Clinical Implications

- Use of dipeptidyl peptidase-4 inhibitors (gliptins), especially vildagliptin, markedly increases the risk for bullous pemphigoid (BP).
- It is currently unclear whether gliptin-associated BP has specific immunological or phenotypical properties that are distinct from those of BP in individuals who have not received gliptins.
- Although the effect of cessation of gliptin treatment on the clinical outcome of BP is not known at the moment, it may be prudent to replace gliptins with another diabetes medication.


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Large-Giant Congenital Melanocytic Nevi: Moving Beyond NRAS Mutations

Mitchell S. Stark

Large-giant congenital melanocytic nevi have been well characterized clinically, yet questions remain about the heterogenous phenotypes observed. Martins da Silva et al. (2018) highlight the genotypic diversity between “classic” and “spilus-like” congenital melanocytic nevi by analyzing multiple biopsy sites and matching satellite nevi. This study provides evidence for alternative modes of development beyond the well-established NRAS mutation paradigm.


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Clinical Implications

- Somatic mosaicism contributes to congenital melanocytic nevi (CMN) formation and data from Martins da Silva et al. (2018) point to mosaicism occurring within the lesion, which may contribute to lesion heterogeneity.
- Large-giant CMN have a higher malignant transformation rate compared to common acquired nevi, which cannot be explained by the presence of mutations in MAPK pathway genes, including NRAS.
- The post-zygotic initiating event remains to be discovered in ~40% of all large-giant CMN.

Present at birth, congenital melanocytic nevi (CMN) range in size from small, medium, or large to giant, and are historically known to be associated with a high frequency of NRAS mutations. On the other hand, common acquired melanocytic nevi (AMN) are considered to have mutually exclusive mutations in BRAF (85%) or NRAS (15%) (Tan et al., 2018). Despite these frequent somatic mutations, acquired nevi rarely transform into melanoma, whereas large-giant CMN have a relatively high transformation rate (10–15%) (Kinsler et al., 2017). Small to medium CMN also have higher transformation rates than AMN. Importantly, people with large-giant CMN are also prone to melanoma in unaffected skin as well as in the central nervous system (Kinsler et al., 2017). AMN were recently discovered to be largely induced by UVR, sharing a common UV signature with cutaneous melanoma (Stark et al., 2018). The genomic events that transition an AMN to a melanoma have been determined (Shain et al., 2018). However, the same cannot be said for CMN, as NRAS mutations are not sufficient to induce malignant transformation. Additionally, what remains to be thoroughly investigated is whether CMN harbor the same somatic alterations across the large CMN and associated satellite nevi. Martins da Silva et al. (2018) aimed to study multiple biopsied regions within large-giant CMN as well as matching satellite nevi. They utilized a focused approach of ultra-deep targeted panel next-generation sequencing to identify the presence or absence of NRAS mutations (plus other genes, such as BRAF, KRAS, KIT, and GNAQ) together with fusion gene analysis (via RNA sequencing) in an attempt to move “beyond NRAS,” as their title suggests.

Importantly, in five additional biopsy sites, no mutations (e.g., NRAS or BRAF) or fusion genes were detected, which clearly indicates congenital melanocytic nevi develop via alternative mechanisms.

WHY THE FOCUS ON NRAS?

If we step back in time, before the advent of next-generation sequencing technologies that enable an unbiased, comprehensive assessment, generally speaking, genomics analysis has been restricted to candidate gene approaches based upon a priori knowledge. In the 1980s, the RAS pathway genes—HRAS, KRAS, and NRAS—were identified as some of the earliest genes involved in neoplastic transformation and, as such, have been the focus of research in a number of malignancies, including melanoma. In sum, NRAS mutations are common in melanoma, whereas HRAS or KRAS mutations are rare, which suggests melanocyte specificity. Evidence for this can be observed in studies involving Ink4a/Arf-deficient mice (CDKN2A locus) with dominant-active human NRAS (N-RasQ61K) targeted to the melanocyte lineage, whereby 90% of mice develop melanoma at 6 months (Ackermann et al., 2005). In contrast to this, in an earlier study, Chin et al. (1997) used Ink4a/Arf-deficient mice with HRAS as the dominant mutation, which resulted in 50% of melanoma development over the same timeframe. To help explain the differences in disease penetrance, Whitvam et al. (2007) assessed all RAS genes and revealed that the NRAS mutation in Ink4a/Arf-deficient melanocytes led to increased cellular proliferation. Importantly, however, despite the activation of the RAS pathway, the take-home message is that NRAS mutations in isolation are not sufficient to cause malignant transformation and require loss of function alterations to occur in tumor suppressor genes, such as CDKN2A.

A BRIEF HISTORY OF MUTATION ANALYSIS OF CONGENITAL MELANOCYTIC NEVI

The earliest report of the involvement of the NRAS oncogene with CMN was in a study by Carr and Mackie (1994), who found NRAS to be mutated in 12 of 43 (28%) specimens. In a follow-up study, Papp et al. (1999) found that 10 of 18 (56%) CMN had an NRAS mutation, and that this mutation was not always present in multiple CMN from the same person. At the time, this study suggested that NRAS mutations were not an essential primary event in CMN formation (Papp et al., 1999). Since then, in a study of large-giant CMN, Charbel et al. (2014) used a variety of sensitive detection methods and concluded that NRAS was the sole recurrent somatic mutation present in 95% of assessed specimens. The authors further assessed five specimens using whole-exome sequencing and found that NRAS was likely to be the initiating event, as no other mutations were found to be at a higher mutation frequency (Charbel et al., 2014). Like AMN, CMN are heterogeneous in many aspects of their clinical appearance (e.g., size, color, and shape). Often congenital nevi are too large to be removed surgically, so for diagnostic and research purposes, a small biopsy is taken for pathologic and molecular analysis. In comparison to AMN, the molecular analyses of CMN have been limited, and each study varies in sample size and heterogeneity of clinical specimens, and they have a diverse range of detection methods. As such, no studies are directly comparable. However, across all studies of CMN, a common theme emerged, with mutations in NRAS being the most common UV signature with cutaneous melanocytes, which suggests melanocyte specificity. Evidence for this can be observed in studies involving Ink4a/Arf-deficient mice (CDKN2A locus) with dominant-active human NRAS (N-RasQ61K) targeted to the melanocyte lineage, whereby 90% of mice develop melanoma at 6 months (Ackermann et al., 2005). In contrast to this, in an earlier study, Chin et al. (1997) used Ink4a/Arf-deficient mice with HRAS as the dominant mutation, which resulted in 50% of melanoma development over the same timeframe. To help explain the differences in disease penetrance, Whitvam et al. (2007) assessed all RAS genes and revealed that the NRAS mutation in Ink4a/Arf-deficient melanocytes led to increased cellular proliferation. Importantly, however, despite the activation of the RAS pathway, the take-home message is that NRAS mutations in isolation are not sufficient to cause malignant transformation and require loss of function alterations to occur in tumor suppressor genes, such as CDKN2A.

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commonly known somatic alteration, and it has long been suggested that it is the initiating, post-zygotic mutational event that is the cause of congenital nevus development. Moreover, because NRAS mutations are considered to be present during development, the pattern that the CMN forms on the skin must be the result of mosaicism (Kinsler et al., 2017), as NRAS mutations are not detectable in the unaffected skin.

MOVING BEYOND NRAS MUTATIONS

While targeted panel sequencing is limited to a set number of genes, it does allow for ultra-high depth of coverage to detect rare mutational events in known genes like NRAS, which reduces the possibility of false negatives. Martins da Silva et al. (2018) analyzed 21 large-giant CMN, which were further classified as classic (57%) or spilus-like (43%), depending on their clinical appearance. There were a total of 54 biopsies panel-sequenced from the 21 study participants and, in most cases, multiple biopsies per participants were available (Supplementary Table S1 online summarizes the data presented in Martins da Silva et al., 2018). While nevus cellularity was not assessed in this study, one can safely assume that it was the dominant cell type in the biopsied material. Taking into account the mutant allele frequency (MAF), if a given CMN was initiated and driven by a post-zygotic NRAS mutation, the MAF should be relatively high. Classic CMN ranged from 20% to 57% MAF in the main lesion and the spilus-like lesions ranged from 3.6% to 59%. In biopsies with an MAF of <10%, these mutation events are unlikely to be the initiating event. With this in mind, classic lesions had 7 of 12 (58%) NRAS mutations and were likely driven by this mutation. In the classic lesions, when NRAS mutations were not present, novel fusion genes (ZEB2-ALK and SOX5-RAF1 fusion) were found in the main lesions, as well as matching biopsies (samples 3 and 4). Spilus-like CMN were overall more diverse, with 5 of 9 (56%) driven by NRAS/KRAS/BRAF. Samples 14 and 21 were both NRAS-mutant, but had an MAF of 3.6% and 5%, respectively, which are unlikely to be initiating events. Importantly, in combination, six (28%) lesions harbored no known mutation or had low MAF mutations or non-initiating mutations present. The total frequency of NRAS mutations is in line with one of the original studies that postulated that NRAS might not be required for CMN development (Papp et al., 1999). Martins da Silva et al. (2018) proposed that the differing MAFs observed, or the detection of no mutations, might be the result of low-nevus cell number present within the biopsied tissue; however, this is not very likely. While there are no histopathologic images to confirm the cellularity of each specimen, one would assume that the cellularity would be sufficient, given the size of the overall lesion. Even if the cellularity was as low as 1% nevus cell, the depth of sequencing was more than sufficient (median coverage approximately ×19,000) to detect somatic mutations. Importantly, Martins da Silva et al. (2018) found evidence for non-uniform or mosaic-like development of CMN with assessable mutation frequencies available across the main CMN and/or matching satellite nevi. From the 14 assessable study participants (samples 1, 3–5, 7–10, 13–17, and 20) that had detectable mutations or fusion-genes, three had different mutations present across the biopsies, or were not detected (samples 16, 17, and 20), and seven had the same somatic alteration (samples 1, 5, 9, 15, 16, and 20), but had differing MAFs (see Supplementary Table S1). For example, sample 9, which has a classic CMN phenotype, has the NRAS mutation present in the main lesion at 57% MAF, whereas in two different biopsy sites, the NRAS MAF is only 35%. The matching satellite lesion is 27% for the same NRAS mutation. Using this example and assuming that the nevus cellularity was equal, the biopsy with 57% MAF must have developed first, followed by the other biopsied regions and then the satellite lesion. Another example is sample 16 (spilus-like), which has a BRAF G464E mutation present at 24% MAF and then in another biopsy site, a different BRAF mutation is present (L584F). Importantly, in five additional biopsy sites, no mutations (e.g., NRAS or BRAF) or fusion genes were detected, which clearly indicates CMN develop via alternative mechanisms. Next, Martins da Silva et al. (2018) assessed whether the phenotypic characteristics (e.g., patterns of anatomic distribution, rugosity, hypertrichosis, and number of satellites) of the large-giant CMN were associated with NRAS mutation. The study numbers are relatively small but no associations with the presence of an NRAS mutation were identified.

WHERE TO FROM HERE?

NRAS mutations play an important role in CMN, but there is still much to be learned from CMN. Martins da Silva et al. (2018) performed whole transcriptome sequencing to detect fusion genes. It is anticipated that these data will expand upon our current paradigm to provide novel insights into the signaling pathways involved in CMN development. We presume that the MAPK pathway will be a dominant player; given the proportion of RAS/RAF mutations, but at this juncture a conclusion cannot be drawn. This study has highlighted that classic and spilus-like CMN are both phenotypically and genotypically diverse. Furthermore, it can be concluded that phenotypic characteristics, such as patterns of anatomial distribution, rugosity, hypertrichosis, and number of satellites, are not related to common point mutations in NRAS or BRAF. Perhaps the transcriptome analysis will assist in determining causality? Lastly, because large-giant CMN are prone to melanoma development, the underlying mechanisms remain to be elucidated, which may pave the way for novel therapeutic targets.

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CONFLICT OF INTEREST

The author states no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.10.003.

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