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Multifocal BRAF^{V600E}-Mutated Melanoma in situ on the Foot

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Key Words
Malignant melanoma in situ · BRAF^{V600E} mutation · PD-L1

Abstract
Melanoma is an aggressive skin cancer that originates from melanocytes, and about one half of melanoma cases possess a BRAF mutation. Together with PD-L1 expression, the BRAF^{V600E} mutation is one of the optimal therapeutic targets for the treatment of melanoma. In this report, we describe a case of multifocal melanoma in situ on the foot, which carried the p.V600E mutation in the BRAF gene. Interestingly, the spotted melanoma lesion is demarcated by normal skin, and in all spotted pigmented lesions, there were no signs of dermal invasion of melanoma cells or spontaneous regression. Our case presented atypical clinical features, which might correlate with the local mutations of BRAF gene and the immunological expression of PD-L1.

Introduction

Melanoma is an aggressive skin cancer that originates from melanocytes and shows malignant transformation distributed throughout the epidermis. Recent reports suggested that about one half of melanoma cases possess a targetable BRAF mutation promoting RAS-ERK pathway activation [1–3]. Among them, the BRAF^{V600E} mutation is one of the optimal therapeutic targets for the treatment of melanoma [4]. Interestingly, multiple melanomas with atypical clinical features, such as intraepidermal epidermotropic metastatic melanoma and microdissected melanoma, were reported as BRAF^{V600E}-mutated melanomas [5, 6]. In this report, we describe a case of multifocal melanoma in situ on the foot that carried the p.V600E mutation in the BRAF gene and a faint expression of PD-L1.

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Case Report

A 61-year-old Japanese woman visited our outpatient clinic with a 20-year history of multiple black plaques. On her initial visit, physical examination revealed multiple, black, pigmented plaques on the lateral side of her left dorsal foot (fig. 1a). Dermoscopic findings revealed an atypical reticular pattern, asymmetric globules and dots, irregular streaks, and blue-white veil (fig. 1b). A biopsy specimen showed markedly atypical melanocytes arranged in irregular nests and solitary units in all levels of the epidermis, which is demarcated by normal skin (fig. 2). Notably, in all spotted, pigmented lesions, there were no signs of dermal invasion of melanoma cells or spontaneous regression. Immunohistochemical staining revealed that these atypical melanocytes were positive for Melan A (fig. 3a) and S100 (data not shown). From the above findings, we diagnosed this patient as having multifocal melanoma in situ and excised the tumor with a 5-mm margin. We screened for possible internal malignancy with computed tomography and found no evidence of metastasis. In addition, a genomic analysis of BRAF^{V600E} mutation in melanoma cells by immunohistochemical staining was performed (fig. 3b), and immunohistochemical staining for PD-L1 revealed a faint expression of PD-L1 on melanoma cells compared with the surrounding keratinocytes (fig. 3c).

Discussion

Previous reports have suggested immunogenicity of the mutant BRAF protein in melanoma [1, 7]. Indeed, Anderson et al. [7] reported human leukocyte antigen-restricted cytotoxic T-lymphocyte (CTL) responses against a synthetic mutant BRAF in patients with melanoma. These reports clearly indicated that tumor-infiltrating leukocytes (TILs) in BRAF^{V600E} melanoma should contain substantial numbers of CTLs against the melanoma. On the other hand, several reports also suggested that BRAF^{V600E} melanoma cells promote an immunosuppressive tumor microenvironment by several pathways [1, 8, 9]. For instance, BRAF^{V600E} melanoma cells produce immunosuppressive mediators [interleukin (IL)-6, IL-10, and vascular endothelial growth factor], which lead to the recruitment of immunosuppressive TILs, such as myeloid-derived suppressor cells and regulatory T cells in the lesional skin of melanoma [1, 8], resulting in the upregulation of the expression of PD-L1 on tumor stromal cells [10]. In addition, BRAF^{V600E} melanoma cells increase the expression of PD-L1 by IL-1-dependent pathways [9]. Notably, previous reports suggested that PD-L1 expression in melanoma determines the progression of melanoma patients [11] and even determines the therapeutic effects of the anti-PD-1 antibody, nivolumab [12]. In the aggregate, the BRAF^{V600E} mutation not only promotes carcinogenesis but also immunomodulation of the tumor microenvironment.

In this report, we describe a case of melanoma in situ on the foot with an atypical clinical presentation. Notably, we histologically examined all spotted areas of the melanoma, and there was no dermal invasion of melanoma cells or spontaneous regression. Although previous reports of multifocal melanoma are limited [5, 6], based on the above findings, we ruled out possible local recurrence or local metastasis of an acral melanoma. In addition, immunohistochemical staining revealed that melanoma cells carried the BRAF^{V600E} mutation but did not express PD-L1. One of the possible mechanisms for this unusual clinical feature might be explained by the BRAF^{V600E} mutation and PD-L1 expression. Depending on the BRAF^{V600E} mutation, TILs might contain substantial numbers of CTLs that react with BRAF^{V600E}-mutated melanoma. Indeed, as presented in figure 2, our patient's melanoma contained substantial numbers of TILs. In contrast to conventional BRAF^{V600E}-mutated melanoma, our case

did not express PD-L1, which suggests that the TILs in our case were not exhausted and reacted as anti-BRAF^{V600E}-mutated melanoma. Since we did not directly assess the CTL functions and antigen specificities of these TILs, further analysis of the mechanisms underlying this phenomenon could offer fundamental insights into the mechanisms of our case. To confirm this hypothesis, further cases and studies will be necessary in the future.

Statement of Ethics

The patient gave written informed consent.

Disclosure Statement

The authors declare no conflicts of interest.

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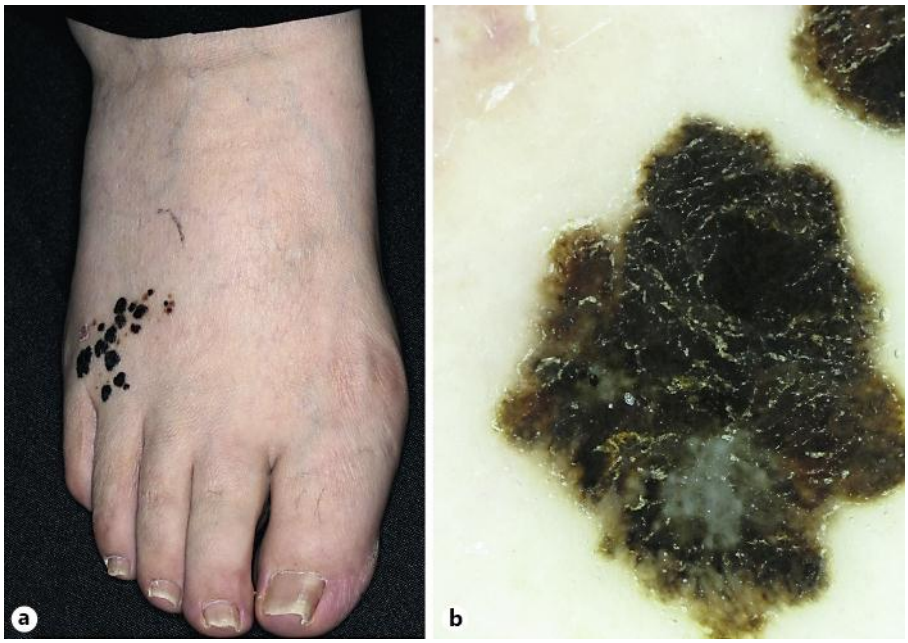

Fig. 1. Multiple, black, pigmented plaques on the lateral side of the left dorsal foot (**a**). Dermoscopic findings: atypical reticular pattern, asymmetric globules and dots, irregular streaks, and blue-white veil (**b**).

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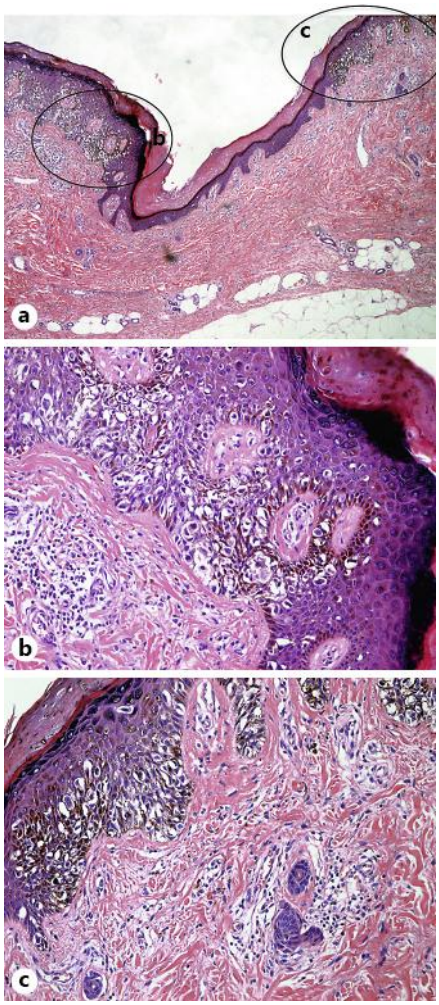

Fig. 2. Markedly atypical melanocytes arranged in irregular nests and solitary units in all levels of the epidermis, which is demarcated by normal skin (**a**). At the spotted tumor areas, there are no signs of dermal invasion of melanoma cells and no spontaneous regression (**b**, **c**). Original magnification: ×50 (**a**), ×200 (**b**, **c**).

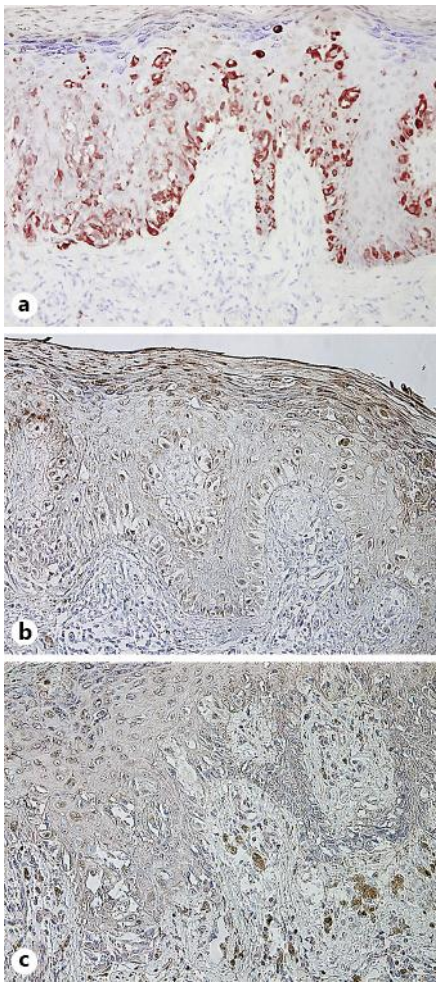

Fig. 3. Paraffin-embedded tissue samples were deparaffinized and stained with anti-Melan A antibody (**a**), anti-BRAF^{V600E} antibody (**b**), and anti-PD-L1 antibody (**c**). The sections were developed with liquid permanent red (**a**, **c**) or with 3,3′-diaminobenzidine tetrahydrochloride and its enhancer (**b**). Original magnification: ×200.