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CASE REPORT

Eruptive melanocytic nevi with malignant transformations and the connection to BRAF and p16 mutations

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ABSTRACT

Eruptive melanocytic nevi (EMN) is a rare phenomenon with an unclear pathogenesis. Mutation on a molecular basis has been proposed, but insufficient data are available to confirm this hypothesis. Theoretically, the possibility of malignant transformation is high, but no previous definite cases had been reported. Herein, we report the first case of a patient with EMN in whom hypopharyngeal low-grade melanocytic malignancies were detected using a fibrolaryngoscope. BRAF activation and p16 mutations were discovered through real-time polymerase chain reaction and immunohistochemical staining on EMN tissue samples, respectively. The significance of these findings has yet to be determined.

Introduction

Eruptive melanocytic nevi (EMN) is a rare phenomenon in which hundreds of melanocytic nevi appear suddenly. This condition has been associated with multiple dermatological diseases and suppression of the immune system.[1] Although EMN is associated with a higher risk of malignant transformation, no previous study has reported a concurrent melanocytic malignancy. BRAF and p16 mutations have been implicated in the pathogenesis of EMN,[2,3] but few studies have examined samples of EMN for these markers. Herein, we report a rare case of EMN associated with multiple concurrent low-grade melanocytic malignancies, for which we performed an immunohistochemistry (IHC) study for p16 mutation and used real-time polymerase chain reaction (PCR) to identify BRAF mutation in tissue samples of EMN.

Case Report

A 32-year-old man presented with multiple asymptomatic purplish and blackish papules (1–4 mm in diameter) over his trunk, axilla, and upper extremities (Figures 1A and 1B). There were more than 100 of these lesions, all of which had abruptly erupted in the 6 months prior to the visit. Other skin findings and symptoms were unremarkable. The patient was previously healthy and denied any history of drug use. A skin biopsy was performed on one of the lesions on his right abdomen. Microscopic examination revealed that it was a Spitz nevus (Figures 1C and 1D). Under the impression of EMN, we arranged a thorough examination. Two additional skin biopsies were performed on lesions on the left abdomen and right wrist, both of which were proven histopathologically to also be Spitz nevi. His serum biochemical and hematologic panels were all within normal limits, as were his levels of prostate-specific antigen, cortisol, and adrenocorticotropic hormone. An anti-HIV antibody test was negative. Chest radiography, panendoscopy, colonoscopy, whole-body computed tomographic scan, and positron emission tomography scan were all normal. However, fibrolaryngoscopy detected a pigmented lesion over the hypopharynx (Figure 2A), which was then removed through laryngomicrosurgery. The tissue was sent for pathology, which revealed a heavily pigmented papule confined to a superficial connective tissue composed of

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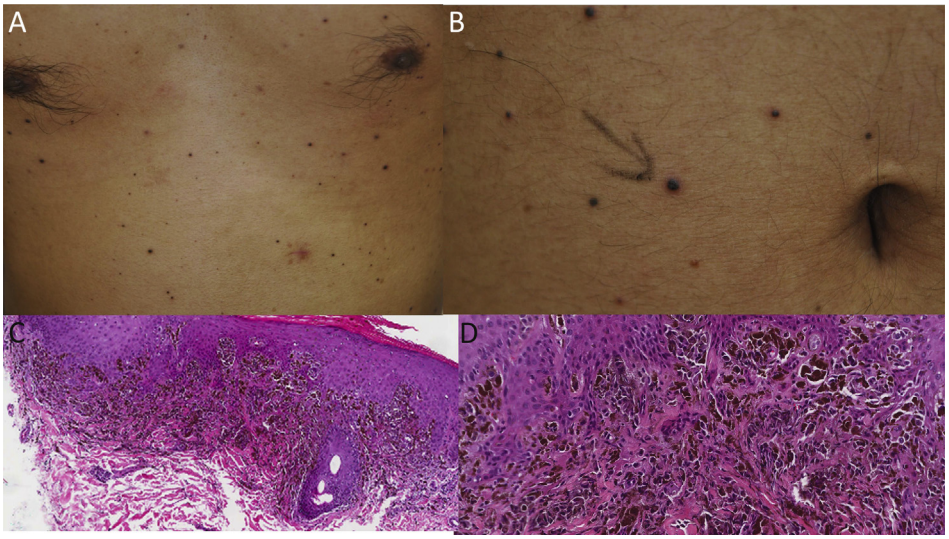

Figure 1 (A) Numerous erupted melanocytic nevi in a 32-year-old man. (B) Close-up view of abdominal region. Nevi are 1–4 mm in diameter, mildly dome-shaped, and blackish. (C) Microscopically, the lesion demonstrates features of a Spitz nevus, including a compound structure with a well-circumscribed outline, and heavy pigmentation (hematoxylin and eosin 40×). (D) High-power view showing large epithelioid and spindle nevus cell aggregation. (hematoxylin and eosin 100×).

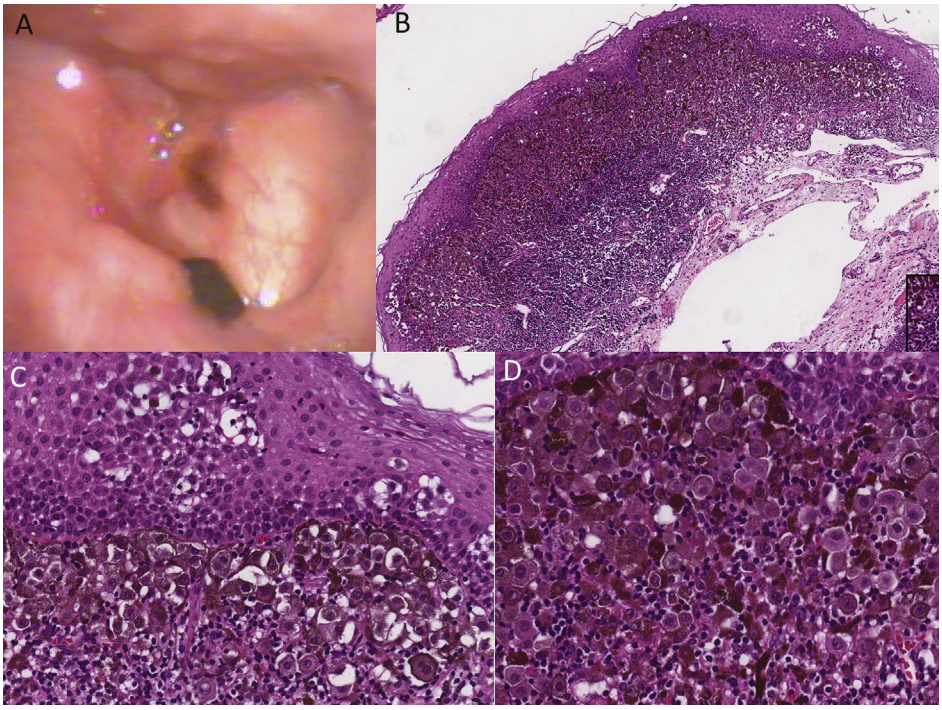

Figure 2 (A) A pigmented lesion detected under a fibrolaryngoscope over the hypopharynx. Three newly erupted pigmented lesions are detected 1 month later in the same region (not shown). (B) Histologic examination of the hypopharyngeal lesion reveals well-demarcated but slightly asymmetrical proliferation with heavy pigmentation under the scanning view (hematoxylin and eosin 40×). (C) Frequent pagetoid spread of nevus cells, singly and in nests are noted, they even located in the upper half of the epithelium (hematoxylin and eosin 200×) (D) Close-up view: the lesion is composed of confluent large epithelioid nevus cells. The cells show mild pleomorphism and nuclear atypia. No mitotic figures or atypical mitosis are detected throughout the lesion (hematoxylin and eosin 400×).

predominantly epithelioid nevus cells with prominent central eosinophilic nucleoli, focal hyperchromatism and mild nuclear atypia (Figure 2B–D). The lesion revealed both benign characteristics, including a well-circumscribed architecture with maturation and no mitotic activity, and troubling characteristics, including a slightly asymmetrical architecture, confluent cell nests, and frequent pagetoid extensions of the nevus cell into the surface epithelium, even in the upper half of the epithelium. These histologic features led to a diagnosis of melanocytic tumor with uncertain malignant potential (MELTUMP). After 1 month, the patient returned for follow-up. The number of skin lesions had increased and some of them had become more raised and enlarged. He received a fibrolaryngoscopy again, and three newly erupted pigmented nevi were detected over the hypopharynx. All of them were excised, sent for pathology, and proven histologically to be MELTUMPs. We detected BRAF V600E mutations by using a real-time melting curve analysis of PCR on four of the excised tissues and using IHC to identify p16 in five of the excised tissues. The PCR

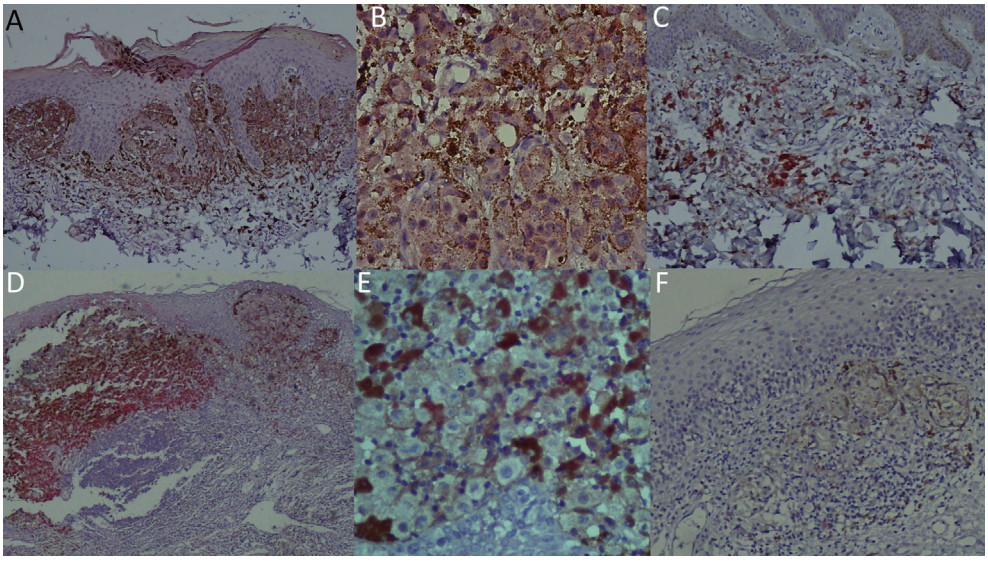

Figure 3 (A) Immunohistochemistry (IHC) staining for p16 performed on the excised cutaneous Spitz nevus of the right abdomen. In the scanning view, there is no significant staining with p16. As we used 3-amino-9-ethylcarbazole as chromogen to develop the reactions, the color of the p16 staining should be red. The brown color in the section thus represents melanin pigment (p16 stain 100×). (B) Close-up view showing very weak or negative p16 expression mixed with melanin pigment (p16 stain 400×). (C) IHC staining for p16 performed on the excised cutaneous Spitz nevus of the left abdomen. The nevus cells exhibit an extremely low percentage of positivity (p16 stain 100×). (D) IHC staining for p16 performed on one of the excised hypopharyngeal MELTUMPs. In the scanning view, the melanocytic cells express diffuse positivity on the left side of the field. An abrupt loss of p16 positivity on the right side is noted (p16 stain 40×). (E) Close-up view of the right side of the field demonstrating only scattering p16 expression in the melanocytic cells (p16 stain 400×). (F) Another hypopharyngeal MELTUMP showing very weak or negative p16 positivity (p16 stain 100×).

result indicated the presence of BRAF V600E mutations in three of the four excised tissues(Supplementary Table 1). The IHC staining for the p16 revealed variable decreased positivity in all the five excised samples, namely two cutaneous Spitz nevi and three hypopharyngeal MELTUMPs (Figure 3). The sites of the excised lesions and corresponding examination results are shown in Table 1.

After complete staging, no lymph node or distant metastasis was detected, thus, the treatment plan was mainly observation. On the basis of the MELTUMP diagnosis, we arranged an intense follow-up plan for the patient according to the guideline for malignant melanoma, including a thorough skin examination for additional peculiar lesions, palpation of lymph nodes, and fibrolaryngoscopy, with particular attention paid to the hypopharynx every 3 months. The number of the nevus stopped increasing 1 year after the initial eruption, but there was no sign of spontaneous regression. During the subsequent 6 months of follow-up after the removal of the hypopharyngeal MELTUMPs, there was no newly detected melanocytic malignancy.

Discussion

EMN is a rare phenomenon in which hundreds of melanocytic nevi appear suddenly.[1] The lesions of EMN can include banal nevi, blue nevi, and Spitz nevi.[4] EMN may arise in the setting of blistering skin disease, immunosuppression, cytokine administration, biological agent administration, chemotherapy,[1] Addison disease,[5] paraneoplastic phenomena,[6] pregnancy, and idiopathic causes.[1,7] The proposed pathogenesis of EMN includes diminished immune surveillance, genetic susceptibility, and drug adverse effects,[1] although the exact pathogenesis is not clearly understood. The chance of potential malignant transformation is assumed to be theoretically higher in patients with EMN. Regular screenings for dysplastic nevi or melanoma have been recommended by many authors.[1–6] However, to our knowledge, no melanocytic malignancy has been reported following an EMN eruption in the English-language literature.

Table 1 Excision site, pathologic diagnosis, and corresponding examination result.

Site	Pathology	BRAF mutation	Percentage of p16 positive cells
Right abdomen	Spitz nevus	Present	< 5% (Figures 3A & 3B)
Left wrist	Spitz nevus	Absent	Test not performed
Left abdomen	Spitz nevus	Test not performed	< 5% (Figure 3C)
Hypopharynx	MELTUMP	Present	50–60% (Figures 3D & 3E)
Hypopharynx	MELTUMP	Test not performed	Test not performed
Hypopharynx	MELTUMP	Present	< 5% (Figure 3F)
Hypopharynx	MELTUMP	Test not performed	< 5% (Supplmentary Figure 1)

MELTUMP = melanocytic tumor with uncertain malignant potential.

The concept of a MELTUMP was first proposed in 2004 as "borderline" melanocytic lesions that are difficult for dermatopathologists to classify as malignant or benign because of their ambiguous morphology.[8] In the present case, the pathology of the hypopharyngeal lesions exhibited both benign characteristics (well-circumscribed architecture with maturation and no mitotic activity) and also troubling characteristics (a slightly asymmetrical architecture, confluent cell nests, and frequent pagetoid spread even in the upper half of the epithelium). These lesions could not be classified as merely benign nevi, but they also failed to meet the criteria for melanoma, which is a constellation of multiple atypical findings. Through consultations with dermatopathologists in Taiwan, we concluded that the optimal diagnosis for these lesions is a MELTUMP.

Two recent large case series of MELTUMPs have reported a low risk of nodal metastasis (6–15%) and mortality from distant metastasis during follow-up (1%).[9,10] These results suggest that a MELTUMP is a low-grade malignant melanocytic tumor and should be treated according to the guidelines for invasive melanoma.[9,10]

We performed a careful workup but could not identify any possible underlying diseases or medications related to EMN in this patient. Indeed, most of the cases of EMN have been associated with underlying diseases or medications,[1,5,6] of which the disease courses flare and abate in parallel with the activity of the underlying diseases. However, as in the present case, a few cases of EMN have been reported as being idiopathic, of these cases, the

documentation of the nature course has generally been incomplete or lacking.[1,7] These idiopathic cases further highlights the importance of investigating the molecular mechanisms underlying the development of EMN. Sekulic et al[2] proposed that the presence of an activating BRAF mutation may play a role in the genesis of EMN because of the finding that a BRAF V600E mutation was identified in 85% of 20 examined EMN lesions. In another study, however, BRAF V600E mutations were also found in > 80% of benign nevi.[11] This apparent paradox was resolved by the discovery that most nevi display the hallmarks of senescence and stain highly for the tumor suppressor p16.[3,12] P16 is a tumor suppressor that inhibits the proliferation of cells through the G1 phase of the cell cycle. A normal-functioned p16 may lead to growth arrest in nevi harboring BRAF V600E mutations. Thus, a p16 mutation may lead to bursts of cell proliferation and has been implicated in the melanogenesis of EMN, especially in those harboring BRAF V600E mutations.[3]

In previous studies, p16 stains for cutaneous or mucosal benign nevi expressed a consistent pattern of diffuse positivity.[13–15] Unlike in previous studies,[13–15] all the cutaneous Spitz nevi and hypopharyngeal MELTUMPs in this case showed considerably decreased percentages of p16 positivity, indicating an underlying aberration of normal senescence pathway.

According to our preliminary results, this is the first study to demonstrate both BRAF and p16 mutation in the same excised EMN tissue, whether these mutations play a combined role in the initiation and subsequent melanogenesis of EMN remains unknown. Because of the limited number of EMN cases, large-scale studies are warranted to establish the consistency of these findings. We hope that these findings can contribute to future studies and treatment options for EMN.

In conclusion, this is the first case of EMN found to have concurrent melanocytic malignancies. We suggest thorough and careful examinations and intense follow-up for patients with EMN, and that fibrolaryngoscopy should always be used as part of the tumor work-up. The BRAF V600E and p16 mutations were speculated in the excised EMN tissues, the significance of which has yet to be determined.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dsi.2016.05.001.