WHAT IS YOUR DIAGNOSIS?

Figure 1. Image courtesy of Emily Y. Chu, Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Editorial note: Welcome to the Journal of Investigative Dermatology (JID) SnapshotDx Quiz. In this monthly online-only quiz, the first question (“What is your diagnosis?”) relates to the clinical image shown, while additional questions concern the findings reported in the JID article by Chang et al. (2020) (https://doi.org/10.1016/j.jid.2020.01.027).

Detailed answers and a list of relevant references are available after the Quiz Questions below.

QUIZ QUESTIONS

1. What is your diagnosis?
   a. Atypical fibroxanthoma
   b. Proliferative nodule
   c. Blue nevus
   d. Metastatic breast cancer
   e. Metastatic melanoma
2. According to the article by Chang et al. (2020), the absence of a presumed truncal driver mutation among one or more metastatic tumors arising from a primary mutation may be due to which of the following?
   a. A primary tumor composed of genetically distinct subclones, each capable of metastatic spread.
   b. An undiagnosed second primary tumor.
   c. A treatment effect from a targeted therapy.
   d. A polyclonal tumor subject to selective pressure.
   e. The acquisition of de novo mutations in metastatic tumors.

3. Which of the following is the most frequent somatic mutation observed in malignant melanoma?
   a. NRAS
   b. BAP1
   c. BRAF
   d. NF1
   e. MITF

See the following pages for detailed answers
The skin is the most common site of melanoma metastasis and frequently the first to be involved (Patterson, 2017). Clinically, cutaneous melanoma metastases present as multiple black, bluish, or pink papules or nodules often located between the site of the primary lesion and the regional lymph nodes. On histopathology, metastatic melanoma typically displays a nodular aggregate of atypical cells in the dermis, with variable pigmentation, and usually without significant epidermal connection, although epidermotropic metastases may be seen. Intravascular tumor deposits may be observed. The lesional cells may stain positively with S-100, MART-1, and/or SOX10 stains, among others.

**Discussion of incorrect answers:**

a. **Atypical fibroxanthoma:** Atypical fibroxanthoma (AFX) is a rare, low-grade superficial sarcoma arising predominantly on the head and area of the elderly, with men affected more than women (Mentzel et al., 2017). AFX presents as a superficial, rapidly growing, gray to pink or red solitary nodule, often dome shaped, which may ulcerate and bleed (Patterson, 2017). On histopathology, AFX appears as a well-circumscribed, nonencapsulated, highly cellular tumor centered in the dermis. The overlying epidermis is often atrophic and may be ulcerated. The lesional cells are plump and spindle shaped with prominent nuclei and aggregate in poorly arranged fascicles. In the photomicrograph shown in this paper, the tumor cells are epithelioid and heavily pigmented rather than spindled, making the choice of AFX incorrect.

b. **Proliferative nodule:** Proliferative nodules (PNs) are benign lesions that may develop from congenital nevi and their variants (Patterson, 2017). The large epithelioid cells seen in PNs may be misdiagnosed as melanoma, but PNs are most often located within or adjacent to conventional nevus cells. Commonly around 5 mm in diameter, a PN can share features with a deep-penetrating nevus, a balloon nevus, or a Spitz nevus (Murphy et al., 2008). Immunohistochemical analysis of the cells away from the PNs has found expression of c-kit (CD 117) in almost all PNs but not in the adjacent congenital nevus (Patterson, 2017).

c. **Blue nevus:** Blue nevi are small blue–black to slate-blue macules or papules usually found on the extremities (Patterson, 2017). On histopathology, blue nevi are composed of elongated, pigmented, sometimes finely branched melanocytes in the interstices of the dermal collagen of the middle and upper dermis with a few melanophages, unlike the epithelioid atypical cells seen in the photomicrograph. Approximately 3% of blue nevi cases are termed amelanotic or hyperpigmented blue nevi owing to the minimal pigment being present (Patterson, 2017).

A potential diagnostic problem facing blue nevi is their resemblance with some cutaneous melanoma metastases. The blue nevus–like metastatic melanoma is a rare form of metastatic melanoma that closely resembles a blue nevus. The lesions are usually seen in the same region as the primary tumor. These lesions are composed of pigmented melanocytes and melanophages in a blue nevus–like growth pattern (Busam, 1999). A problematic scenario arises if there is no clinical history of a previous melanoma to facilitate the diagnosis. The clue to the diagnosis can sometimes be the presence of an inflammatory reaction at the periphery of the lesion. In addition to features that favor melanoma, such as nuclear pleomorphism, mitotic activity, and associated inflammation, FISH studies can be helpful.

d. **Metastatic breast cancer:** On histopathology, metastatic breast cancer (MBC) usually shows the characteristics of a poorly differentiated adenocarcinoma. MBC cells may appear to intercalate between collagen bundles in the dermis or may form small ductal or glandular structures (Patterson, 2017). Increased mucin in the dermis is a common feature. Sclerosis of dermal collagen may be present, especially on scalp lesions—a common location for MBC. Compared with metastatic melanoma, MBC typically has far less melanin pigmentation and may exhibit gland formation and stains positively with cytokeratin stains, including cytokeratin 7. MBC is negative for melanocytic markers, including MART-1, S100, and SOX10 stains.

2. According to the article by Chang et al. (2020), the absence of a presumed truncal driver mutation among one or more metastatic tumors arising from a primary mutation may be due to which of the following?

**CORRECT ANSWER:** a. A primary tumor composed of genetically distinct subclones, each capable of metastatic spread.

In the article by Chang et al. (2020), mutational heterogeneity was observed in the three most commonly mutated genes in melanoma. According to the authors, the results suggest that known driver mutations may be subclonal in primary...
melanomas, although likely in a minority of cases. Moreover, they suggest that the development of subclones may be inherent to melanoma even in the absence of targeted therapeutic pressure. In the current model of branched evolution used to describe melanoma, clones stem from a driver or truncal mutation such as BRAF, and subclones (i.e., branches) are defined by the acquisition of subsequent mutations such as TERT (Davis et al., 2017; Shain et al., 2015). The article by Chang et al. (2020) and other previously published reports support the concept of subclones within melanoma tumors, although they raise questions regarding the truncal nature of BRAF mutations in all cases. Examples of patients who appear to acquire BRAF mutations after the development of metastases have been described because they were not detected in the paired primary tumors from the same patients (Chang et al., 2020). Although initially considered to be consistent with a tumor evolution model characterized by the acquisition of mutations, because of lack of sampling of mutant cells in the primary tumor, this finding raises the possibility that BRAF mutations are not necessarily truncal mutations in all cases. A truncal mutation should be present in all cells and is rarely missed if tumor cells were sampled from primary tumors of patients in which BRAF mutant metastases arose.

Discussion of incorrect answers:

b. An undiagnosed second primary tumor: To address the concern regarding second-order primary melanomas, the authors of the study obtained the tumor samples from a prospective clinical-pathologic biospecimen that included complete clinical data and protocol-driven follow-up and included second primary tumors in the few patients in whom they occurred (Chang et al., 2020). The authors found BRAF, NRAS, and TERT mutational heterogeneity in 7%, 3%, and 13% of these patients, respectively. Previous studies have used whole-exome sequencing to assess either for heterogeneity between primary and metastatic lesions or for intra-tumor heterogeneity and observed none (Harbst et al., 2016; Sanborn et al., 2015). As was documented by Chang et al. (2020), these studies had the problem of having insufficient samples to be able to appropriately detect mutational heterogeneity that may occur at the rates of 18% or less.

c. A treatment effect from targeted therapy: Although targeted therapy has been shown to cause inter-tumor mutational heterogeneity, this is believed to arise from either a polyclonal tumor being subject to selective pressure or the acquisition of de novo mutations in line with clonal evolution and does not account for the absence of a truncal driver mutation among one or more metastatic tumors arising from a primary mutation, according to the study by Chang et al. (2020). In this study, there were two patients who presented with both BRAF and NRAS mutations. One patient developed the NRAS mutation later in their disease course, whereas the other patient had both mutations in the same metastasis, but only the NRAS mutation persisted among later metastases. NRAS mutations are known to confer resistance to BRAF-targeted therapies; however, neither patient received BRAF-targeted therapy at any time during their treatment, which points to the possibility that the development of multiple subclones throughout the tumor evolution, even in the absence of exogenous therapeutic pressure, may be part of the intrinsic nature of melanoma.

d. A polyclonal tumor subject to selective pressure: A polyclonal tumor subject to selective pressure could explain intertumor mutational heterogeneity among patients undergoing targeted therapy. Yet, it cannot explain the absence of a presumed truncal driver mutation among metastatic tumors arising from a primary mutation (Chang et al., 2020). The selective pressure induced by targeted therapies can result in the dominance or acquisition of additional driver mutations or molecular aberrations in tumor subclones (Shi et al., 2014). The heterogeneity present in a given population defines its capacity to respond, at a population level, to selective pressure (Maleý et al., 2017). Diversity can be a proxy for the likelihood that a resistant clone is present in a neoplasm. Rapid tumor progression after chemotherapy, for example, may be driven by pre-existing resistant variants. Chemotherapy can be mutagenic and can select for hypermutated clones, generating new clones and more diversity. For this reason, therapeutic failure may be more likely in tumors with more subclonal diversity. Tumors under selective pressure evolve rapidly and can generate and maintain new clones at a high rate.

e. The acquisition of de novo mutations in metastatic tumors: Although it is true that de novo mutations lead to heterogeneity in melanoma, this statement does not explain the authors’ findings, which indicate that primary tumors may be composed of genetically distinct subclones capable of metastatic spread, even in the absence of exogenous therapeutic pressure. It is worth noting that de novo mutations in metastatic tumors are not the same as de novo metastatic tumors, which refers to tumors that are diagnosed as metastatic from the start (Zhang et al., 2020).

3. Which of the following is the most frequent somatic mutation observed in malignant melanoma?
BRAF is a serine/threonine kinase that is commonly activated by a somatic point mutation (Davies et al., 2002). Patients with BRAF-mutated melanomas are generally younger than patients with other melanoma subtypes (Cancer Genome Atlas Network, 2015). BRAF mutations do not seem to be related to the effects of UV light because the T→A nucleotide change observed in BRAF is distinct from the C→T changes associated with pyrimidine dimer formation after UV light exposure. It has been documented that around 60% of melanomas on nonchronic sun-damaged skin had BRAF mutations (Curtin et al., 2005). More than 90% of melanomas with a mutated BRAF contain a V600E single-base nucleotide change (Davies et al., 2002). It is worth noting that the BRAFV600E mutation has also been found in over 80% of melanocytic nevi, which suggests that mutation activation of the MAPK/extracellular signal–regulated kinase (ERK) pathway is a critical step in the initiation of melanocytic neoplasia (Pollock et al., 2003).

Discussion of incorrect answers:

a. **NRAS:** Activating NRAS mutations occur in almost 30% of malignant melanomas and are the second most common oncogenic driver mutation in the disease (Cancer Genome Atlas Network, 2015). As part of the RAS subtype family, NRAS is involved in the transduction of extracellular growth signals through both the MAPK and phosphatidylinositol-3/ protein kinase B pathways. In contrast to BRAF mutations, NRAS melanomas are correlated with the nodular subtype and found in chronic sun-damaged skin (Lee et al., 2011). In addition, NRAS expression has been linked with a lower grade of tumor-infiltrating lymphocytes and a higher tumor stage (Thomas et al., 2015). The overall survival is unclear because shorter survival has been reported from NRAS-mutated melanomas, whereas others have reported no difference between wild-type and NRAS-mutated melanomas (Ellerhorst et al., 2011; Jakob et al., 2012). Another difference between melanomas in which NRAS and BRAF are mutated is the higher mutational load in NRAS-mutated melanomas. Targeted therapy has shown limited effectiveness, and for this reason, chemotherapy and other immune therapies are used. MAPK/ERK kinase inhibitors have also been the focus of research as a potential therapy for NRAS mutants (Chang et al., 2020).

b. **BAP1:** BAP1 is a tumor suppressor gene involved in cell-cycle progression, and germline mutation of BAP1 leads to uveal melanoma and, to a lesser degree, cutaneous melanoma (Bononi et al., 2017). The BAP1 tumor syndrome is also associated with the development of mesothelioma, spitzoid neoplasms (BAPomas), renal cell carcinoma, and basal cell carcinoma. In melanoma specimens, somatic BAP1 mutations are not nearly as frequently observed as BRAF mutations, making this answer choice incorrect.

c. **NF1:** NF1 is a ubiquitous multidomain protein found mostly in the CNS that functions as a regulator of activated RAS proteins (Philpott et al., 2017). The highest frequency of somatic NF1 mutations are found in desmoplastic melanoma (Wiesner et al., 2015) and has been cited as the third most common somatic mutation overall in melanoma (Krauthammer et al., 2015).

d. **MITF:** MITF has been shown to promote melanoma cell growth and metastasis and serves as a melanoma-survival prognostic marker (Dar et al., 2016). MITF has also been shown to serve as a marker for differentiating melanoma from non-melanocytic tumors, and low expression has been associated with increased invasiveness of melanoma (Carreira et al., 2006). Germline MITF mutations have also been associated with increased susceptibility to the cooccurrence of melanoma and renal cell carcinoma (Bertolotto et al., 2011). Recognized as a critical regulator of metabolism and the DNA damage response, MITF binds to DNA to regulate transcription of its target genes (Goding and Arnheiter, 2019). Its function is also to control the development and differentiation of melanocytes, especially in response to UVR. MITF is a melanocytic lineage-specific transcription factor that plays a critical role in malignant melanoma (Hartman and Czyz, 2015). Yet, mutation-dependent alterations in MITF expression and activity have been found in a relatively small subset of melanomas.

**REFERENCES**


