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

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INTRODUCTION

Malignant melanoma (MM) is the 5th most common cancer in the United States across all ages; however, it ranks third among patients aged 15–39- also known as adolescents and young adults (AYA) (National Cancer Institute, 2015; Weir *et al.*, 2011). There are intrinsic differences in AYA and older melanomas, with AYA having female predominance, increased likelihood of developing superficial spreading melanoma, and presence of somatic BRAF and N-RAS 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 and develop mutations in NF-1 and kit, and overexpress p53.

Despite its potential importance in elucidating different pathways in melanomagenesis, no comparison of genomic alterations between AYA 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 (CNA) has not been well studied. We aim to investigate differences in genomic alterations in melanoma tumors from AYA and older patients and explore the association between genomic characteristics and melanoma staging using publically available datasets.

RESULTS

Across all studies, 94 patients were AYA (TCGA:50; Hodis:32; Krauthammer:5; Gartner:7), while 507 were older (TCGA:311; Hodis:88; Krauthammer:86; Gartner:22) (Table S1). Overall age range was 15–39 in AYA and 40–94 in older patients (Figure S1). Females

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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comprised 50% of AYA 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 AYA and older patients in the combined studies ($p=0.56$, Table 1). Interestingly, 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 *AHNAK2* ($p=0.01$, Table S2) and *NF1* ($p=0.001$) were significantly more likely to occur among older patients, while *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 ($p<0.001$, Table S3).

Fraction of CNAs did not differ between AYA 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 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 UV radiation did not differ between the AA 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 AYA and older patients. Surprisingly, even normal skin carries thousands of mutations, with the key differentiating factor from tumors being a lower number of driver mutations per cell in normal skin (Martincorena et al., 2015). Another study found that invasive melanomas have a higher mutational burden compared to benign lesions, as well as 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, 2015). Our finding that *BRAF* mutations were more likely to occur among AYA patients while *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 which regulates the MAP kinase/ERK signaling pathway (Kim et al., 2015). Mutations in *BRAF* are well described in MM, but have been identified in benign nevi (Shain et al., 2015). Higher incidence of *BRAF* mutations among patients <50 has been previously described (Kim et al., 2015).

NF1 is a protein coding gene which 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).

Lastly, AHNAK2 is a protein coding gene containing 4300 amino acids not previously identified to have a relationship with melanoma (Han and Kursula, 2014; Shtivelman *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 utilizes different sequencing and computational analyses, as demonstrated by different median mutation rates amongst the studies. However, our finding that mutational burden is similar in the AYA and older age groups is found in all studies. Nevertheless, the differences in individual gene mutation rates are not always consistent among studies- as seen with regards to AHNAK2 which is found at a higher mutation rate in older individuals in TCGA and Hodis but not reproduced in Gartner. 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 utilizing the TCGA dataset is that the samples consist of thick primaries, 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 develop 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, while the other is 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 N-RAS mutations, and carry germline MC1R variants, while 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 copy number alterations did not differ between AYA 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

Publically accessible, genomic datasets with mutational burden of MM were identified. Four studies were identified: the Cancer Genome Atlas (TCGA), Hodis, Krauthammer and Gartner (Gartner *et al.*, 2013; Hodis *et al.*, 2012; Krauthammer *et al.*, 2012; Tomczak *et al.*, 2015). Each study was accessed and data regarding clinical variables such as age at diagnosis and procurement, gender, race, site of lesion, Breslow depth, ulceration, mitotic rate and stage were extracted. Genomic data were assessed for mutational burden, specifically nonsynonymous genetic mutations, and fraction of CNAs. Melanoma genetic subtypes were defined as: BRAF subtype contains BRAF hot-spot mutations; RAS subtype contains hotspot mutations in N-, K-, and H-RAS; NF1 contains mutations in NF1; triple wild subtype lacks BRAF, RAS and NF1 mutations. Mutations due to UV radiation (C->T

change) were assessed from TCGA, Hodis and Gartner (Gartner *et al.*, 2013; Robles-Espinoza *et al.*, 2016). Supplementary materials 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 (Figures S2–S3). Categorical data were evaluated using Chi-Square test or Fisher Exact test. ANOVA was used to assess differences in mutational burden or CNAs by stage. An $\alpha=0.05$ was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

AYA	Adolescent and Young Adult
CNA	Copy Number Alterations
MM	Malignant Melanoma
TCGA	The Cancer Genome Atlas

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Figure 1.

Distribution of gene mutations and copy number alterations by stage and age group (Staging present in TCGA only) Please note that the stage at diagnosis was utilized for this analysis. However, some of the samples utilized were later metastases collected in these patients.

Figure 1a. Mutational burden by stage at diagnosis

Figure 1b. Mutational burden by stage at diagnosis for patients under 40.

Figure 1c. Mutational burden by stage at diagnosis for patients aged 40 or older.

Figure 1d. Copy number alterations by stage at diagnosis

Figure 1e. Copy number alterations by stage at diagnosis for patients under 40.

Figure 1f. Copy number alterations by stage at diagnosis for patients aged 40 or older

Table 1

Gene mutations and copy number alteration characteristics by age group. Data are presented as median and interquartile range for mutational burden, and mean and standard deviation for copy number alterations.

Characteristic	<40 (n=94)	40+ (n=507)	p-value
Mutational Burden			
TCGA	263.5 (185.75–388.75)	291 (110–555.5)	0.84
Hodis	355.5 (188.75–492)	333 (189–583)	0.68
Krauthammer	112 (8–176)	120.5 (26–287.5)	0.64
Gartner	142 (92.5–305)	163 (115.25–322.25)	0.5
Overall	285.5 (143.75–412.75)	268.5 (104.25–550.25)	0.56
Copy Number Alterations *			
TCGA	0.35±0.21	0.32±0.21	0.3

* Available in TCGA only