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Unfolding the Mutational Landscape of Human Melanoma

Diwakar Davar[1], **Yan Lin**[2], and **John M. Kirkwood**[1]

[1]Department of Medicine, Division of Hematology-Oncology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

[2]Biostatistics Facility, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania, USA

Abstract

Over the preceding two decades, sophisticated sequencing techniques have been used to characterize the genetic drivers of adult melanoma. However, our understanding of pediatric melanomas is still rudimentary. In this report, we comment on a thorough multi-platform analysis of common pediatric melanoma subsets, including pediatric conventional melanoma (CM), congenital nevus-derived melanoma (CNM), and Spitzoid melanoma (SM), contributed by Lu *et al.*

Data from whole-genome and -exome sequencing (WGS/WES) and The Cancer Genome Atlas (TCGA) have revealed multiple new insights to the nature of cutaneous melanoma, etiology, and potential avenues of therapy. First, the role of oral BRAF/MEK inhibitors (based upon the frequency of ~50% with UV signature activating mutations in *BRAF* and the *MAPK* pathway) has become abundantly apparent. Further, the mutational landscape and high frequency of mutations observed in cutaneous melanoma, which exceeds those of all other solid tumors, have also provided support for our pursuit of immunotherapies that historically have been singularly successful in treating melanoma. The prospects for the therapy of melanoma, with two BRAF inhibitors, one MEK inhibitor, and the first combination of these agents, as well as five immunotherapies approved to date, have expanded remarkably in the past several years. A neglected but important aspect of the problem of melanoma is with respect to the patients who develop this disease at or before puberty. These pediatric and young adult (PAYA) melanomas have been studied clinically and demographically, but until recently they have not been characterized with respect to the fundamental drivers of melanoma (Averbook *et al.*, 2013; McCormack *et al.*, 2014). The paradoxes of the childhood melanomas are many—beginning with the entity of spindle cell or spitzoid lesions, which are often difficult to define pathologically or clinically, and the fraction of melanomas that are derived from congenital garment–type nevi, which have been the focus of much attention but few rigorous studies. However, what has been termed “conventional melanoma” in the PAYA population is clinically indistinguishable from the adult counterpart of the disease, and it remains the largest fraction. Here, the role of UV

Correspondence: John M. Kirkwood, Department of Medicine, Division of Hematology-Oncology, University of Pittsburgh Medical Center, 5150 Centre Avenue, Pittsburgh 15232, Pennsylvania, USA. kirkwoodjm@upmc.edu.

CONFLICT OF INTEREST

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mutagenesis might have been presumed to be less than that for adults—given the shorter potential for UV exposure. Further, the role and meaning of lymphatic traffic have been of interest, given the regular pursuit of sentinel node biopsy in lesions over stage IA among adults. However, the role and meaning of lymphatic pathways of dissemination have been confounded by the paradox that the melanomas of the young are more likely to be detected in regional nodal basins thickness-for-thickness than among older patients (Sondak *et al.*, 2004)—hence, the question of the biological consequences of nodal disease detected in the pediatric and adolescent/young adult populations remains unanswered.

Our understanding of genomic drivers in melanoma has paralleled the increasing sophistication of sequencing techniques over the last two decades. Early studies utilized pyrosequencing, a modification of the Sanger “chain termination” method, and mapped melanoma susceptibility loci to individual chromosomal alterations on chromosomes 9p and 10q—since determined to be the loci of tumor suppressor genes CDKN2A and PTEN, respectively (Herbst *et al.*, 1994; Kamb *et al.*, 1994; Guldberg *et al.*, 1997). First generation studies relied on hotspot sequencing and required some knowledge of where mutations were located; these were consequently limited in bandwidth and throughput. Development of less expensive high-throughput sequencing technologies spurred interest in genome-wide techniques such as comparative genomic hybridization, permitting detection of DNA copy number variants (CNV) on formalin-fixed paraffin-embedded (FFPE) tissues. Various CNVs, including loss of 9p/10q, focal loss of 6q/8p, and gains/amplifications of 1q/2/4q/6p/7/8/11q/17/20, were frequently found in melanoma but absent in benign nevi (Bastian *et al.*, 1998). Classification of melanomas was advanced significantly by Curtin *et al.* (2005) who accurately subclassified cutaneous melanomas on the basis of the number of chromosomal aberrations, and *BRAF/NRAS* mutational status, into cutaneous melanomas with associated chronic sun damage (CSD), those without CSD, and mucosal and acral melanomas (also without solar damage). Subsequently, Bastian and colleagues established that mutations and/or copy number increases in *KIT* and *GNAQ11/GNAS* were important drivers of mucosal and uveal melanoma respectively (Curtin *et al.*, 2006; Van Raamsdonk *et al.*, 2009). These findings have directly parsed with the development of inhibitors targeting the mitogen-activated protein kinase (MAPK) pathway and the regulatory approval of BRAF inhibitors vemurafenib (Zelboraf, Genentech) and dabrafenib (Tafinlar, GlaxoSmithKline) and MEK inhibitor trametinib (Mekinist, GlaxoSmithKline) as well as the combination of dabrafenib and trametinib for treatment of *BRAF* mutant melanoma.

Identification of oncogenic drivers in adult cutaneous melanoma has been complicated by the unprecedented high baseline frequency of somatic mutations in melanoma tumor tissue (~14 mutations per megabase). This rate is higher compared with that of any other solid tumor and partially reflective of the etiologic role of UV radiation exposure (Lawrence *et al.*, 2013). Hence, approaches that rely on statistical methods to distinguish mutational hotspots from mutationally quiescent areas are unable to reliably distinguish driver from passenger mutations in this disease. Exposure to UV radiation is associated with single base mutations (typically cytidine to thymidine (C → T) substitutions in pyrimidines), which constitute a “UV radiation–induced mutational signature” (Pfeifer *et al.*, 2005; Greenman *et al.*, 2007). More recently, high-throughput approaches with next-generation sequencing

(NGS) have been utilized to analyze WGS/WES, while compensating for elevated baseline mutation frequencies and the prevalence of UV mutational signatures, and have identified novel genes, including ARID2, PPP6C, RAC1, SNX31, STK19, and TACC1 in melanoma tumorigenesis (Hodis *et al.*, 2012; Krauthammer *et al.*, 2012). These genes are involved in canonical cellular pathways such as RB, p53, and Aurora kinase and their dysregulation suggest new potential targets for rational drug development.

Contrary to the situation in adult melanoma, much less is known about pediatric melanoma. Pediatric melanomas are rare—accounting for only 2% of all malignancies in patients below 20 years of age, the majority of which (75%) were seen to occur in patients between 15 and 19 years in a 2006–2010 SEER analysis (Howlader *et al.*, 2014). For reasons that likely include increasing recreational sun exposure, the incidence of pediatric melanoma is rising, especially in the cohort of patients aged 15–19. The actual incidence may be higher because of the inherent diagnostic challenges associated with pigmented skin lesions in pediatric patients. At least three distinct melanocytic lesions have been identified in this age cohort, each with a unique pattern of clinical behavior and a distinct molecular profile. Similar to adult melanoma, pediatric patients with distant metastatic disease have a high mortality rate exceeding 80% (Lange *et al.*, 2007). A recent report of a cooperative group registry of melanomas and atypical melanocytic lesions in PAYA patients has shown that many of the same prognostic features that have been of use in prognosticating risk in adult melanoma do apply, without significant modification for the younger population (Averbook *et al.*, 2013).

It is against this background that Lu *et al.* (2014) have characterized the mutational landscape of what has been designated “conventional melanoma” (CM), in relation to congenital nevus–derived melanoma (CNM) and the PAYA entity known as spitzoid melanoma (SM) in a cohort of 23 pediatric patients using several platforms, including WGS/WES, the molecular inversion probe assay, and targeted sequencing (Lu *et al.*, 2014). The authors are to be commended for conducting the first large-scale well-annotated analysis of pediatric patients using clinically relevant tumor subtypes. In CM, the authors noted a high somatic mutation burden (14.36 mutations per megabase) that mirrors adult melanoma, with a high percentage (>80%) of single base mutations (typically C → T or A → G transitions), most (>90%) of which occurred 3’ to pyrimidine base, a high ratio of synonymous to silent (1.27–2.22) mutations, and a lower mutation frequency on the transcribed compared with the untranscribed strand. Most CM patients had mutations in *BRAF* (11/13 patients) and *TERT-p* (12/15 patients), while approximately half had PTEN copy number changes. Interestingly, *TERT-p* and *BRAF* mutations were not mutually exclusive. In CNM and SM, *BRAF* mutations were not commonly identified, although *NRAS* mutations were identified in both CNM patients. *TERT-p* mutations were uncommon in both CNM and SM, although the sole SM patient whose disease spread hematogenously had a *TERT-p* mutation. Two of the five SM patients had novel kinase fusions (*NTRK1* and *BRAF*), whereas all three CNM patients (and only these patients in this series) had *NRAS* mutations. Although enticing and thought provoking, the low numbers of patients in the CNM and SM samples—3 CNM and 2 SM respectively—preclude significant conclusions being drawn.

In summary, the authors of this paper are to be commended for performing the first large-scale, well-annotated genetic and molecular analysis of pediatric melanomas. It is self-

evident that pediatric CM and adult cutaneous melanoma are essentially the same disease. Similar to adult melanoma, pediatric CM is associated with a high somatic mutation load, high frequencies of activating *BRAF* mutations, and PTEN copy number changes, with resulting activation of MAPK and PI3K/AKT cellular signaling pathways. Given the poor prognosis of these patients, pediatric CM patients should be staged and followed in a manner similar to adult patients. Promising trials of immunotherapy, targeted therapy, and/or combinations hitherto restricted to adult patients aged 18 and above should consider enrolling PAYA patients with advanced melanoma.

Pediatric CNM and SM are distinct clinical entities. The high incidence of *NRAS* mutations, high somatic mutation burden, and grim prognosis in CNM contrasts distinctly with the absence of *BRAF/NRAS* mutations, low-somatic mutation burden, and potentially more benign prognosis of SM. Further study is required to characterize the natural history of SM and specifically the prognostic role of *TERT-p* mutations as markers of the subset of these tumors that have the capacity for distant metastasis. The observation of novel kinase fusions in SM is supported by earlier data reported by Boris Bastian at ASCO 2013 (Stephens *et al.*, 2013). The kinase fusions observed (Lu *et al.*, NTRK1/BRAF; Stephens *et al.*, NTRK1/BRAF/ROS1/ALK/RET) are constitutively expressed and result in active oncogenes that drive malignant transformation. Should these observations be corroborated, the clinical implications will be tremendous both in terms of diagnosis/prognosis and in the management of advanced disease.

The unraveling of the mutational landscape in adult melanoma in the last decade is a standout success of the genomic revolution in molecular biology and oncology—both in terms of understanding a particularly thorny disease entity, and in terms of developing actionable targets that have improved patient survival. We appear to be on the cusp of a similar revelation in pediatric melanoma, with potentially even greater diagnostic, prognostic, and therapeutic implications associated with the longer life horizons of pediatric patients. The findings of Lu *et al.* (2014) should sound a tocsin to galvanize prospective studies and clinical trial enrolment of patients with PAYA cutaneous melanoma, of the three variants that have now been distinguished: conventional, congenital nevus-derived, and Spitzoid.

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Clinical Implications

- Pediatric CM and adult melanoma are both associated with high rates of somatic mutations and activating BRAF/TERT-p mutations.
- CNM and SM are distinct entities: CNM is characterized by NRAS mutations, a high somatic mutation burden, and a poor prognosis, whereas SM is distinguished by novel kinase fusions, a low somatic mutation load, and a relatively benign prognosis.
- Enrollment of pediatric CM patients in adult melanoma trials and tumor banking of pediatric CNM/SM to further characterize the natural history and etio-pathogenetic features should be a priority.