



Oncogenic signaling pathways and hallmarks of cancer in Korean patients with acral melanoma

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ABSTRACT

Acral melanoma (AM), a rare subtype of cutaneous melanoma, shows higher incidence in Asians, including Koreans, than in Caucasians. However, the genetic modification associated with AM in Koreans is not well known and has not been comprehensively investigated in terms of oncogenic signaling, and hallmarks of cancer. We performed whole-exome and RNA sequencing for Korean patients with AM and acquired the genetic alterations and gene expression profiles. *KIT* alterations (previously known to be recurrent alterations in AM) and *CDK4/CCND1* copy number amplifications were identified in the patients. Genetic and transcriptomic alterations in patients with AM were functionally converge to the hallmarks of cancer and oncogenic pathways, including 'proliferative signal persistence', 'apoptotic resistance', and 'activation of invasion and metastasis', despite the heterogeneous somatic mutation profiles of Korean patients with AM. This study may provide a molecular understanding for therapeutic strategy for AM.

1. Introduction

Acral melanoma (AM) is a rare subtype of cutaneous melanoma (CM) that occurs in ultraviolet (UV)-protected acral locations (e.g., palms, soles, and nail apparatus [subungual]) [1,2]. Although the incidence rate of AM is only 2–3% of all melanomas in Caucasians [1,2], it accounts for half of all melanoma incidences in Asian populations [3]. AM has worse prognosis than other CMs, and Asian patients with AM, in particular, have poorer prognosis than other ethnicities [3].

With the advent of next-generation sequencing (NGS), our understanding of the genomic environment of melanoma has improved [4,5]. Especially, NGS has been applied extensively to CM, and the latter has

been divided into four genomic subtypes, according to the status of mutated genes, namely “*BRAF* subtype,” “*RAS* (N/H/K) subtype,” “*NF1* subtype,” and “Triple wild-type (WT) subtype” [4]. The genomic profiles of AM have been far less revealed than those of CM, mostly due to the rarity of AM. Interestingly, AM has distinct genomic profiles. Patients with AM have a lower mutational burden compared to those with CM [1, 5]. Structural rearrangements and copy number alterations (CNAs) occur slightly more frequently in AM than in CM [1,2,5]. Genes mutated in AM include *BRAF*, *NRAS*, and *NF1*, and mutations and amplification of *KIT* also occur frequently. In addition, amplification of *CCND1*, *CDK4*, and *TERT* affects tumorigenesis in AM [1,2].

For the majority of patients with AM, genetic and transcriptomic

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changes are yet to be revealed. In addition, only a few studies on AM have explored the genetic and transcriptomic changes [1,2]. Nevertheless, efforts have been made to inspect genetic alterations (e.g., mutations, CNAs), mainly using targeted-panel sequencing [6–9], which, however, does not reveal the genetic landscape of whole protein-coding genes. Recently, whole-exome sequencing (WES) has been applied to AM studies [1,10–13] for simply enumerating mutations and CNAs. However, genetic alterations in AM have not been systematically investigated in terms of oncogenic signaling [14]. Moreover, how the second-generation hallmarks of cancer [15] are shaped in AM biological networks based on RNA-sequencing (RNA-Seq) remains to be elucidated.

Treatment and diagnosis in AM are still difficult, and clinical trials of massive drug responsiveness in patients are unethical and inefficient [16]. Drug repurposing is promising for discovering safe and effective drugs [17]. With the development of pharmacogenomics and computational technology, diverse attempts have been made to build drug response prediction models using the molecular characteristics of patients [16,18,19]. However, no studies have yet applied deep learning models to explore potential drug repurposing candidates to AM.

In this study, we aimed to analyze not only the WES of four Korean patients with AM but also the RNA-Seq data of two of them. We explored the differences and similarities in genetic events, including single nucleotide variants (SNVs) and CNAs, between the patients with AM in this study and those in previous studies. Biological networks of patients with AM using RNA-Seq were constructed to inspect associations between gene expression profiles of patients with AM and global hallmarks of cancer [15]. Finally, we applied a deep learning (DL) method to the gene expression profiles from RNA-Seq of patients with AM to predict potential drug candidates in AM.

2. Materials and methods

2.1. Study subjects and samples

This study included four patients with AM at the Gil Medical Center (GMC). Primary tumor and matched blood samples were collected from the patients (GMC-AM1, -AM2, -AM3, and -AM4) for mutation profiling. For two of four patients (GMC-AM2, and -AM4), additional matched normal adjacent tissues were collected to confirm mRNA expression profiling (Table S1).

Patient demographic information is summarized in Table 1. All four patients were Korean females. Three patients (GMC-AM2, -AM3, and -AM4) had lesions on the subungual area of the fingers with positive Hutchinson sign, while one patient (GMC-AM2) had melanoma on the sole side of great toe. One patient (GMC-AM4) had an additional malignancy of advanced gastric cancer with hepatic metastases. Skin biopsy was performed from the darkest part.

2.2. Whole-exome sequencing (WES)

We performed WES on tumor tissue and matched blood samples for mutation profiling from four patients. Exome libraries were prepared

according to the manufacturer's instructions by the SureSelect XT V5 Library Prep Kit (Agilent, Santa Clara, CA, USA). The prepared library was sequenced by Illumina sequencing equipment with a coverage of 200X for tumor samples and 100X for matched normal blood samples.

Sequence data (FASTQ) were quality trimmed ($q > 30$) by Sickle (version 1.33) and aligned to the human genome hg19 using BWA-mem (version 0.7.15). Duplicate readings were verified and corrected by MarkDuplicates in the Picard tools (version 2.5.0). The Genome Analysis Toolkit (GATK, version 3.6) [20] was used to correct alignment errors caused by insertion-deletions (indels) and to calibrate the quality score. Somatic SNVs and indels were called from mutect2 [20] using rearranged and recalibrated BAM files. To remove low-quality variation, 'PASS' was selected. Annotated somatic SNVs and Indels using Annovar (version 2016-02-01) [21]. We excluded somatic variants with at a minor allele frequency of $\geq 1\%$ in any of the three germline population variant databases (1000 Genomes Project (version 2015), ESP6500, the Exome Aggregation Consortium dataset (release 0.3)) [21] for the identification of somatic mutations. Somatic coding mutations were identified by selecting missense, nonsense, and indel from exonic and splice sites. We calculated tumor mutation burden (TMB) as the sum of somatic mutations (SNVs and indels) including synonymous SNVs per megabase of the coding region (exon, splice region) [22]. Driver missense mutations were identified using cancer-specific high-throughput annotation of somatic mutations (CHASM) annotations (P value < 0.05) from Cancer-Related Analysis of Variants Toolkit (CRAVAT), an online tool [23].

2.3. Transcriptome sequencing (RNA-Seq) analysis

We performed RNA-Seq for two patients (GMC-AM2 and GMC-AM4) using matched tumor and adjacent normal tissues. The RNA-Seq library was prepared using TruSeq RNA library preparation kit (Illumina, San Diego, CA, USA) and sequenced by Illumina sequencers (Theragen Inc., Suwon, Korea). Adapters and low-quality reads trimmed ($q > 20$) from sequencing reads using BBDuk from the BBTools suite (v37.90). It was aligned to the human genome (GENCODE Release 19 (GRCh37)) using STAR (version 2.5.3a) [24], and the expression level of each gene was estimated using RSEM (v1.2.28) [25]. The gene expression level was measured in Transcripts Per Million (TPM).

2.4. Collection of publicly available AM cohorts

SNVs and indels mutation information and mRNA expression levels of two patients with primary AM were downloaded from The Cancer Genome Atlas Skin Cutaneous Melanoma (TCGA – SKCM) project [26]. Clinical information of the patients with AM in TCGA SKCM was obtained through the University of California Santa Cruz's Xena platform [27]. For another cohort, we also downloaded SNVs and indels mutation information, mRNA expression levels, and CNAs information for 17 patients with primary AM from Translational Genomics Research Institute (TGen) [1].

2.5. Mutational signatures

We confirmed mutation signatures using MUTation AnaLyIS toolkit (Mutalisk) [28], a web-based tool that can comprehensively analyze somatic DNA mutations using genomic regulatory elements and DNA sequence context. Mutation signatures were decomposed using single-base substitution (SBS) signatures (v3.2) [29] provided in the catalog of somatic mutation in cancer [30]. The relative contribution of the signature was calculated using a linear regression method and was calculated for all 78 SBS signatures [29].

2.6. Human leukocyte antigen (HLA) typing and neoantigen prediction

We used Polysover [31] from WES of our four patients to determine

Table 1
Patient demographics.

Patient	Sex/ Age	Anatomic location	Duration (years)	Tumor stage
GMC-AM1	F/50	Left great toe	10	Melanoma in situ
GMC-AM2	F/73	Right second finger, subungual	2	Melanoma in situ
GMC-AM3	F/75	Left thumb, subungual	16	T4b
GMC-AM4	F/69	Right third finger, subungual	3	T2a

the HLA type with 4 digit resolution in class I HLA genes (HLA-A, HLA-B, HLA-C). Mutations in the HLA class I gene were performed using paired tumor and normal WES data for our four patients according to the Polysolver pipeline. We analyzed with pVAC-Seq [32] using VEP annotated VCF files to predict neoantigens. In the pVAC-Seq pipeline, NetMHC (version 4.0) and NetMHCpan (version 4.1) binding prediction algorithm (≤ 500 nM binding strength) [32] were applied, and the binding affinity to the Major Histocompatibility Complex (MHC) was measured for all peptides were. We additionally used the ScanNeo [33] pipeline to detect neoantigens from the RNA-Seq data of our two patients (GMC-AM2 and GMC-AM4). Binding affinities between MHC and peptides in a length 8–10 amino acids were obtained by either pVec-Seq [32] or ScanNeo [33]. Peptides in a length 8–10 amino acids were selected, such that the peptides appeared only in cancer tissues and that had MHC binding affinity less than 500 nM. Neoantigens with a binding affinity ≤ 50 nM were defined as strong affinity; ≤ 150 nM as medium affinity; and ≤ 500 nM as weak affinity.

2.7. Copy number analysis

We called the somatic CNAs in the WES bam file according to the workflow recommended by VarScan (v2.4.4) [34]. We used the online software package GenePattern [35] to identify recurrent events through GISTIC 2.0 (version 6.15.28) [36] analysis and to identify changes in the number of copies at the gene level.

2.8. Differential expression network of genes and the hallmark of cancer

Gene networks were constructed using differentially expressed cancer-associated genes in our two patients (GMC-AM2 and GMC-AM4). The gene expression level was transformed into $\log_2$ (TPM+1). Common differentially expressed genes (DEGs) between two patients were selected in the following conditions: (i) $\log_2$ fold change (FC) of tumor over adjacent normal tissues greater than or less than one, and (ii) the same signs of the $\log_2$ FCs in the two patients. 1,154 common DEGs (584 upregulated and 570 downregulated genes in tumor versus adjacent normal tissues) in the two patients were obtained. We constructed a network using cancer-related genes provided by the Cancer Gene Census (CGC) [30] among differentially expressed genes. Protein–protein interaction networks were constructed using the STRING tool [37] and we manually annotated the genes encoding the proteins in the networks by associating with 10 hallmarks of cancer [15].

2.9. Performance comparison between ResNet and a transformer network, DeepTTA

Cancer cell lines are the most commonly used model in cancer biology research [38]. We chose two deep learning models of regression, the ResNet [16] and the transducer network DeepTTA models [18], to predict drug responsiveness (i.e., cell viability $\ln(\text{IC}_{50})$) in a variety of cancer cell lines. The Cancer Cell Line Encyclopedia (CCLE) [38] provides transcriptome data from 504 cancer cell lines and experimentally measured values of the drug responsiveness $\ln(\text{IC}_{50})$ (i.e., the natural logarithm cell viability half maximal inhibitory concentration [IC_{50}]) of each of these cancer cell lines for 24 drugs.

In a previous study [16], these transcriptome data from 504 cancer cell lines were transformed through z-standardization for 18,989 genes. The properties of the 24 drugs were expressed as 2,325 pharmacological vectors in PaDEL [39], and individual features were processed using the min-max normalization method [16]. For every combination of cell line and drug, the z-standardized gene expression data of the cell line and the feature vector of the drug were concatenated together to be used as input to the ResNet model, and the numerical $\ln(\text{IC}_{50})$ value was obtained as output [16]. The published ResNet model we used (including architecture, weights, and parameters) randomly split the response data set 9:1 into a training set and a test set for model

training [16]. The training and test sets contained data for 10,224 and 1,136 of the possible combinations of drug treatments and cancer cell lines, respectively [16]. The ResNet model [16] was trained on the training set and evaluated on the test set, resulting in a regression model that can predict the drug response $\ln(\text{IC}_{50})$ of cancer cell lines for 24 drugs.

We utilized the ResNet model, in our previous publication [16], to predict $\ln(\text{IC}_{50})$ s of drugs. The input type of the model consisted of a drug chemical vector and basal gene expression profiles (18,988 genes) of a cancer cell line, and the output of the model predicted an $\ln(\text{IC}_{50})$ value for the drug treatment on the cancer cell line [16]. Gene expression profiles were transformed by z-normalization, and numericized drug chemical property vectors were generated by PaDEL [39] which took drug structures as input. The ResNet model consisted of 22 convolutional layers with eight skip connections, eight neural layers with one skip connection, and seven drop-out layers [16]. The ResNet model has seven dropout layers to which the drop-out method can be applied in addition to hold-out, which was used independently by the training and test sets to prevent overfitting. The learning rate, batch size, and optimizer in the ResNet were $2e-4$, 100, and Adam, respectively [16]. We transformed the gene expression data of patients with AM following the method described in previous studies (z-normalization for 18,989 genes) [16]. Additionally, the chemical properties of 24 drugs were determined using data provided by Park et al. [16].

It is noted that, previously, we evaluated the ResNet model and compared the model with other models (i.e., CDRScan [19], lasso, random forest, XGBoost, support vector regression (SVR), and ridge) in our publication [16], demonstrating better performance of ResNet over the other models in the test set. The evaluation measurements included determination of R^2 and root mean squared error (RMSE) between experimentally obtained ground truth $\ln(\text{IC}_{50})$ s and predicted $\ln(\text{IC}_{50})$ s. The lower RMSE and the higher R^2 indicate better performance.

In this study, we also utilized a transformer network, DeepTTA [18], to predict $\ln(\text{IC}_{50})$ s. The input type of DeepTTA took basal gene expression profiles (18,988 genes) of a cancer cell line and a drug chemical structure represented by the Simplified Molecular Input Line Entry System (SMILES) format as input [18]. Gene expression profiles were processed by z-normalization. DeepTTA consisted of three main parts [18]. In the first part, the drug chemical property vector was obtained from the SMILES of a drug through a transducer encoder [18]. The transformer encoder used 8 parallel attention layers and consisted of a stack of 6 identical layers [18]. The second part extracted features from gene expression profiles through four-layer neural network [18]. In the final part, the drug feature extraction part (i.e., the first part) and the gene expression feature extraction part (i.e., the second part) were concatenated together and the concatenation was fed into a classifier network consisting of four fully connected layers [18]. The learning rate, batch size, and optimizer in DeepTTA were $1e-3$, 128, and Adam, respectively [18].

2.10. ResNet-based drug responsiveness prediction in our patients with AM

We normalized the RNA expression TPM values of two GMC patients (GMC-AM2 and -AM4) by z-normalization. The normalized gene expression profiles of the 15 TGen patients with AM were downloaded from the cBioPortal [1,40]. We transformed each patient's expression profile by z-normalization at 18,988 genes. Pharmacological vectors generated in PaDEL [39] for 24 drugs were collected in a previous study [16]. We predicted the drug response $\ln(\text{IC}_{50})$ in the ResNet model with the gene expression profile of patients with AM and the drug's pharmacological vector as inputs.

Also, to identify explainable features (i.e., genes) of the ResNet models in predicting drug responsiveness for the two GMC patients (GMC-AM2 and -AM4), we applied an explainable artificial intelligence (XAI) method, local interpretable model-agnostic explanation (LIME)

[41], to the ResNet. We obtained important genomic features (i.e., genes). In the LIME analysis, we utilized the lime package in Python.

3. Results

3.1. WES revealed the genomic landscape of Korean patients with AM

We performed WES on four patients with AM (GMC-AM1 through -AM4) at GMC. RNA-Seq was performed for two of them (Table S1). In the four patients, coding somatic mutations identified 259 SNVs and 21 indels, including exons and splice sites (Fig. 1a and Table S2). The number of SNVs and small indels in the coding regions ranged from 14 to 161, with a median of 53 (Table S3).

We explored the somatic SNVs and small indels across the patients, and identified 272 mutated genes in the four patients (Fig. S1). No mutation was detected in *BRAF*, *N/H/KRAS*, or *NF1*. The *KIT* (L576P) mutation, reported in previous melanoma studies [1,42], was identified

in patient GMC-AM2. *FGFR2* mutations had been reported in 10% of patients with melanoma and melanoma cell lines in a previous study [43], and our current analysis revealed a frameshift insertion in *FGFR2* in patient GMC-AM2. Patient GMC-AM1 had a *CBL* mutation that was involved in melanoma cell proliferation, migration, and invasion [44]. *BRIP1* mutations were identified in patient GMC-AM3 and had been reported in 5% of patients with melanoma in a previous study [26]. *BRIP1* mutations increase the risk of ovarian cancer [45]. None of the other mutated genes, except *PCDHGA6*, overlapped across the four GMC patients with AM. Of the mutated genes, 191 were recurrent, reported in previous AM studies [1,2,5,6,10,11,26,46,47], whereas 81 mutated genes were novel (Fig. S1).

TERT, *KIT*, *MDM2*, *GAB2*, *CDK4*, *CCND1*, and *PAK1* showed frequent CNAs in AM [1]. Patient GMC-AM1 showed copy number amplification or gain in *TERT*, *MDM2*, *CDK4*, *CCND1*, and *PAK1* (Fig. 1a). Patient GMC-AM3 showed copy number amplification in *KIT* (Fig. 1a). Patient GMC-AM4 showed copy number amplification or gain in *GAB2*, *CDK4*,

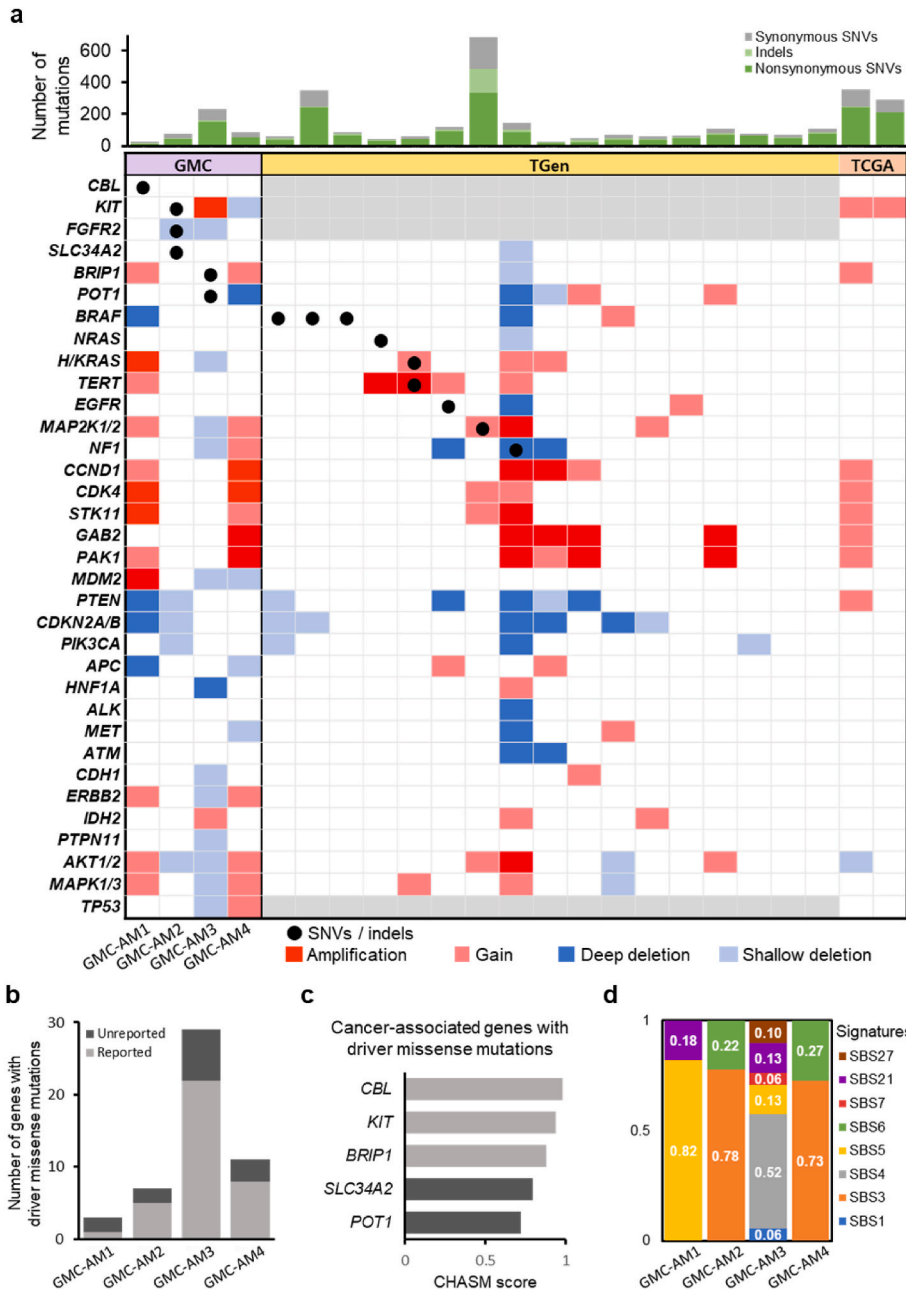


Fig. 1. The genomic landscape of acral melanoma in our GMC and other AM cohorts. (a) A summary of somatic alterations in patients with AM (GMC, TGen, and TCGA cohorts) is shown; the number of mutations in the coding region (top), and somatic mutations and copy number alterations in selected genes (bottom). Gray indicates non-availability of CNAs. **(b)** Number of genes with putative driver missense mutations per patient is shown. **(c)** Five genes with driver mutations were identified (CHASM, cancer-specific high-throughput annotation of somatic mutations). Genes reported in previous AM studies are shown in dark gray and novel genes are shown in light gray. **(d)** Contribution of the single substitution (SBS) mutation signatures identified per patient.

CCND1, and *PAK1* (Fig. 1a). We also identified recurrent focal loss in the 3q23 region of our four patients. An association between 3q23 loss and carcinogenicity has not yet been reported in AM (Fig. S2).

We used CHASM [23] for 259 SNVs to identify the driver mutations. Fifty genes with driver mutations were identified in our four patients (ranging from 3 to 29) (Fig. 1b), of which 14 were novel and had not been reported in previous AM studies. We used CGC [30] annotations to confirm the association across genes, for which driver mutations were identified. Cancer-associated mutant genes included *POT1* and *SLC34A2*, which had not been identified in previous AM studies, in addition to *KIT*, *CBL*, and *BRIP1*, which had been reported previously [1, 2,5,6,10,11,26] (Fig. 1c and Fig. S1).

We performed a SBS mutational signature (SBS signature) [29,48] analysis for our patients using the Mutalisk [28] (Fig. 1d, Fig. S3). SBS signatures 6 and 21, associated with defective DNA mismatch repair and microsatellite instability, were identified in all patients (13–27% contributions) (Fig. 1d). SBS signature 5 (Fig. 1d), associated with aging and cancer [29], was present at the highest level (82% contribution) in patient GMC-AM1 compared to that in others. It was also detected in patient GMC-AM3. SBS signature 3 (Fig. 1d), associated with deficiency of double-strand break repair via homologous recombination or *BRCA 1/2* mutations [29,48], was found to be at high levels (>70% contribution) in patients GMC-AM2 and GMC-AM4. *BRCA1* and *BRCA2* mutations were not identified in the patients. Patient GMC-AM3 had a high level of smoking-related SBS signature 4 (52% contribution) [29]. Interestingly,

the UVR-related signature (SBS signature 7 in Fig. 1d) contributed to GMC-AM3 (6%), unlike the other patients. Overall, our study suggests the heterogeneous somatic mutation profiles of Korean patients with AM.

Mapping of genetic alterations in our patients with AM to previously known melanoma pathways revealed oncogenic signaling pathways in AM.

We mapped mutations (SNVs and indels), CNAs, and DEGs from our patients to the pathways commonly found altered in previous melanoma studies [1,4,5], namely MAPK/AKT, CDK4/CCND1, MDM2/TP53, EP300/MITF, TERT, PPP6C, and IDH1 pathways (Fig. 2). All four patients showed the MAPK/AKT pathway, associated with proliferation/survival, affected; patients GMC-AM1, -AM2, and -AM4 showed the CDK4/CCND1 and EP300/MITF pathways (associated with cell cycle progression and survival) affected, patients GMC-AM1, -AM3, and -AM4 showed the MDM2/TP53 pathway (associated with apoptosis and senescence) affected, patient GMC-AM1 showed the TERT pathway (associated with telomere maintenance) affected, patients GMC-AM2 and -AM3 showed the PPP6C pathway (associated with chromatid segregation and genetic stability) affected, and patient GMC-AM1 showed the IDH1 pathway, related to epigenetic regulation, affected. We inspected whether the somatic genomic alterations of our GMC patients with AM were involved in 13 canonical cancer pathways [49]. Out of the 13 pathways, 'PI3K-AKT-MTOR signaling' and 'RTK signaling family' were commonly affected by the somatic genetic alterations of all

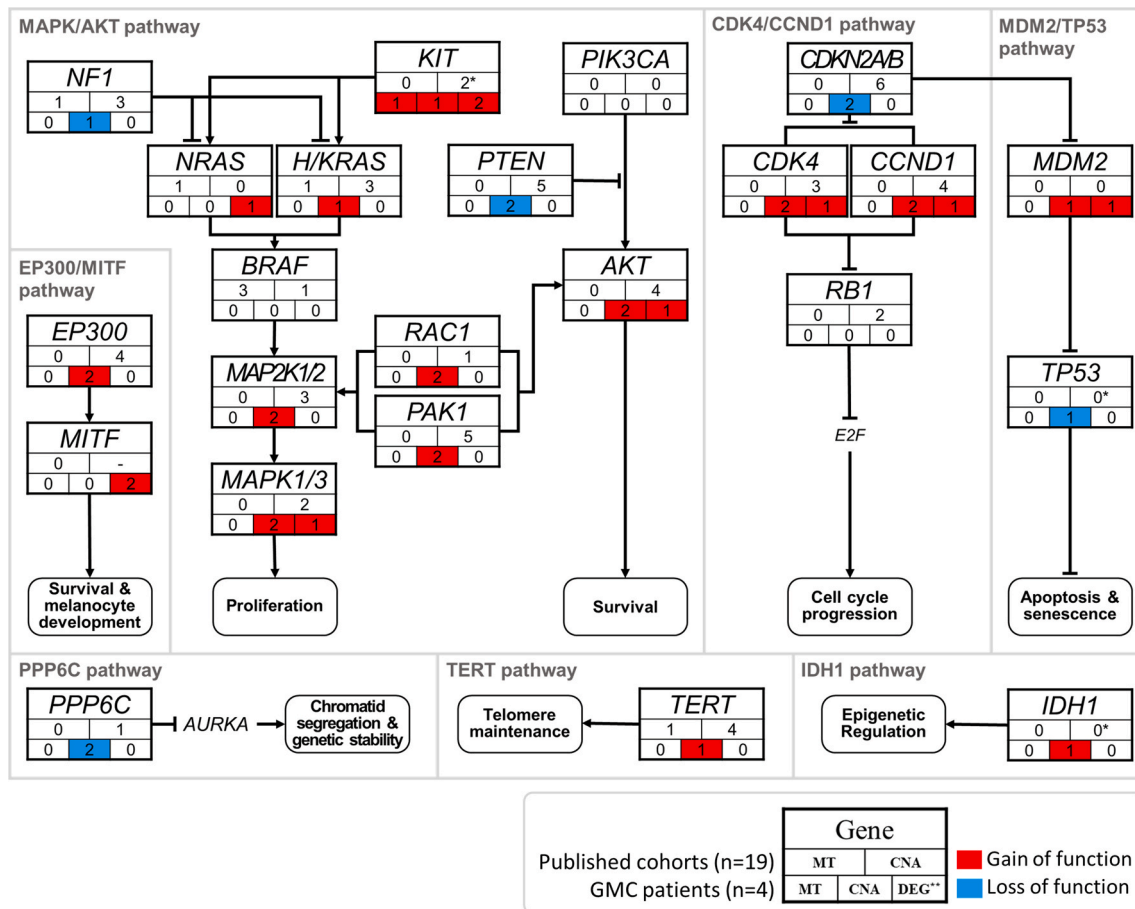


Fig. 2. Mapping genetic events of our patients with AM and of other published AM cohorts to commonly altered melanoma pathways. We mapped genetic events, including mutations, CNAs, and DEGs to the commonly altered pathways. The upper row in the rectangle indicates the gene name; the middle row indicates the number of patients with genetic events as published (TGen and TCGA), with mutations on the left and CNAs on the right; lower row indicates the number of our patients (with AM) with genetic events, with mutations on the left, CNAs in the center, and DEGs on the right. Somatic genetic alterations in our GMC patients with AM were involved in MAPK/AKT, CDK4/CCND1, MDM2/TP53, EP300/MITF, PPP6C, TERT, and IDH1 oncogenic signaling pathways. MT, mutation; *, TGen cohort missing; **, RNA-Seq was available only for our two patients, and not for the published AM cohorts.

GMC patients with AM (Fig. S4). Interestingly, each patient was associated with multiple signaling pathways, rather than a single pathway. Molecular networks from the gene expression profiles of our patients with AM revealed associations between the networks and hallmarks of cancer.

We identified the DEGs in tumor versus normal adjacent tissues of two patients (GMC-AM2 and GMC-AM4) for whom RNA-Seq data were available. We further narrowed down the DEGs to cancer-related genes using CGC [30] annotation. We then identified molecular networks of differentially expressed cancer-associated genes using the STRING [37] tool. Subsequently, we manually annotated genes associated with the hallmarks of cancer, proposed by Hanahan et al. [15], from the networks. We obtained comprehensive networks of associations between gene expression profiles of the two patients and the hallmarks of cancer (Fig. 3a). The networks included 15 genes that promote or inhibit the hallmarks of cancer. The prevalent hallmarks of cancer affected by the 15 genes were “invasion and metastasis,” “escaping programmed cell

death” and “proliferative signaling.” *CTNNB1* promoted seven hallmarks of cancer, and *KIT*, *CSF3R*, *ERBB3*, *HIF1A*, *MYD88*, *PAX3*, and *DDB2* were cross-talk genes that promoted three or more hallmarks of cancer (Fig. 3b).

3.2. Neoantigen candidates in our patients with AM were revealed

HLA mutations are associated with evasion of immune surveillance in tumors [31]. No HLA gene mutation was found in the four patients. We used RNA-Seq data from two patients (GMC-AM2 and GMC-AM4) to determine the expression levels of HLA class I genes. The expression profiles of HLA-A, -B, and -C in the tumor tissues were similar to those in the normal tissues of both patients (Fig. S5).

Mutational burden is known to strongly correlate with neoantigen burden [50], which also correlates with the response to immune checkpoint inhibitor therapy [50]. Therefore, we measured the TMB in our four patients, values ranging from 0.68 to 6.05, with a median of

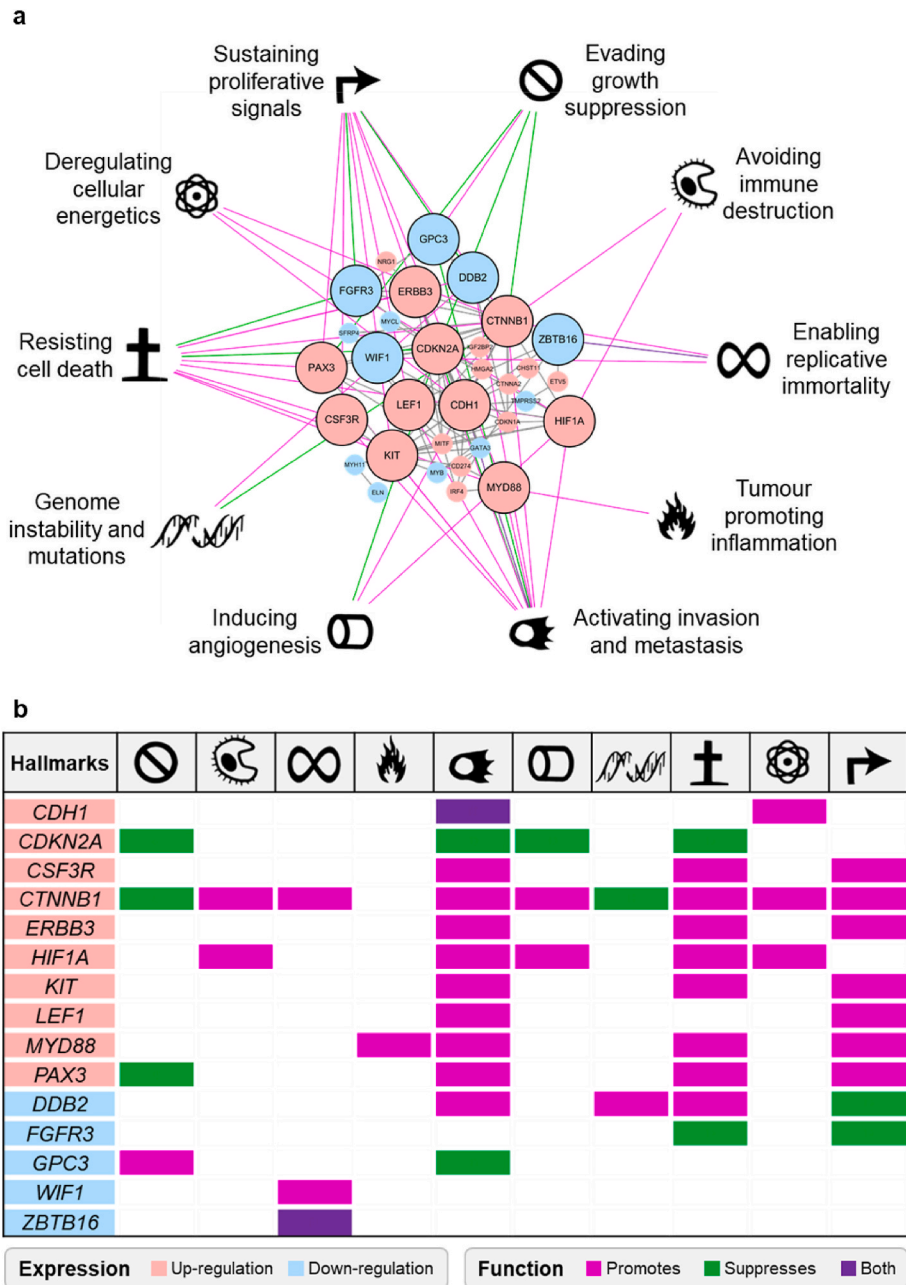


Fig. 3. Differentially expressed cancer-related gene networks and the hallmarks of cancer in our patients with AM. (a) The networks for our patients included the hallmarks of cancer, differentially expressed cancer-associated genes, and their interactions. A big node indicated the gene to be associated with the hallmark of cancer while a small node indicated a non-association. (b) DEGs associated with the hallmarks of cancer. For our two patients with AM, genes (nodes) with higher expression in tumor tissues than in normal tissues are indicated in coral, and genes (nodes) with lower expression are shown in light blue. The magenta and green edges indicate the adjacent gene (node) to promote and suppress a hallmark of cancer, respectively. The purple edge is involved in both promotion and suppression.

2.21 (Fig. 4a). TMB in our patients was relatively lower than that in patients with non-acral melanoma in TCGA, consistent with previous results that observed low TMB in AM [2,5]. Interestingly, the TMB of patient GMC-AM3 was 6.05, which was more than three times higher than that of the other three patients. Neoantigens with a binding affinity of less than 500 nM were detected by pVAC-Seq [32] analysis using WES data from all four patients. One neoantigen with medium affinity (≤ 150 nM) was detected in the GMC-AM2 cells. No neoantigen was detected in the other three patients (GMC-AM1, -AM3, and -AM4). Additional neoantigens were detected by ScanNeo [33] analysis using RNA-Seq data from two patients (GMC-AM2 and GMC-AM4). Neoantigens with binding affinity ≤ 500 nM amounted to 89 and 107 in patients GMC-AM2 and GMC-AM4, respectively (Fig. 4b). Sixteen and 25 strong-affinity neoantigens (binding affinity ≤ 50 nM) were detected in GMC-AM2 and GMC-AM4, respectively (Fig. 4b and c). Interestingly, all detected neoantigens were novel and had not been reported previously.

3.3. Comparison between ResNet and DeepTTA in the test set from CCLE

We selected two deep learning models for predicting drug responsiveness (i.e., cell viability $\ln(\text{IC}_{50})$): ResNet [16] and a transformer network, DeepTTA [18]. The two models took drug chemical information and gene expression profiles (of a cancer cell line or cancer cells of a patient) as input. We compared the performances of the two models by using the test set from CCLE database [38]. In the comparison, the root mean squared error (RMSE) and R^2 between predicted $\ln(\text{IC}_{50})$ s of the two models and actual $\ln(\text{IC}_{50})$ in the test set were obtained as evaluation measurements. As a result, ResNet demonstrated better

performance over DeepTTA (Table S4).

3.4. Application of ResNet-based drug responsiveness prediction to patients with AM

Owing to the lack of effective therapeutics for patients with AM, drug repurposing is necessary [51]. Therefore, we predicted the drug responsiveness of cancer cells in patients with AM for 24 drugs, including chemotherapeutics, from CCLE pharmacogenomics database [38]. We used gene expression data from 17 patients with AM (our two patients and 15 TGen patients) as input to our published ResNet model [16] to predict drug responsiveness (i.e., IC_{50} values) for the 24 drugs. Overall, the drug responses of all 17 patients with AM were similar to those of 39 melanoma cell lines from CCLE [38]. We also identified potential drug candidates based on a drug sensitivity cutoff, $\text{IC}_{50} < 1 \mu\text{M}$ [52]; cancer cells of patients with AM were found to be “responsive” (i.e., $\text{IC}_{50} < 1 \mu\text{M}$) to five drugs (17-AAG, irinotecan, paclitaxel, panobinostat, and topotecan) (Fig. 5). Given the drug sensitivity cutoff, two drugs (AZD6244 and PD-0325901) were predicted to be “non-responsive” ($\text{IC}_{50} \geq 1 \mu\text{M}$) in patients with AM while being “responsive” in melanoma cell lines (Fig. 5). This indicated that discrepancies between patients and cancer models do exist despite good agreement between the responses of cancer cell lines and those of the patients.

Also, we aimed at identifying important features (genes) relating to responsive drugs in our two patients with AM (i.e., GMC-AM2 and -AM4). We applied a local interpretable model-agnostic explanation (LIME) [41] to the ResNet model. As a result, we obtained the top 20 explainable features for the five responsive drugs (i.e., 17-AAG,

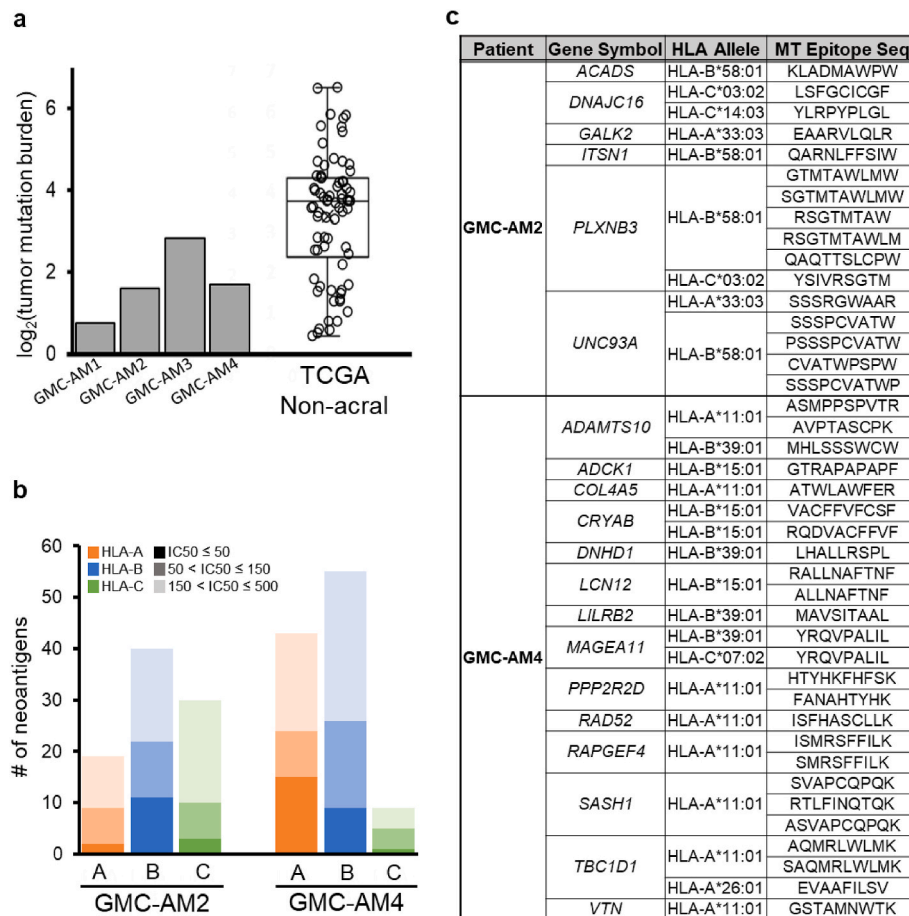


Fig. 4. HLA typing and neoantigen prediction in our patients with AM. (a) TMB of our patients with AM (bar plot) and TMB distribution in patients with non-acral melanoma from TCGA (box plot). Y-axis indicates $\log_2(\text{TMB}+1)$. (b) Number of neoantigens binding to HLA-A, -B, and -C in patients GMC-AM2 and GMC-AM4. (c) High-affinity ($\text{IC}_{50} \leq 50$ nM) neoantigens were predicted by ScanNeo. MT, mutation.

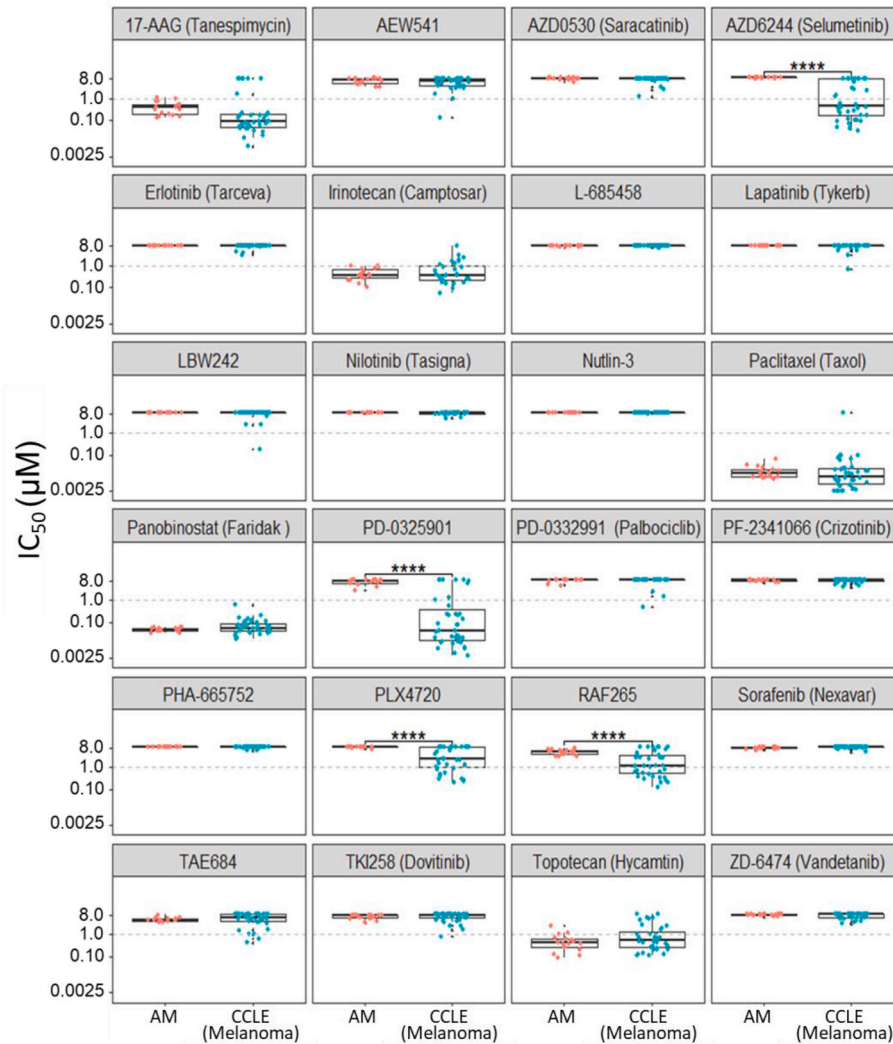


Fig. 5. Prediction of drug responses (IC_{50}), for 24 drugs, in patients with AM using a DL model. Boxplots show the predicted drug responsiveness of 17 patients with AM (pink) and the drug responsiveness of skin cell lines provided by CCLE (cyan). Adjusted P values were obtained by Bonferroni corrections, and adjusted P value < 0.05 was considered significant. ****, P value < 0.0001.

irinotecan, paclitaxel, panobinostat, and topotecan) from the two patients (GMC-AM2 and AM4). Among them, 8 features (*GAPDH*, *KRT14*, *KRT16*, *KRTDAP*, *RPL10*, *RPL37A*, *S100A8*, and *S100A9*) were common to both patients with AM (Fig. S6a). We compared feature weights (from the LIME) of the genes between the five responsive drugs and the other non-responsive drugs in the two patients. Interestingly, the feature weights of the genes showed opposite signs between responsive and non-responsive drugs in our two patients (Fig. S6b). Therefore, it indicates that these eight genes are considered as the important molecular basis for drug responsiveness in AM. In particular, *S100A8* and *S100A9* were reportedly associated with drug responsiveness in acute myeloid leukemia [53].

4. Discussion

In this study, based on the genomic and transcriptomic data of our Korean patients with AM, we provided molecular characteristics of AM while validating the previous results through comparison with other studies [1,26]. *KIT* alterations (GMC-AM2 and GMC-AM3) and *CCND1/CDK4* CNAs (GMC-AM1 and GMC-AM4), the representative recurrent alterations known in AM [1,2], were observed in our patients (Fig. 1a). Five cancer-associated genes (*KIT*, *CBL*, *BRIP1*, *POT1*, and *SLC34A2*), with driver mutations, were identified (Fig. 1c). In our

patients, the UVR-related signature was not dominant compared to other signatures, as in the study by Liang et al. [1] (Fig. 1d). Mutations, CNAs, and DEGs in our patients were involved in the pathways commonly altered in melanoma (Fig. 2). In addition, through transcriptome analysis of our patients with AM, comprehensive networks combining the hallmarks of cancer and DEGs were revealed (Fig. 3).

KIT alterations were found in two of our patients (GMC-AM2 and GMC-AM3). *KIT* plays an important role in carcinogenesis by activating the MAPK/PI3K-AKT pathway, which is involved in cell proliferation, differentiation, and survival [54]. Occasionally, *KIT* alterations may be regarded as a therapeutic target in patients with melanoma [54].

There was a gain or amplification of *CCND1* and *CDK4* in two of our patients (GMC-AM1 and GMC-AM4). Cyclin D1 (*CCND1*) and cyclin dependent kinase 4 (*CDK4*) are involved in the regulation of cell proliferation and cell cycle progression, and alterations in *CCND1* and *CDK4* play important roles in tumor formation and growth [55]. *CDK4/6* inhibitors show promising activity in some AMs with abnormalities in the *CCND1/CDK4* pathway [55].

The Protection of Telomeres 1 (*POT1*) is involved in telomere protection and maintenance, and its mutations may contribute to cancer development [56]. *POT1* mutation has been reported as a predisposing gene for familial melanoma [57]. However, the therapeutic value of targeting *POT1* is currently unknown [56].

SLC34A2 is known to be both a suppressor and a promoter of cancer [58]. *SLC34A2* mutations have been identified in 7% of CM [26], but their role in melanoma remains unknown. Research regarding diagnostic and prognostic markers and therapeutic targets for breast cancer, non-small cell lung cancer, and thyroid cancer is still ongoing [58].

We studied our patients for the commonly altered pathways observed in previous melanoma studies [1,4,5] and identified mutations, CNAs, and DEGs accordingly (Fig. 2). No mutation belonging to these pathways were observed in our cohort, except for the *KIT* mutation in one patient (GMC-AM2). In other AM studies, TGen [1] and TCGA [26], no frequently mutated gene was found in the commonly altered pathways of melanoma, with the exception of *BRAF* (16%) (Fig. 2). This highlighted the need for further research, such as on CNAs and DEGs, besides on mutations, to understand the oncogenic characteristics of AM, which is known to have a low mutational burden.

In this study, we identified a network of cancer-associated differentially expressed genes in the two patients and found the hallmarks of cancer, in the two patients (GMC-AM2 and GMC-AM4) (Fig. 3). Common mutations were not identified between the two patients, and their CNA profiles were different (Fig. 1a). In other words, identification of common functional contexts between the two patients, in terms of mutations and CNAs, was difficult. In terms of gene expression profiles, genes with the same expression patterns were identified by RNA-Seq of the two patients, and common functional contexts were subsequently identified. We confirmed, through the transcriptome analysis, that the hallmarks of cancer [15], such as “activating invasion and metastasis,” “resisting cell death,” and “sustaining proliferative signals,” were commonly promoted in both the patients.

Cancer immunotherapy is a very promising therapeutic approach for tumors; however, evidence for clinical benefit in AM is still limited. Improved therapeutic efficacy has been confirmed in melanoma through a combination of neoantigen vaccine and checkpoint immunotherapy of programmed cell death protein-1 (PD-1) [59]. Neoantigen vaccines are also applicable to tumors with a relatively low mutational burden, such as glioblastomas [59]. A literature survey of neoantigen candidates (Fig. 4c) showed *CRYAB* and *PLXNB3* to be associated with cancer. *CRYAB* is involved in diverse signaling pathways in cancer, such as apoptosis, oxidative stress, and inflammation [60]. *PLXNB3* is primarily associated with an aggressive phenotype in several carcinomas [61]. Further studies would be required to develop a therapeutic target using a personalized neoantigen vaccine [59].

In drug response prediction for AM (Fig. 5), patients with AM and melanoma cell lines had similar drug response patterns for chemotherapeutics, except for a few drugs. This indicated that melanoma cell line models could mimic the drug responses of patients with AM, despite discrepancies between cell lines and patients [62]. A literature survey for predicted potential drug candidates confirmed the efficacy of combination therapy with 17-AAG, paclitaxel, and panobinostat [63–65]. In response prediction, the IC_{50} values of 19 out of 24 drugs were relatively high ($IC_{50} > 1 \mu M$) in patients with AM, urging development of new therapeutic agents in combination therapies with conventional chemotherapies [66].

First, this study has a limitation that its sample size was small; it was due to the low incidence of AM in Korean population [6,11,13]. To overcome this limitation, we augmented the publicly available AM datasets in our analysis. This study should be validated in a larger Korean patient cohort with AM. Secondly, validation on molecular basis for further therapeutic developments for patients with AM should be experimentally performed in future.

5. Conclusion and future prospects

In this study, we systematically analyzed WES and RNA-Seq for four Korean patients with AM. We revealed that somatic genomic alterations and gene expression profiles of the patients were associated with oncogenic signaling pathways. The novelty of this study is that (i) deep

learning models were applied to potential drug repurposing under a lack of effective drug treatments in AM; and (ii) an explainable artificial intelligence (XAI) technique, LIME, revealed a potential molecular basis (i.e., *S100A8* and *S100A9*) for drug responsiveness in AM. Thus, the molecular basis should be considered for further drug developments. In the future, combining machine learning in various OMICs (e.g., proteomics [67], metabolomics [68] and pharmacometabonomics [69]) and experimental validation should be necessarily required for drug development in AM, finally leading to personalized medicine in patients with AM.

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Author contributions

Conceptualization: SN, JYR; Data curation: SM, HJK, YL, YJL, SJ, JSL, SHH; Formal Analysis: SM; Funding acquisition: JYR, SN; Investigation: SM, HKJ, YL, KK; Supervision: SN, JYR; Validation: SM, HJK; Visualization: SM, YL; Writing-original draft: SM, SN; Writing – review & editing: SM, HJK, SJ, JSL, SHH, KK, JYR, SN.

Institutional review board statement

The study was approved by the GMC Ethics Committee (GAIRB2017-173) and conducted in accordance with the Declaration of Helsinki.

Informed consent statement

All participants provided written informed consent.

Data availability statement

The WES and RNA-Seq data generated in this study were deposited in the NCBI Sequence Read Archive (SRA), accession PRJNA77736 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA777362>). The source codes, training, and test sets of DeepTTA and ResNet were deposited in the GitHub (https://github.com/labnams/acral_melanoma).

Declaration of competing interest

KK is an employee of VUNO Inc., and all authors declare no potential conflicts.

Abbreviations

AM	acral melanoma
CGC	The Cancer Gene Census
CM	cutaneous melanoma
CNA	copy number alteration
DEG	differentially expressed genes
DL	deep learning
FC	fold change
GMC	Gachon University Gil Medical CenterHLA, human leukocyte antigen
IC_{50}	half-maximal inhibitory concentration
indel	insertion-deletions
MHC	major histocompatibility complex
NGS	next-generation sequencing
SBS	single base substitution

SKCM	skin cutaneous melanoma
SNV	single nucleotide variant
TCGA	The Cancer Genome Atlas
TGen	Translational Genomics Research Institute
TMB	tumor mutation burden
TPM	transcripts per million
UVR	ultraviolet radiation
WES	whole-exome sequencing
WT	wild-type

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.compbiomed.2023.106602>.

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