CANCER GENOMICS & PROTEOMICS *18*: 83-92 (2021)
doi:10.21873/cgp.20243

Genomic Landscapes of Acral Melanomas in East Asia

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Abstract. *Background/Aim: Acral melanomas (AM) represent a rare subgroup of melanomas with poor clinical outcomes and are enriched in Asian populations. Recent advances in next generation sequencing have provided opportunities to apply precision medicine to AM. Patients and Methods: Here, we present a series of 13 patients with melanomas from Taiwan and Singapore, including 8 patients with AM profiled using whole exome sequencing and summarize the recent studies on the genomic landscape of AM. Results: We identified mutually exclusive mutations in BRAF, NRAS, HRAS, NF1 and KIT in 6 AM cases. In addition, recurrent copy number gains in CCND1 and CDK4, as well as recurrent deletions in CDKN2A/CDKN2B, ATM and RAD51 were observed, supporting the potential use of CDK4/6 or PARP inhibitors in the treatment of these patients. Conclusion: The genomic landscape of AM provides an important resource for applying novel targeted therapies in this rare disease.*

Acral melanomas (AM) are a subset of melanomas that arise from non-hair bearing glabrous skin on the palms and soles, or on the nail apparatus (1). Despite global rarity, AM is the commonest subtype of melanoma in Asian populations (2). Notably, patients with AM harbor worse prognosis as compared with cutaneous melanomas, and survival outcomes remain dismal despite modern advances in the therapeutic landscape of melanomas (3, 4).

Recently, next generation sequencing (NGS) technologies, involving whole exome (WES) (5-7) or genome sequencing (WGS) (8-14), have enhanced the molecular understanding of AM. At the molecular level, AM is a distinct disease as compared with cutaneous melanomas, defined by few point or indel mutations and high degrees of complex structural rearrangements and focal copy number alterations (8-10). Unlike cutaneous melanomas, the tumor mutation burden (TMB) is consequently lower and mutational signatures of ultraviolet damage are infrequent (11). At the individual gene level, hotspot mutations in *BRAF and NRAS* occur in over 50% of cutaneous melanomas, whereas their occurrence in AM is considerably lower (approximately 10-25%). On the other hand, mutations in *NF1* and *KIT*, as well as oncogenic amplification of genes such as *CCND1*, *CDK4*, and *TERT* have been demonstrated to be common events in AM (5, 8). These unique genomic alterations harbor therapeutic implications - small molecule inhibitors of KIT and other tyrosine kinases, including imatinib, nilotinib and dasatinib, have demonstrated significant (albeit modest) efficacy against *KIT*-mutant AM (15-18). Similarly, CDK4/6 inhibitors have also shown promising activity in AM (19). Taken together, the unique genomic landscape of AM offers an opportunity for the application of precision medicine in this rare disease and warrants further investigation.

In this article, we present a series of patients with AM from Taiwan and Singapore profiled using whole exome sequencing and summarize the recent studies on the genomic landscape of AM using NGS, extending our current understanding of this Asian-prevalent subtype of melanoma.

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Key Words: Genomics, precision oncology, rare cancers, targeted therapy.

Table I. *Characteristics of the patients included in the study.*

Patient ID	Subtype	Primary Site	Age at diagnosis	Gender	Country of origin	Stage at diagnosis
Mel-01	Acral lentiginous	Left sole	85	Male	Taiwan	IIC
Mel-02	Acral lentiginous	Left big toe	78	Male	Taiwan	II
Mel-03	Acral lentiginous	Left foot	65	Male	Taiwan	II
Mel-04	Acral lentiginous	Right sole	60	Male	Taiwan	IIIC
Mel-05	Acral, nodular pattern	Right big toe	97	Male	Singapore	IIC
Mel-06	Acral lentiginous	Right heel	56	Male	Singapore	IIIC
Mel-07	Acral, unspecified pattern	Left foot	51	Female	Singapore	IIIC
Mel-08	Acral, nodular pattern	Right sole	61	Male	Singapore	IIIC
Mel-09	Mucosal	Nasopharynx	41	Female	Singapore	IIC
Mel-10	Mucosal	Anus	75	Female	Singapore	IIIC
Mel-11	Mucosal	Vagina	44	Female	Singapore	IIC
Mel-12	Cutaneous, unspecified pattern	Chest wall	39	Male	Singapore	IIIB
Mel-13	Cutaneous, nodular pattern	Left upper arm	80	Male	Singapore	IIC

Patients and Methods

Study design and participants. A total of 13 patients with histologically-proven melanoma from the National Cancer Centre Singapore (Singapore) and Chang Gung Memorial Hospital at Linkou (Taiwan, R.O.C.) were included in the study. Clinical information collected included sex, age, stage at diagnosis (20) and primary tumor location. Written informed consent was obtained in accordance with the Declaration of Helsinki. The study was approved by the Institutional Review Boards of all participating hospitals. All authors had access to the study data and had reviewed and approved the final manuscript.

Whole exome sequencing. Genomic DNA isolated from formalin-fixed paraffin-embedded or snap frozen tissue with adequate tumor content, as well as from their paired normal tissue, were selected for whole exome sequencing. A qualified pathologist provided the initial microscopic evaluation and assessment of tumor content. Whole exome sequencing was performed with hybrid selection using the Human All Exon kit SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA, USA) version 6 and sequenced on the Illumina HiSeq X platform (Illumina, San Diego, CA, USA) as paired-end 150-base pair reads. Read pairs were aligned to the human reference genome NCBI GRC Build 37 (hg19) using Burrows-Wheeler Aligner (BWA MEM) (Wellcome Genome Campus, Hinxton, Cambridge, UK) (21). Optical duplicates were marked with Picard followed by base score recalibration using GATK version 4.1.4 (Broad Institute, Cambridge, MA, USA) for post alignment data processing (22). Somatic variants from the resulting normal and tumor BAM files were identified using Mutect2, and subsequently annotated and prioritized using VEP (Wellcome Genome Campus, Hinxton, Cambridge, UK) (23). Tumor mutation burden was estimated based on the proportion of nonsynonymous variants over the region of interest (ROI) of the exome panel used. Mutational signature identification was performed using SigProfiler Bioinformatics Tools (Wellcome Genome Campus, Hinxton, Cambridge, UK) (24). Biologically significant copy number changes were identified with GISTIC 2.0 (Broad Institute, Cambridge, MA, USA) and copy-number segmentations were processed with TitanCNA v1.17.1 (University of British Columbia, Vancouver, Canada) (25, 26).

Literature review criteria. Literature review was performed by searching the PubMed database for articles published from 2010 to 2020. Only articles published in English were considered. Search terms included "acral melanoma" and "genomics". Full articles were retrieved, and further information was obtained from relevant references. We focused on relevant primary articles rather than reviews to compile this review. Final inclusion criteria included studies profiling AM using whole exome sequencing and/or whole genome sequencing. Case reports, commentaries, review articles, meta-analyses were excluded.

Results

Patient demographics. A total of 13 patients were included in the study based on tissue availability (Singapore cases, n=9; Taiwan cases, n=4). The median age was 61 years (range=39-97 years) and there was a male predominance (n=9; 69.2%). Eight cases were acral melanomas, all arising from the feet. Three were mucosal melanomas of the nasopharynx, anus and vagina. Two cases of cutaneous melanomas arising from the chest wall and upper arm were also included for comparison. None of the patients had distant metastases at diagnosis. Table I summarizes the clinical characteristics of all patients in the study cohort.

Somatic mutational landscape and COSMIC mutational signatures. We performed whole exome sequencing of all 13 melanomas with matched non-tumor tissues. Tumor samples were sequenced to a median coverage of 134X (range=100X-270X) and normal samples to a median coverage of 89X (range=50X-100X). The median tumor mutational burden was 1.1 mutations per megabase (range=0.4-75.6). We identified a total of 919 somatic exonic mutations, including

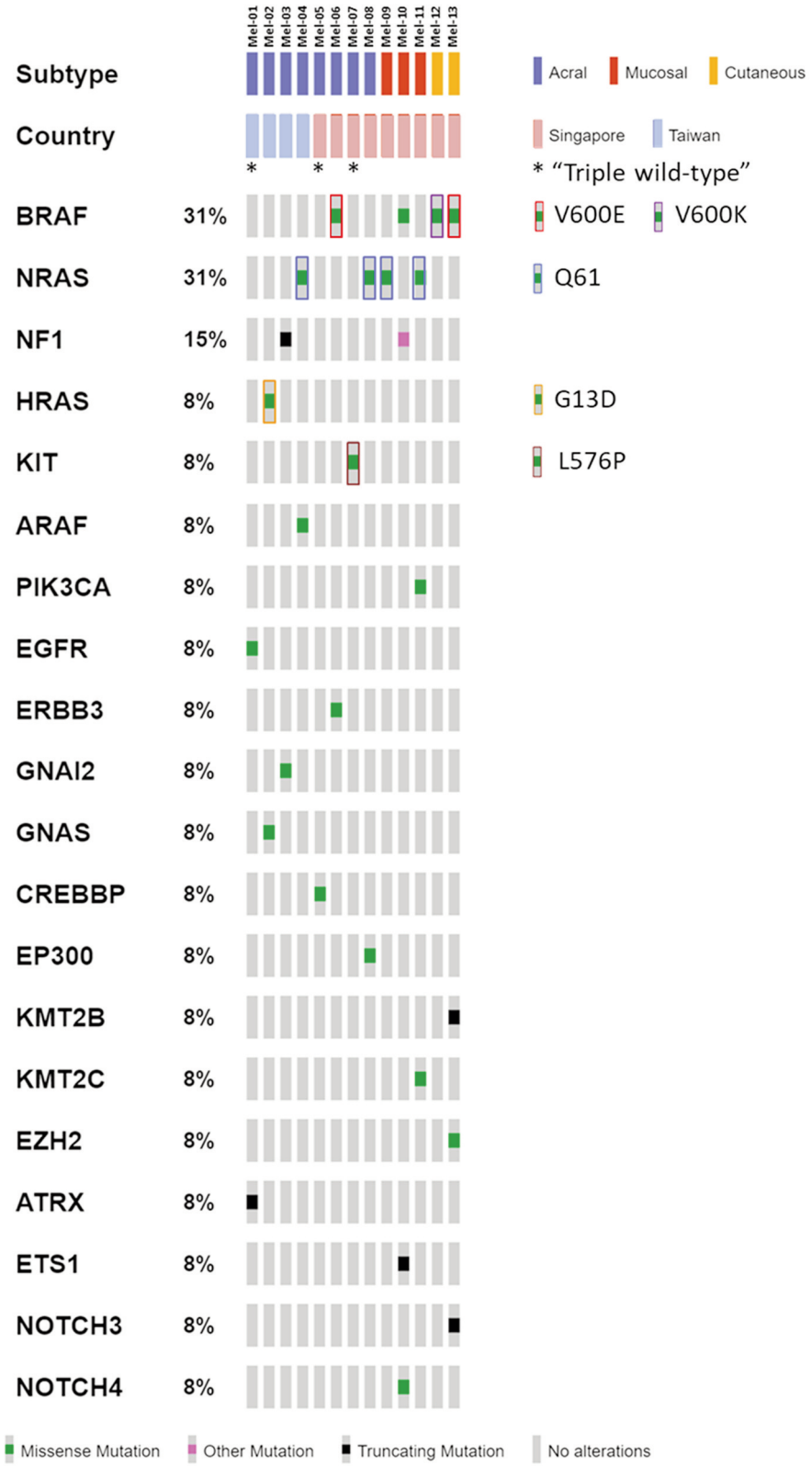

Figure 1. *Somatic mutation landscape of acral, mucosal and cutaneous melanoma of East Asian origin. Variants of interest are represented in an oncoplot, including recurrent mutations in genes such as BRAF, NRAS and NF1. Three of 11 non-cutaneous melanomas were "triple wild-type" – one of which was characterized by a KIT exon 11 L576P mutation.*

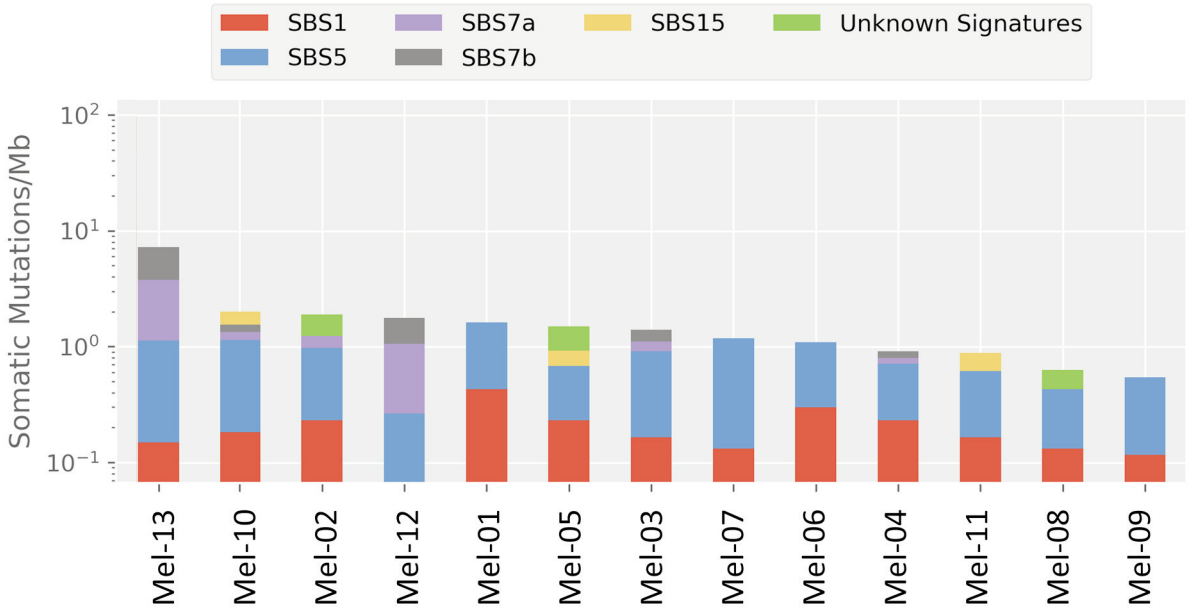

Figure 2. *Mutational signatures of melanomas in the cohort. The proportions of mutations conferred by each inferred mutational signature in individual cases are as shown.*

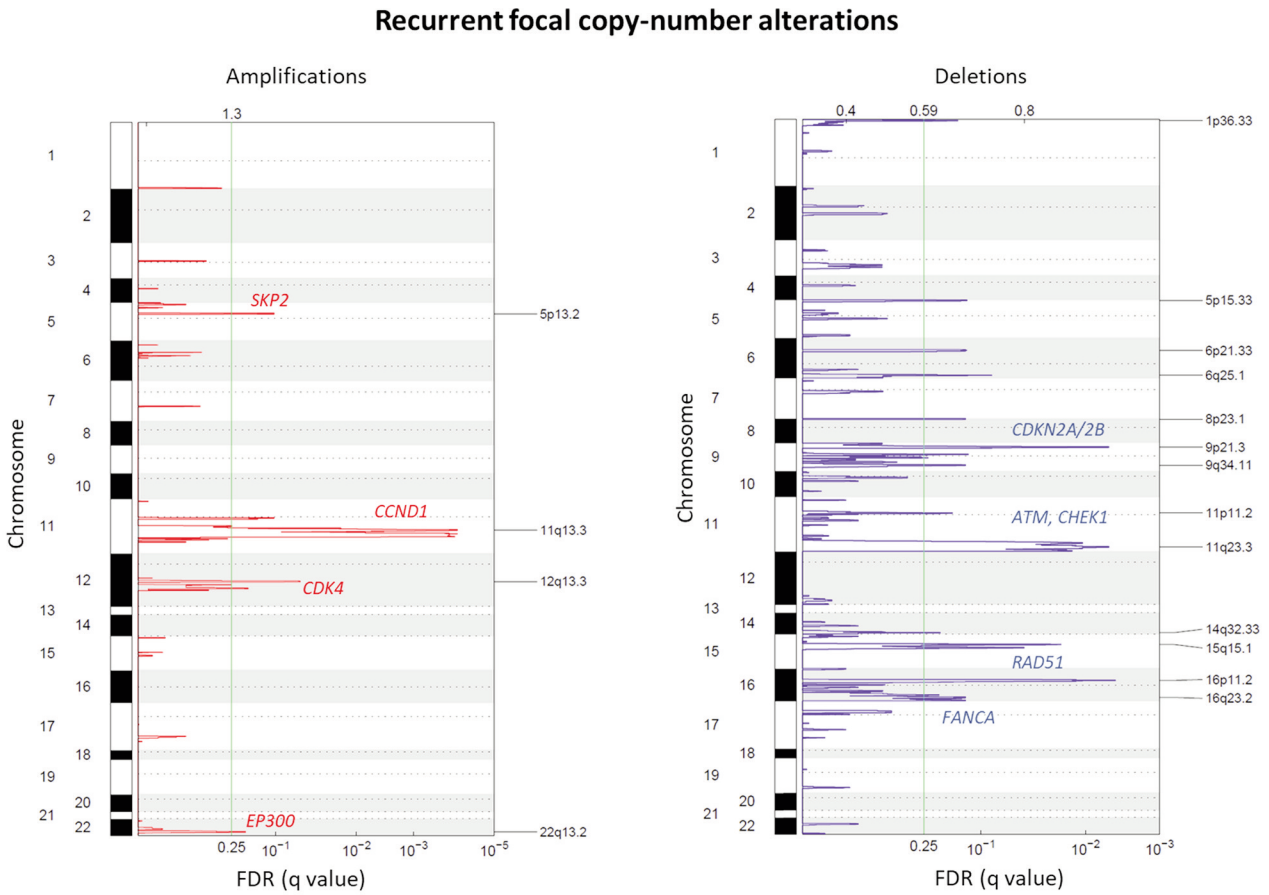

Figure 3. *Copy number alterations in the global melanoma cohort. Analysis of somatic copy number alterations identified 4 gained and 13 lost genomic regions. The involved regions and important cancer-related genes within are highlighted.*

Table II. *Overview of studies on the genomic landscape of acral melanoma.*

Reference	Platform	n	Main findings
Newell *et al*. (8)	WGS RNAseq	87	• Subset of cases (mostly subungual) harbor ultraviolet mutational signatures • Whole genome duplication, aneuploidy and complex rearrangements • Amplification of *TERT, CDK4, MDM2, CCND1, PAK1* and *GAB2*
Hadi *et al*. (9)	WGS	35	• Complex rearrangement involving amplified fold-back inversions ("Tyfonas") commonly observed in AM
Pan-Cancer Analysis of Whole Genomes Consortium (10)	WGS	20	• Chromotripsis and kataegis frequent, and commonly co-occur with gene amplification (*CCND1, TERT*)
Hayward *et al*. (11)	WGS	35	• Genome dominated by structural variations • Significant mutations include *BRAF, NRAS, NF1, KIT, MAP2K2* • 18-fold fewer mutations than cutaneous melanomas
Liang *et al*. (12)	WGS/WES RNAseq	34	• Somatic alterations dominated by structural variations • Only 38% harbor *BRAF, NRAS* or *NF1* mutations • Somatic TERT alterations present in 41% of cases
Rawson *et al*. (13)	WGS	35	• 3 cases harbored ultraviolet mutational signatures (mostly subungual), and were associated with younger age and higher mutational load
Furney *et al*. (14)	WGS	5	• Early work demonstrating fewer mutations but more frequent structural variations than cutaneous melanomas
Forschner *et al*. (5)	WES	31	• Somatic mutations in *NF1* (18%), *NRAS* (18%), *BRAF* (7%), *KIT* (11%) • Amplification of *MYC, TERT, CCND3, RICTOR* and *CDK4* • Deletions of CDKN2A/2B, PTEN
Lee *et al*. (6)	WES	2	• Missense mutations *BRAF* V600E and *NRAS* Q61R in 2 cases

614 missense single nucleotide variants (SNVs), 37 nonsense SNVs, 25 indels and 243 silent mutants. Somatic nonsynonymous variants of interest are represented in an oncoplot, including recurrent mutations in known melanoma-associated genes such as *BRAF* (31%), *NRAS* (31%), *NF1* (15%) and *HRAS* (8%) (Figure 1). *BRAF* mutations were all missense and present in 1 of 8 AM cases (p.V600E), 1 of 3 mucosal melanomas (p.G534R), and both cutaneous melanomas (p.V600E and p.V600K). *NRAS* mutations were all missense in the p.Q61 hotspot for 2 of 8 AM cases and 2 of 3 mucosal melanoma cases. Notably, 3 of 11 (27.3%) non-cutaneous melanomas were "triple wild-type" – one of which was a *KIT* exon 11 L576P mutant.

The estimated proportions of mutations contributed by inferred mutational signatures in individual melanoma cases were examined. Signatures 1 and 5, which are related to aging and observed in most cancer types, were present in most of the cases. The signatures for ultraviolet DNA mutagenesis - Single Base Substitution (SBS) 7a and SBS 7b, as characterized by a majority of C>T mutations, were observed in 6 cases (2 cutaneous, 1 mucosal, and 3 acral melanomas), though the relative contribution per case was minor (Figure 2).

Somatic copy number alterations. Analysis of somatic copy number alterations identified 4 gained genomic regions (5p13.2, 11q13.3, 12q13.3, 22q13.2). We further identified 13 deleted regions (1p36.33, 5p15.33, 6p21.33, 6q25.1, 8p23.1, 9p21.3, 9q34.11, 11p11.2, 11q23.3, 14q32.33, 15q15.1, 16p11.2, 16q23.2) (Figure 3). Further introspection of individual cases revealed several important gained regions of interest, including chromosome 11q and 12q – containing oncogenes *CCND1* and *CDK4*, respectively (Figure 4). Gene-level copy number analysis revealed recurrent copy number gains/amplifications in *CCND1* (46%), *CDK4* (31%), *SKP2* (15%) and *EP300* (15%), and recurrent deletions in *CDKN2A/CDKN2B* (54%), *ATM* (38%), *RAD51* (38%) and *FANCA* (15%) (Figure 5).

Recent genomic studies on acral melanoma. A total of 92 articles were screened. After excluding review articles (n=9), meta-analyses (n=1), case reports (n=4), commentaries (n=5) and other studies (n=63), 10 articles remained and were included in the final analysis. The study design and main findings are summarized in Table II.

Discussion

Newell *et al*. have recently reported the largest series of AM profiled using whole genome sequencing (n=87). The authors observed several significantly mutated genes including *BRAF*, *NRAS*, *NF1*, *NOTCH2*, *PTEN* and *TYRP1*, as well as *KIT* alterations. Mutational signature analysis revealed a subset of tumors, mostly subungual, with an

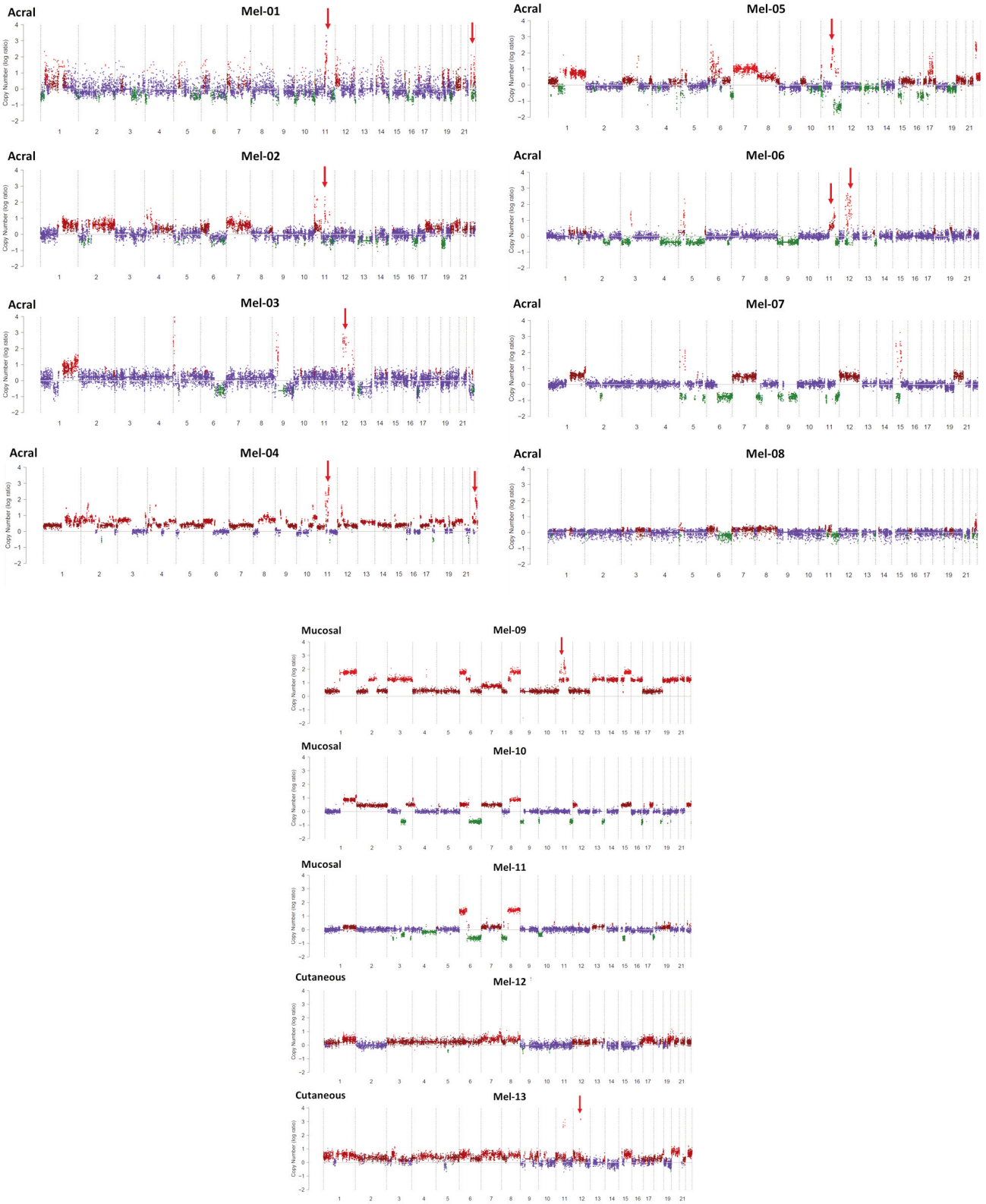

Figure 4. *Copy number landscape of individual melanoma cases. Red arrows mark selected gained regions of interest, including chromosome 11q and 12q – containing oncogenes CCND1 and CDK4, respectively.*

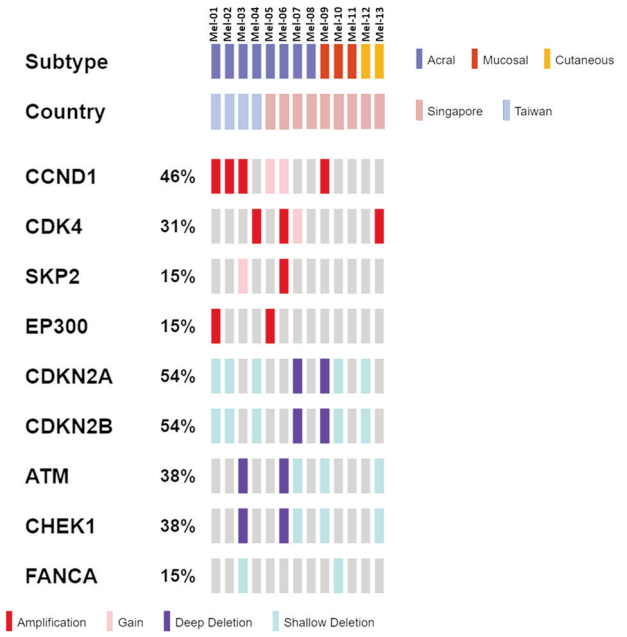

Figure 5. *Genomic gains and amplifications of CCND1/CDK4, as well as deletions of CDKN2A/CDKN2B are the commonest copy number alterations. Additionally, deletions of genes involved in DNA damage repair including ATM, RAD51 and FANCA are also frequently observed.*

ultraviolet radiation signature. Recurrent complex rearrangements were observed on chromosomes 5, 6, 7, 11 and 12, associated with amplification of *TERT*, *CDK4*, *MDM2*, *CCND1*, *PAK1* and *GAB2*. In keeping with previous reports (11, 12), structural alterations including whole genome duplication, aneuploidy and complex rearrangements (such as breakage-fusion-bridge and chromotripsis) are common in AM (8). A unique form of complex rearrangement involving amplified fold-back inversions, termed "Tyfonas", were commonly observed in AM (40%) but rarely seen in cutaneous melanomas, and it has been hypothesized that they provide an alternative source of neoantigens through the generation of expressed protein-coding fusions (9). By analyzing SNVs, regions of *CCND1* amplification were found to harbor low or even no mutations at a high variant allele fraction in AM. This is in contrast to cutaneous melanomas, in which a large number of mutations typically pre-date amplification and are thus present at a high variant allele fraction. This suggests that both chromothripsis and subsequent gene amplification occur early in the evolution of AM (10).

In the present study, we examined the genomic landscape of AM derived from 2 East Asian countries (Taiwan and Singapore), and also included mucosal and cutaneous melanoma cases for comparison. In the AM cases, somatic nonsynonymous variants in known melanoma-associated genes were present in a mutually-exclusive manner, including *BRAF* V600E (n=1), *NRAS Q61* (n=2), *HRAS G13D* (n=1) and *NF1* (n=1). Three of the AM cases were "triple wild-type" – one of which harbored a *KIT* exon 11 L576P mutant. This *KIT* mutation has been previously reported to confer sensitivity to imatinib (27). In terms of mutational signatures, signatures 1 and/or 5 were present in all cases of AM, which is consistent with previous analyses (8). Signatures for ultraviolet mutagenesis, though present in 3 cases, were not the predominant contributor of the mutations, in keeping with prior observations that ultraviolet signatures, if present, are more likely to contribute to AMs of subungual origin (8, 13). In addition to these observations, nearly all cases of AM in our cohort harbored *CCND1* or *CDK4* amplification, and or *CDKN2A/2B* deletion. Interestingly 2 of the cases contained deep deletions of *ATM*. Altogether, the somatic alterations of AM may suggest potential avenues of therapeutic susceptibility.

Contemporary treatment options for advanced *BRAF* mutant melanomas commonly involve the use of one or more small molecule tyrosine kinase inhibitors (TKI) (28, 29) or checkpoint immunotherapy (30, 31). While an attractive target, *BRAF* mutations occur in only 15% of AM as compared to 50% of cutaneous melanomas. The presence of other therapeutically-tractable mutations such as *KIT* may indicate additional treatment options using other TKIs such as imatinib (32), dasatinib (33) or nilotinib (16). Aberrations in the CDK4 pathway, including amplifications of *CDK4* and *CCND1*, as well as deletions of *CDKN2A,* may indicate the potential utility of CDK4/6 inhibitors in AM (34-36). Interestingly, our data revealed *ATM* deep deletions, with or without concurrent shallow deletions of *RAD51* and *FANCA* in 3 of 8 (37.5%) AM patients, supporting the use of PARP inhibitors for their treatment (37). Recent real-world data suggests that the efficacy of immune checkpoint inhibitors is significantly lower in patients with AM as compared to cutaneous melanomas (38). While the lack of high TMB may in part explain this dismal result, genetic gains of *CDK4* or *CCND1*, as well as *CDKN2A* loss have been identified in melanoma patients with innate resistance to anti-PD1 checkpoint immunotherapy (39). Yu *et al*. have provided further evidence that this innate resistance may be mediated by the lack of IFNγ and TNFα-NFκB signaling responses in CDK4 pathway-defective tumors, and that the addition of the CDK4/6 inhibitor palbociclib may enhance the efficacy of immunotherapy by upregulating PD-L1 (39).

Our current study is limited by the small patient cohort. Nonetheless, the results are consistent with previously published studies and lend confirmatory evidence to support a therapeutic target landscape for AM. In addition, we described the loss of genes involved in homologous recombination repair in a significant proportion of AM, supporting the use of PARP inhibitors in the treatment of

these patients. Taken together, the recent data suggesting that complex structural alterations represent early events unique to AM pathogenesis opens up further avenues that can be exploited for therapy.

In conclusion, the genomic landscape of AM presents a unique opportunity for applying novel therapies to this group of patients. Future studies are warranted for the direct translation of these findings to the clinic.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

JYC analyzed the data and drafted the manuscript; CCN processed tissue and performed sequencing experiments; AHL performed the bioinformatic analyses; JYC, CEW, and JWC obtained patient samples and data; BTT, JWC designed the study; JYC, BTT, JWC interpreted the results, and revised the manuscript; JJH was responsible for the CORPG3J0151~2 grant application, sample collection, DNA preparation, clinical data confirmation, and manuscript revisions; and all authors read and approved the final version of the manuscript.

Acknowledgements

This work was supported by the Singapore Ministry of Health's National Medical Research Council under its Singapore Translational Research Investigator Award (NMRC/STAR/0006/2009), and Research Training Fellowship (NMRC/Fellowship/0054/2017), as well as SingHealth Duke-NUS Academic Medical Centre and Oncology ACP Nurturing Clinician Scientist Scheme (08-FY2017/P1/14-A28), SHF-Foundation Research Grant (SHF/FG653P/2017). This work was also supported by a grant from Chang Gung Memorial Hospital (CORPG3J0151~2).

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Received December 14, 2020
Revised December 29, 2020
Accepted December 30, 2020