BRAF Wild-Type Melanoma in Situ Arising In a BRAF V600E Mutant Dysplastic Nevus

Jean-Marie Tan, MB, BCh, BAQ (Hons); Lynlee L. Lin, BSc; Duncan Lambie, BDSc, MBBS, FRCPA; Ross Flewell-Smith, BCom, BEng; Kasturee Jagirdar, MSc; Helmut Schaider, MD; Richard A. Sturm, PhD; Tarl W. Prow, BS, MSc, PhD; H. Peter Soyer, MD, FACD

IMPORTANCE The BRAF V600E mutation accounts for the majority of BRAF mutations found in cutaneous melanoma and is also commonly found in nevi. We used dermoscopy-targeted sampling and a microbiopsy device coupled with DNA sequence analysis to highlight BRAF V600E heterogeneity within a multicomponent melanocytic proliferation. This sampling technique demonstrates the prospect of in vivo application in a clinical setting.

OBSERVATIONS A man in his 50s with Fitzpatrick skin type II presented with an irregularly pigmented melanocytic lesion on his back that met melanoma-specific dermoscopic criteria, and diagnostic shave excision of the lesion was performed. Histopathologic analysis revealed a melanoma in situ arising in a dysplastic nevus. Dermoscopy-targeted microbiopsy specimens were taken across the lesion, and genotyping was carried out on extracted DNA samples for BRAF and NRAS mutations. The melanoma in situ showed only BRAF wild-type results, while the dysplastic nevus showed both BRAF wild-type and BRAF V600E mutations. Sequencing in all DNA samples revealed NRAS wild-type genotype.

CONCLUSIONS AND RELEVANCE Dermoscopy-targeted sampling and genotyping of a melanoma in situ arising in a dysplastic nevus revealed a phenotype-genotype paradox that confounds the exclusive significance of BRAF and NRAS mutations in melanoma pathogenesis. Further studies are required to investigate the importance of other candidate genes linked to melanomagenesis.

Report of a Case

This study was approved by the Princess Alexandra Hospital human research ethics committee. The patient provided written informed consent in accordance with the Declaration of Helsinki.

A man in his 50s with Fitzpatrick skin type II and a family history of melanoma presented with a 5 × 3-mm irregularly pigmented melanocytic lesion on his left lower back detected on routine skin examination. The patient underwent imaging with the FotoFinder system (FotoFinder Systems Inc) of all nevi larger than 5 mm and also those between 2 and 5 mm on the back as part of a study on nevus morphology. The total nevi count was 42, with 36 nevi on the back and 6 nevi on the rest
of the body; 35 of 36 of the nevi on the back measured between 2 and 5 mm. The patient’s germline MC1R genotype was wild-type.

Dermoscopy unveiled a pigmented macule with multi-component pattern (Figure 1A, Figure 2A, and Figure 3A)—a normal reticular pattern on the left side of the lesion, some confluent blue areas at the center of the lesion, and a well-circumscribed area measuring 1 × 1 mm and characterized by an atypical pigment network and a few irregular streaks on the right side of the lesion at the 4-o’clock corner. The lesion met the 3-point checklist criteria for a melanoma with the presence of 2 features in that it had asymmetry and an atypical pigment network. It also showed melanoma-specific local criteria (atypical pigment network and irregular streaks), and so a diagnostic shave excision of the lesion was performed.

The excised lesion was evaluated by 2 independent board-certified dermatopathologists (D.L. and H.P.S.). Histopathologic findings of the left side of the lesion were consistent with a dysplastic nevus characterized by elongated pigmented rete ridges, discrete nests of melanocytes, and few single melanocytes mostly along the dermoepidermal junction; a microbiopsy defect can be seen in this frame (arrowhead).

A, Circled area of the dermoscopy image reveals a normal reticular pattern without any dermoscopic criteria suggestive for melanoma; dermoscopy-targeted microbiopsy samples were taken from this circled area; vertical line corresponds to the site of the specimen shown in panel B. B, Photomicrograph of the histologic specimen reveals a rather conventional dysplastic nevus; boxed area corresponds to the magnified area shown in panel C. C, Higher magnification of the boxed section displays elongated pigmented rete ridges, discrete nests of melanocytes, and few single melanocytes, mostly along the dermoepidermal junction; a microbiopsy defect can be seen in this frame (arrowhead).

A, Circled area of the dermoscopy image reveals a slightly more pigmented typical pigment network and subtle blue areas; dermoscopy-targeted microbiopsy samples were taken from this circled area; vertical line corresponds to the site of the specimen shown in panel B. B, Photomicrograph of the histologic specimen reveals a dysplastic nevus without any remarkable histopathologic findings; boxed area corresponds to the magnified area shown in panel C. C, Higher magnification of the boxed section exhibits a slightly increased number of melanophages in the papillary dermis, most probably reflecting the subtle bluish pigmentation at the center of the lesion.
cal melanocytes at all levels of the epidermis corresponding to the well-circumscribed area at the 4-o’clock corner arising in an otherwise conventional predominantly junctional dysplastic nevus (Figure 3).

We used a new miniaturized biopsy device to sample lesional tissue for molecular analysis. This sampling technique has been shown not to cause significant disruption to the lesion architecture and thus would not compromise the histopathologic assessment. First, a transparent plate with a pinhole was coupled with a dermoscope to visualize the desired area for sampling. The dermoscope was removed once the area was identified through the pinhole, leaving the plate in contact with the lesion and rendering the area for sampling accessible to the biopsy device microneedle. The microbiopsy device applicator was then aligned with the pinhole allowing for targeted sampling of the tissue. Three dermoscopy-guided microbiopsy specimens were taken from each of the described dermoscopic areas in the excised lesion using this technique (Figure 4A).

Samples of DNA were extracted from the microbiopsy tissue specimens using the QIAamp DNA Micro Kit followed by whole genomic amplification carried out with REPlI-g Single Cell Kit according to manufacturer’s protocols (QIAGEN). The quality and integrity of amplified DNA was validated using the Bioanalyzer DNA 12000 kit (Agilent Technologies Inc). Genespecific forward and reverse primers flanking BRAF exon 15 and NRAS exon 2 mutation hotspots were used for polymerase chain reaction. Sanger sequencing was then carried out on the DNA amplified products for BRAF and NRAS mutation detection, with BRAF V600E mutation detection confirmed using a MALDI-TOF mass spectrometry assay (eFigure in the Supplement).

The microbiopsy-extracted DNA samples from the left side and center of the lesion revealed BRAF V600E heterogeneity, while no BRAF V600E mutation was found in any of the samples taken from the melanoma in situ (Figure 4B). Of note, molecular sequencing of all microbiopsy specimens across the lesion revealed no NRAS mutation at codon 61. Thus, somatic genomic profiling and molecular landscaping of this lesion revealed the paradoxical finding of a melanoma in situ with BRAF wild-type DNA arising in a dysplastic nevus heterogeneous for the BRAF V600E mutation.

**Discussion**

We describe herein the use of a miniaturized biopsy device to evaluate a melanoma in situ with a BRAF wild-type DNA sequence developing within a heterogeneous BRAF V600E mutant dysplastic nevus. A dermoscope was used as a targeting tool to improve the sampling of distinct dermoscopic areas from within this multicomponent lesion. We observed that all samples from the melanoma in situ were concordantly BRAF wild-type, whereas the BRAF V600E mutation was detected in the other 2 dermoscopic areas within the same melanocytic lesion.

Mutation of the BRAF gene in melanoma arising within nevi was recently studied using DNA extraction of 1 or multiple laser-capture-microdissected, formalin-fixed, paraffin-embedded, 5-μm-thick histologic sections that were hematoxylin-eosin stained and then subjected to Sanger sequencing and immunohistochemical analysis. This study observed 5 cases (about 11%) of BRAF wild-type superficial spreading melanoma adjacent to a BRAF V600E mutated nevus, which was not statistically significant. Additionally, the investigators found melanoma-associated nevi to be either strictly dermal or compound type, leading Tschandl et al to conclude that their phenotypic and genotypic findings did not mirror the traditional model of stepwise tumor progression. In comparison, we were able to demonstrate this phenomenon in a thinner melanoma using dermoscopy-targeted sampling with a microbiopsy device coupled with DNA sequence analysis, accentuating the potential role of this minimally invasive tech-
nique in providing insight into intraleisonal heterogeneity in melanocytic lesions in vivo. Moreover, the phenotypic characteristics of our melanocytic lesion fits with the Clark clinicopathologic model in that the melanoma developed within a dysplastic nevus, though this is undermined by the paradox in our lesion’s genetic signature.

Although histopathologic criteria have traditionally been the practical reference standard in melanoma diagnosis and staging, recent discovery of tumors harboring different oncogenic mutations has implicated the existence of molecular subgroups of melanoma. The evaluation of early cutaneous melanomas in particular would benefit from a shift toward an integrated phenotype-genotype melanoma classification system, instead of using morphologic criteria alone, because this enables future development of melanoma-specific biomarkers to aid in melanoma detection independent of disease stage. Other candidate genes linked to melanogenesis such as cKIT, GNAQ, GNA11, RAC1, TERT, PTEN, and NF1 remain to be studied in the lesion described herein.

Similarly, BRAF and NRAS gene mutations have been used as genetic markers in studies attempting to integrate genetic and morphologic features to improve melanoma classification. However, the high concordance rate (80.4%) of BRAF mutation between melanoma and its associated nevus counterpart demonstrated by Tschandl et al as well as the occurrence of BRAF wild-type melanoma arising in a BRAF V600E mutated nevus suggest that other molecular signatures are involved in melanoma development. One may argue that our findings might result from the microneedle deviating from the exact desired location despite an attempt to improve sampling accuracy using a dermoscope, as well as the remote possibility that the microneedle did not sample melanocytes and so consequently yielded the wild-type sequence. Nonetheless, conventional methods such as laser-capture microdissection also present a probability of not sampling BRAF-mutant melanocytes.

Conclusions

Our observation supports other reports on the limitations of current melanoma biomarkers in deconstructing the molecular events responsible for tumorigenesis. Further large-scale studies are required to investigate the significance of other candidate genes linked to melanogenesis. Translation of a minimally invasive in vivo microbiopsy device in sampling for melanoma-specific biomarkers may have a prospective role in the next phase of melanoma diagnosis, risk stratification, and personalized therapeutics.
In The Jungle, 1 author Upton Sinclair uses cold exposure injuries to illustrate exploitation of America’s laboring classes by ruthlessly venal industrialists during the early 20th century. In the book, working-class immigrants in Chicago’s meatpacking industry suffer varying degrees of cold injury as a consequence of hostile working conditions. Injuries range from milder forms, including frostnip (superficial, local paresthesias without tissue destruction), chilblains (painful edematous erythematous lesions due to acute or repetitive exposure to near-freezing cold), to more serious conditions, such as frostbite (tissue destruction). There was no heat upon the killing beds; the men might exactly as well have worked out of doors all winter. For that matter, there was very little heat anywhere in the building, except in the cooking rooms and such places—and it was the men who worked in those who ran the most risk of all, because whenever they had to pass to another room they had to go through ice-cold corridors, and sometimes with nothing on above the waist except a sleeveless undershirt. On the killing beds you were apt to be covered with blood, and it would freeze solid. (chapter 7)

Stanislovas came … screaming with pain. They unwrapped him, and a man began vigorously rubbing his ears; and as they were frozen stiff, it took only two or three rubs to break them short off. (chapter 7)

All that they knew how to do was to hold the frozen fingers near the fire, and so little Stanislovas spent most of the day dancing about in horrible agony. (chapter 12)

In his novel, Sinclair 1 hoped to expose rampant health violations and unsanitary work practices, and to convey the hopelessness and widespread exploitation of America’s working class during the waning years of America’s Gilded Age and the early 20th century. Public response ultimately pressured Congress to enact the Meat Inspection Act and the Pure Food and Drug Act in 1906, which strengthened regulation of the meatpacking industry and improved treatment of its employees. 2 Great advances in public health have been made since publication of The Jungle, but according to Human Rights Watch, meatpacking remains the “most dangerous factory job in the United States.” 3 In addition, the demography of meatpacking workers has shifted from mainly southern and eastern European immigrants during the early 20th century (eg, The Jungle’s protagonist, Jurgis Rudkis, whose family immigrated from Lithuania) to today’s meatpacking workforce, where most laborers come from Mexico and Central and South America. Although threats of severe cold exposure injuries may evoke a distant era, the workforce of America’s meat and poultry industry allegedly still endures unsafe working conditions, 3 suggesting further public advocacy to prevent hazardous (although probably both legal and profitable) practices that perpetuate unsafe working conditions.

Author Affiliations: Georgetown University School of Medicine, Washington, DC (Banzon); Georgetown University–Washington Hospital Center, Department of Dermatology, Washington, DC (Norton).

Corresponding Author: Tina M. Banzon, Georgetown University School of Medicine, 3900 Reservoir Rd, Washington, DC 20007 (tmb43@hoyamail.georgetown.edu).