to multiple organs, including the brain, lungs, liver, left adrenal gland and peritoneum. Molecular testing revealed genetic aberrations in BRAF, MYB, p16, MYC and PTEN. The small resection margin, nodular histological type and number of genetic aberrations may be strongly associated with high-metastatic potential, resistance to targeted therapy and poor prognosis in this case.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YCY detected the gene mutation, collected the pathological images, drafted the manuscript and analyzed the data for the literature review. YL and YP contributed to the pathological diagnosis and clinical data collection. BG contributed to the analysis and interpretation of the case report and revised the manuscript. YCY, YL, YP and BG confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the First Affiliated Hospital of Dali University (approval no. 20221202001). Informed consent was obtained from the patient's family.

Patient consent for publication

Consent for publication was obtained from the patient's family.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA Cancer J Clin 63: 11-30, 2013.
2. Shashanka R and Smitha BR: Head and neck melanoma. ISRN Surg 2012: 948302, 2012.
3. Deep NL, Glasgow AE, Habermann EB, Kasperbauer JL and Carlson ML: Melanoma of the external ear: A population-based study. Am J Otolaryngol 38: 309-315, 2017.
4. Hao M, Zhao G, Du X, Yang Y and Yang J: Clinical characteristics and prognostic indicators for metastatic melanoma: Data from 446 patients in north China. Tumour Biol 37: 10339-10348, 2016.
5. Amando García L, Suárez Nieto C, Madrigal Rubiales B and García García J: External ear melanoma. Acta Otorrinolaringol Esp 54: 89-93, 2003 (In Spanish).
6. Hannan SA and Parikh A: Malignant melanoma of the external ear canal. Lancet 368: 1680, 2006.
7. Gowthami C, Kumar P, Ravikumar A, Joseph LD and Rajendiran S: Malignant melanoma of the external auditory canal. J Clin Diagn Res 8: FD04-FD06, 2014.
8. Toia F, Garbo G, Tripoli M, Rinaldi G, Moschella F and Cordova A: A systematic review on external ear melanoma. J Plast Reconstr Aesthet Surg 68: 883-894, 2015.
9. Frost J, Dunne JA and Powell BW: External ear melanoma: A 10 year assessment of management and outcomes. J Plast Reconstr Aesthet Surg 70: 551-552, 2017.
10. Harrison C, Mikhail M, Potter M, Gore S and Cassell O: Sentinel lymph node biopsy for external ear melanoma: A 17 year experience with long term survival data. J Plast Reconstr Aesthet Surg 70: 1301-1303, 2017.
11. Straccia P, Martini M and Pierconti F: Pleural metastasis from auricular melanoma: A brief report. Diagn Cytopathol 48: 376-379, 2020.
12. Truong A, Hyngstrom JR, Andtbacka RHI, Noyes RD, Wright M, Snyder J, Winters A, Sause WT, Grossmann KF, Khong HT, et al: Recurrence risk of early-stage melanoma of the external ear: An investigation of surgical approach and sentinel lymph node status. Melanoma Res 30: 173-178, 2020.
13. Fiorio LM, Diniz LM, Spelta K and Badaró BA: Ear melanoma: A four-case series. An Bras Dermatol 96: 64-67, 2021.
14. Pająk J, Lelonek E, Chlebicka I and Szepietowski JC: Nodular melanoma of the external ear: A rare tumour successfully treated with simple excision. Postepy Dermatol Alergol 39: 635-636, 2022.
15. Davis GH and Zollinger RW: Metastatic melanoma of the stomach. Am J Surg 99: 94-96, 1960.
16. Morini S, Bassi O and Colavolpe V: Malignant melanoma metastatic to the stomach: Endoscopic diagnosis and findings. Endoscopy 12: 86-89, 1980.
17. Mimica M and Tomić I: Endoscopic diagnosis of malignant melanoma metastatic to the stomach. Am J Gastroenterol 97: 1572-1573, 2002.
18. Malladi V, Palanivelu C, Mathew S, Rajan PS, Jani K, Senthilkumar R, Senthilkumaran S and Kavalkat AJ: Malignant melanoma metastatic to the stomach and duodenum. Indian J Gastroenterol 24: 133, 2005.
19. Matsubayashi H, Takizawa K, Nishide N and Ono H: Metastatic malignant melanoma of the gastric mucosa. Intern Med 49: 1243-1244, 2010.
20. Wu PR, Yen HH and Chen CJ: Gastrointestinal: Primary esophageal melanoma with gastric metastasis. J Gastroenterol Hepatol 26: 1338, 2011.
21. Law ST, Li MK and Lo WH: Gastric melanoma. Hong Kong Med J 17: 503-504, 2011.
22. Eivazi-Ziaei J and Esmaili H: Metastatic malignant melanoma affecting stomach. J Cancer Res Ther 10: 733-736, 2014.
23. El-Sourani N, Troja A, Raab HR and Antolovic D: Gastric metastasis of malignant melanoma: Report of a case and review of available literature. Viszeralmedizin 30: 273-275, 2014.
24. Zhao L, Yan J, Li L, Wei J, Li L, Qian X, Liu B and Zou Z: Gastric metastasis from sphenoid sinus melanoma: A case report. Oncol Lett 9: 609-613, 2015.
25. Ruiz-Cuesta P, Hervás-Molina AJ, Villar-Pastor CM, Jurado-García J and Barrera-Baena P: Late gastric metastasis from cutaneous melanoma. Gastroenterol Hepatol 37: 564-565, 2014 (In Spanish).
26. Morita S, Suda T and Terai S: Gastric metastasis from uveal melanoma. Clin Gastroenterol Hepatol 7: A20, 2019.
27. Farshad S, Keeney S, Halalau A and Ghaith G: A case of gastric metastatic melanoma 15 years after the initial diagnosis of cutaneous melanoma. Case Rep Gastrointest Med 2018: 7684964, 2018.
28. Santos-Seoane SM, Pérez-Casado L, Helguera-Amezua C and Diez-Fernández M: Metastatic melanoma of the stomach. Cir Esp (Engl Ed) 97: 51, 2019 (In English, Spanish).
29. Sachdeva S, Dalal A, Kumar A and George R: Multifocal gastric metastasis of malignant melanoma: An ominous endoscopic appearance: Gastric metastasis of malignant melanoma. Dig Liver Dis 52: 1512, 2020.
30. Anandabaskaran S, Fewtrell M, Ismail K and Prakoso E: A case of delayed metastatic melanoma to the stomach. Intern Med J 50: 1590-1591, 2020.
31. Groudan K, Ma W and Joshi K: Metastatic melanoma presenting as a gastric mass. Cureus 12: e11874, 2020.
32. Yoshimoto T, Okamoto T and Fukuda K: Giant gastric metastasis of malignant melanoma. Oxf Med Case Reports 2021: omab050, 2021.
33. Monti M, Guidoboni M, Oboldi D, Bartolini G, Pieri F, Ruscelli S, Passardi A, Ridolfi L, De Rosa F, Sullo FG and Frassineti GL: Melanoma metastasis mimicking gastric cancer: A challenge that starts from diagnosis. Therap Adv Gastroenterol 14: 1756284821989559, 2021.
34. Zhu M, Zhang DY, Zhang GJ, Wang ZB and Lid MY: Amelanotic metastatic gastric malignant melanoma: A case report. Anticancer Drugs 33: e808-e812, 2022.
35. Maksimaityte V, Reivytyte R, Milaknyte G, Mickys U, Razanskiene G, Stundys D, Kazenaite E, Valantinas J and Stundiene I: Metastatic multifocal melanoma of multiple organ systems: A case report. World J Clin Cases 10: 10136-10145, 2022.
36. Bharwad A, Shah H and Salyers WJ Jr: Malignant melanoma of the stomach. Eur J Case Rep Intern Med 9: 003640, 2022.
37. Patel TD, Chin OY, Baredes S, Eloy JA and Ying YM: A population based analysis of melanoma of the external ear. Otol Neurotol 39: e137-e142, 2018.

38. Oliver JD, Boczar D, Sisti A, Huayllani MT, Restrepo DJ, Spaulding AC, Gabriel E, Bagaria S, Rinker BD and Forte AJ: National Analysis of Patients With External Ear Melanoma in the United States. J Craniofac Surg 30: e787-e790, 2019.
39. Augenstein AC, Capello ZJ, Little JA, McMasters KM and Bumpous JM: The importance of ulceration of cutaneous melanoma of the head and neck: A comparison of ear (pinna) and nonear sites. Laryngoscope 122: 2468-2472, 2012.
40. Kang S, Barnhill RL, Graeme-Cook F, Randolph G, Nadol JB and Sober AJ: Primary malignant melanoma of the external auditory canal: A case report with presentation as an aural polyp. Am J Otol 13: 194-196, 1992.
41. Hoshikawa H, Miyashita T and Mori N: Surgical procedures for external auditory canal carcinoma and the preservation of postoperative hearing. Case Rep Surg 2012: 841372, 2012.
42. López RG, Santomé PM, Porto EI, Moreiras MI, Gómez CG, Villanueva JM, Canosa MA, Veiga OR, Gutiérrez AE, Ares IM and Val FA: Intestinal perforation due to cutaneous malignant melanoma mestastatic implants. Rev Esp Enferm Dig 103: 386-388, 2011 (In English, Spanish).
43. Lens M, Bataille V and Krivokapic Z: Melanoma of the small intestine. Lancet Oncol 10: 516-521, 2009.
44. Benmeir P, Baruchin A, Weinberg A, Nahlieli O, Neuman A and Wexler MR: Rare sites of melanoma: Melanoma of the external ear. J Craniomaxillofac Surg 23: 50-53, 1995.
45. Appelbaum EN, Gross ND, Diab A, Bishop AJ, Nader ME and Gidley PW: Melanoma of the external auditory canal: A review of seven cases at a tertiary care referral center. Laryngoscope 131: 165-172, 2021.
46. Jahn V, Breuninger H, Garbe C and Moehrle M: Melanoma of the ear: Prognostic factors and surgical strategies. Br J Dermatol 154: 310-318, 2006.
47. Ohsie SJ, Sarantopoulos GP, Cochran AJ and Binder SW: Immunohistochemical characteristics of melanoma. J Cutan Pathol 35: 433-444, 2008.
48. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, *et al*: Mutations of the BRAF gene in human cancer. Nature 417: 949-954, 2002.
49. Melis C, Rogiers A, Bechter O and van den Oord JJ: Molecular genetic and immunotherapeutic targets in metastatic melanoma. Virchows Arch 471: 281-293, 2017.
50. Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, Hughes TM, Thompson JF, Scolyer RA and Kefford RF: Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. J Clin Oncol 29: 1239-1246, 2011.
51. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH Jr, Kaempgen E, *et al*: Dabrafenib in BRAF-mutated metastatic melanoma: A multicentre, open-label, phase 3 randomised controlled trial. Lancet 380: 358-365, 2012.
52. Sarkisian S and Davar D: MEK inhibitors for the treatment of NRAS mutant melanoma. Drug Des Devel Ther 12: 2553-2565, 2018.
53. Robert C, Lewis KD, Gutzmer R, Stroyakovskiy D, Gogas H, Protsenko S, Pereira RP, Eigentler T, Rutkowski P, Demidov L, *et al*: Biomarkers of treatment benefit with atezolizumab plus vemurafenib plus cobimetinib in BRAF^{V600} mutation-positive melanoma. Ann Oncol 33: 544-555, 2022.

54. Abd Elmageed ZY, Moore RF, Tsumagari K, Lee MM, Sholl AB, Friedlander P, AI-Qurayshi Z, Hassan M, Wang AR, Boulares HA and Kandil E: Prognostic role of BRAF^{V600E} cellular localization in melanoma. J Am Coll Surg 226: 526-537, 2018.
55. Cheng L, Lopez-Beltran A, Massari F, MacLennan GT and Montironi R: Molecular testing for BRAF mutations to inform melanoma treatment decisions: A move toward precision medicine. Mod Pathol 31: 24-38, 2018.
56. Bandarchi B, Jabbari CA, Vedadi A and Navab R: Molecular biology of normal melanocytes and melanoma cells. J Clin Pathol 66: 644-648, 2013.
57. Greulich KM, Utikal J, Peter RU and Krähn G: c-MYC and nodular malignant melanoma. A case report. Cancer 89: 97-103, 2000.
58. Parrella P, Caballero OL, Sidransky D and Merbs SL: Detection of c-myc amplification in uveal melanoma by fluorescent in situ hybridization. Invest Ophthalmol Vis Sci 42: 1679-1684, 2001.
59. Su J, Yu W, Liu J, Zheng J, Huang S, Wang Y, Qi S, Ma X, Chen J and Zhang Y: Fluorescence in situ hybridisation as an ancillary tool in the diagnosis of acral melanoma: A review of 44 cases. Pathology 49: 740-749, 2017.
60. Meese E, Meltzer PS, Witkowski CM and Trent JM: Molecular mapping of the oncogene MYB and rearrangements in malignant melanoma. Genes Chromosomes Cancer 1: 88-94, 1989.
61. Romano RC, Shon W and Sukov WR: Malignant melanoma of the nail apparatus: A fluorescence in situ hybridization analysis of 7 cases. Int J Surg Pathol 24: 512-518, 2016.
62. Straume O, Sviland L and Akslen LA: Loss of nuclear p16 protein expression correlates with increased tumor cell proliferation (Ki-67) and poor prognosis in patients with vertical growth phase melanoma. Clin Cancer Res 6: 1845-1853, 2000.
63. Ince FA, Shariev A and Dixon K: PTEN as a target in melanoma. J Clin Pathol jclinpath-2021-208008: 9 May, 2022 (Epub ahead of print).
64. Giles KM, Rosenbaum BE, Berger M, Izsak A, Li Y, Illa Bochaca I, Vega-Saenz de Miera E, Wang J, Darvishian F, Zhong H and Osman I: Revisiting the clinical and biologic relevance of partial PTEN loss in melanoma. J Invest Dermatol 139: 430-438, 2019.
65. Aguissa-Touré AH and Li G: Genetic alterations of PTEN in human melanoma. Cell Mol Life Sci 69: 1475-1491, 2012.
66. Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky WE Jr, You MJ, DePinho RA, McMahon M and Bosenberg M: Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet 41: 544-552, 2009.
67. Cabrita R, Mitra S, Sanna A, Ekedahl H, Lövgren K, Olsson H, Ingvar C, Isaksson K, Lauss M, Carneiro A and Jönsson G: The role of PTEN loss in immune escape, melanoma prognosis and therapy response. Cancers (Basel) 12: 742, 2020.