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Nomogram based on autophagy related genes for predicting the survival in melanoma

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Abstract

Background: Autophagy, a highly conserved lysosomal degradation pathway, is associated with the prognosis of melanoma. However, prognostic prediction models based on autophagy related genes (ARGs) have never been recognized in melanoma. In the present study, we aimed to establish a novel nomogram to predict the prognosis of melanoma based on ARGs signature and clinical parameters.

Methods: Data from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases were extracted to identify the differentially expressed ARGs. Univariate, least absolute shrinkage and selection operator (LASSO) and multivariate analysis were used to select the prognostic ARGs. ARG signature, age and stage were then enrolled to establish a nomogram to predict the survival probabilities of melanoma. The nomogram was evaluated by concordance index (C-index), receiver operating characteristic (ROC) curve and calibration curve.

Decision curve analysis (DCA) was performed to assess the clinical benefits of the nomogram and TNM stage model. The nomogram was validated in GEO cohorts.

Results: Five prognostic ARGs were selected to construct ARGs signature model and validated in the GEO cohort. Kaplan-Meier survival analysis suggested that patients in high-risk group had significantly worse overall survival than those in low-risk group in TCGA cohort ($P = 5.859 \times 10^{-9}$) and GEO cohort ($P = 3.075 \times 10^{-9}$).

We then established and validated a novel promising prognostic nomogram through combining ARGs signature and clinical parameters. The C-index of the nomogram was 0.717 in TCGA training cohort and 0.738 in GEO validation cohort.

TCGA/GEO-based ROC curve and decision curve analysis (DCA) demonstrated that the nomogram was better than traditional TNM staging system for melanoma prognosis.

Conclusion: We firstly developed and validated an ARGs signature based-nomogram for individualized prognosis prediction in melanoma patients, which could assist with decision making for clinicians.

Keywords: autophagy; melanoma; survival; nomogram.

Introduction

Cutaneous melanoma (thereafter as “melanoma”) is one of the most aggressive skin malignancies, characterized by its high potential for invasiveness and metastasis, and limited response to treatment¹. It is estimated that there is almost 287,723 new melanoma cases and 60,712 related deaths globally in 2018². Despite considerable improvement in the treatments for melanoma, there are still several factors contributing to the poor prognosis of melanoma, including delayed diagnosis and acquired resistance to targeted therapy and immunotherapy³. Prognostic prediction is necessary to help clinicians optimize therapeutic strategies. However, until now, the prognostic prediction still relies too much on the American Joint Committee on Cancer’s (AJCC) staging system for tumor-node-metastasis (TNM), which remains limitations because melanoma patients at the same stage vary widely in the survival outcomes^{4,5}. Therefore, it is imperative to elucidate prognostic predictors for melanoma.

Autophagy, a highly conserved lysosomal degradation pathway that supports nutrient recycling and metabolic adaptation, has been implicated as a double-edged sword in carcinogenesis⁶. In the pre-malignant cells, autophagy assists in sustaining physiological tissue homeostasis and avoids early-stage development of cancer through eradicating damaged organelles⁷. On the other hand, in established cancer, active autophagic flux provides energy and macromolecular precursors for tumor cell survival and growth even under harsh microenvironmental conditions⁸. Numerous studies have reported the involvement of autophagy in melanoma prognosis⁹⁻¹². For

example, down-regulated ATG5 contributed to tumorigenesis in the early-stage melanoma and were correlated with a reduced progression-free survival¹³. Also, Atg7 deficiency could prevent melanoma development by BrafV600E and allelic Pten loss and extend mouse survival¹⁴. These findings substantiate the close correlation between autophagy and melanoma, suggesting that autophagy-related genes (ARGs) are promising predictors for melanoma prognosis¹⁵. Considering that these studies mainly focused on assessing the function of one single gene, global expression patterns based on all the ARGs could increase the accuracy of prognostic prediction. To our best knowledge, no prognostic models have been established before based on multiple ARGs expression for melanoma.

Gene expression profiling and bioinformatics analysis have been used to explore the prognostic markers in many cancers. In our study, using these high-throughput expression data, we aimed to construct and validate a novel prognostic nomogram with more accuracy than traditional TNM staging system for melanoma patients through combining ARGs signature and clinical parameters.

Materials and methods

Data collection and processing

All the RNA sequencing data were extracted from The Cancer Genome Atlas (TCGA) dataset (<https://portal.gdc.cancer.gov/>), Gene Expression Omnibus (GEO) dataset (<https://www.ncbi.nlm.nih.gov/geo/>) and the Genotype-Tissue Expression (GTEx) project using the University of California Santa Cruz (UCSC) Xena website

(<https://xenabrowser.net/datapages/>). The 222 ARGs were extracted from the Human Autophagy Database (HADb, <http://www.autophagy.lu/project.html>)¹⁶. TCGA data including 471 melanoma samples and 1 normal sample and GETx data including 812 normal tissue samples were used to identify the differentially expressed ARGs with $\log_2 | \text{fold-change (FC)} | > 2$ and adjusted P-value < 0.05 using R package “limma”. 460 melanoma samples in TCGA including overall survival were used for the following univariate and multivariate Cox analysis as the TCGA training cohort. 79 melanoma samples in GSE54467 (GEO validation cohort) was used for external validation. Before Cox analysis, the overlapped genes in TCGA and GEO cohorts were extracted and their expressions were normalized using “limma” and “sva” package in R.

Functional annotation and pathway enrichment analysis

Gene ontology (GO), including biological process, cellular component and molecular function, and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway analysis were performed for these differentially expressed ARGs using “org.Hs.eg.db”, “clusterProfiler”, “enrichplot”, “ggplot2”, “GOplot” packages in R. Adjust P value less than 0.05 was considered statistically significant.

Construction and evaluation of ARGs-based prognostic signature

Univariate Cox analysis was conducted to screen these differential expressed ARGs related to overall survival in TCGA melanoma training cohort. Then, least absolute shrinkage and selection operator (LASSO) COX regression analysis and multivariate COX regression analysis were used to select the prognostic ARGs¹⁷. The optimal

prognostic ARGs were determined to construct ARGs signature based on the Akaike information criteria (AIC). ARGs signature = $\beta_1 \times \text{expression of Gene}_1 + \beta_2 \times \text{expression of Gene}_2 + \dots + \beta_n \times \text{expression of Gene}_n$, where β is the corresponding coefficients generated by multivariate Cox analysis. The ARGs signature of each patient from the TCGA and GEO cohorts was calculated based on the above formula. The median signature from TCGA training cohort was regarded as the cutoff for both TCGA and GEO cohorts. All patients were strictly separated to high- and low-risk group with the cutoff. The survival difference for each cohort was evaluated by the Kaplan-Meier curve and log-rank test. Furthermore, to determine whether the ARGs signature could act as an independent prognostic factor, univariate and multivariate Cox analysis were performed in TCGA and GEO cohorts based on ARGs signature and clinicopathological factors including age, sex, body mass index (BMI), ulceration, Breslow depth and TNM stage. R package “glmnet”, “survminer” and “survival” were used in these analyses.

Verification of the differential expression of prognostic ARGs

Gene expression profiling interactive analysis (GEPIA) has been used widely to explore the gene expression (<http://gepia.cancer-pku.cn/index.html>) between tumors and normal samples¹⁸. GSE15605 including 46 primary melanoma samples and 16 normal samples, and GSE46517 including 31 primary melanoma samples and 7 normal samples were used to validate the differential expression of prognostic ARGs.

Establishment and validation of nomogram

ARG signature, age and stage were enrolled to establish a nomogram in TCGA

training cohort. The concordance index (C-index) and area under curve (AUC) in receiver operating characteristic (ROC) curve were generated to evaluate the discrimination of our nomogram. Calibration curve was plotted to measure the accuracy of the nomogram. Decision curve analysis (DCA) is widely used to evaluate the clinical value of models by integrating the preferences of the patients into analysis^{19, 20}. DCA was performed to assess the clinical benefits of the nomogram and TNM stage model in both TCGA and GEO cohorts. The packages of R used in the analyses are as follows: “rms”, “foreign”, “survival”, and “stdca.R”.

Results

Screening of differentially expressed ARGs and enrichment analysis

The overall design and workflow for the study is presented in **Figure 1**. Out of all ARGs, 15 differentially expressed ARGs were identified and visualized by volcano plot analysis (**Figure 2A**). Boxplot and heatmap further demonstrated that seven ARGs have higher expression while eight ARGs have lower expression in melanoma than in normal skin (**Figure 2B and 2C**). GO analysis was performed on these differentially expressed ARGs which mainly enriched in response to xenobiotic stimulus, ubiquitin protein ligase binding and autophagosome membrane (**Figure 2D**). Moreover, KEGG pathway analysis revealed that these DE-ARGs were significantly enriched in autophagy, PI3K-Akt signaling pathway and HIF-1 signaling pathway (**Figure 2E**).

Figure 1. A flowchart of the study.

Figure 2. Screening of differentially expressed autophagy-related genes (ARGs) and enrichment analysis. **(A-C)** Volcano plot **(A)**, boxplot **(B)** and heatmap **(C)** of differentially expressed ARGs between melanoma and normal samples in TCGA cohort with $\log_2 |\text{fold-change (FC)}| > 2$ and adjusted P-value < 0.05 . **(D-E)** GO **(D)** and KEGG pathway analysis **(E)**.

Identification of prognostic ARGs

Through univariate Cox analysis, we identified seven prognostic ATGs which have

significant prognostic value ($P < 0.05$) (**Figure 3A**). To avoid collinearity, we performed a LASSO logistic regression with tenfold cross-validation and finally six candidate ATGs were selected (**Figure 3B and 3C**). Then, multivariable Cox analysis further showed that APOL1 (HR = 0.86, 95% CI: 0.78 - 0.95), ATG16L2 (HR = 0.72, 95% CI: 0.54 - 0.97), DAPK2 (HR = 0.58, 95% CI: 0.29 - 1.16) were considered as protective genes, while ATG9B (HR = 1.41, 95% CI: 1.04 - 1.92) and EGFR (HR = 1.24, 95% CI: 1.07 - 1.45) were risk genes for melanoma overall survival (**Figure 3D**). To validate the differential expression of the above five genes, we analyze their expression in other GEO datasets. The results confirmed that APOL1 have higher expression in GSE46517 and ATG16L2, DAPK2, ATG9B and EGFR have lower expression in GSE15605 for primary melanoma compared with normal skin (**Figure 4A**). Interestingly, these differential expressions were independent of the status of key melanoma mutations including BRAF, NF1, RAS mutations and triple wild type using GEPIA database (**Figure 4B-4F**).

A

	p value	Hazard ratio
ITGB4	0.274	0.949 (0.863-1.043)
GABARAP	0.689	1.061 (0.795-1.416)
CTSD	0.559	1.040 (0.912-1.187)
DAPK2	0.004**	0.392 (0.208-0.739)
ATG16L2	0.001**	0.652 (0.502-0.846)
CXCR4	0.004**	0.858 (0.775-0.951)
EGFR	0.041*	1.164 (1.007-1.347)
HSP90AB1	0.269	1.132 (0.908-1.411)
BIRC5	0.056	1.178 (0.996-1.395)
VEGFA	0.062	0.884 (0.776-1.006)
ATG9B	0.009**	1.475 (1.102-1.975)
CFLAR	<0.001***	0.613 (0.486-0.773)
EIF4EBP1	0.084	1.134 (0.983-1.307)
APOL1	<0.001***	0.798 (0.734-0.868)
HSPA5	0.320	0.905 (0.744-1.102)

B

C

D

Figure 3. Identification of prognostic ARGs. **(A)** Univariate Cox analysis of 15 differentially expressed ARGs in TCGA cohort. **(B)** Selection of the optimal

parameter (λ) in the LASSO model via 10-fold cross-validation in TCGA cohort. **(C)**

LASSO coefficients produced by the regression analysis. **(D)** Multivariate Cox

analysis of the candidate ARGs obtained from LASSO regression. $P < 0.05$ was

regarded as statistically significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 4. Verification of the differential expressions of prognostic ARGs. (A) The expression of APOL1 in GSE46517, and the expression of ATG16L2, DAPK2, ATG9B, and EGFR in GSE15605. $N(T)=31$ and $N(N)=7$ in GSE46517; $N(T)=46$ and $N(N)=16$ in GSE15605. *, $P < 0.05$; ***, $P < 0.001$. (B-F) The expression of APOL1 (B), ATG16L2 (C), DAPK2 (D), ATG9B (E) and EGFR (F) in three mutational signatures (BRAF, NF1 and RAS) and wild types (WT) of melanoma. The number of sorts: $N(T)=147$ and $N(N)=558$ in BRAF mutation; $N(T)=27$ and $N(N)=558$ in NF1 mutation; $N(T)=91$ and $N(N)=558$ in RAS mutation; $N(T)=47$ and $N(N)=558$ in WT. T=tumor, N=normal skin.

Construction and evaluation for ARGs signature model

ARG signature model was established based on the regression coefficients of gene expression in multivariable Cox analysis with following formula: ARGs signature = $(-0.146 \times \text{expression of APOL1}) + (0.344 \times \text{expression of ATG9B}) + (-0.329 \times \text{expression of ATG16L2}) + (-0.546 \times \text{expression of DAPK2}) + (0.217 \times \text{expression of EGFR})$. The ARGs signature of each patient was calculated and all the patients were divided into high-risk ($n = 230$) and low-risk ($n = 230$) groups with the median risk score as the cutoff (-1.270) in the TCGA training cohort. The patients' survival time, life status, and ARGs expressions were shown in **Figure 5A**. K-M survival analysis demonstrated that patients in high-risk group had significantly poorer overall survival than those in low-risk group ($P=5.859 \times 10^{-9}$) (**Figure 5B**). To verify the robustness of the ARGs signature model, the same formula and cutoff obtained from TCGA cohort

was applied to GEO validation cohort. In line with our TCGA training cohort, patients in high-risk group (n = 38) had more death patients, poorer overall survival, increased risk gene expression and decreased protective gene expression than those in low-risk group (n = 41) in GEO cohort (**Figure 5C and 5D**).

Figure 5. Construction and evaluation for ARGs signature model. (**A-D**) The risk score distribution, survival status and gene expression profiles in TCGA cohort (**A**) and GEO validation cohort (**C**). K-M survival curve of the ARGs signature for

patients' overall survival in the TCGA cohort (**B**) and GEO validation cohort (**D**).

(**E-F**) Univariate Cox analysis of ARGs signature and clinical parameters in TCGA cohort (**E**) and GEO validation cohort (**F**).

Determination of ARGs signature as an independent prognostic factor

To evaluate whether the prognostic values of the ARGs signature was independent of clinical parameters, ARGs signature, age, sex, BMI, Breslow depth, ulceration and TNM stage were analyzed with univariate Cox analysis in TCGA cohort. The results demonstrated that ARGs signature (HR = 2.968, 95% CI: 2.181 - 4.040), age (HR = 1.023 95% CI: 1.013 - 1.032), Breslow depth (HR = 1.027, 95% CI: 1.014 - 1.041), ulceration (HR = 2.084, 95% CI: 1.498 – 2.899) and TNM stage (HR = 1.390, 95% CI: 1.185 - 1.630) were significantly associated with overall survival (**Figure 5E**). In GSE validation cohort, only age, stage, sex and ARG signature were available, in which ARGs signature (HR = 6.498, 95% CI: 2.571 - 16.421), age (HR = 1.031, 95% CI: 1.010 - 1.051) and TNM stage (HR = 1.655, 95% CI: 1.119 - 2.446) were also significantly associated with overall survival (**Figure 5F**). Through multivariate Cox regression analysis, we found that the ARGs signature (HR = 3.174, 95% CI = 1.874 - 5.376), TNM stage (HR = 1.790, 95% CI=1.192 - 2.690) and age (HR = 1.023, 95% CI = 1.004 - 1.043, P = 0.020) were independent prognostic predictors in TCGA cohort (**Figure 6A**). These results were consistent in GEO cohort (**Figure 6B**). To compare the predictive ability of our ARGs signature model, we plotted ROC curve. The ARGs signature model showed satisfactory predictive ability for 3- and 5-year overall survival rates, with AUC value of 0.715 and 0.731 respectively in TCGA

cohort (**Figure 6C**). In GEO cohort, the ARGs signature model also demonstrated a satisfactory predictive ability for the 3- and 5-year overall survival rates, with AUC value of 0.655 and 0.730 respectively (**Figure 6D**).

Figure 6. Prognostic performance of ARGs signature. (**A-B**) Multivariate Cox analysis of ARGs signature and clinical parameters in TCGA cohort (**A**) and GEO validation cohort (**B**). (**C-D**) ROC curve for predicting overall survival of 3-year (red) and 5-year (purple) in the TCGA cohort (**C**) and GEO validation cohort (**D**).

Development and validation of a prognostic nomogram

A prognostic nomogram based on the independent prognostic predictors including ARGs signature, age and TNM stage in TCGA training cohort were constructed to

predict the overall survival of melanoma patients at 3 and 5 years (**Figure 7A**). The C-index of the nomogram was 0.717 for predicting melanoma overall survival. The nomogram showed a better predictive ability for the 3- and 5-year overall survival rates with AUC values of 0.790 and 0.760 than ARGs signature (0.715 and 0.731), TNM stage system (0.672 and 0.592), or age (0.607 and 0.613) alone (**Figure 7B and 7C**). Moreover, calibration plots showed excellent agreement between the prediction of our nomogram and actual prognosis for 3 - and 5-year overall survival rates (**Figure 7D and 7E**). In addition, in GEO validation cohort, the C-index for the nomogram was 0.738. For the 5-year overall survival rates, the ROC curve demonstrated that the nomogram (AUC = 0.844) has more favorable predictive ability than other models (**Figure 7F**); moreover, the calibration plot showed excellent agreement between prediction and observation curve (**Figure 7G**). Furthermore, the DCA, for clinical usefulness evaluation, showed that the nomogram achieved the better net benefit than traditional TNM stage system in predicting the survival for melanoma patients for 3 - and 5-year overall survival rates in TCGA and GEO validation cohort (**Figure 7H-7J**).

Figure 7. Development and validation of a prognostic nomogram based on ARGs signature. **(A)** Development the nomogram based on ARGs signature and independent clinical parameters. **(B-G)** The ROC curves for nomogram, age, stage and ARGs signature for predicting the overall survival at 3-year **(B)** and 5-year **(C)** in the TCGA cohort and 5-year **(F)** in the GEO validation cohort. The calibration curves of the nomogram for predicting overall survival at 3-year **(D)** and 5-year **(E)** in the TCGA cohort and 5-year in the GEO validation cohort **(G)**. **(H-J)** Decision curve analysis of

the nomogram and TNM stage system at 3-year (**H**) and 5-year (**I**) in the TCGA cohort and 5-year in the GEO validation cohort (**J**).

Discussion

Autophagy is a highly-conserved dynamic process that deliver cellular proteins and damaged organelles to the lysosome for degradation. Recent studies showed that ARGs could regulate or be regulated by multiple signaling pathways such as PI3K/AKT/mTOR, P53/DRAM, RAS signaling pathway, which are essential for melanoma development and progression²¹⁻²⁵. Therefore, ARGs are promising prognostic predictors in melanoma. In our study, we utilized high-throughput expression profiling of autophagy related genes to generate an ARGs signature model and a novel prognostic nomogram, which could guide individualized treatment for melanoma patients in high-risk group at the early stage.

In our study, we first screen out 15 differentially expressed ARGs based on TCGA and GETx databases and then confirmed five prognostic ARGs through univariate, lasso and multivariate Cox analysis. ApoL1, a BH3-only protein, is the major apoprotein of high density lipoprotein and has never been studied in melanoma, but APOL1 is overexpressed in a variety of cancer cell types to induce autophagy and autophagy-associated cell death²⁶⁻²⁸. Liu et al. reported that APOL1 might be clinically relevant biomarkers for the diagnosis of pancreatic cancer²⁹. Moreover, a recent study demonstrated that APOL1 could predict the prognosis of pancreatic

cancer³⁰. ATG16L2, a ubiquitously expressed homologue of ATG16L1, plays pivotal roles in autophagy pathway tumorigenesis by interacting with ATG5³¹. ATG16L2 have been found to be prognostic marker for clear-cell renal cell carcinoma and stages I-III colon cancer^{32, 33}. DAPK2 is a Ca²⁺-regulated serine/threonine kinase and phosphorylates mTORC1 through direct interaction, and promotes autophagy induction through suppressing mTOR activity³⁴. In line with our finding, Li et al reported that DAPK2 is a protective gene for melanoma prognosis³⁵. As for ATG9B, which is located on chromosome 7 in humans, have been reported to be involved in the regulation of autophagy³⁶. Studies have reported that ATG9B was overexpressed in clear-cell renal cell carcinoma but down-regulated in hepatocellular carcinoma, and was associated with the cancer prognosis^{33, 37}. Our study showed that increasing ATG9B predicted poor prognosis. EGFR is well studied in melanoma and has been found to activate autophagy and melanoma cell mobility^{38, 39}. Another interesting finding is that HER3, a member of the EGFR family, is able to reactivate RAS-ERK signaling, allowing tumor cells to escape from the inhibitory effects of BRAF inhibitors⁴⁰. That suggests that combination of EGFR and BRAF inhibitor shows synergistic effects in BRAF-mutant human melanoma in preclinical model⁴¹. All these studies supported our finding that EGFR is a risk gene for melanoma prognosis. In summary, these five genes may serve as the prognostic biomarkers and targets for melanoma therapy through modulating autophagy.

We next constructed and validated a novel AGRs signature model based on the five genes to predict the prognosis of melanoma patients. Compared with the TNM stage

system, ARGs signature model showed satisfactory predictive ability of the 3- and 5-year overall survival rates, with higher AUC value. Patients with higher risk score had significantly poorer overall survival than those with lower risk score in both TCGA and GEO cohort. Moreover, the prognostic values of the ARGs signature was independent of clinical parameters through univariate and multivariate Cox analysis. That means ARGs signature is important for the prediction of melanoma prognosis. More precise individual treatment strategies should be taken for the patients in with high risk score including more aggressive treatment and closer follow-up.

Lastly, we developed a prognostic nomogram based on the clinical parameters and ARGs signature. Nomogram have been widely used in oncology and medicine, which is a steady and credible tool through combining independent risk factors in a certain disease for their intuitive presentation⁴²⁻⁴⁶. Based on individual patients' TNM stage, age and ARGs signature, our nomogram generates a numerical possibility for the overall survival. More importantly, this is the first nomogram to incorporate ARGs signature for the prediction of melanoma prognosis and has a better ability to predict the 3- and 5-year overall survival rates with higher AUC values than ARGs signature, TNM stage system or age alone. DCA is a well-established tool to evaluate the clinical value of models across a range of threshold probabilities to facilitate decisions about test selection and use^{19, 20}. DCA also showed that our nomogram added more net benefit than traditional TNM stage system in melanoma prognosis prediction for 3 - and 5-year overall survival rates.

Admittedly, our study had several limitations. First, the clinical characteristics from

TCGA and GEO datasets were limited. Some information such as therapy and tumor pathological feature was not involved in our study. Second, an external validation based on multicenter, large-scale clinical trials was necessary to confirm the prediction ability of the nomogram. Finally, we used differentially expressed ARGs to construct the ARGs signature model and nomogram, which could leave out some ARGs with prognostic value but without difference in their expression between melanoma and normal skin.

Conclusions

We detected and validated an ARGs signature model which could independently predict melanoma prognosis. Furthermore, we established and validated a novel prognostic nomogram with more accuracy than traditional TNM staging system for melanoma patients through combining both ARGs signature and clinical parameters.

Ethics approval and informed consent

Not applicable

Data availability statement

The data that support the findings of this study are openly available in TCGA dataset (<https://portal.gdc.cancer.gov/>), GEO dataset (GSE15605 and GSE46517) (<https://www.ncbi.nlm.nih.gov/geo/>) and the Genotype-Tissue Expression (GTEx) project using the University of California Santa Cruz (UCSC) Xena website (<https://xenabrowser.net/datapages/>).

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Competing interests

None declared.

Authors' contributions

FRZ and GTD conceived and designed the study. GTD and WHW analyzed the data.
GTD and FRZ wrote the manuscript. YYL and HYS revised the manuscript. All
authors read and approved the final manuscript

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Consent of publication

Not applicable

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Figure legends

Figure 1. A flowchart of the study.

Figure 2. Screening of differentially expressed autophagy-related genes (ARGs) and enrichment analysis. (A-C) Volcano plot (A), boxplot (B) and heatmap (C) of differentially expressed ARGs between melanoma and normal samples in TCGA cohort with $\log_2 |\text{fold-change (FC)}| > 2$ and adjusted P-value < 0.05 . (D-E) GO (D) and KEGG pathway analysis (E).

Figure 3. Identification of prognostic ARGs. (A) Univariate Cox analysis of 15 differentially expressed ARGs in TCGA cohort. (B) Selection of the optimal parameter (λ) in the LASSO model via 10-fold cross-validation in TCGA cohort. (C) LASSO coefficients produced by the regression analysis. (D) Multivariate Cox analysis of the candidate ARGs obtained from LASSO regression. $P < 0.05$ was regarded as statistically significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 4. Verification of the differential expressions of prognostic ARGs. (A) The expression of APOL1 in GSE46517, and the expression of ATG16L2, DAPK2, ATG9B, and EGFR in GSE15605. $N(T)=31$ and $N(N)=7$ in GSE46517; $N(T)=46$ and $N(N)=16$ in GSE15605. *, $P < 0.05$; ***, $P < 0.001$. (B-F) The expression of APOL1 (B), ATG16L2 (C), DAPK2 (D), ATG9B (E) and EGFR (F) in three mutational signatures (BRAF, NF1 and RAS) and wild types (WT) of melanoma. The number of sorts: $N(T)=147$ and $N(N)=558$ in BRAF mutation; $N(T)=27$ and $N(N)=558$ in NF1 mutation; $N(T)=91$ and $N(N)=558$ in RAS mutation; $N(T)=47$ and $N(N)=558$ in WT. T=tumor, N=normal skin.

Figure 5. Construction and evaluation for ARGs signature model. **(A-D)** The risk score distribution, survival status and gene expression profiles in TCGA cohort **(A)** and GEO validation cohort **(C)**. K-M survival curve of the ARGs signature for patients' overall survival in the TCGA cohort **(B)** and GEO validation cohort **(D)**. **(E-F)** Univariate Cox analysis of ARGs signature and clinical parameters in TCGA cohort **(E)** and GEO validation cohort **(F)**.

Figure 6. Prognostic performance of ARGs signature. **(A-B)** Multivariate Cox analysis of ARGs signature and clinical parameters in TCGA cohort **(A)** and GEO validation cohort **(B)**. **(C-D)** ROC curve for predicting overall survival of 3-year (red) and 5-year (purple) in the TCGA cohort **(C)** and GEO validation cohort **(D)**.

Figure 7. Development and validation of a prognostic nomogram based on ARGs signature. **(A)** Development the nomogram based on ARGs signature and independent clinical parameters. **(B-G)** The ROC curves for nomogram, age, stage and ARGs signature for predicting the overall survival at 3-year **(B)** and 5-year **(C)** in the TCGA cohort and 5-year **(F)** in the GEO validation cohort. The calibration curves of the nomogram for predicting overall survival at 3-year **(D)** and 5-year **(E)** in the TCGA cohort and 5-year in the GEO validation cohort **(G)**. **(H-J)** Decision curve analysis of the nomogram and TNM stage system at 3-year **(H)** and 5-year **(I)** in the TCGA cohort and 5-year in the GEO validation cohort **(J)**.

Figures

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A

	p value	Hazard ratio
ITGB4	0.274	0.949 (0.863-1.043)
GABARAP	0.689	1.061 (0.795-1.416)
CTSD	0.559	1.040 (0.912-1.187)
DAPK2	0.004**	0.392 (0.208-0.739)
ATG16L2	0.001**	0.652 (0.502-0.846)
CXCR4	0.004**	0.858 (0.775-0.951)
EGFR	0.041*	1.164 (1.007-1.347)
HSP90AB1	0.269	1.132 (0.908-1.411)
BIRC5	0.056	1.178 (0.996-1.395)
VEGFA	0.062	0.884 (0.776-1.006)
ATG9B	0.009**	1.475 (1.102-1.975)
CFLAR	<0.001***	0.613 (0.486-0.773)
EIF4EBP1	0.084	1.134 (0.983-1.307)
APOL1	<0.001***	0.798 (0.734-0.868)
HSPA5	0.320	0.905 (0.744-1.102)

B

C

D

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