Concurrent *BRAF* and *PTEN* mutations in melanoma of unknown origin presenting as a breast mass

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Abstract

Background: Metastases represent a small percentage of the malignancies affecting the breast, and only 5% of melanomas originate from non-cutaneous sites. Multiple genetic aberrations have been associated with the development of melanocytic lesions, including *BRAF* V600E mutation. Mutations in *PTEN* gene have also been related to the pathogenesis of multiple malignancies.

Purpose/Method: This is the case of a 28-year-old female who presented with a tender, palpable mass in the upper outer quadrant of the right breast. Ultrasound showed a 1-cm solid mass, initially diagnosed as invasive ductal carcinoma on biopsy. During pre-operative workup, a second mass was identified and biopsied. Immunohistochemical stains performed on the second mass biopsy demonstrated that the neoplastic cells were positive for cytokeratin AE1/3, pan-melanoma, tyrosinase, and SOX-10 and negative for CK7, CAM5.2, and GATA-3. Subsequent workup showed widespread metastatic disease involving the liver, lungs, bones, and brain. The brain metastasis tested positive for *BRAF* p.V600E and *PTEN* p.R130Efs*4 mutations. Thorough skin and eye examination did not reveal a primary melanoma.

Conclusion: Only few reports have been published of melanoma presenting as a breast mass. This is an interesting case due to the clinical presentation, diagnostic challenges, and genetic mutations profile.

Keywords

Melanoma, carcinoma, breast, BRAF, PTEN

Introduction

It is estimated that there will be approximately 76,380 new diagnoses and 10,130 deaths due to melanoma in the United States during 2016.[1] Although melanoma of the skin is frequently diagnosed among people in the sixth to seventh decade, 5.9% of the cases occur in patients aged 20–34 years.[1] Metastasis of melanoma is more frequently located in the lungs, liver, central nervous system, and bones.[2]

Metastases to the breast represent less than 1.3% of breast malignancies.[3,4] The most common primary tumors include hematologic malignancies, melanomas, and carcinomas of lungs and ovaries, among others.[3,5,6] Even though melanomas metastasizing to the breast have been widely described, they typically present after a diagnosis of primary cutaneous melanoma. It is rare to diagnose melanoma presenting initially as a breast mass without a known previous lesion.

Multiple genetic alterations are associated with the development of melanoma. *BRAF* is the most commonly mutated gene present in approximately 37%–50% of melanomas.[7,8] The most frequent *BRAF* mutation is a T>A transversion, a substitution at nucleotide 1799 that causes a valine to glutamic acid change in codon 600 at exon 15 (p.V600E). The V600E mutation accounts for 80% of *BRAF* mutations in melanoma.[7,8] Neuroblastoma RAS viral oncogene homologue (*NRAS*) is the second most commonly mutated oncogene in melanoma present in 13%–25% of cases.[7,9] Mutations

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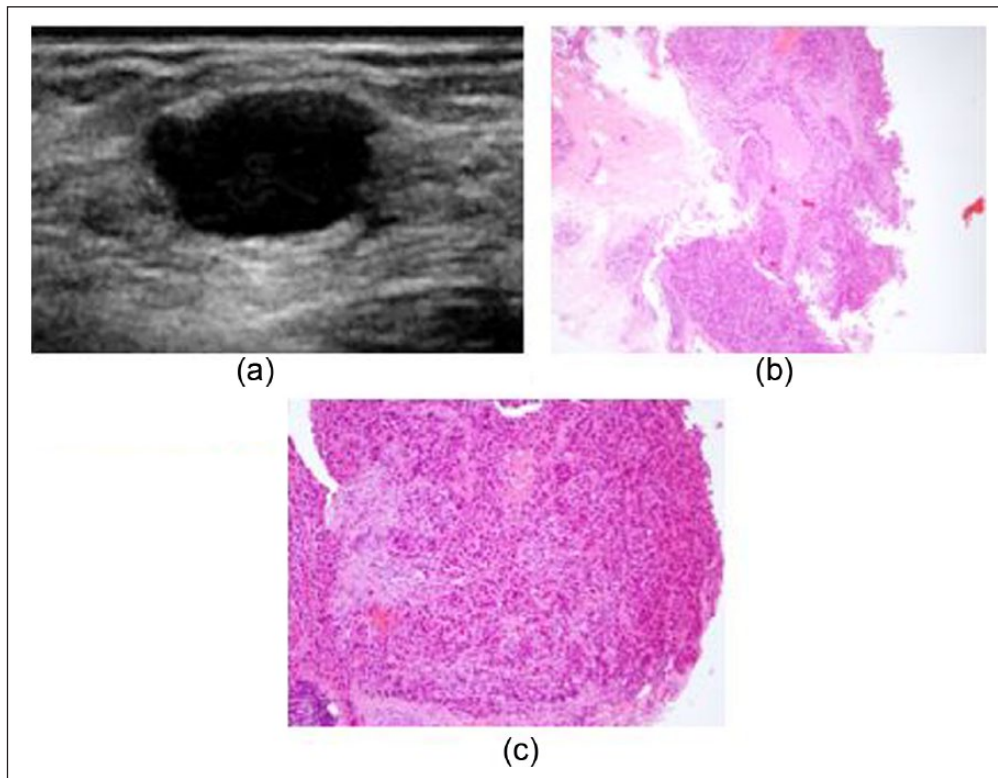Email: Laila.khazai@moffitt.org

Figure 1. (a) Ultrasound demonstrated a solid mass at the site of palpable concern in the right breast upper outer quadrant. Core biopsy showed sheets of (b) high-grade neoplastic cells, with (c) focal comedonecrosis.

in these two genes are considered drivers of melanoma development since they can be found with similar frequencies in benign melanocytic lesions.[10] In addition, mutations in the telomerase reverse transcriptase (*TERT*) gene promoter have been identified in primary and metastatic melanoma with a frequency of 33% and 85%, respectively.[11] Other genetic changes, including tumor suppressor gene alterations, can be found with lesser frequency.

Herein, we report the case of a female patient presenting with breast masses diagnosed as melanoma, with no evidence of cutaneous or ocular lesions. In addition, this neoplasm proved to be positive for concomitant *BRAF* and *PTEN* mutations.

Case report

A 28-year-old Caucasian female presented to our institution with a 1-month history of a mildly tender, palpable lump in the upper outer quadrant of the right breast. The patient did not have any personal or family history of breast carcinoma. Her past medical history was only significant for prior biopsies of benign skin nevi. Targeted ultrasound of the area of palpable concern in the right breast upper outer quadrant demonstrated a corresponding 1-cm solid mass (Figure 1(a)). A core needle biopsy was performed. Histologically, tumor was characterized by sheets and nests of highly pleomorphic, mitotically active neoplastic cells infiltrating the breast

parenchyma, with focal comedonecrosis (Figure 1(b) and (c)). An initial diagnosis of poorly differentiated breast carcinoma was made. Further studies showed the tumor to be negative estrogen receptor, progesterone receptor, and HER-2/neu oncogene, consistent with a triple-negative invasive ductal carcinoma.

During pre-operative workup, an additional solid mass was identified in the inferior right breast. Core needle biopsy of this second mass showed cytologically similar neoplastic cells, with a prominent single-cell growth pattern. In addition, the tumor appeared to be well circumscribed with a thin rim of lymphoid tissue on one edge, suggestive of a replaced intra-parenchymal lymph node (Figure 2(a) and (b)). At this point, considering the patient's age and multi-centricity of the disease, together with the somewhat unusual growth pattern of neoplastic cells in the second biopsy specimen, immunohistochemical stains were performed. The neoplastic cells were positive for cytokeratin AE1/3 (patchy), pan-melanoma, tyrosinase, and SOX-10 and negative for CK7, CAM5.2, and GATA-3 (Figure 2(c)–(e)). The same immunohistochemical panel was then performed on the tissue specimen from the first mass, with similar results. Therefore, the initial diagnosis of breast ductal carcinoma was revised to melanoma. In light of this diagnosis, the patient's prior skin nevi biopsy results were reviewed and the absence of malignancy confirmed. In addition, thorough skin and eye examination failed to reveal any evidence of primary melanoma.
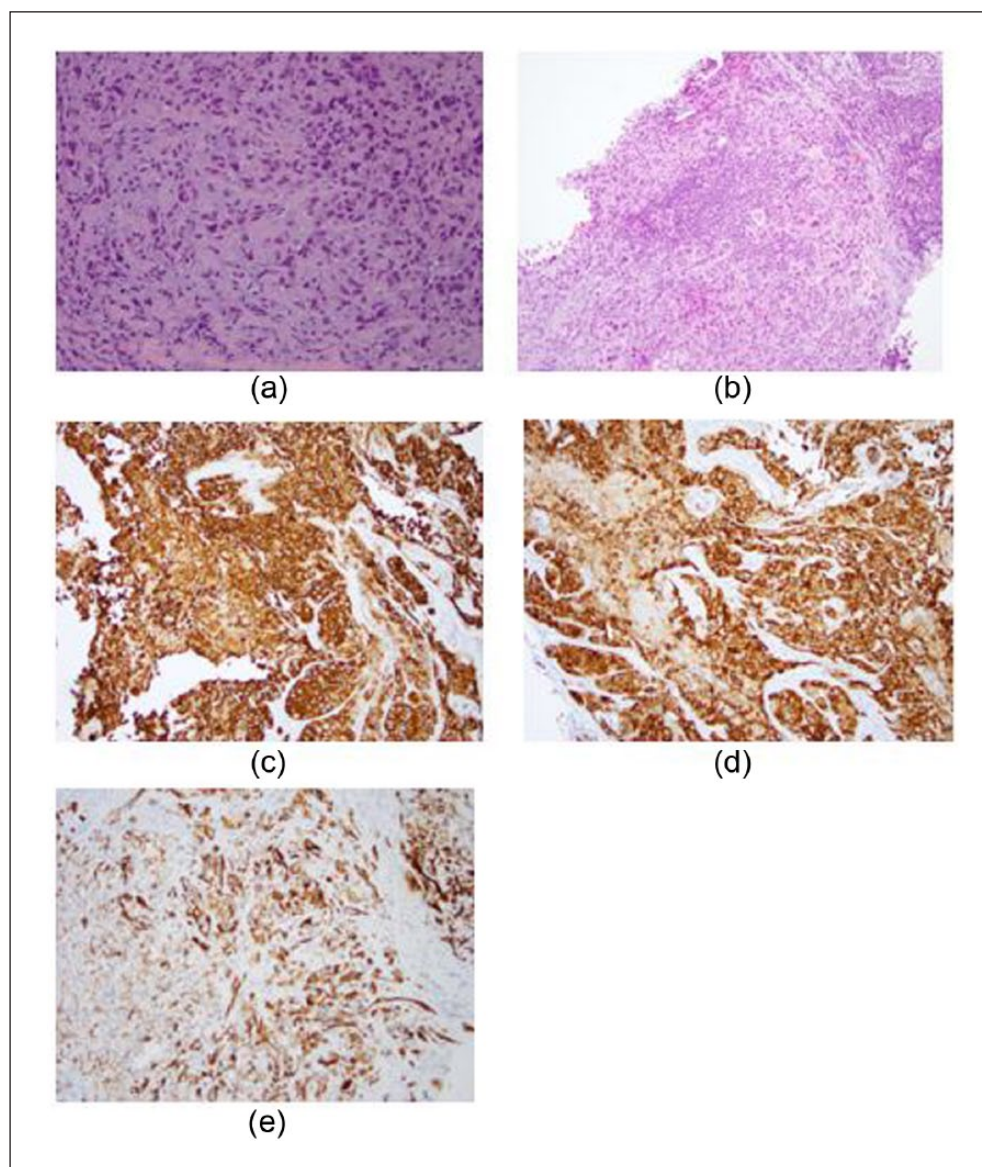
Figure 2. Biopsy of the second mass featuring diffusely infiltrating pleomorphic tumor cells (a) with a prominent single-cell growth pattern and (b) with a thin rim of lymphoid tissue suggestive of an underlying lymph node structure. (a and b) Diffuse staining with (c) pan-melanoma and (d) tyrosinase and patchy staining with (e) AE1/3 cytokeratin immunohistochemical stains.

Subsequent positron emission tomography–computed tomography (PET/CT) scan showed widespread metastatic disease to liver, lungs, and bones. Brain magnetic resonance imaging (MRI) demonstrated left temporal lobe metastasis, which was also histologically confirmed (Figure 2(b)). Genetic counseling was recommended, and the patient was tested with a gene panel for hereditary cancers, which was negative for germ line mutations. Targeted next-generation sequencing was performed, which revealed that the neoplastic cells harbored somatic *BRAF* p.V600E and *PTEN* p.R130Efs*4 mutations. Of note, *TERT* gene promoter mutation analysis was not performed.

In view of her advanced disease, the patient was enrolled in a clinical trial and was started on a combination of ipilimumab plus nivolumab, which was later changed to a combination of dabrafenib plus trametinib due to severe neurotoxicity (quadriparesis). This second chemotherapy course was complicated by severe neutropenia and hemorrhagic transformation of a temporal lobe lesion requiring craniotomy. Unfortunately, her disease continued to progress, and she died 8 months after the initial diagnosis.

Discussion

Malignant melanoma is known for its aggressive clinical behavior and propensity for metastasis. Approximately 5% of melanomas originate primarily from non-cutaneous sites.[12] There have been several reports of melanomas arising

from the skin of the breast.[13–15] In retrospective studies, melanoma has been found to account for up to 38.5% of metastasis from solid neoplasms affecting the breast.[6,16–18] However, only few cases have been described in the literature of melanoma presenting as a breast mass without evidence of a primary cutaneous lesion.

Biswas et al. reported the case of a 32-year-old female who presented with a 4-cm lower inner quadrant lump in the left breast. Diagnosis of melanoma was made by fine-needle aspiration biopsy.[19] Physical examination failed to reveal any primary cutaneous lesions, and metastatic workup showed no evidence of systemic metastasis. The diagnosis was confirmed on wide local surgical excision. Only 1 out of 13 lymph nodes was positive for metastatic melanoma. The patient received radiotherapy and immunotherapy and was reported to be alive 9 months after surgery.[19] Similar cases of melanoma affecting the breast without demonstrable cutaneous lesions or systemic metastasis have been described, predominantly in females aged 45–56 years old.[12,20] No molecular studies were performed on any of these previously described cases.

Presentation of widespread metastatic melanoma as breast masses is very rare. Our case was originally diagnosed as poorly differentiated breast carcinoma, with completely different therapeutic implications. Expression of breast carcinoma tumor markers and therefore tumor molecular subtypes classification have been associated with important clinical and prognostic features. Triple-negative breast carcinomas have been identified as one of the factors associated with metastasis and shorter overall survival (hazard ratio = 2.24) in breast cancer patients.[21] Triple-negative breast tumors have also been associated with black race and older age at diagnosis.[21] Triple-negative carcinoma were recently reported to be diagnosed in 22.2% of patients younger than 35 years.[22] It was also found that in the younger patients, triple-negative breast carcinoma cases may have a shorter disease-free survival but no significant difference in overall survival when compared to older patients.[22]

Another interesting fact in our case is the presence of concurrent *BRAF* and *PTEN* somatic mutations. *PTEN* is a tumor suppressor gene located in chromosome 10q23.[23,24] It modulates cell cycle progression and survival by regulating phosphoinositide-3-kinase (PI3K) and the protein-SER/Thr kinase (AKT) signaling pathway.[25,26] *PTEN* mutations have been described in advanced stages of glioblastomas, prostate, and breast carcinomas, among other neoplasms.[27,28] This tumor suppressor gene has been implicated in the etiology of Cowden syndrome, which shows an inherited predisposition for breast and thyroid carcinomas in the affected patients.[29] Mutations and deletions of *PTEN* have been reported in melanoma cell lines as well as uncultured melanomas. A higher incidence of *PTEN* mutations have been reported in melanoma cell lines than primary melanomas and metastatic melanoma biopsies, with a frequency of 27.6%,

7.3%, and 15.2%, respectively.[30] Celibi et al.[31] observed loss of heterozygosity of *PTEN* gene in 7 out of 21 (33%) melanoma cases.

BRAF is a proto-oncogene located in chromosome 7q34 that helps transmit extracellular signals to the cell nucleus as part of the RAS/mitogen-activated protein kinase (MAPK) pathway.[32] This pathway controls several important cell functions such as cell growth and division. *BRAF* mutational activation is detected in more than 50% of patients affected by melanoma and is one of the earliest and most common genetic alterations in its development.[8] *BRAF* mutations are also detected in benign nevi and other non-malignant melanocytic lesions.[33] Besides the RAS/MAPK pathway, PI3K, Rho GTPases, JAK-STAT, and WNT/B-catenin pathways are also contributors to melanoma progression.[10]

Malignant progression of *BRAF* p.V600E–expressing melanocytes is frequently promoted by silencing of the tumor suppressor *PTEN*.[34] Dankort et al.[35] demonstrated that the induction of *BRAF* p.V600E expression in mice, combined with *PTEN* tumor suppressor gene silencing, elicited development of melanomas and also predisposed to metastasis. This phenotype was not observed in *BRAF* p.V600E–induced mice in the absence of *PTEN* alterations.

Our case is interesting due to the unusual clinical presentation (a breast mass in a woman with no other known primary malignancies) and diagnostic challenge it posed from the diagnostic standpoint (sheets of non-pigmented epithelioid cells with focal comedonecrosis). These unusual histologic features were further compounded by keratin positivity of the neoplastic cells, which is known to occur in only approximately 5% of melanomas.[36] Furthermore, targeted next-generation sequencing analysis showed concurrent a *BRAF* and *PTEN* mutation profile, making the case even more interesting and unique. Only few reports have been published of malignant melanomas presenting as a breast mass, and to our knowledge, none has shown concurrent *BRAF* and *PTEN* mutations.

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