Anoikis-related gene signature predict the prognosis and immune infiltration characterization of lung adenocarcinoma

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Abstract

**Background:** Anoikis is a mechanism utilized by organisms to defend against foreign cellular infiltration in various cancers. Anoikis-related genes (ARGs) in lung adenocarcinoma (LUAD) are still poorly understood.

**Methods:** ARGs were extracted from The Cancer Genome Atlas (TCGA) database. Concurrently, enrichment analysis were conducted using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Cox regression analysis was employed to identify prognostic genes of significance, which were subsequently utilized to establish a predictive model and calculate risk scores for individual patients. To forecast the likelihood of patient survival at different time intervals, a nomogram was constructed. Additionally, the relationship between ARGs and immunogenomic features of LUAD was elucidated using Single-sample GSEA (ssGSEA), while the Human Protein Atlas (HPA) database was utilized to verify the protein expression levels of the underlying ARGs. Ultimately, the association between LATS2 expression levels and TP53 mutation status, survival, clinical outcomes as well as the immune infiltration was explored.

**Results:** The results of functional enrichment analysis demonstrated a significant enrichment of differentially expressed ARGs in pathways associated with the cell cycle. Based on the findings from the cox regression analysis conducted in this study, a six-ARGs expression signature comprising TIMP1, SLC2A1, TRAF2, LDHA, LATS2, and HOXA10 was established. The consistency between the nomogram and actual observations was remarkably high, suggesting a high level of predictive accuracy. Noteworthy differences in immune-cell and immune-checkpoint markers were observed between the low-risk and high-risk cohorts. The hypothesis was validated by the HPA database, which confirmed that the molecules implicated in the risk model exhibit distinct expression patterns in tumors compared to normal tissues. Within the set of six analyzed ARGs, a significant association between LATS2 and immune infiltration was identified.

**Conclusions:** We discovered a 6 genes expression pattern related to anoikis. The risk model developed in this study may be useful in the prediction of patient survival.

Introduction

Lung cancer claims more than 350 lives every day, surpassing the total number of deaths resulting from breast, prostate and pancreatic cancers combined, which is the second leading cause of cancer fatalities [1]. Lung adenocarcinoma (LUAD) constitutes the most prevalent subtype, accounting for over 40% of all lung cancers [2]. With a survival rate of less than 20%, LUAD has one of the highest invasiveness and lethality rates among all cancer types globally [3]. LUAD originates in cells that produce surfactant components, with acinar, papillary, solid, micropapillary, and invasive mucinous patterns being the most common morphological classifications [4]. Multiple treatments strategies for LUAD including radiation, surgery, targeted therapy, chemotherapy and immunotherapy, either individually or in combination, have
shown promise [5, 6], while a significant percentage of LUAD cases still with poor prognosis and high mortality. At present, prognostic factors for LUAD are being explored with the widely use of next-generation sequencing and bioinformatics, which may help to predict prognosis [5, 7].

In 1993, researchers demonstrated matrix detachment-induced apoptosis or anoikis for the first time [8]. Cells can undergo programmed cell death, termed anoikis, after losing access to their extracellular matrix (ECM) or neighboring cells [9]. In the light of the relevant literature, the blockade of anoikis is an early step in the metastatic spread of cancer to distant organs. Last few years, anoikis-related genes (ARGs) have been studied in various cancers such as breast cancer, gastric cancer and colorectal cancer. [10–12]. In this way, there is a lack of relevant studies on anoikis in LUAD, the delineation of anoikis phenotypes would strengthen our perception of the mechanisms of transformation in LUAD.

The tumor microenvironment (TME) is the complex balance and mutual interaction of tumor cells, immune cells and supportive tissue cells within the tumor, which has been extensively implicated in tumorigenesis, as tumor cells harbour tumor cells that interact with surrounding cells through the vascular and lymphatic systems and influence cancer development and progression [13]. Evidence suggests that the immune phenotype defined by TME components has a concomitant effect on outcome and response to immunotherapy [14]. Research has shown a significant improvement in survival for patients with LUAD in recent years with immune checkpoint inhibitors (ICIs) [15]. Therefore, it is important to clarify the role of anoikis for the occurrence and progression of in LUAD[16].

Anoikis is a type of programmed cell death that results from prolonged suspension of cells, caused by the detachment of cells from the extracellular matrix. Resistance to anoikis can render tumor cells immune, safeguarding them while in circulation through the lymphatic and circulatory systems [17, 18]. Given the vital role of anoikis in tumor, predicting survival outcomes of LUAD based on an anoikis model has great potential. The current study aims to investigate the biological characteristics of LUAD and develop a prognostic model that integrates genomic characteristics and clinical risk factors to accurately predict survival rates at different time points. This research may pave the way for prognostic strategies at the cellular and molecular level for the management and diagnosis of LUAD.

## Materials and Methods

### Data collection and validation

Somatic mutation data, mRNA expression data, corresponding clinicopathological information and documents were pooled from the TCGA-LUAD database. The Genecards database was used to extract anoikis-related genes.

### Identification of differentially expressed genes

According to the information of TCGA database, 598 RNA-seq cases in LUAD were initially extracted and 558 cases (including 58 normal and 500 luad) were obtained for differential and survival analysis. The
TCGA gene expression data analysis was conducted using the DEseq2 package in R software, with a significance threshold of $p < 0.05$ and $\log_2|\text{fold change}| > 1$.

**Development of a model for LUAD**

To distinguish between the train and validate cohorts, the risk assessment model was systematically randomised in a 7:3 ratio. To identify genes that were related to prognosis, univariate Cox regression analysis and Lasso Cox regression analysis were employed. A prognostic risk model was established via multivariate Cox regression analysis. Using the selected genes, a clinical prediction model was developed, and a nomogram was constructed. To evaluate the accuracy of the prediction model, a calibration curve of the nomogram was generated.

**Functional enrichment analysis**

In order to elucidate the mechanism and to find potential targets in the ARGs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were carried out. Briefly, GO enrichment analysis predicts target gene function, and KEGG, a widely used systematic pathway analysis database.

**Establishment and verification of ARGs for LUAD patients**

To evaluate the prognostic signature of 6 ARGs prognostic signature, individuals with LUAD from TCGA were segregated into high-risk and low-risk groups according to the median of risk scores. The Kaplan-Meier survival analysis was utilized to identify any differences in survival rates between the two groups. The discriminative capability of the signature was tested by calculating the Receiver Operating Characteristic (ROC) and Area Under Curve (AUC). Univariate and multivariate Cox regression analysis were performed to determine whether the relationship among risk score and other clinicopathological characteristics, including age, gender, TNM stage and clinical stage. Furthermore, principal component analysis (PCA) and t-Distributed Stochastic Neighbor Embedding (t-SNE) were performed to examine the clustering ability of the risk signatures.

**Extraction of immunogenomic characteristics in LUAD**

According to the TCGA transcriptome expression data, gene expression levels in the samples were classified from high to low using ssGSEA. For different immune cell types, differences between two risk groups were examined. As immune checkpoint-related gene levels may be associated with responders to immune-checkpoint-inhibiting therapies, expression differences between the two groups were examined at different immune checkpoints. To determine the model's ability to forecast treatment response in lung adenocarcinoma, we calculated the half-maximal inhibitory concentration (IC50) of commonly used chemotherapeutic drugs.

**Protein expression levels of anoikis-related genes**

Using the HPA database, we examined protein expression levels of ARGs in normal lung and lung adenocarcinoma tissues. The database of HPA currently contains more than 26,000 antibodies. All
results are immunohistochemically stained and have had expert confirmation. It is accurate and reliable to validate our previous analysis.

**Validation of prognostic ARGs in LUAD**

In our study, we validated the prognostic value of the 6 well-researched prognostic ARGs using GEPIA and Kaplan-Meier Plotter. We also examined LATS2 expression levels, LATS2 expression distribution in LUAD subgroups in TIMER, TISIDB. To explore this further, we performed an in depth analysis to determine the predictions of the potential biological role of LUAD in Tumor Infiltrating Lymphocytes(TILs).

**Cell culture and qRT-PCR**

We procured human LUAD cells (H1975 and PC9) and normal bronchial epithelial cell (16HBE) from Cell Bank, Institute of Life Sciences, Chinese Academy of Sciences Cell Bank (Shanghai, China). The cells were authenticated using short tandem repeat (STR) profiling. The 16HBE, H1975 and PC9 cells were grown in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS). The cells were incubated in a humidified atmosphere at 37°C and 5% CO2. Total cellular RNA was isolated using RNAfast200 (Shanghai, China), and its quantity estimated by NanoDrop Lite spectrophotometer (Thermo Scientific). We performed reverse transcription on the total RNA to generate cDNA using the EvoM-ML RT Premix for qPCR (Changsha, Hunan, China), following the manufacturer's instructions. We determined relative mRNA expression levels via qRT-PCR in quadruplicate on the CFX-Connect TM Real-Time System (cfx connect) using the SYBR Green Pro Taq HS qPCR Kit. All qRT-PCR steps followed the manufacturer's guidelines. Melting curves were generated at the end of amplification to ensure the PCR product's specificity. We used GAPDH as an internal control and calculated the relative expression of each mRNA using the $2^{- \Delta \Delta C_t}$ method. qRT-PCR analysis was used to measure LATS2. The primers' sequences are provided in Table 1.

**Results**

**Identification and exploration of different expressed genes**

The objective of this research was to build an anoikis-based prediction model using the TCGA database. The research process is depicted in Fig. 1. Differential expression analysis yielded a summary of 5445 ordered genes, which 1914 genes were down and 3531 genes were up-regulated compared to normal samples. 48 lung adenocarcinoma-related anoikis genes were found, of these 30 were up-regulated and 18 were down in tumor after matching with 143 anoikis genes in the genecards database. A volcano map (Fig. 2A) illustrates differential expression of these 48 genes.

**Genetic landscape of the ARGs in LUAD**

The next step was to determine the frequency of somatic mutations and CNVs of the 48 ARGs. According to Fig. 2B, 219 of 616 (35.55%) LUAD samples showed genetic mutations, and results indicated ZEB1 as the most mutated gene, followed by CENPF and ADCY10 among 48 ARGs. In addition, we investigate the
incidence of CNV mutations. The chromosomal locations of 48 ARGs CNVs are shown in Fig. 2C. We explored the CNV mutation incidence, which showed that 48 ARGs showed obvious CNV alterations (Fig. 2D). To sum up, our results indicate that CNV might act as a regulator for ARGs expression. This is demonstrated by the significant variation in both ARGs expression levels and genomic backgrounds between LUAD and normal samples (Fig. 2E). Based on the significant differences observed in expression levels of ARGs and genomic backgrounds between LUAD and normal samples, our findings suggest that ARGs may have a possible role in LUAD tumorigenesis.

**Functional enrichment analysis of ARGs.**

We conducted a GO and KEGG enrichment analysis to identify the biological functions of the 48 ARGs. The top five GO terms associated with the genes were: Mitotic cell cycle phase transition (p = 1.31E-07), Tissue homeostasis (p = 3.96E-07), Anatomical structure homeostasis (p = 1.32E-06), Regulation of protein localization to the nucleus (p = 1.03E-06) and regulation of mitotic cell cycle phase transition (p = 1.38E-06), according to both the number of functional genes and the p-value (Fig. 3A). Table 2 also shows the top 25 GO terms. KEGG analysis revealed that the 48 ARGs were highly enriched in cell cycle, renal cell carcinoma, human T-cell leukaemia virus 1 infection and cellular senescence (Fig. 3B). Table 3 shows the top 25 KEGG terms.

**Building a predictive signature**

Patients with LUAD were randomised in a ratio of 7:3 to a treatment cohort (n = 352) or a validation cohort (n = 148). To determine the predictive value of certain anoikis-associated genes, a statistical analysis called univariate Cox regression was conducted. This analysis identified a total of 46 candidate genes that could predict prognosis for LUAD (Table 4). To visualize the relationship between these genes and LUAD prognosis, a network plot was created (Fig. 2F). Using LASSO regression analysis, nine variables, DAPK2, CENPF, TRAF2, LDHA, HMGA1, LATS2 and HOXA10, were identified as prognostic signal variables (Fig. 3C,D). Next, a predictive model for LUAD was constructed using a multivariable Cox proportional hazards model. The model incorporated 6 genes: TIMP1, SLC2A1, TRAF2, LDHA, LATS2, and HOXA10. The risk score for this model was calculated as follows: risk score = (0.000187963 x TIMP1 expression) + (0.002028102 x SLC2A1 expression) + (0.01912884 x TRAF2 expression) + (0.00094403 x LDHA expression) + (0.02107372 x LATS2 expression) + (0.033884251 x HOXA10 expression).

**Creating a prognostic nomogram**

A prognostic nomogram was developed to estimate the likelihood of survival for patients with LUAD. As the risk score had a strong correlation with prognosis, clinical factors were included in the nomogram (Fig. 3E). The individualized prediction model generated by the nomogram accurately predicted survival outcomes for LUAD patients. The calibration curve demonstrated satisfactory overlap between the nomogram-predicted probabilities and actual observations in the training cohort, indicating optimal prediction accuracy (Fig. 3F-H).
To validate the prognostic model, univariate Cox regression analysis was performed and revealed a significant association between the risk score and overall survival (HR = 1.386, 95%CI = 1.261–1.524, P < 0.001) (Fig. 4A). In the multivariate Cox analysis, the risk score was identified as an independent prognostic indicator for LUAD (HR = 1.426, 95%CI = 1.278–1.591, P < 0.001) (Fig. 4B). PCA and t-SNE analysis (Fig. 4C, D) confirmed that the risk signature could effectively classify patients. The Kaplan-Meier curves indicated distinct differences in the probability of survival between the low and high-risk groups in the training cohort (P < 0.001) (Fig. 5A), with the prognostic value being significantly better in the low-risk group. And in the validation group, the survival difference was also statistically meaningful (P = 0.003) (Fig. 5B). The AUC values for the one-, three- and five-year OS rates in the train group were 0.7274, 0.7059 and 0.6552, respectively. 0.7109, 0.7213 and 0.6071 in the validation one, which showed that this model had a good sensitivity and a good specificity (Fig. 5C, E). The AUC values for prediction of 1-year OS showed that the risk score performed better than gender, stage T, N, M and age (Fig. 5D, F). As we can see, the mortality rate for LUAD patients grows as the risk score also rises (Fig. 5G, H).

Furthermore, the heatmap of these 6 independent risks genes shows the differential expression levels between the two groups.

**Analysing LUAD's immune microenvironment**

To investigate the relationship between the 6 genes and the immunogenomic features of LUAD, we used single-sample gene set enrichment analysis (ssGSEA) to evaluate the infiltration rates of different subpopulations of human immune cells in two groups. Figure 6A shows a significant difference in the enrichment of most immunocytes between the two clusters. Enrichment levels of activated B cells, activated CD8 T cells, eosinophils, immature B cells, immature dendritic cells, mast cells, monocytes, plasmacytoid dendritic cells, and T follicular helper cells were significantly higher in the high-risk group than in the low-risk group. Conversely, enrichment levels of activated CD4 T cells, CD56 dim natural killer cells, memory B cells, neutrophils, and type 2 T helper cells were lower in the high-risk group. These findings suggest that our prognostic model is an effective approach for characterizing the immunoregulatory network in LUAD. Given the importance of checkpoint-based immunotherapy, we also compared the expression levels of several checkpoint-related genes between the two groups. Figure 6B shows a difference in the levels of TNFRSF9, CD40LG, CD276, CD48, TNFSF4, CD274, TNFSF15, and BTLA expression between the two groups.

**Drug sensitivity and ARGs**

We looked at whether there was an association between a predictive signature and overall chemotherapy effectiveness in LUAD. We found that the IC50 of etoposide, erlotinib, gefitinib, docetaxel, gemcitabine, paclitaxel, PF.02341066, vinorelbine, doxorubicin was lower in high-risk than in the other one. This is useful for exploring individualised treatment regimens that are appropriate for the patient (Fig. 7A-I).

**Verification of gene expression in LUAD tissue**
To determine the differences in expression of the 6 selected genes (TIMP1, SLC2A1, TRAF2, LDHA, LATS2, and HOXA10) between LUAD and normal lung tissue, we examined their protein expression levels using data from the HPA database (Fig. 8A-F). We found that the protein expression levels of TIMP1, SLC2A1, TRAF2, LDHA, and HOXA10 were higher in LUAD tissue than in lung tissue. However, LATS2 is lower in LUAD samples (Fig. 8A-F). These differences were consistent with what we predicted using the TCGA database. By studying the mechanism of these risk model genes in LUAD, it may provide ideas for therapeutic targets in lung adenocarcinoma.

**Validation 6- ARGs prognostic model in lung adenocarcinoma**

Validation of 6-ARG expression levels in TCGA and GTEx using GEPIA (Supplementary Fig. S1) showed that SLC2A1 and LDHA were high, whereas LATS2 was downregulated in LUAD patients. However, the differences among TIMP1, TRAF2, and HOXA10 were statistically insignificant. To further evaluate the prognostic value of the 6 selected ARGs, we conducted a Kaplan-Meier survival analysis. The results showed that high expression levels of TIMP1 (P = 0.00011), SLC2A1 (P = 3.3e-08), LDHA (P = 4e-14), LATS2 (P = 6.2e-11), and HOXA10 (P = 1e-09) were significantly associated with poor prognosis in LUAD patients. TRAF2 did not show significant prognostic value and was excluded from further investigation. Among the five significant genes, LATS2 was selected for further analysis.

**Further exploration of LATS2**

In order to investigate the relationship between LATS2 expression and tumor-infiltrating lymphocytes (TILs), we utilized the TIMER and TISIDB databases. Initially, we examined the expression of LATS2 across various cancers and observed that its expression was different in LUAD compared to normal lung tissue in BRCA, BLCA, KIRP, KICH, LUSC, LUAD, UCEC, and THCA (P < 0.001) (Fig. 9A). We also investigated the correlation between TP53 mutations and immune cell infiltration levels and found that TP53 mutation was associated with increased CD8 + T cell (P < 0.010), neutrophil (P < 0.01), and dendritic cell (P < 0.05) infiltration (Fig. 9B). When we examined the relationship between LATS2-SCNA expression level and immune cell infiltration, we observed that B cell infiltration level was linked to higher ARM score (p < 0.050)(Fig. 9C), macrophage infiltration level related to deep erasure (p < 0.001) and ARM score increase (p < 0.01), and CD4 + T cell infiltration level was connected with ARM score increase (p < 0.001). There did not appear to be an association with LATS2 SCNA levels for dendritic cell infiltration and CD8 + T cell infiltration. To investigate this further, we carried out an integrated analysis to predict LATS2’s potential biological role on LUAD TILs (Fig. 9D). The results showed that with increasing tumor purity, LUAD expression was favourably associated with B cells (partial.cor = 0.007, p = 8.73e-01), CD8 + T cells (partial.cor = 0.242, p = 6.36e-08), CD4 + T cells (partial.cor = 0.255, p = 1.33e-08), macrophages (partial.cor = 0.369, p = 3.97e-17), neutrophils (partial.cor = 0.398, p = 9.22e-20) and dendritic cells (partial.cor = 0.32, p = 4.30e-13) infiltration rates in the TIMER database. Using the TISIDB database, we sought to correlate LATS2 expression with TILs (Fig. 10). We also looked at the prognostic value of high TIL expression levels compared to the low. Kaplan-Meier analysis revealed a significant difference in
cumulative survival between low and high B cell (p = 0.00027) and dendritic cell (p = 0.048) infiltration and LATS2 expression (p = 0.024) (Fig. 9E). In addition, LATS2 was expressed in different clinical presentations of LUAD. The data indicated that LATS2 expression was lower in stage 1 than in other stages (Fig. 9F). In addition, LATS2 was highly expressed in C6 in different LUAD subtypes (Fig. 9G). Finally, our qRT-PCR analysis revealed that the expression of LATS2 was downregulated in LUAD cells (Fig. 9H), confirming the accuracy of our bioinformatics analysis.

**Discussion**

In this study, we initially identified ARGs from the TCGA and Genecards databases by the DEseq2 package in R software. To refine the potential roles of these ARGs, we performed functional enrichment analysis. We then used Cox regression analysis to select 6 ARGs for further investigation, and based on these genes, we constructed a risk model. We assessed the diagnostic efficacy of the predictive model, investigated the relationship between the 6 genes and the immune microenvironment and analysed the sensitivity of the risk genes to chemotherapy. Following the construction of the risk model based on the 6 selected ARGs, we analyzed their expression levels in LUAD and assessed their relationship with patient survival. We identified LATS2 as a promising candidate for further investigation. Additionally, we utilized the TIMER and TISIDB databases to investigate the correlation between immune cell infiltration and LATS2 expression, and performed PCR to confirm that LATS2 expression in tumor cells was consistent with our predictions. In conclusion, we have developed a risk model linked to ARG to monitor immune cell infiltration levels and assess prognosis in LUAD.

Anoikis refers to a particular form of programmed cell death that takes place when cells detach from the extracellular matrix [19]. Disruption of anoikis can lead to anchorage-independent cell growth and predisposition of malignant cells to metastasis during cancer development [20]. A large proportion of cancer-related mortality occurs after metastatic disease [21, 22]. Metastatic cancer cells undergo changes in phenotype that enable them to leave the primary tumor site, enter the bloodstream, survive harsh conditions, and eventually establish a metastasis in a distant location [23]. Anoikis resistance is a crucial factor in facilitating tumor metastasis by allowing cancer cells to spread through the bloodstream to other organs. When detached from the extracellular matrix and cell-cell contacts, tumor cells exhibit resistance to anoikis through various mechanisms,[24, 25] both paracrine and non-paracrine, thereby enabling them to survive and regain their ability to attach, invade, and metastasize. If it is normal cells, generally occur anoikis, but some tumor cells have tenacity vitality, can use all conditions to survive (including mutual support, survival signals to each other is a way), the formation of metastasis.

There are various mechanisms through which cancer cells develop resistance to anoikis. These include modifying their integrin repertoire to enable growth in different environments, activating pro-survival signals via oncogene activation, sustained autocrine loops, growth factor receptor overexpression, and mutation/upregulation of enzymes involved in integrin or growth factor receptor signaling. The tumor microenvironment plays a significant role in promoting anoikis resistance by altering matrix stiffness, generating oxidative stress, producing pro-survival factors, inducing epithelial-mesenchymal transition
and self-renewal ability, and causing metabolic deregulation [26]. Multiple studies have investigated the mechanisms associated with anoikis [27–29]. However, the use of next-generation sequencing for ARGs and the establishment of predictive risk models has not been well explored. Given the poor prognosis associated with LUAD, identifying new prognostic biomarkers using various methodologies is crucial.

Over the last few years, there has been a development of potential prognostic biomarkers for ARGs. In one study, glioblastoma (GBM) risk scores based on 27 anoikis-associated genes were systematically generated and evaluated in patients with GBM and these patterns were associated with TME [30]. To predict prognosis and response to immunotherapy in LUAD, a risk score model was used. Conducting a systematic evaluation of risk scores could deepen our understanding of anoikis and enhance the development of more personalized and accurate treatment approaches. Here, we identified 6 ARG by Cox regression analysis and further evaluated the risk scores for each gene by coefficient values and gene expression values. Based on their risk scores, patients with LUAD were divided into two groups using the median cut-off. The analysis of survival showed that the group with low risk had significantly better prognoses as compared to the high-risk group. The results indicate that these 6 genes can function as independent indicators of prognosis, and there is a need for further research on their patterns of prognostication in LUAD.

Recent functional studies implicate TIMP1 in poor prognosis in almost all cancers, contributing to cancer progression and metastasis [31]. SLC2A1 is involved in various modalities of cell death and has been suggested to be involved in the prognosis and immune microenvironment of a variety of tumors [32]. TRAF2 has been identified as an important contributor to limit the killing of cancer cells after immune checkpoint blockade using cytotoxic T cells. Alternatively, TRAF2 may act as a tumor suppressive factor in a context-dependent manner, presumably through its inhibitory effect on the alternative NFκB pathway [33]. LDHA is up-regulated in a number of tumor types and is involved in tumor development, maintenance and invasion, so inhibiting it can reduce the energy supply to tumor cells, thereby reducing their potential to metastasise and invade [34]. Based on relevant literature, HOXA10 plays a crucial role in regulating metabolism, cell junction and lysosome, DNA replication and repair, and signal transduction [35]. LATS2, on the other hand, can inhibit cell growth during the G1/S transition by reducing cyclin E/CDK2 kinase activity. It has also been shown to induce apoptosis by downregulating apoptosis inhibitors such as Bcl-2 and Bcl-xL, which is associated with epithelial-mesenchymal transition and carcinogenesis [36].

The important role of TILs and their microenvironment in cancer growth and dissemination has been highlighted in recent studies [37]. In melanoma, breast cancer, head and neck cancer, ovarian cancer, colorectal cancer and lung cancer, high immune cell infiltration has been shown to be associated with a favourable clinical outcome [38]. Furthermore, in the immunotherapy era, TILs are speculated to be a potential predictive marker for treatment efficacy [39, 40]. Earlier studies have demonstrated that the prognostic significance of TILs varies remarkably among non-small cell lung cancer (NSCLC) patients, dependent on histology and other associated factors [41]. We hypothesised that TILs may be affected by anoikis, which may affect the prognosis of those suffering from LUAD, because we have noticed, in
collaboration with previous studies, that anoikis is closely related to TILs. We selected LATS2 for further evaluation of its specificity. Furthermore, we observed a significant correlation between the expression of LATS2 and the infiltration levels of CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells. However, according to the Kaplan-Meier analysis, only infiltration scores of B-cells and dendritic cells had a significant correlation with LUAD outcome.

There are a few limitations to our current research. Firstly, it is a retrospective study that utilizes the TCGA database, which may not be able to obtain some of the specific clinical information of the patients. Second, our study requires further verification in other databases, cells or tissue samples. Lastly, the biological value of the differential genes has not been fully investigated.

**Conclusions**

In summary, we have successfully established a robust predictive signature for anoikis. Moreover, the signature contributes to a deeper understanding of anoikis. Additionally, this study paves the way for a promising approach towards anti-tumor immunotherapy in the future.

**Abbreviations**

ARGs  Anoikis-related genes  
AUC  Area Under Curve  
GBM  glioblastoma  
GO  Gene Ontology  
HPA  the Human Protein Atlas database  
IC50  the half-maximal inhibitory concentration  
ICIs  immune checkpoint inhibitors  
KEGG  Kyoto Encyclopedia of Genes and Genomes  
LUAD  lung adenocarcinoma  
PCA  principal component analysis  
ROC  Receiver Operating Characteristic  
ssGSEA  Single-sample GSEA  
TCGA  The Cancer Genome Atlas database
Declarations

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

This study was conducted based on publicly available databases, including

Genecards (https://www.genecards.org/),

GEPIA (http://gepia.cancer-pku.cn/),

HPA (https://www.proteinatlas.org/),

KM (https://kmplot.com/analysis/),

TCGA (https://www.cancer.gov/ccg/research/genome-sequencing/tcga),

TIMER (https://cistrome.shinyapps.io/timer/),


The detailed databases used in the study were described in the method section.

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Author contributions

YQ-Q designed and supervised the study. XH-K, MY-Z and RL performed analysis and drafted the manuscript. TT-L, HY-B, FS-Z, and ZG-N collected and assembled the data, collected the literature, edited figures, and checked the manuscript. YQ-Q and MY-Z revised the manuscript. All authors contributed to the article and approved the final version. All authors read and approved the final manuscript.


**Tables**

Tables 1 to 4 are available in the Supplementary Files section.

**Figures**
Flowchart of the current study. TCGA-LUAD, the Cancer Genome Atlas Lung Adenocarcinoma; Genecards, Genecards database; PCA, principal component analysis; ssGSEA, the single sample gene set enrichment analysis; HPA, The Human Protein Atlas database; GEPIA, Gene Expression Profiling Interactive Analysis; KM, Kaplan–Meier plotter; TILs, tumor-infiltrating lymphocytes;
Figure 2

Identification of differentially expressed ARGs and Genetic mutational landscape in LUAD. (A) Volcano plot of the 48 ARGs analysis. There were 30 upregulated genes and 18 downregulated genes. X-axis: log 2-fold change; Y-axis: −log10 P for each probe. (B) Genetic alteration on a query of ARGs. (C) Circus plots of chromosome distributions of ARGs. (D) Frequencies of CNV gain, loss, and non-CNV among ARGs.
(E) The PPI network acquired from the STRING database among the ARGs. (F) A network of correlations including ARGs in the TCGA cohort.

**Figure 3**

Functional enrichment analysis and Construction and validation of a nomogram. (A,B) GO and KEGG enrichment analyses of anoikis-related genes. (C,D) Construction of a prognostic-related risk model in
LUAD using LASSO regression. (E) Nomogram for predicting the 1-, 3-, and 5-year OS of LUAD patients in the train cohort. (F-H) ROC curves for predicting the 1-, 3-, and 5-year ROC curves in the entire cohort.

Figure 4

The prognostic risk model based on ARGs in LUAD. (A) Univariate Cox regression analysis of the correlation between OS and various clinicopathological features including risk score. (B) Multivariate Cox regression analysis revealed that the risk score was independent prognostic factor for predicting the OS of LUAD patients (C,D). The principle component analysis and t-SNE analysis of the prognostic anoikis-related genes in LUAD patients.
Figure 5

The prognostic value of the risk signature including 6 ARGs in training set and validation set. (A,B) Kaplan–Meier analysis of the OS in training set (P<0.001) and validation set (P=0.003). (C,D) ROC curves and the time–dependent ROC curves of the nomograms compared for 1−, 3−, and 5−year OS in training set. (E,F) ROC curves and the time–dependent ROC curves of the nomograms compared for 1−, 3−, and 5−year OS in validation set. (G) risk score plot; Survival status; Two-dimensional hierarchical clustering of
the significant 6 ARGs in training set. (H) risk score plot; Survival status; Two-dimensional hierarchical clustering of the significant 6 ARGs in validation.

**Figure 6**

Immune infiltration analysis. (A) Results for ssGSEA scores. (B) Expression of immune checkpoints among high and low risk groups. ns not significant; *P < 0.05; **P < 0.001; ***P < 0.0001;
Figure 7

Comparison of treatment drugs sensitivity between high- and low-risk groups. (A-I) Docetaxel, Erlotinib, Etoposide, Gefitinib, Gemcitabine, Paclitace, Vinorelbine, PF.02341066, Doxorubicin expression in high and low risk groups. IC50, half-maximal inhibitory concentration.
Figure 8

Results of immunohistochemistry. (A–F) Immunohistochemical images from the HPA database showing TIMP1, SLC2A1, TRAF2, LDHA, LATS2, and HOXA10 protein expressions in lung tissues (N) and LUAD (T) tissues.
Figure 9

Validation of LATS2 expression level and its associations with OS. (A) LATS2 expression level in various cancers compared with normal tissues. (B) TP53 mutation rates were associated with CD8+ T cell, neutrophil, and dendritic cell infiltration levels. (C) Correlation between SCNA level of LATS2 and immune cell infiltration level. (D) Correlation between LATS2 expression levels and immune cell infiltration levels; (E) Kaplan–Meier survival analysis by different LATS2 expression levels of LUAD. (F) LATS2 expression level in various cancers compared with normal tissues.
level in different stages of LUAD. (G) LATS2 expression distribution across LUAD subtype. (H) Validation the expression of LATS2 \( *P < 0.05; **P < 0.01; ***P < 0.001. \)

**Figure 10**

LATS2 expression level associated with immune cell infiltration levels. (A-C) Correlation between LATS2 expression level and dendritic cell infiltration levels. (D-F) Correlation between LATS2 expression level and B cell infiltration levels

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure.docx
- Table.zip