Melanoma Tumor Characteristics: An Analysis of Mutational Burden and Copy Number Alterations by Patient Age and Stage


TO THE EDITOR

Malignant melanoma is the fifth most common cancer in the United States across all ages; however, it ranks third among patients aged 15–39 years, those also known as adolescents and young adults (AYAs) (National Cancer Institute, 2015; Weir et al., 2011). There are intrinsic differences in AYAs and older patients with melanomas, with AYAs having female predominance, increased likelihood of developing superficial spreading melanoma, and presence of somatic BRAF and NRAS mutations and germline MC1R mutations (Weir et al., 2011). In contrast, older patients have a male predominance, are more likely to develop lentigo maligna melanoma, develop mutations in NF1 and KIT, and overexpress p53.

Despite its potential importance in elucidating different pathways in melanomagenesis, no comparison of genomic alterations between AYAs and older patients exists (Chalmers et al., 2017). Furthermore, melanoma stage is the key prognostic factor in outcomes of melanoma, and the relationship between stage and mutational burden and copy number alterations (CNAs) has not been well studied. We investigated differences in genomic alterations in melanoma tumors from AYAs and older patients and explored the association between genetic characteristics and melanoma staging using publically available datasets.

Results

Across all studies, 94 patients were AYAs (The Cancer Genome Atlas [TCGA]; n = 50; Hodis et al., 2012: n = 32; Krauthammer et al., 2012: n = 5; Gartner et al., 2013: n = 7), and 507 were older (TCGA: n = 311; Hodis et al., 2012: n = 88; Krauthammer et al., 2012: n = 86; Gartner et al., 2013: n = 22) (see Supplementary Table S1 online). Overall age range was 15–39 years in AYAs and 40–94 years in older patients (see Supplementary Figure S1 online). Females made up 50% of AYAs and 37.5% of older patients. The extremities were the most commonly affected site in both groups.

There were no differences in mutational burden between AYAs and older patients in the combined studies (P = 0.56) (Table 1). More advanced stages of melanoma were not correlated with a higher mutational burden across all ages (P = 0.75) or in younger (P = 0.52) and older (P = 0.65) age when analyzed separately (Figure 1a–c).

Mutations in AHNK2 (P = 0.01) (see Supplementary Table S2 online) and NF1 (P = 0.001) were significantly more likely to occur among older patients, whereas BRAF mutations were more
likely in AYA patients ($P < 0.001$). Mutational burden was higher among RAS ($P < 0.001$) and NF1 ($P < 0.001$) melanoma genetic subtypes but was lower among the triple-wild subtype ($P < 0.001$) (see Supplementary Table S3 online).

Fraction of CNAs did not differ between AYAs and older patients ($P = 0.3$). CNAs were not correlated with melanoma stage across all ages ($P = 0.97$), in AYA patients ($P = 0.21$), and in older patients ($P = 0.9$) (Figure 1d–f). CNA fraction did not vary among the genetic subtypes of melanoma. Finally, total number of mutations ($P = 0.74$) and mutations due to UVR did not differ between AYAs and older patients (84.2% vs. 86.1%, $P = 0.09$).

### Discussion

Normal cellular proliferation is known to result in accumulation of various mutations with age, and melanoma is known as one of the cancers with the highest mutational burden (Martincorena et al., 2015). However, we found that mutational burden of melanoma does not differ between AYAs and older patients. Even normal appearing skin carries thousands of mutations, with the key differentiating factor from tumors being a lower number of driver mutations per cell in healthy skin (Martincorena et al., 2015). Another study found that invasive melanomas have a higher mutational burden compared with benign lesions and increased presence of CNAs; however, they did not examine differences by stage (Shain et al., 2015).

In an effort to classify melanoma based on mutational pattern, TCGA network identified four potential subtypes: the RAS, BRAF, NF1, and triple-wild subtype (Cancer Genome Atlas Network, 2015). Our finding that BRAF mutations were more likely to occur among AYA patients and that NF1 mutations were more likely to be present among older patients are in agreement with this classification, indicating that distinct clinical patterns exist.

BRAF is a proto-oncogene that regulates the MAP kinase/ERK signaling pathway (Kim et al., 2015). Mutations in BRAF are well described in malignant melanoma but have been identified in benign nevi (Shain et al., 2015). Higher incidence of BRAF mutations among patients younger than 50 years has been previously described (Kim et al., 2015).

NF1 is a protein-coding gene that acts as a negative regulator in the Ras pathway (Yap et al., 2014). Approximately 14% of melanomas carry mutations in NF1, and recent reports show that NF1-mutated tumors have a higher mutational burden and occur more often in older patients (Cirenajwis et al., 2017).

Finally, AHNAK2 is a protein-coding gene containing 4,300 amino acids not previously identified to have a relationship with melanoma (Han and Kursula, 2014; Shitivelman et al., 1992). It is expressed in all muscular cells and is involved in cytoarchitecture and calcium signaling by interacting with proteins such as S100B (Han and Kursula, 2014).

Each individual study used different sequencing and computational analyses, as shown by different median mutation rates among the studies. However, mutational burden was similar in the AYA and older age groups in all studies. Nevertheless, the differences in individual gene mutation rates are not always consistent among studies, as seen with regard to AHNAK2, which is found at a higher mutation rate in older individuals in TCGA (Cancer Genome Atlas Network, 2015) and Hodis et al. (2012) but not reproduced in Gartner et al. (2013). Given the differences in study technique and multiple comparisons we have performed, this finding may not be reproducible, and its significance is uncertain.

A limitation of using the TCGA dataset is that the samples consist of thick primary, regional, and distant metastatic sites, and our findings may not be representative of thinner tumors.

We hypothesize that environmental factors and/or germline genetic host factors likely predispose AYA individuals to developing melanoma at an earlier age. It is postulated that two divergent pathways for development of melanoma exist: one caused by intermittent bursts of sun exposure and the other associated with chronic sun exposure (Anderson et al., 2009). Molecular studies found that persons without chronically sun-damaged skin have melanomas with somatic BRAF or NRAS mutations and carry germline MC1R variants, whereas those with significant chronic sun exposure are more likely to have melanomas with somatic mutations in KIT and overexpression of p53 (Anderson et al., 2009).

In conclusion, we found that mutational burden and CNAs did not differ between AYAs and older patients, nor did these characteristics vary by stage. Future studies should explore factors associated with melanoma development in the AYA population, and additional studies sequencing AYA melanoma specifically are needed.

### Methods and materials

#### Study selection.

Publicly accessible genomic datasets with mutational burden of malignant melanoma were identified

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### Table 1. Gene mutations and copy number alteration characteristics by age group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>&lt;40 Years (n = 94)</th>
<th>≥40 Years (n = 507)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutational burden, median (interquartile range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCGA</td>
<td>263.5 (185.75–388.75)</td>
<td>291 (110.00–555.50)</td>
<td>0.84</td>
</tr>
<tr>
<td>Hodis et al., 2012</td>
<td>355.5 (188.75–492.00)</td>
<td>333 (189.00–583.00)</td>
<td>0.68</td>
</tr>
<tr>
<td>Krauthammer et al., 2012</td>
<td>112 (8.00–176.00)</td>
<td>120.5 (26.00–287.50)</td>
<td>0.64</td>
</tr>
<tr>
<td>Gartner et al., 2013</td>
<td>142 (92.50–305.00)</td>
<td>163 (115.25–322.25)</td>
<td>0.5</td>
</tr>
<tr>
<td>Overall</td>
<td>285.5 (143.75–412.75)</td>
<td>268.5 (104.25–550.25)</td>
<td>0.56</td>
</tr>
<tr>
<td>Copy number alterations, mean (standard deviation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCGA</td>
<td>0.35 ± 0.21</td>
<td>0.32 ± 0.21</td>
<td>0.3</td>
</tr>
</tbody>
</table>


Available in TCGA only.

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Mutations in AYA Melanoma
Four studies were identified: TCGA (Cancer Genome Atlas Network, 2015), Hodis et al., 2012; Krauthammer et al., 2012; and Gartner et al., 2013. Each study was accessed, and data regarding clinical variables such as age at diagnosis and procurement, sex, race, site of lesion, Breslow depth, ulceration, mitotic rate, and stage were extracted. Genomic data were assessed for mutational burden, specifically non-synonymous genetic mutations, and fraction of CNAs. Melanoma genetic subtypes were defined as (i) BRAF subtype contains BRAF hotspot mutations; (ii) RAS subtype contains hotspot mutations in NRAS, KRAS, and HRAS; (iii) NF1 contains mutations in NF1; and (iv) triple-wild subtype lacks BRAF, RAS, and NF1 mutations. Mutations due to UVR (C>T change) were assessed from TCGA (Cancer Genome Atlas Network, 2015), Hodis (Hodis et al., 2012), and Gartner (Gartner et al., 2013; Robles-Espinoza et al., 2016). The Supplementary Materials online contain additional methodology.

**Statistical methods.** If age at diagnosis was unavailable, age at sample procurement was used. Normally distributed continuous data were analyzed using t test; otherwise, Wilcoxon ranked sum test was used (see Supplementary Figures S2 and S3 online). Categorical data were evaluated using chi-square test or Fisher exact test. Analysis of variance was used to assess differences in mutational burden or CNAs by stage. An α = 0.05 was considered statistically significant.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.
Role of the Ceramide-CD300f Interaction in Gram-Negative Bacterial Skin Infections

TO THE EDITOR
Acute bacterial skin and skin structure infections (ABSSIs), including cellulitis and erysipelas, wound infections, and abscesses, are the most common infections in health care. Gram-positive organisms predominate among a variety of pathogens that cause ABSSI, but Gram-negative bacterial infections are also a major clinical problem for immunocompromised patients. Although antibiotics are the criterion standard therapy, the prevalence of antibiotic-resistant bacteria prompts us to develop different therapies for ABSSI (Colonna, 2003; Itani and Shorr, 2014; Modlin, 2012; Rittirsch et al., 2008).

CD300f, also called leukocyte mono-immunoglobulin–like receptor 3 (LMIR3) or CMRF-35–like molecule-1 (CLM-1), is an inhibitory receptor that is mainly expressed in myeloid cells, including mast cells and neutrophils (Chung et al., 2003; Izawa et al., 2007). Mouse CD300f recognizes extracellular ceramide in the surroundings of tissue mast cells, thereby suppressing IgE-mediated allergic responses or adenosine triphosphate-mediated colonic inflammation (Izawa et al., 2012; Matsukawa et al., 2016). In addition, the ceramide-CD300f binding inhibits lipopolysaccharide (LPS)-induced skin edema and neutrophil accumulation in mice (Shiba et al., 2017). Conversely, disrupting the ceramide-CD300f interaction prevents cecal ligation and puncture-induced septic peritonitis by promoting the recruitment of neutrophils to sites of infection that efficiently engulf and kill Escherichia coli (Izawa et al., 2017). However, whether CD300f regulates human mast cell and neutrophil activation in response to E. coli or LPS and Gram-negative bacterial skin infections in mice and humans remains elusive.

First, we asked if human CD300f can suppress skin edema induced by...