Brief Report

Molecular Profile of Subungual Melanoma: a MelaNostrum consortium Study of 68 Cases Reporting BRAF, NRAS, KIT, and TERT promoter status.

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Short Title: *BRAF, NRAS, KIT,* and *TERT* promoter mutations in subungual melanoma.

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Number of Tables: 3

Number of Figures: -

Word count: 1,180

Keywords: subungual; melanoma; mutations

Abstract

Background: Subungual melanoma (SM) is an unusual type of melanocytic tumor affecting the nail apparatus. The mutational prevalence of the most prominently mutated genes in melanoma has been reported in small cohorts of SM, with unclear conclusions on whether SM is different from the rest of melanomas arising in acral locations or not. Hence, the molecular profile of a large series of SM is yet to be described.

Objectives: The aim of this study was to describe the molecular characteristics of a large series of SM and their association with demographic and histopathological features.

Methods: Patients diagnosed with SM between 2001 and 2021 were identified from six Spanish and Italian healthcare centers. The mutational status for *BRAF, NRAS, KIT,* and the promoter region of *TERT (TERTp)* were determined either by Sanger sequencing or Next-Generation Sequencing. Clinical data were retrieved from the hospital databases to elucidate potential associations.

Results: A total of 68 SM cases were included. Mutations were most common in *BRAF* (10.3%) and *KIT* (10%), followed by *NRAS* (7.6%), and *TERTp* (3.8%). Their prevalence was similar to that of non-subungual acral melanoma, but higher in SM located on the hand than on the foot.

Conclusions: To date, this study represents the largest cohort of SM patients with data on the known driver gene mutations. The low mutation rate supports a different etiopathogenic mechanism for SM in comparison of non-acral cutaneous melanoma, particularly for SM of the foot.

Introduction

Subungual melanoma (SM) is a very rare subtype representing between 0.7% and 3.5% of all melanomas worldwide [1]. It includes melanocytic tumors arising from structures within the nail apparatus of both at hands and feet [2]. Despite having a similar incidence among ethnicities, the lower incidence of other melanoma types in individuals of non-European descent increases the relative prevalence of SM in these populations [3]. The role of UV radiation in the development of these SM is under debate. Studies published hitherto lean towards an independence from sun exposure – consistent with low melanocyte content of the nail matrix–, but recent findings show UV-induced mutations contributing to the mutational burden of SM [4,5]. A history of trauma has also been suggested over the years as a potential risk factor for SM, possibly related to fibroblast growth factors released in healing wounds which may activate both MAPK and PI3K/AKT pathways in melanocytes [6,7]. Given the rarity of this type of melanoma, collecting many SM patients with available clinical data has been challenging throughout the years. Thus, the etiology of these tumors remain unclear[8,9].

Genetic alterations are known to drive melanoma development. Mutations such as those found in the coding regions of *BRAF*, *NRAS*, or *NF1*, and in the promoter region of *TERT* (*TERTp*) are the most prominently found [10], while other mutations, e.g., in *KIT* or *GNAQ/11* in acral/mucosal or uveal melanoma, respectively are less frequent[11]. Melanoma classification based on molecular subtypes have led to the development of targeted therapies, highlighting the value of molecular biology testing [12]. However, little is known about SM molecular profile, often reported as a subset of large acral melanoma series [5]. Some studies have raised the question of whether SM should be considered as a distinct form from acral melanoma, as a different mutational prevalence was found reporting *KIT* mutations more frequently in SM and *BRAF* mutations in non-subungual acral melanoma[2,13]. This highlights the importance of molecularly characterizing this subtype.

This study aims to describe a large series of SM, focusing on the mutational prevalence of *BRAF, NRAS, KIT,* and *TERTp* genes.

Materials and Methods

All cases of subungual melanoma consecutively diagnosed between 2000 and 2021 with available information about *BRAF*, *NRAS*, *KIT*, and *TERTp* mutational status were collected from 6 European centers: Fundación Instituto Valenciano de Oncología, Hospital Universitari Arnau de Vilanova, Hospital de Bellvitge, Veneto Institute of Oncology, San Gallicano Dermatological Institute, Policlinico

Universitario Fondazione Agostino Gemelli, and University of L'Aquila. These centers are members of "MelaNostrum", an international consortium focused on studying genetic, environmental, and clinical determinants of melanoma risk and progression in Mediterranean populations.

From each melanoma, the following clinical, pathological, and demographic information was retrieved: sex, age, histological type, ulceration, stage, and Breslow thickness. In addition, two groups were defined based on the site of the primary tumor (hand vs. foot).

These samples had undergone mutational determinations according to the following procedures. DNA was extracted from three formalin-fixed paraffin-embedded (FFPE) sections, each 10 μm thick, using the QIAamp DNA Investigator kit (QIAGEN®). Mutational screening for *BRAF*, *NRAS*, *KIT*, and *TERT*p was performed either by Sanger sequencing as described elsewhere[12], or by NGS using the Solid Tumor Solution® by Sophia Genetics© (Supplementary Table 1), focusing on the regions covered by the primers shown in Supplementary Table 2 . A cutoff was established at 15% for the variant allele frequency (VAF). The variants identified were annotated using COSMIC, OncoKb, and My Cancer Genome databases, and only those with a pathogenic or likely pathogenic effect were considered. The statistical analyses were performed using SPSS from IBM Corp. Released 2011 (IBM SPSS Statistics for Macintosh, IBM Corp, version 20.0. Armonk, NY, USA). A chi-square test was applied to evaluate differences among the groups, using a value of $p<0.05$ to define significance.

Results

Sixty-eight SM were included in the study (Table 1). They were similarly distributed between men and women (54.4% vs. 45.6%) and were more common in patients older than 60 years of age (60%). Anatomic location was the foot in 54.4% of cases, and the hand in 45.6%. The histological pattern of SM was prominently acral lentiginous melanoma (95.6%), with only one case displaying a superficial spreading melanoma pattern (1.5%) and two with a nodular melanoma pattern (2.9%). Most SM patients had localized disease (stage I or II) (67.2%), seven patients presented *in situ* SM, and the median Breslow thickness for invasive SM was 2.8 mm (range 0.5-19.25mm; Table 1).

Sanger sequencing data was reported for sixty-two samples (91.2%), while NGS data was reported for six samples (8.8%). Mutational analyses identified *BRAF* (10.3%) and *KIT* (10.0%) as the most frequently mutated genes, followed by *NRAS* (7.6%) and *TERT*p (3.8%). The details on the mutations are shown in Table 2. All *KIT* mutations were identified in ulcerated melanomas (17.9% vs. 0%; p=0.023).

Per anatomical location, hand SM had a higher prevalence of mutations than foot SM (41.4% vs. 16.1%; p=0.045). The mutational frequencies of each gene differed between hand and foot: 19.4% vs. 2.7% (*BRAF*), 10.0% vs. 5.6% (*NRAS*), 13.8% vs. 6.5% (*KIT*), and 7.7% vs. 0% (*TERT*p), respectively, being the difference in *BRAF* mutation prevalence statistically significant (p=0.041) (Table 3). Foot SM presented more frequently in older patients than hand SM, although differences did not reach statistical significance (median age 68 vs. 61; p=0.056) (Table 3).

Discussion/Conclusion

In this study, we have characterized the prevalence of *BRAF*, *NRAS*, *KIT*, and p*TERT* gene mutations in a series of 68 subungual melanomas. The mutational prevalence is similar to that in non-subungual acral melanomas [14–16].Interestingly, we found a similar prevalence of *BRAF* and *KIT* mutations in SM in contrast with previous studies that reported different frequencies in SM [17] or a higher prevalence of *KIT* than *BRAF* mutations in SM vs. non-subungual acral melanomas [13,18].

Interestingly, we showed that mutations in all four genes were more likely to be present in SM of the hand rather than SM of the foot. It could be hypothesized that this fact results from the higher degree of exposure to UV radiation of the hand compared to the feet, which are usually covered by footwear. However, even the mutational prevalence of *BRAF* – which was significantly higher in SM of the hand (19.4%) – was lower than that reported for non-acral cumulative solar damage (CSD) melanomas (37.8%) [10]. Moreover, sunburns are hardly found on the nail plate based on our clinical experience. Hence, the subungual region of the hand seems to have a certain degree of protection that prevents UV-related damage as previously suggested [19,20]. One could speculate that while SM of the hands have more single point mutations, foot SM have higher structural variants or other genomic alterations [15] resulting from traumatic stress, but further studies are needed to confirm this hypothesis.

In conclusion, SM display a mutational profile in four major driver genes similar to that of non-SM acral melanoma, but SM of the hand showed a higher frequency of mutations compared to that of the foot. Additional research is required covering more genes and other genomic alterations in large cohorts to further characterize the SM molecular landscape.

Statement of Ethics

This study is covered by the resolution 39-20 of the Ethics Committee at the Fundación Instituto Valenciano de Oncología. Written informed consent was obtained from participants to participate in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work has been partially funded by the Spanish Ministry of Science (ISCIII-PI19/00667), the Generalitat Valenciana (Prometeo21/067), and the European Academy of Dermatology and Venereology (PPRC-36-2018).

Author Contributions

The authors participated in the design of the study (DME, ZGC, EMS, AG, CR, EN), performing experimental and analyses processes (AM, IR, CTVL, RMP, SP, MRB, PR, ADS, CP, MCF, KP, CC, CM), and writing and review of the manuscript (MTL, EN, DME, ZGC).

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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