An autophagy-related prognostic signature associated with immune microenvironment features of uveal melanoma

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Abstract

Autophagy is involved in cancer initiation and progression but its role in uveal melanoma (UM) has rarely been investigated. Herein, we built an autophagy-related gene (ARG) risk model for UM patients using univariate Cox regression and the least absolute shrinkage and selection operator regression model and filtered out 9 prognostic ARGs based on a cohort from The Cancer Genome Atlas (TCGA). Survival and receiver operating characteristic curve analysis of TCGA and four other independent UM cohorts (GSE22138, GSE27831, GSE39717 and GSE84976) demonstrated that the ARG-signature possessed robust and steady prognostic predictive ability. Risk scores were calculated for patients included in our study and patients with higher risk scores showed worse clinical outcomes. The expression of 9 ARGs were significantly associated with clinical and molecular features (including risk score) and overall survival of UM patients. Furthermore, we utilized univariate and multivariate Cox regression analysis to determine the independent prognostic ability of the ARG-signature. Functional enrichment analysis showed the ARG-signature was correlated with several immune-related processes and pathways like T cell activation and the T cell receptor signaling pathway. Gene set enrichment analysis identified tumor hallmarks including angiogenesis, oxidative phosphorylation, and the IL6-JAK-STAT3-signaling and reactive oxygen species pathways and were enriched in high-risk UM patients. Finally, infiltration of several immune cells and immune-related scores were found to be significantly associated with the ARG-signature. In conclusion, the ARG-signature might represent a strong predictor for evaluating the prognosis and
immune infiltration of UM patients.

**Keywords:** Uveal melanoma, Autophagy, Prognostic signature, Immune infiltration, Biomarker.
**Introduction**

Uveal melanoma (UM) is a malignant intraocular cancer derived from melanocytes with a high metastasis rate and poor prognosis[1]. Approximately 50% of patients with UM present liver metastases within 10 years from the first diagnosis, and the median survival time of patients with metastatic lesions is about 5 to 7 months[2, 3]. These data indicated that UM is an aggressive tumor with fetal malignancy. Nonetheless, despite the rapid advancements in cancer diagnosis and targeted therapy in past decades, the metastasis and death rates due to UM have not declined[3]. Currently, given the rapid development of biomedical technologies and bioinformatics, studies based on cancer expression profiles have contributed to identify diagnostic and therapeutic biomarkers for cancer, but similar studies in UM have been rare. Therefore, the identification of novel and effective prognostic and therapeutic biomarkers remains a priority for UM-tailored therapy.

Autophagy, a cellular programmed-digestion mechanism, has been reported to be associated with several pathological processes including progression of malignancy. Autophagy is a special mechanism used by cancer cells to obtain enough energy and nutriment in the hostile conditions of hypoxia, oxidative stress, and nutrient starvation[4, 5]. In recent years, numerous autophagy-related cancer studies and sufficient evidence have revealed that the autophagy process plays a crucial role in the occurrence and progression of various cancers[6-9]. Several ARGs were identified to be associated with the prognosis of cancer patients and have been defined as potential biomarkers for predicting survival and therapeutic effects of cancer patients[9, 10].
the field of UM research, several new autophagy-related prognostic biomarkers, such as \textit{P16INK4a}[11] and \textit{ABCB5}[12], have been studied, albeit there has been less attention compared with other cancer types and relative biomarkers or prognostic signatures need to be identified.

In the present study, we collected five independent UM cohorts (1 training cohort and 4 validation cohorts) and established an autophagy-related prognostic signature by using univariate Cox regression analysis and the least absolute shrinkage and selection operator (Lasso) Cox regression model in TCGA training cohort. A risk score was calculated for each patient and using the median value of risk scores, patients can be dichotomized into low- and high-risk subgroups having different overall survival (OS) and disease-free survival (DFS). Furthermore, functional enrichment analysis between low- and high-risk UM patients has revealed malignancy-related processes, pathways, and signatures associated with high-risk patients. Immune microenvironment features, including multiple immune cells and machine-learning based scores have also been investigated for their associations with the ARG-signature in UMs.

\textbf{Methods and materials}

\textbf{Data acquisition}

RNA-seq profiles and related clinical information relative to TCGA cohort were obtained from the website of TCGA (https://portal.gdc.cancer.gov/) and the related RNA expression and clinical information files of four additional cohorts (including GSE22138, GSE27831, GSE39717 and GSE84976) were downloaded from the Gene Expression Omnibus (GEO) repository (https://www.ncbi.nlm.nih.gov/geo/) and
previously published studies[13-16]. The baseline information of patients included in the present study are summarized in Table 1. The ARGs included in this study were acquired from the Human Autophagy Database (HADb, http://autophagy.lu/clustering/index.html)[17] and the GO_AUTOPHAGY gene set in the Molecular Signatures Database v6.2 (MSigDB, http://software.broadinstitute.org/gsea/msigdb)[18]. We intersected genes in the five databases and obtained a list of shared 423 ARGs for further analysis.

Data processing

For TCGA RNA-seq cohort, the Fragments Per Kilobase of transcript per Million (FPKM) values were transformed to Transcripts Per Kilobase Million (TPM) values according to an algorithm acquired in previously published studies[19, 20]. For the microarray data in the GSE22138 (GPL570), GSE27831(GPL570), GSE39717, (GPL6098) and GSE84976 (GPL10558) cohorts, background adjustments, and quantile normalization were performed on the microarray raw data using a robust multiarray averaging method (RAM) with the R packages “affy” [21] and “simpleaffy”[22]. The TPM and RAM expression values of ARGs in each cohort were utilized in following data analysis.

Construction of the ARG-signature

Using TCGA cohort as the training cohort, we performed univariate Cox regression analysis with the 423 ARGs based on the survival information in TCGA cohorts. We screened out 133 OS-related ARGs (p<0.05) including 39 protective ARGs (HR <1) and 94 risk ARGs (HR >1). These OS-related ARGs were used to further construct the
prognostic signature by Lasso Cox regression analysis, a dimension reduction analysis method. Finally, a 9-ARG risk signature was established and a risk score calculating formula based on the related expression value and coefficients of the 9 ARGs, was developed. The formula developed was as following:

$$\text{risk score} = \sum_{i=1}^{n} \text{Coef}_i \times x_i$$

in which the $\text{Coef}_i$ is the coefficient of each ARGs, and $x_i$ is the TPM value or RMA value of each ARG in each UM cohort.

Based on the formula above, a risk score was calculated for each patient and UM patients were divided into low- and high-risk subgroups by the median risk score in each cohort.

**Functional enrichment analysis**

Genes whose expression between the low- and high-risk UM patients had $|\log_2 (\text{fold change})| > 1$ and p-value $< 0.05$ were defined as differentially expression genes (DEGs) in this study. We used the R package “limma”[23] to perform differential expression analysis in TCGA cohort and we obtained 2365 DEGs. Then the R package “clusterProfiler”[24] was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the 2365 DEGs identified using the above approach. The software “GSEA” (version 4.0.1) was the main platform to identify significant cancer hallmarks between low- and high-risk UM patients in TCGA cohort based on the following cutoff values of the normalized Enrichment Score (NES) $\geq 1$, normalized p-value (Norm p) $< 0.05$ and the False Discovery Rate (FDR) $< 0.25$. 
Immune cell infiltration and calculation of immune-related scores

Based on the TPM expression values of UM patients in TCGA cohort, the proportion of 22 immune cells infiltration of each UM patient was calculated using the “CIBERSORT” website tool based on the LM22 signature gene file[25]. The ESTIMATE score, immune score, stromal score, and tumor purity values of UM patients in TCGA dataset were obtained from the website of PanCanAtlas Publications (https://gdc.cancer.gov/about-data/publications/panimmune).

Statistical methods

A two-sided log-rank test was applied to compare the OS/DFS between different risk subgroups or expression levels of ARGs of UM patients in the survival curves analysis, and was visualized using the R package “survival”. To evaluate the predictive power of the ARG-signature on OS/DFS, receiver operating characteristic (ROC) curves were plotted using the R package “timeROC”[26] and the area under curve (AUC) was calculated as the main parameter. Based on the clinical and survival information, univariate and multivariate Cox regression were the analytical methods used to determine the independent prognostic role of the ARG-signature. Correlations between risk scores and immune-related scores were determined using Pearson’s correlation analysis. Statistical analysis were implemented based on the R programming language (version 3.6.3, https://www.r-project.org/).

Results

Development of the ARG-signature in UM patients

The workflow of the development of the risk signature is shown in Figure 1. To develop
an ARG-signature that could be applied to all patients in these five cohorts, we first examined the detection statuses of the 531 ARGs in each cohort. The intersection of the detected ARGs in each cohort produced a list of shared 423 ARGs for all the cohorts. Next, univariate Cox regression analysis indicated that 133 of the 423 ARGs were associated with the OS of UM patients in TCGA cohort (p<0.05), of which 39 ARGs were protective genes (HR <1) and 94 ARGs were risk genes (HR >1). The Lasso Cox regression model was subsequently applied to the 133 OS-related ARGs to establish a prognostic model for UM patients in TCGA cohort. We successfully developed a prognostic signature based on 9 ARGs (Figure 2A, B), including *IKBKE*, *BNIP1*, *ITGA6*, *FKBP1A*, *DLC1*, *PRKCD*, *GABARAPL1*, *LMCD* and *TUSC1*. The coefficients of the nine ARGs were visualized from high to low in Figure 2C, and univariate Cox regression analysis of the 9 ARGs are summarized in Table 2.

To confirm the predictive value of the ARG-signature on UM prognosis, 80 UM patients were stratified into 40 low- and 40 high-risk patients by the median risk score in TCGA cohort. The Kaplan-Meier curves (survival analysis) revealed that the low-risk UM subgroup had higher survival rates and longer survival rates compared with the high-risk subgroup (Figure 2D). The patients’ distribution plot revealed that high-risk UM patients had a shorter OS compared with low-risk patients (Figure 2E). Furthermore, ROC analysis indicated that the risk score possessed a very high AUC value for predicting the 1/3/5-year OS of UM patients (Figure 2F, 1/3/5-year AUC=0.907/0.953/1.000), which meant that the ARG-signature exhibited robust predictive accuracy.
Furthermore, associations between the expression of the 9-gene panel and clinical and molecular features (including the risk score) were investigated. UM patients were listed in order of increasing risk score and patients with higher risk scores were associated with lower *DLC1, GABARAPL1, LMCD1, PRKCD*, and *TUSC1* expression; higher *BNIP1, ITGA6 IKBKE* and *FKBP1A* expression; greater Chr3/Chr8q loss; less Chr6p loss; and higher tumor thickness, diagnostic age, and clinical stage (Figure 2G).

**Validation of the ARG-signature in external cohorts**

In general, a robust prognostic signature requires additional validation in other cohorts. Prognostic validation analysis was used to verify the prognostic predictive stability of the ARG-signature. In the GSE84976 cohort, 28 UM patients were divided into low- and high-risk UM groups by the median risk score and survival analysis showed that high-risk UMs were associated lower OS rates and shorter OS (Figure 3A). Additional patient distributions comparing OS and clinical status also confirmed this result (Figure 3B). ROC curves revealed the risk score maintained a high level of prognostic predictive ability in the GSE84976 cohort (Figure 3C, 1/3/5 AUC=0.668/0.74/0.698).

Similar results were also found in the GSE22138 (n=63) UM cohort (Figure 3D-F) and GSE39717(n=30) UM cohort (Figure G-I), which showed that the ARG-signature could effectively distinguish low- and high-risk UMs and estimate a distinct OS time.

In addition, the GSE27831 cohort (n=29) was used to examined whether the ARG-signature could predict the DFS of UM patients. As in the results described above, DFS analysis and patient distributions of DFS indicated that high-risk UM patients had a lower DFS rate and shorter DFS time (Figure 3G, H). ROC curves further demonstrated
that the ARG-signature could effectively predict the 3/5-year DFS of UM patients (Figure 3I).

The prognostic value of the nine ARGs

Survival analysis was used to test the relationship between the OS and the expression of the ARG signature in TCGA cohort. UM patients were divided into low- and high-expression of each genes to compare survival difference between the two subgroups. UM patients with high expression of *IKBKE*, *BNIP1*, *ITGA6* and *FKBP1A* showed worse clinical outcomes, which indicated these genes might represent risk ARGs in UM patients (Figure 4A-D). Further, high expression levels of *DLC1*, *PRKCD*, *GABARAPL1*, *LMCD1* and *TUSC1* were associated with better clinical outcomes of UM patients, the 5 ARGs could be potential protective genes in UM (Figure 4E-I). The results of survival analysis were consistent with the univariate Cox regression results (Table 2).

Independent prognostic ability of the ARG-signature stratification

Univariate and multivariate Cox regression were implemented to examine the independent prognostic role of the ARG-signature in TCGA cohort. We concluded that clinical factors such as sex, age, stage, T and M statuses (N status was excluded due to the 76 N0 UM and 4 Nx UM) in the univariate Cox regression analysis. The results of univariate Cox regression showed higher age (HR:1.046, 95% CI: 1.008–1.085, p=0.019), clinical stage (HR:2.902, 95% CI: 1.149–7.330, p=0.024), M status (HR:49.280, 95% CI: 4.434–547.731, p=0.002), T status (HR:2.439, 95% CI: 1.032–5.763, p=0.042), and risk stratification (HR:3.857, 95% CI: 2.595–5.732, p<0.001)
were significant associated with the prognosis of UM patients, while sex (HR:1.542, 95% CI: 0.651–3.652, p=0.325) was not a prognostic predictor (Figure 5A).

Factors with statistical significance in the univariate Cox regression were included in the multivariate Cox regression analysis and results showed that the M stage (HR:24.99, 95%CI: 1.074–581.502, p=0.045) and the risk stratification (HR:4.243, 95%CI: 2.501–7.196, p<0.001) possessed independent prognostic roles (Figure 5B).

We noticed that half of the UM patients in TCGA received radiotherapy and the other half of UM patients were under chemotherapy. To excluded the effects of different therapy methods, we applied survival analysis in UM patients receiving different therapies and the results indicated that UM patients with higher risk scores had worse clinical outcomes independently of the treatment they received (Figure 5C, D).

Principle component analysis (PCA) showed UM patients were in distinct directions based on all detected genes or 531 ARGs profiles in TCGA cohort (the top 3 principle components were analyzed), which indicated that UM patients with different risk stratification harbored different gene expression and autophagy statuses (Figure 5E, F).

**Functional enrichment analysis**

To investigate the inner molecular distinctions between low- and high-risk UM patients, were compared DEGs of low- and high-risk patients in TCGA cohort and 2369 DEGs were identified.

In order to understand the main functional processes and pathways of the 2369 DEGs, we performed GO and KEGG pathway analysis to identify the dominating processes and functions involved. The results of the GO analysis revealed that functional
enrichments of these DEGs included the biological processes (BP) T cell activation, lymphocyte activation, leukocyte activation and differentiation; cellular components (CC) plasma membrane protein complex, extracellular matrix, collagen-containing extracellular matrix; and molecular functions (MF) receptor regulator activity, channel activity, passive transmembrane transporter activity (Figure 6A). KEGG pathway analysis indicated that cytokine-cytokine receptor interaction, cell adhesion molecules (CAMs), antigen processing and presentation, Th1 and Th2 cell differentiation, natural killer cell mediated cytotoxicity, Th17 cell differentiation, T cell receptor signaling pathway, and the NF-κB signaling pathway were significantly differentially enriched between low- and high-risk UM patients (Figure 6B).

Tumor hallmarks were also investigated between low- and high-risk UM patients in TCGA cohort using the GSEA method. We identified four tumor hallmarks including angiogenesis, oxidative phosphorylation and the IL6-JAK-STAT3 and reactive oxygen species pathways were prominently enriched in high-risk UM patients (Figure 6C).

**ARG-signature related immune microenvironment features**

Considering the results obtained from the functional enrichment analysis, we speculated that the ARG-signature was associated with the immune characterization of UM. The landscape of 22 immune cell infiltration proportions are shown in Figure 7A. Violin plots were used to visualize the differences in immune cell infiltration between low- and high-risk UM samples (Figure 7B). The results showed that B cell naive, resting memory CD4 T cells, monocytes, and resting mast cells significantly infiltrated the low-risk UM samples and CD8 T cells, activated memory CD4 T cells, and
follicular helper T cells showed greater infiltration in high-risk UM patients.

Immune microenvironment related scores, calculated using the ESTIMATE, provided an index of tumor purity, an immune score, and a stromal score for each patient using the associated algorithms. These immune microenvironment related scores were used to perform Pearson correlation analysis with the risk score to confirm the correlation between the ARG-signature and immune microenvironment status. The results showed that the risk score was positively associated with the ESTIMATE score (R=0.43, $p=6.1\times10^{-5}$), immune score (R=0.46, $p=2\times10^{-5}$), and the stromal score (R=0.31, $p=0.0048$), and was negatively associated with the tumor purity (R=−0.43, $p=8.1\times10^{-5}$).

These data demonstrated that UM patients with higher risk scores presented significant infiltration of immune and stromal cells.

**Discussion**

Along with booming evolution in biotechnology and bioinformatics, genomic analysis has been widely applied to search for cancer biomarkers or to develop diagnostic and prognostic models[27]. Some eminent studies have revealed the importance of autophagy and its prognostic role in UM[28, 29], although there have been few studies focusing on the development of prognostic predictive models using ARGs in UM patients. In the present study, we combined a univariate Cox regression model and Lasso Cox regression model to screen OS-associated ARGs and developed a prognostic ARG-signature able to distinguish patients with distinct clinical outcomes. Furthermore, unlike previous studies focusing on the development of prognostic signatures[30], our
ARG-signature was successfully validated in four external independent UM cohorts, which represents the greatest strength of our study and has supports the robustness and stability of our ARG-signature. Nevertheless, the main limitation of our study was the number of patients included in our analysis was fewer than similar studies in other cancer types, although we attempted to search for all available UM cohorts for our validation analysis.

ROC curve analysis was used as the main method to judge the prognostic prediction accuracy of the ARG-signature in our study, and we observed that the AUC value of the 5-year OS prediction in TCGA cohort and the 5-year DFS prediction in the GSE27831 cohort was 1.000, which might raise some concerns about the accuracy of our risk model. These results might be explained by the presence of only 3 patients and 1 patient, respectively in TCGA and the GSE27831 cohorts, who lived longer than 5 years. Conversely, 18, and 17 UM patients lived longer than 5 years in the GSE22138 and GSE84976 cohorts, respectively.

In the present study, we identified 9 prognostic ARGs and found high expression of \textit{IKBKE}, \textit{BNIP1}, \textit{ITGA6}, \textit{FKBP1A} and low expressions of \textit{DLC1}, \textit{PRKCD}, \textit{GABARAP1}, \textit{LMCD1} and \textit{TUSC1} were associated with worse prognosis in UM patients. The inhibitor of nuclear factor kappa B kinase subunit epsilon (\textit{IKBKE}) had previously been reported to be an oncogene and has been shown to overexpressed in over 30% of breast carcinomas samples and cell lines\cite{31}. \textit{BNIP1} could restrain cervical cancer cell proliferation, migration, and invasion by inhibit the mTOR signaling pathway, although it appears to be a risk factor in UM patients\cite{32}. Integrin
subunit alpha 6 (ITGA6), a member of the integrin alpha chain family of proteins, is an efficient early-detection biomarker and prognostic factor for colorectal cancer patients[33]. *FKBP1A* belongs to immunophilin protein family and mediates the immunosuppressive and antitumor effects of rapamycin[34]. *DLC-1*, a Rho GTPase-activating protein (RhoGAP), facilitates melanoma cell invasion and metastasis by cooperating with transcription factor FOXK1 to promote MMP9 expression[35] and enhancing colorectal cancer metastasis by the epithelial-to-mesenchymal transition[36]. Protein kinase C delta (PRKCD) is a member of the protein kinase C family of serine- and threonine-specific protein kinases and it is involved in manipulating tumor repopulation while receiving radiotherapy[37]. Lower *GABARAPL1* expression has also been correlated with poor prognosis of hepatocellular carcinoma and lymph node-positive breast cancer patients[38, 39], which might indicate that *GABARAPL1* is a negative regulator of cancer progression. LIM and cysteine rich domains 1 (LMCD1) have been reported to act as an activator of the E2F1 transcription factor in human cells[40], but its role in cancer has rarely been investigated. TUSC1 tumor suppressor candidate 1 (*TUSC1*) is a putative tumor suppressor gene, which restrains lung cancer and glioblastoma cell growth *in vitro*[41, 42]. Few studies have focused on the role of these 9 ARGs in UM and we believe that our study could help to shed light on their potential roles.

In conclusion, 257 UM patients in five independent cohorts were included in our study and were used to develop and validate a prognostic predictive signature for UM patients. The ARG-signature showed a powerful predictive ability relative to clinical outcomes.
in both the training cohort and in four external validation cohorts. Meanwhile, 9 novel prognostic ARGs were identified to be associated with the clinical outcomes of UM patients and might provide the basis for further study. Our findings revealed that UM patients with higher risk scores showed higher immune cell infiltration levels and enrichment of tumor hallmarks.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Xiaolong Yin and Deng Yan designed this study; Zhuotao Zheng performed the data analysis, prepared the figures, and wrote the manuscript; Lingyue Zhang, Zewei Tu were responsible for the data acquisition and critical reading of the manuscript. All authors have read and approved the final manuscript.

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Data Availability Statement

The data analyzed in this study are available from TCGA (http://cancergenome.nih.gov/) and GEO (https://www.ncbi.nlm.nih.gov/geo/) websites.

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Figure legends

Figure 1. Flow chart of the construction and validation of the ARG-signature.

Figure 2. Construction of the ARG-signature. (A-B) Least absolute shrinkage and selection operator (Lasso) regression was performed to calculate the minimum criteria and coefficients for constructing the ARG-signature. (C) The histogram exhibits the coefficients of the 9 ARGs. (D) The survival curves indicate that UM patients with higher risk scores were significantly associated with worse OS time. (E) The survival distribution plot showing that patients with higher risk scores are associated with shorter OS. (F) Receiver operating characteristic (ROC) curves showing the 1-, 3-, and 5-year OS predictive efficiency of the ARG-signature. (G) The heatmap showed the correlations between the expression levels of the 9 ARGs and clinical and molecular features (including the ARG-signature) of UM patients in the TCGA cohort.

Figure 3. Survival curves, survival distribution plot and ROC curves showed the ARG-signature could accurately predict the OS or DFS of patients and in the GSE84976 (A-C), GSE22138 (D-F), GSE39717(G-I) and GSE27831 (J-L).

Figure 4. (A-D) Survival analysis represented high expressions of IKBKE (A), BNIP1 (B), ITGA6 (C) and FKBP1A (D) were correlated with worse clinical outcomes of UM patients. (E-I) Survival analysis represented high expressions of DLC1 (E), PRKCD (F), GABARAPL1 (G), LMCD1 (H) and TUSC1 (I) were correlated with better clinical outcomes of UM patients.

Figure 5. Prognostic role of the ARG-signature. (A, B) Univariate Cox regression and multivariate analysis showed the ARG-signature was an independent prognostic risky factor of UM patients in the TCGA cohort. (C, D) Survival analysis indicated that the ARG-signature could divide UM patients into low- and high-risk subgroups independent of chemotherapy (C) of radiotherapy (D). (E, F) Principal component analysis (PCA) showed that low-risk and high-risk UM patients could be distinguished based on all genes’ expression or autophagy-related genes’ expression.

Figure 6. Functional enrichment analysis between low- and high-risk UM patients. (A) Gene ontology biological process (GO-BP) analysis of differential expression genes (DEGs) between low- and high-risk UM patients in the TCGA cohort. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of differential expression genes (DEGs) between low- and high-risk UM patients in the TCGA cohort. (C) Gene set enrichment analysis (GSEA) of hallmarks enriched in high-risk UM patients in the TCGA cohort.

Figure 7. Immune cell infiltration analysis of UM samples. (A) Proportion of 22 immune cells calculated by CIBERSORT algorithm of each UM samples were shown between low- and high-risk UM patients. (B) Statistic significance of the proportion of 22 immune cells between low- and high-risk UM patients. (C-F) Pearson correlation analysis showed the risk score was significantly associated with the ESTIMATEScore (C), TumorPurity (D), Immune score (E) and Stromal score (F) calculated using machining learning methods.
Table 1. Baseline information of UM patients included in this study

|                | Training cohort | Validation cohorts |          |          |          |
|----------------|----------------|--------------------|----------|----------|----------|
|                | TCGA(n=80)     | GSE22138(n=63)     | GSE27831(n=29) | GSE84976(n=28) | GSE39717(n=30) |
| OS (year)      |                |                    |          |          |          |
| Range (Median) | 0.01-7.12 (2.08) | 0.01-9.91 (2.61) | /        | 1.17-13.00 (5.50) | 0.00-7.48 (1.85) |
| DFS (year)     |                |                    |          |          |          |
| Range (Median) | /               | /                  | 1.23-5.51 (3.29) | /          | /        |
| Age (year)     |                |                    |          |          |          |
| <=60           | 40 (50%)       | 28 (44.44%)        | /        | 12 (42.86%) | 11 (36.67%) |
| >60            | 40 (50%)       | 35 (55.56%)        | /        | 16 (57.14%) | 19 (63.33%) |
| Gender         |                |                    |          |          |          |
| Male           | 45 (56.25%)    | 39 (61.90%)        | /        | /         | 23 (76.67%) |
| Female         | 35 (43.75%)    | 24 (38.10%)        | /        | /         | 7 (23.33%)  |
| M stage        |                |                    |          |          |          |
| Yes            | 3 (37.50%)     | 35 (55.56%)        | 11 (37.93%) | 14 (50.00%) | 8 (26.67%) |
| No             | 73 (91.25%)    | 28 (44.44%)        | 18 (62.07%) | 14 (50.00%) | 22 (73.33%) |
| NA             | 4 (5.00%)      | 0 (0.00%)          | 0 (0.00%) | 0 (0.00%) | 0 (0.00%)  |
| N stage        |                |                    |          |          |          |
| Yes            | 0 (0.00%)      | /                  | /        | /         | /         |
| No             | 76 (95.00%)    | /                  | /        | /         | /         |
| NA             | 4 (5.00%)      | /                  | /        | /         | /         |
| T stage        |                |                    |          |          |          |
| T2             | 4 (5.00%)      | /                  | /        | /         | /         |
|   | T3   | T4   | NA  |
|---|------|------|-----|
|   | 36(45.00%) | 38(47.50%) | 2(2.50%) |
| Histology | 13(16.25%) | 21(33.33%) | / |
|           | 37(46.25%) | 23(36.51%) | / |
|           | 30(37.50%) | 0(0.00%) | / |
|           | 0(0.00%) | 19(30.16%) | / |
| Thickness (mm) | <=10 | >10 |
|   | 37(46.25%) | 10(15.87%) | / |
|   | 43(53.75%) | 53(84.13%) | / |
| Chr 6p gain | Yes | No |
|   | 23(28.75%) | 57(71.25%) | / |
| Chr 8q gain | Yes | No |
|   | 32(40.00%) | 48(60.00%) | / |
| Chr1 loss | Yes | No |
|   | 9(11.25%) | 71(88.75%) | / |
| Chr3 loss | Yes | No |
|   | 31(38.75%) | 49(61.25%) | / |
Table 2. Univariate Cox regression analysis of the 9 ARGs in the TCGA cohort.

| ARGs    | HR   | HR.95L  | HR.95H  | pvalue  | Coefficient |
|---------|------|---------|---------|---------|-------------|
| TUSC1   | 2.1716 | 1.5322 | 3.0778 | <0.0001 | -0.0606     |
| LMCD1   | 0.6813 | 0.5515 | 0.8418 | 0.0004  | -0.0480     |
| GABARAPL1 | 1.0242 | 1.0125 | 1.0361 | <0.0001 | -0.0411     |
| PRKCD   | 0.8149 | 0.7370 | 0.9010 | 0.0001  | -0.0319     |
| DLC1    | 1.6332 | 1.3017 | 2.0491 | <0.0001 | -0.0146     |
| FKBP1A  | 1.9597 | 1.4198 | 2.7050 | <0.0001 | 0.0035      |
| ITGA6   | 0.1594 | 0.0571 | 0.4447 | 0.0005  | 0.0930      |
| BNIP1   | 0.8626 | 0.7972 | 0.9333 | 0.0002  | 0.1349      |
| IKBKE   | 0.6954 | 0.5867 | 0.8243 | <0.0001 | 0.1870      |
Survival analysis of single ARG in the TCGA cohort

A. IKBKE
   - Expression
     - High
     - Low
   - OS rate
   - p=4.049e-05

B. BNIP1
   - Expression
     - High
     - Low
   - OS rate
   - p=3.612e-04

C. ITGA6
   - Expression
     - High
     - Low
   - OS rate
   - p=1.126e-03

D. FKBP1A
   - Expression
     - High
     - Low
   - OS rate
   - p=2.453e-04

E. DLC1
   - Expression
     - High
     - Low
   - OS rate
   - p=1.148e-04

F. PRKCD
   - Expression
     - High
     - Low
   - OS rate
   - p=1.265e-06

G. GABARAPL1
   - Expression
     - High
     - Low
   - OS rate
   - p=2.94e-05

H. LMCD1
   - Expression
     - High
     - Low
   - OS rate
   - p=1.964e-08

I. TUSC1
   - Expression
     - High
     - Low
   - OS rate
   - p=7.888e-08
A  **Univariate Analysis**

| Variable | p value | Hazard ratio (95% CI) |
|----------|---------|----------------------|
| Gender   | 0.325   | 1.542 (0.651–3.652)  |
| Age      | 0.019   | 1.046 (1.008–1.085)  |
| Stage    | 0.024   | 2.902 (1.149–7.330)  |
| M        | 0.002   | 49.280 (4.434–547.731) |
| T        | 0.042   | 2.439 (1.032–5.763)  |
| Risk     | <0.001  | 3.857 (2.595–5.732)  |

B  **Multivariate Analysis**

| Variable | p value | Hazard ratio (95% CI) |
|----------|---------|----------------------|
| Age      | 0.126   | 1.052 (0.986–1.123)  |
| Stage    | 0.929   | 0.921 (0.154–5.520)  |
| M        | 0.045   | 24.990 (1.074–581.502)|
| T        | 0.182   | 3.324 (0.570–19.384) |
| Risk     | <0.001  | 4.243 (2.501–7.196)  |

**Survival analysis in patients with distinct adjuvant therapy**

**UVM Patients with chemotherapy**

- High risk
- Low risk

\[ p=4.205\times10^{-4} \]

**UVM Patients with radiotherapy**

- High risk
- Low risk

\[ p=3.157\times10^{-7} \]

**Principal component analysis**

**PCA of all genes**

**PCA of autophagy-related genes**
Functional enrichment analysis

**A. GO analysis**
- T cell activation
- Regulation of leukocyte activation
- Regulation of T cell activation
- Regulation of lymphocyte activation
- Leukocyte cell-cell adhesion
- Leukocyte differentiation
- Positive regulation of leukocyte cell-cell adhesion
- Positive regulation of cell activation
- Positive regulation of leukocyte activation
- Plasma membrane protein complex
- Receptor complex
- MHC protein complex
- Side of membrane
- MHC class II protein complex
- Plasma membrane receptor complex
- External side of plasma membrane
- Extracellular matrix
- T cell receptor complex
- Collagen-containing extracellular matrix
- Peptide antigen binding
- Channel activity
- Passive transmembrane transporter activity
- Cytokine receptor binding
- Receptor regulator activity
- MHC protein complex binding
- Cytokine activity
- MHC protein binding
- Metalloproteinase activity
- Receptor ligand activity

**B. KEGG pathway analysis**
- Graft-versus-host disease
- Cytokine-cytokine receptor interaction
- Type I diabetes mellitus
- Cell adhesion molecules (CAMs)
- Allograft rejection
- Rheumatoid arthritis
- Hematopoietic cell lineage
- Viral protein interaction with cytokine and cytokine receptor
- Autoimmune thyroid disease
- Inflammatory bowel disease (IBD)
- Antigen processing and presentation
- Th1 and Th2 cell differentiation
- Viral myocarditis
- Natural killer cell mediated cytotoxicity
- Asthma
- Th17 cell differentiation
- Leishmaniasis
- Intestinal immune network for IgA production
- Staphylococcus aureus infection
- Phagosome
- T cell receptor signaling pathway
- Systemic lupus erythematosus
- Chemokine signaling pathway
- Osteoclast differentiation
- Neuroactive ligand-receptor interaction
- Tuberculosis
- Human T-cell leukemia virus 1 infection
- Pertussis
- NF-kappa B signaling pathway
- Epstein-Barr virus infection

**C. Gene set enrichment analysis (GSEA)**
- **HALLMARK ANGIOGENESIS**
  - NES = 1.581
  - Norm p = 0.03
  - FDR = 0.222
- **HALLMARK_IL6_JAK_STAT5_SIGNALING**
  - NES = 1.699
  - Norm p = 0.02
  - FDR = 0.198
- **HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY**
  - NES = 1.608
  - Norm p = 0.04
  - FDR = 0.228
- **HALLMARK_OXIDATIVE_PHOSPHORYLATION**
  - NES = 1.730
  - Norm p = 0.003
  - FDR = 0.232
A Proportion of immune cells analysis

B Difference of immune cell proportions

C Pearson correlation analysis

D

E

F