Spitz nevi are benign melanocytic neoplasms composed of epithelioid or spindle cell melanocytes. While Spitz nevi have distinct histologic criteria for diagnosis, a subset of Spitz nevi can be clinically and histopathologically difficult to distinguish from malignant melanoma, leading to controversy regarding the nature of these lesions. Some Spitz nevi harbor activating mutations in HRAS (GenBank 3265) and BRAF (GenBank 673), serine-threonine kinases in the mitogen-activated protein kinase pathway that play a critical role in epidermal development, homeostasis, and tumor progression. These mutations can be a favorable prognostic biomarker for Spitz nevi.

Report of a Case

A 25-year-old man presented to the Stanford Pigmented Lesion and Melanoma Clinic with a 4-year history of pink papules emanating in a large congenital pigmented tan patch on his left lower back. Clinical examination revealed a more than 20-cm tan patch speckled with 1- to 2-mm hyperpigmented macules, characteristic of a nevus spilus, and containing fifteen to twenty 4- to 6-mm pink papules, characteristic of Spitz nevi (Figure 1 A and B). The patient was otherwise healthy, with no personal or family history of malignant melanoma. Histopathologic specimens of 2 pink papules revealed symmetric, well-demarcated melanocytic proliferations consisting of spindle cell melanocytes with large vesicular nuclei splayed...
through the dermis, consistent with intradermal Spitz nevi (Figure 1C and D). To identify underlying genetic alterations, we obtained specimens from 2 additional pink papules, with histopathologic features also confirming the diagnosis of Spitz nevi. Our study complied with the Declaration of Helsinki and was approved by the institutional review board at Stanford University School of Medicine. Genomic DNA was isolated from these 2 lesional samples along with the adjacent normal skin, 1 cm outside the boundaries of the nevus spilus, and subjected to exome sequencing (eMethods and eTable in the Supplement).

**Results**

Comparison of recurrent variants from the exome sequencing identified an HRAS point mutation (c.37G→C, p.Gly13Arg) in both Spitz nevi that was absent in the adjacent normal skin (Figure 2A). No other recurrent somatic mutations were detected (eMethods in the Supplement). Sanger sequencing confirmed the presence of the HRAS mutation in both Spitz lesions. We performed Sanger sequencing on DNA derived from 2 additional formalin-fixed, paraffin-embedded Spitz nevi obtained from the same patient that also demonstrated the HRAS point mutation (Figure 2B). Therefore, all 4 Spitz nevi obtained from our patient harbored the same single-nucleotide variation.

To evaluate for copy number changes, we used SeqGene-CNV on the exome sequencing data.13 This algorithm detects regions with abnormal copy number changes using circular binary segmentation. This revealed a copy number increase in chromosome 11p in both Spitz nevi compared with the normal skin control (Figure 2C). We then performed fluorescent in situ hybridization using an HRAS probe that confirmed amplification of HRAS in the melanocytes from 2 Spitz nevi (Figure 2D) and polysomy in the melanocytes from a third Spitz nevus (Figure 2E). No HRAS amplification was detected in adjacent fibroblasts or epidermal keratinocytes (Figure 2F).

Figure 1. Clinical and Histopathologic Features of the Agminated Spitz Nevi Arising in a Nevus Spilus

A and B, Photograph of a large tan patch on the left lower back with 1- to 2-mm hyperpigmented macules and 4- to 6-mm pink papules. C, Pink papule showing plump melanocytes splayed through desmoplasic collagen, consistent with Spitz nevi (hematoxylin-eosin, original magnification ×10). D, Melanocytes with amphophilic cytoplasm in the dermis (hematoxylin-eosin, original magnification ×20).
Figure 2. Activating HRAS Mutations and Amplification of Chromosome 11p in Agminated Spitz Nevi

A. Sanger sequencing of a representative Spitz nevus and adjacent unaffected skin demonstrates a c.37G→C, p.Gly13Arg mutation specific to the lesional tissue. B, Table of HRAS mutations showing the HRAS mutation is present in all 4 Spitz nevi but undetectable in the adjacent normal skin. C, Chromosomal amplifications predicted by SeqGene CNV and displayed with an Integrative Genomics Viewer (http://www.broadinstitute.org/igv/). Both Spitz nevi have a predicted amplification (red bars) over chromosome 11p. D, Dual-color fluorescent in situ hybridization (FISH) with HRAS probe (red signals) and a reference centromeric probe for chromosome 11 (green signals) showing a focus of melanocytes with increased red signal significantly above reference green signals, indicating HRAS amplification (arrows). E, Dual-colored FISH showing a focus of melanocytes with polysomy demonstrated by increased HRAS (red) and centromeric (green) signals in the nucleus (arrows). F, Dual-color FISH showing epidermal keratinocytes and papillary dermal fibroblasts with equivalent red and green signals. G, Sanger sequencing of AciI-digested DNA from a Spitz nevus, nevus spilus, and the adjacent normal skin demonstrating the HRAS mutation in the nevus spilus and Spitz nevus but not in the normal skin. H, Diagram of 2-hit model of a nevus spilus, with the first hit leading to the macular portion of the nevus spilus and the second hit leading to the formation of Spitz nevi. WT indicates wild type.
Spitz nevi are heterogeneous melanocytic tumors, with less than 20% of these lesions harboring HRAS activating mutations and even fewer containing the HRAS point mutation. Thus, it would be highly improbable for all Spitz nevi obtained from our patient to develop identical mutations if they represented independent lesions. We hypothesized that these Spitz nevi arose in an agminated fashion from a common postzygotic clone of melanocytes, likely demarcated by the nevus spilus. To improve our sensitivity to detect this mutation in the nevus spilus, we performed polymerase chain reaction amplification of the genomic DNA followed by enzymatic digestion with AciI, which digests the wild-type sequence but not the mutant sequence (eMethods and eFigures 1 and 2 in the Supplement). Subsequent Sanger sequencing reproducibly detected the HRAS point mutation in the nevus spilus and Spitz nevi but not in the adjacent normal skin (Figure 2G). This implicates the HRAS point mutation as the initiating mutation predisposing melanocytes to develop into Spitz nevi. In this model, a “second hit” may be required for the formation of Spitz nevi (Figure 2H). Our data support HRAS amplification as a secondary change because its mechanism was not identical in all Spitz nevi, with 1 nevus demonstrating polysony of chromosome 11.

Discussion

Multiple Spitz nevi can occur rarely in agminated and disseminated forms, but the genetic alterations that lead to these occurrences are unknown. To our knowledge, this is the first report demonstrating mosaicism in agminated Spitz nevi and identifying an activating HRAS mutation in agminated Spitz nevi. Mosaic HRAS mutations were recently recognized in the nevus sebacei and nevus spilus in patients with phacomatosis pigmentokeratotica. This report extends this finding by demonstrating an HRAS mutation in a sporadic nevus spilus. Interestingly, the HRAS point mutation, in particular, has been detected in a variety of benign skin neoplasms, including epidermal and sebaceous nevi, Spitz nevi, and nevus spilus, providing a unique example of genetic pleiotropy within the ectodermal lineage.

Our data indicate that multiple Spitz nevi may have a similar pathogenesis to that of solitary Spitz nevi since a subset of solitary Spitz nevi also harbors activating mutations in HRAS and copy number increases in chromosome 11p. However, similar to solitary Spitz nevi, other genetic alterations also may play a role in the pathogenesis of multiple Spitz nevi. Gantner et al recently demonstrated the absence of HRAS-activating mutations in a patient with eruptive Spitz nevi, suggesting that alternate genetic alterations may be responsible for the lesions in this patient. It is tempting to speculate that many cases of multiple Spitz nevi may result from an early clonal mutation, as demonstrated in our patient.

Recently, significant progress has been made in understanding the genetic alterations in cutaneous tumors, in part due to the advances in sequencing technology. Many of these technologies rely on a large number of samples to determine recurrent mutations. This approach may be difficult in solitary Spitz nevi since the lesions are uncommon and possess heterogeneous mutations. Identifying clonal mutations in patients with multiple Spitz nevi presents a promising approach to distinguish genetic alterations in all Spitz nevi. Insight into these genetic changes is critical to improve our ability to diagnose and manage these controversial lesions.
NOTABLE NOTES

Euphorbia peplus: 18th-Century Insights on a 21st-Century Therapy

Navya S. Nambudiri, MBBS; Vinod E. Nambudiri, MD, MBA

In 2012, the US Food and Drug Administration approved a new therapeutic agent, ingenol mebutate, for the topical treatment of actinic keratoses. Ingenol mebutate is a diterpene ester with the chemical formula C_{25}H_{34}O_{6} and is extracted from the sap of the plant species Euphorbia peplus, also known as the petty spurge. Euphorbia peplus extract has been used for centuries as a topical agent for the treatment of a variety of skin conditions in traditional medicine systems from around the world.

Euphorbia peplus was first taxonomically categorized in the Western scientific community by Carl Linnaeus in the 1750s and presented in a thesis defended by his student Johannes Wiman at Uppsala University in Sweden. Linnaeus described a variety of medicinal uses for the genus of Euphorbia plants as topical treatments and systemic agents for gastrointestinal tract purging. Members of this genus were known to cause skin irritation on contact with the plant’s sap. The genus was named after the ancient Greek physician Euphorbus, who in the first century AD documented the laxative properties of the spurges.

A monograph published in London, England, circa 1770 highlights specific insights into several plants, including E peplus. The manuscript (Figure), published in both Latin and English, likely represents one of the earliest documentations of the dermatologic applications after Linnaean classification. The monograph authors describe “the milky fluid which it abounds with, is by some applied to Warts, which it is said to destroy.” The other members of the Euphorbia genus, particularly Euphorbia helioscopia, or sun spurge, were also recognized to have sap with similar properties in the monograph.

A later selection from the same monograph discusses the sun spurge or “wart-wort” species in greater detail, including its toxicity. “My friend Mr William Wavell lately informed me of a case which fell under his notice in the Isle of Wight, where from the application of the juice of this Spurge [E helioscopia] to some Warts near the eye of a little girl, the whole face became inflamed to a very great degree,” noted the author of the monograph.

Consistent with these case reports from more than 2 centuries earlier, most patients enrolled in clinical trials demonstrating the efficacy of ingenol mebutate for actinic keratoses developed clinically significant erythema at the site of application. It is also notable that a lower concentration of the drug is approved for treatment of the face and that the most common adverse effects of ingenol mebutate in the aforementioned clinical trials were pruritus, irritation, and pain—echoing the cautionary case described in the monograph. As future work unfolds examining additional applications for topical ingenol mebutate, looking back into the past may help uncover other natural remedies awaiting our rediscovery.

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