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Prediction of lung adenocarcinoma prognosis and clinical treatment efficacy by telomere-associated gene risk model

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Abstract

Background The most prevalent cause of cancer-related death in China and across the globe is lung adenocarcinoma (LUAD). Telomere shortening (TS) has been found to contribute to the development of LUAD. Therefore, our aim is to investigate the impact of telomere-related genes (TRGs) on immunotherapy and clinical prognosis prediction in LUAD.

Materials and methods TRGs were obtained from TelNet, while RNA-seq and clinical information were retrieved from the GEO and TCGA databases. TelNet preserves a series of genes known to be engaged in telomere maintenance and also provides information on the type of telomere maintenance mechanism in which the gene is involved. Data pertinent to RNA sequencing and clinical parameters were accessed from two widely-accessed electronic repositories- the GEO and TCGA databases, respectively. We conducted univariate Cox regression analysis in order to recognize prognostic TRGs and employed multivariate Cox regression analysis to develop a risk model for these TRGs. The patients were stratified into high-risk and low-risk groups based on the first quartile of the risk score. The predictive ability and stability of the model were subsequently verified through Kaplan-Meier analysis, ROC curve, and C-index. We investigated the immune landscapes of different risk groups and predicted their responses to immunotherapy. Lastly, we evaluated the sensitivity of different groups to commonly used chemotherapeutic and targeted drugs through drug sensitivity analysis.

Results Univariate Cox analysis identified 12 prognostic TRGs, while a signature consisting of 4 prognostic TRGs was constructed through multivariate Cox analysis. Survival analysis indicated a significantly shorter survival time in the high-risk group. The predictive immunotherapy analysis suggested that patients in the high-risk group may have a more favorable response to immunotherapy. Finally, we identified 28 appropriate chemotherapeutic and 51 targeted drugs for different patient groups.

Conclusion The study has successfully developed a prognostic model for LUAD prediction that takes into account TRGs and predicts both prognosis and response to immunotherapy.

Keywords Lung adenocarcinoma, Telomere, Signature, Prognosis, Immune, Drug sensitivity

1 Introduction

Lung cancer (LC) is the leading cause of cancer-related deaths in both China and globally [1]. Despite the proven effectiveness of evidence-based screening in reducing cancer-related mortality, advanced LC presentations still persist [2]. Approximately 55% of LC cases with localized dissemination survive for five years, while only 1% of cases with distant metastasis do [3]. Small cell LC (SCLC) comprises 2% of LC cases, while non-small cell LC (NSCLC) makes up 15% of all cases. Lung adenocarcinoma (LUAD), squamous LC, and large cell LC are the three main histological forms of NSCLC, representing 35%, 12%, and 15% of all NSCLC cases, respectively [4]. LUAD is a prevalent variety of NSCLC and one of the most grave and lethal tumors worldwide, posing a significant threat to human health [5]. Surgery, radiation therapy, and chemotherapy are common treatment modalities for LC [6]. However, the diagnosis of LUAD is presently challenging, with the majority of patients being diagnosed at an advanced stage. Consequently, surgery becomes more limited, and the distant metastasis of cancer cells may have adverse health effects [7]. Hence, a logical treatment strategy should aim to elevate the standard of LUAD treatment. Nowadays, a non-invasive method for estimating patient survival involves utilizing tumor risk score predictive signals. These signals are increasingly incorporated into clinical practice and can assist in accurately predicting prognosis [7, 8]. Consequently, it is critical to establish prognostic indicators capable of precisely forecasting the long-term survival of LUAD patients.

Currently, the clinical management of LUAD is an integrated and complex process. In the early stage, surgical resection is the main treatment and can achieve a high cure rate [9]. However, for advanced patients, surgery alone is often difficult to achieve the ideal effect, and traditional treatments such as chemotherapy and radiotherapy are needed. Although chemotherapy can inhibit the growth of tumor cells to some extent, it will bring serious side effects, and some patients will develop drug resistance [10]. Radiotherapy also has problems such as damage to normal tissues and limited effect of local control. Immunotherapy as an emerging treatment, brings new hope for LUAD patients. It activates the body's own immune system to fight tumors, and has better tolerance and durable efficacy than traditional therapy [11]. However, in immunotherapy, there are also many challenges, such as only some patients can benefit from it, how to accurately select patients suitable for immunotherapy, and finding more effective immunotherapy targets have become key issues.

Finding new immunotherapeutic targets is crucial. On the one hand, the discovery of new targets can expand the beneficiaries of immunotherapy and allow more LUAD patients to receive effective treatment. On the other hand, it helps to develop more targeted immunotherapy strategies to improve the therapeutic effect and reduce the adverse effects [1]. Our study focuses on telomere-related genes, and is expected to provide new targets for immunotherapy in LUAD, and bring new opportunities for improving patient outcomes, and we will elaborate on this association in subsequent introductions. Telomeres are structures found at the ends of chromosomes that comprise repeating TTAGGG DNA sequences and shelterin complexes [12]. Chromosome

stability relies on telomeres, which shorten after cell division and in certain diseases [13]. Furthermore, abnormalities in telomeres can result in several conditions, including congenital dyskeratosis, heart disease, cancer, and mental health issues [13, 14]. The role of telomeres in the onset and progression of cancer [15]. Investigations have revealed that telomere shortening (TS) can modulate cancer development in two ways [15, 16]. Firstly, TS may exert antitumor effects by impeding cell proliferation. Secondly, it could lead to significant genetic instability, hastening the advancement of cancer [17]. Nanzheng Chen et al. discovered that SNPs in TRG-ACYP2 were linked to an elevated risk of LC [18]. High DKC1 expression is indicative of a poor prognosis for LUAD, and downregulation of DKC1 leads to cellular senescence and apoptosis associated with telomeres. Their study suggests that LUAD is caused by the suppression of DKC1, which in turn causes telomere-associated senescence and apoptosis [19, 20]. The length of telomeres in cancer has been extensively researched, and previous investigations have established its correlation with cancer prognosis. Earlier studies found that altered telomere length (TL) at the chromosome ends was prominently related to LC risk, and the combination of altered TL and mean TL may help in screening people at high risk of LC, and play a more pronounced role in patient populations younger than 60 years [21, 22]. Studies have observed that TL is significantly shorter in LUAD patients than in healthy controls, and that the risk of developing LC increases with shorter TL. Further analysis revealed that the effect of TL on the risk of LC was more pronounced in SCLC patients than in LUSC and LUAD patients. The TS may be a risk factor for LC [23]. However, as of yet, the precise influence of telomere-related genes (TRGs) on the prognosis of LUAD has not been fully investigated. Extensive studies are needed to adequately comprehend the role of TRGs in shaping the prognosis of LUAD, which may help recognize novel therapeutic targets and prognostic markers for LC research. There is a complex and tight link between immune signatures and telomerase shortening [24]. Numerous studies have shown that the activation or inhibition state of the immune system affects the proliferation and aging process, and telomerase as a key enzyme in maintaining telomere length, its activity changes are closely related with the proliferative capacity. For example, under immune stress, the massive activation and proliferation of immune cells may lead to changes in telomerase activity, which in turn affects telomere length [25]. Several inflammatory factors are able to modulate the expression of telomerase-related genes, indirectly affecting telomerase function, leading to accelerated telomere shortening. In addition, the senescence of immune cells also interacts with the telomere shortening. If the telomere length of the aged immune cells shortens, their immune function will also decline, thus affecting the balance of the entire immune system. Inflammatory factors play an important regulatory role in this process [26]. They can influence the expression of telomerase-related genes through multiple signaling pathways. For example, the nuclear factor- κ B (NF- κ B) signaling pathway is widely activated in the inflammatory response, and many inflammatory factors such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) can activate NF- κ B [27]. Once NF- κ B is activated, it enters the nucleus and binds to the promoter regions of telomerase-related genes to regulate the transcriptional process of the genes. We found that TNF- α can upregulate the expression of telomerase reverse transcriptase (TERT) gene in some cells, which may contribute to the maintenance of telomere length in the short term. However, long-term high TNF- α stimulation may lead to excessive cellular stress response, disrupt the normal

regulatory mechanism of telomerase and accelerate telomere shortening. IL-6 can similarly affect TERT gene expression through the JAK-STAT signaling pathway, and subsequently affect telomerase function [28].

In the study, we identified prognostic TRGs and developed a risk assessment signature. Subsequently, we examined the immunologic features and responsiveness to immunotherapy of various groups and evaluated different chemotherapy and targeted drugs for their clinical management in LUAD. To summarize, this study has successfully developed a prognostic model for LUAD to forecast the prognosis and response to immunotherapy based on TRGs for LUAD.

2 Methods

2.1 Preparation of data

The RNA-seq, clinical, and mutation information for LUAD were obtained from TCGA-LUAD and GEO-GSE68465 [29]. To ensure the reliability of our analyses, we excluded data that had missing values or a survival time of less than 30 days [30]. TRGs were obtained from TelNet (Score > 2; Tab S1) [31].

2.2 Development and validation of model

Univariate Cox analysis was performed to recognize prognostic TRGs ($p < 0.001$). In Cox univariate analysis, relevant patient information and observation time were first collected. Then, a Cox proportional hazards model was applied to estimate the effect of each factor on survival or recurrence, here mainly TRGs. The key idea of the Cox proportional hazards model is to quantify the effect of factors on survival or recurrence by estimating the hazard ratio (hazard ratio) [32]. A multivariate Cox analysis was used to develop a risk model for these TRGs [33]. We computed the risk score for each LUAD patient using a formula: $\sum_{i=1}^{k} \beta i Si$. The external validation set, GEO-GSE68465, was employed to verify both the predictive ability and stability of the model [29]. Survival rates of different groups were assessed using Kaplan-Meier analysis and log-rank test [34]. The time-dependent ROC curves and the AUC were employed to estimate the reliability of survival prediction.

To evaluate the applicability of the model across patients with diverse clinical characteristics, we examined survival disparities between different risk score groups within each subgroup. Our differential genetic analysis of the high-risk and low-risk groups. Univariate and multivariate Cox regression analyses were employed to validate the model as an independent prognostic predictor. The C-index was used to assess the accuracy of the model. Furthermore, we constructed a nomogram that incorporates both the model and clinical characteristics to predict the 1-, 3-, and 5-year survival rates for patients with LUAD.

We identified DEGs between the different groups ($|\log FC| > 1$ and $FDR < 0.05$). Subsequently, GO and KEGG analyses were performed on these DEGs to explore potential functional and pathway differences ($p < 0.05$) [35]. We evaluated the overall expression levels of TRGs using the ssGSEA algorithm.

2.3 Evaluation of immune landscape

Both groups were assessed for immune landscape based on the developed characteristics classified as high risk and low risk groups. Mutational analysis was performed to

identify the number of gene mutations, and TIDE and TMB scores were computed in order to predict response to immunotherapy [36, 37]. The TIDE algorithm evaluates the presence of tumor immune evasion mechanisms and predicts immunotherapy response, while the TMB score quantifies the total burden of genetic mutations within the tumor, serving as a measure of neoantigen load. The various algorithms were used to calculate immune cell infiltration [38–43]. Through the utilization of these algorithms, we were able to obtain a comprehensive and quantitative analysis of immune cell infiltration patterns. We performed a single-sample ssGSEA to evaluate the variances in immune function and examined the expression levels of distinct immune checkpoint genes.

2.4 Identification of anti-tumor drugs

We utilized the "pRRophetic" R package to compute the IC50 values of drugs and compared the IC50 to evaluate the efficacy of conventional chemotherapeutic and targeted drugs used in clinical management of LUAD ($p < 0.05$) [44].

2.5 Statistical analyses

We performed statistical analyses and generated figures for the research using R software version 4.1.3, a widely recognized open-source tool for statistical analysis. We used the Wilcoxon test to compare and assess gene expressions between various groups. The significance level for determining statistical significance was set at $P < 0.05$.

3 Result

3.1 Construction and validation of risk assessment signature

Initially, 12 candidate prognostic TRGs were recognized through a univariate Cox analysis (Fig. 1A). Further analysis using a multivariate Cox model led to the development of a signature consisting of 4 select prognostic TRGs (Fig. 1B). The survival analysis revealed a statistically significant decrease in survival time among individuals classified as high-risk based on the developed signature ($p < 0.001$; Fig. 1C). This finding was consistent across both the traning set and the independent testing set (GSE68465) ($p = 0.002$; Fig. 1D). The validation set (GSE68465) serves as an external confirmation cohort, further supporting the clinical relevance and potential applicability of the signature in real-world settings. The agreement between the findings in the validation set and the initial dataset demonstrates the robustness and reproducibility of the signature's prognostic performance. The model was employed to forecast the 1-, 3-, and 5-year survival rates of LUAD patients, and the AUC values were 0.642, 0.677, and 0.666 (Fig. 1E). The AUC of the model was higher than other clinical features, indicating that it is more reliable (Fig. 1F). The ssGSEA results showed that TRG scores were significantly higher in the low-risk group, suggesting that patients may have longer TLs (Fig S1).

According to the various subsets, the low-risk group patients had a longer survival time, implying that the signature is relevant to patients with a variety of clinical characteristics (Fig. 2A). The risk score was found to be an independent prognostic factor in Cox analysis ($p < 0.001$; Fig. 2B). The C-index demonstrated that the model outperformed conventional clinical criteria in predicting LUAD prognosis (Fig. 3A). The observed 1-, 3-, and 5-year survival rates demonstrated a strong agreement with the projected rates in the correlation plot (Fig. 3B). A nomogram was constructed incorporating the developed signature along with carefully selected clinical features, aiming to enhance

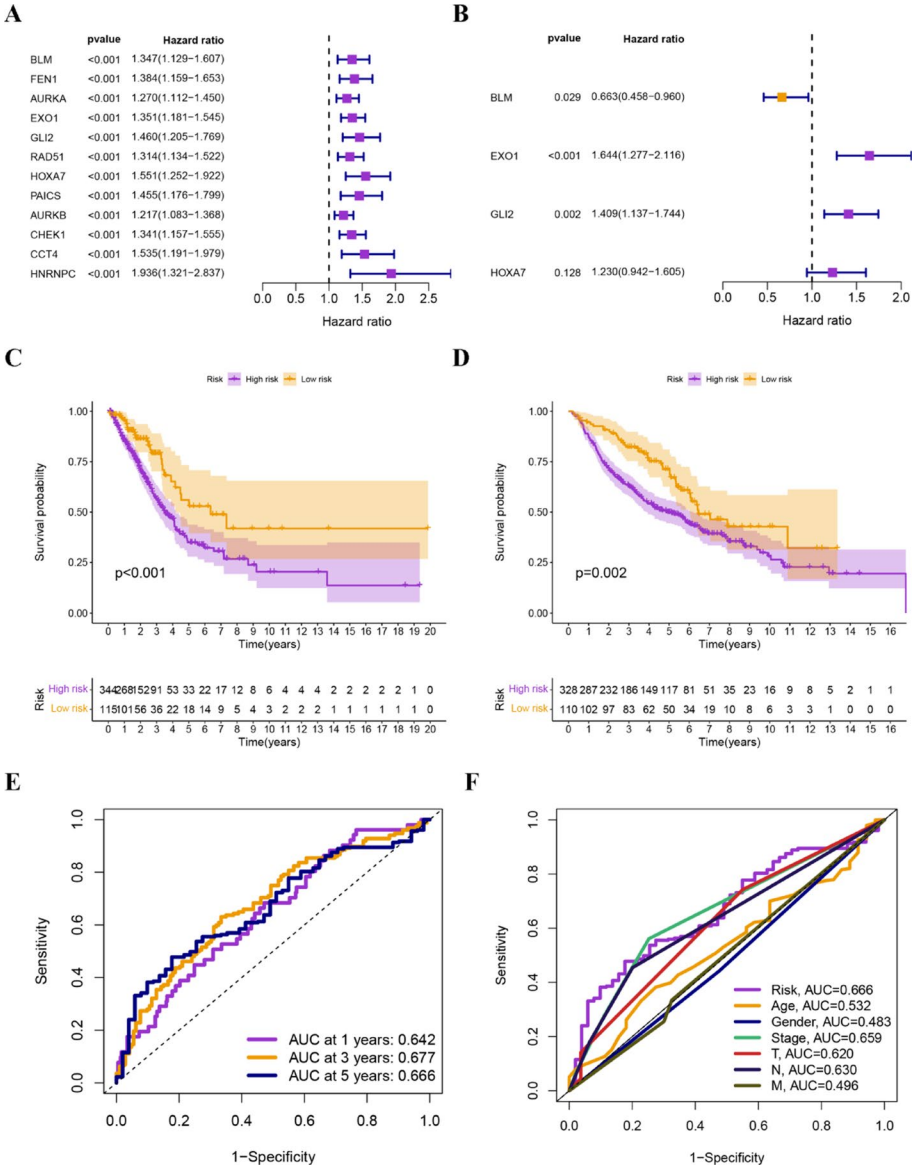

Fig. 1 Construction and validation of this prognostic model. (A) Univariate Cox analysis. (B) Multivariate Cox analysis. (C and D) The survival analysis from TCGA-LUAD and GSE68465. (E) The AUC values for the model. (F) The AUC of the model was also higher than other clinical features

the accuracy of survival predictions for patients with LUAD (Fig. 3C). This tool allows clinicians to evaluate multiple factors simultaneously and derive a personalized estimation of the patient's prognosis.

3.2 Enrichment analysis

To explore the diverse molecular mechanisms underlying different groups, we employed a comprehensive analysis to identify 689 DEGs between these groups (Tab S2). The GO analysis showed that the BP terms of the DEGs were primarily associated with mitotic nuclear division, nuclear division, and extracellular matrix organization. In terms of CC, the DEGs were found to be related to the collagen-containing extracellular matrix, chromosome, centromeric region, and condensed chromosome, centromeric region. Furthermore, the MF terms were predominantly associated with extracellular matrix

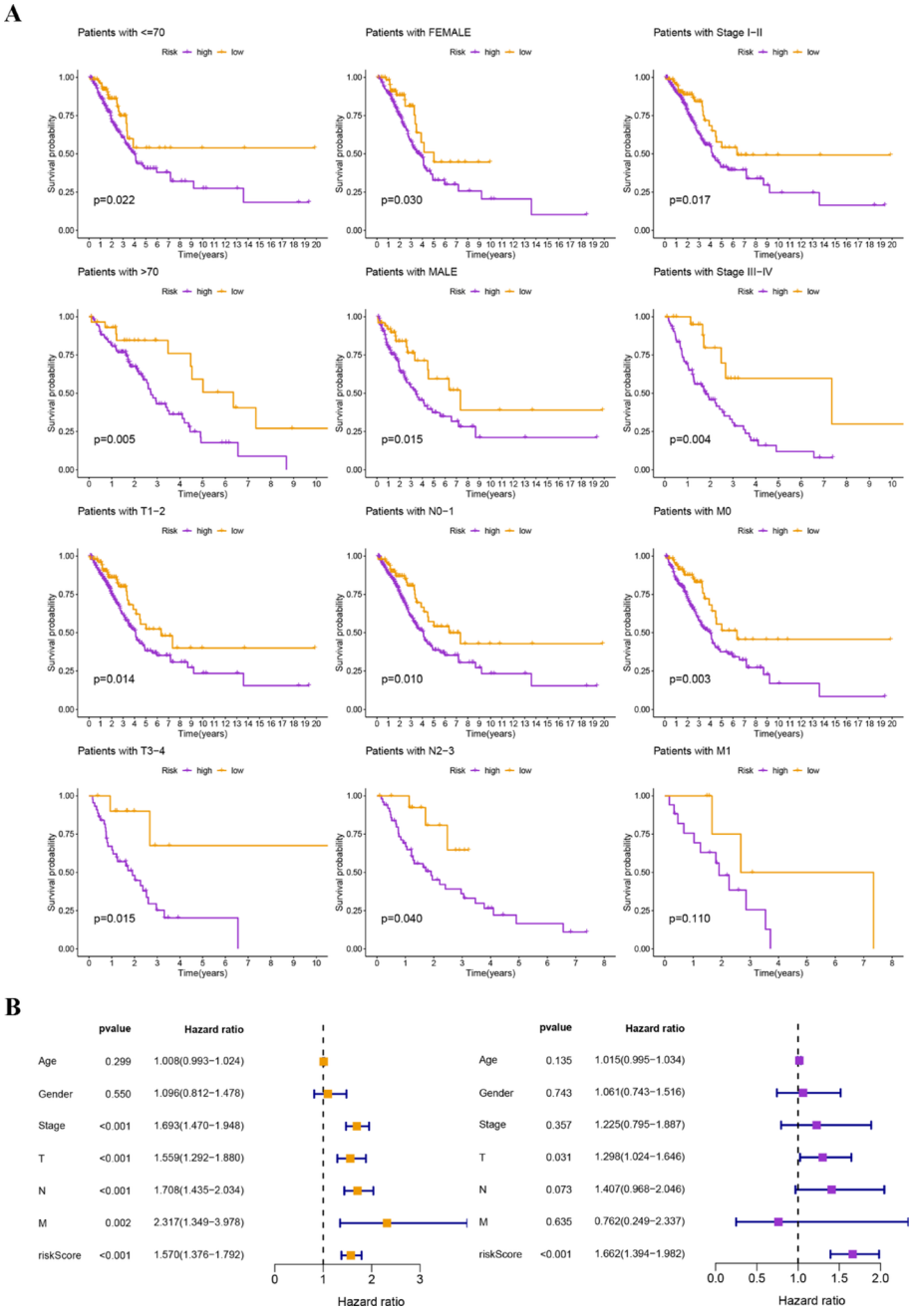

Fig. 2 Evaluation of the prognostic model. (A) According to the various clinical subgroups, patients in the low-risk group had a longer survival time. (B) The risk score was found to be an independent prognostic factor

structural constituent, extracellular matrix structural constituent conferring tensile strength, and serine-type endopeptidase inhibitor activity (Fig. 4A, C, E and Tab S3). Additionally, KEGG analysis was performed to recognize enriched pathways among the DEGs. This analysis unveiled various pathways that were significantly enriched, including protein digestion and absorption, cell cycle, ECM-receptor interaction, p53 signaling pathway, oocyte meiosis, and focal adhesion (Fig. 4B, D, F and Tab S4).

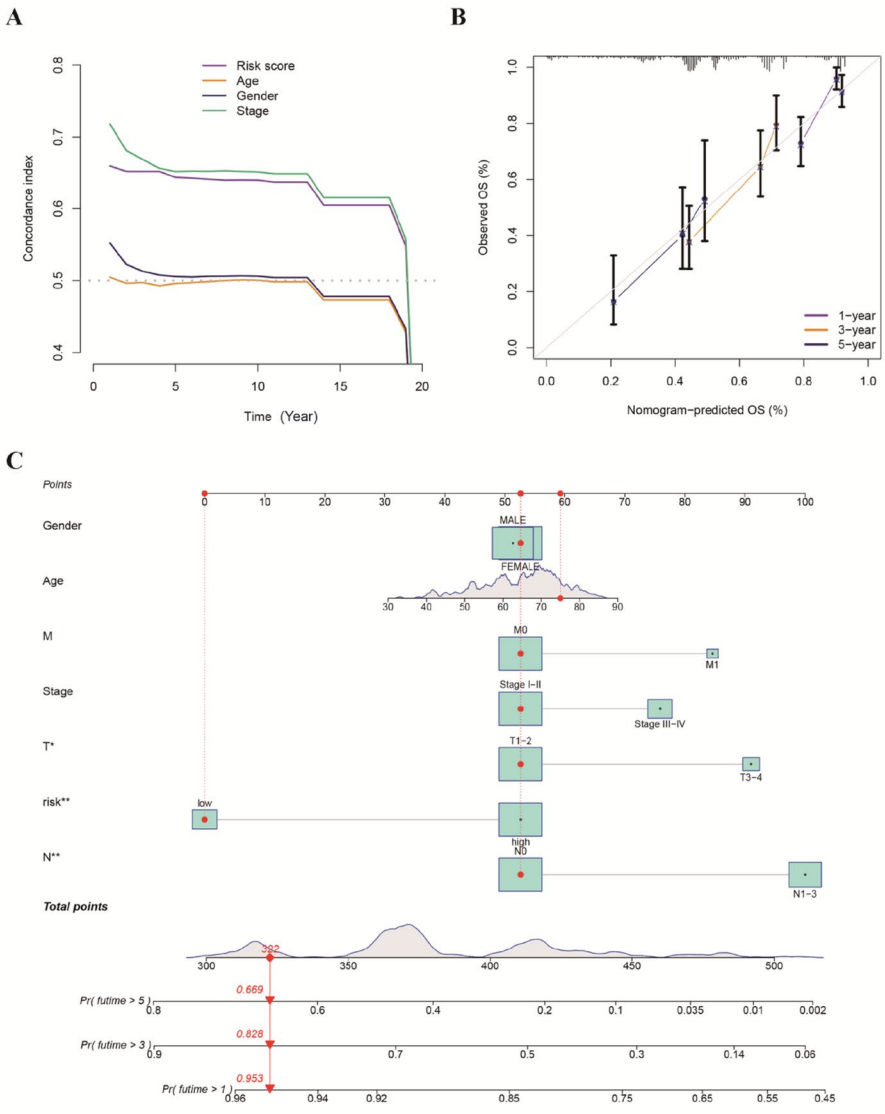

Fig. 3 Construction of the nomogram. (A) The model outperformed conventional clinical criteria in predicting LUAD prognosis. (B) The observed 1-, 3-, and 5-year survival rates demonstrated a strong agreement with the projected rates in the correlation plot. (C) A nomogram containing the model and clinical features

3.3 Assessment of immunological landscape

The frequency of gene mutations was substantially higher in the high-risk group (Fig. 5A and B). The high-risk group demonstrated markedly lower TIDE scores ($p = 0.0045$; Fig. 5C), indicating a more sensitive response to immunotherapy. Conversely, the high-risk group exhibited significantly higher TMB scores ($p < 0.001$; Fig. 5D), further supporting their potential suitability for immunotherapy. Incorporating TMB scores as a covariate improved the predictive accuracy of LUAD patient prognosis, as indicated by statistically distinct survival rates among different TMB and risk score groups (Fig. 5E and F).

As shown by the immune scores for the high-and low-risk groups, the risk score was positively connected with the enrichment of M0 macrophages, M1 macrophages, myeloid dendritic cells, neutrophils and CD4 + T cells, while negatively connected with the enrichment of B cells, mast cells and CD8 + T cells (Fig. 6A). Some immunological

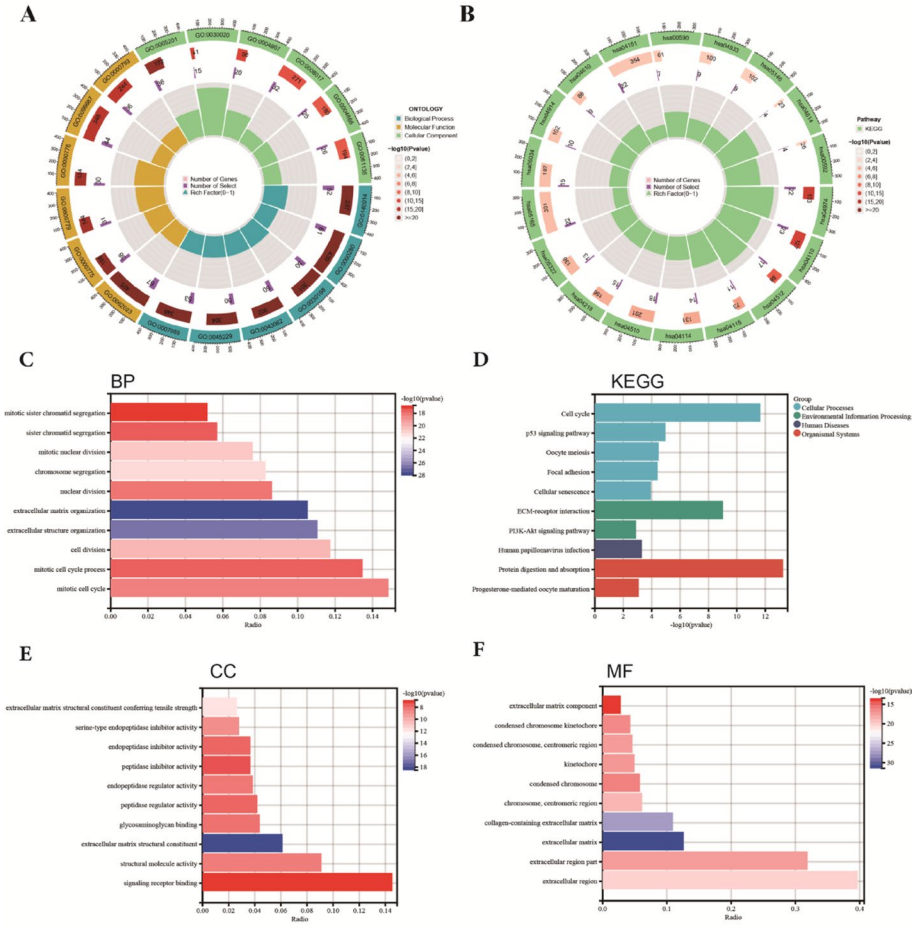

Fig. 4 Functional enrichment analysis. (A and B) The GO and KEGG analyses for 986 DEGs. The BP, CC, MF for GO specific results (C, E, F), The KEGG specific results (D)

activities, such as APC co-inhibition, CCR, check-point, inflammation promoting, MHC class I, parainflammation, and T cell co-inhibition, differed statistically between risk groups (Fig. 6B). Immune checkpoint-related gene expression, including CTLA-4, PDCD1, LAG3, TIGIT, and CD274, was also statistically different (Fig. 6C). Selection of immune checkpoint related genes is based on two considerations. First, from the biological theory, immune checkpoint plays a central role in the process of tumor immune escape. Immune checkpoint molecules, such as programmed death receptor 1 (PD-1) and its ligand (PD-L1), help tumor cells escape the surveillance and attack of the body's immune system by inhibiting the activity of immune cells. In the study of lung cancer, especially LUAD, the abnormal expression of immune checkpoint is closely associated with the development of tumors, and the prognosis of patients. Secondly, combined with the purpose of our study, we aimed to explore the connection of telomere-related genes and immune features in LUAD, immune checkpoint-related genes as key nodes of immune regulation, and the inclusion study helps to more comprehensively dissect the potential association between tumor immune microenvironment and telomere biology.

3.4 Selection of anti-tumor drugs

Besides immunotherapy, our research aims to identify chemotherapeutic drugs and novel targeted drugs for patients in various risk groups. Our analysis revealed 28

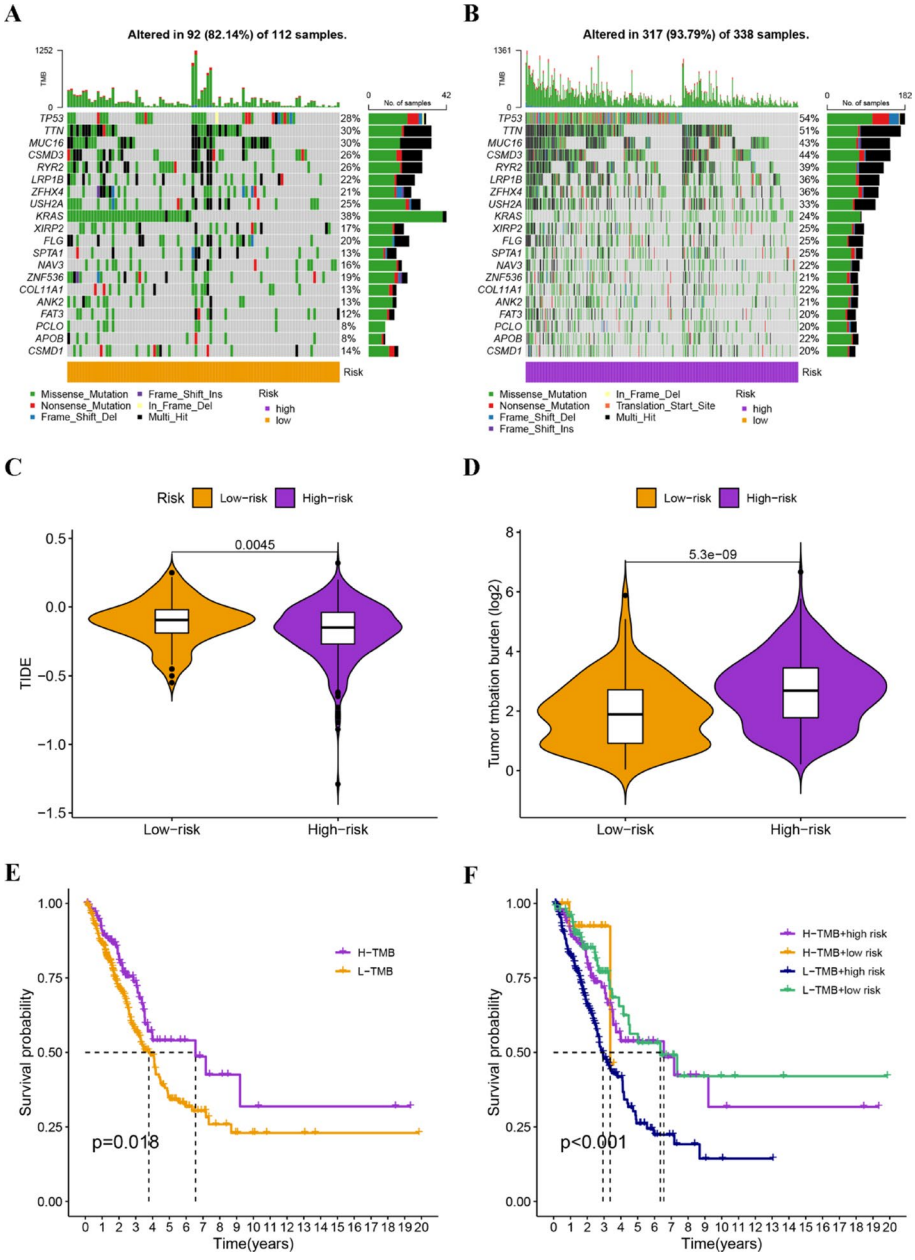

Fig. 5 Evaluation of immunotherapy. (A and B) The specific mutated genes varied greatly in different groups. (C and D) The high-risk group had lower TIDE score and higher TMB scores. (E) Survival rates were significantly lower in high-TMB groups. (F) Survival rates were significantly different between the four groups

chemotherapy drugs and 51 targeted drugs, including Cisplatin, Gemcitabine, Paclitaxel, Vinblastine, AKT inhibitor VIII, WH.4.023, and XMD8.85 ($p < 0.001$; Figs. 7 and 8). These findings offer valuable insights into potentially effective therapeutic options for patients in different risk groups and may inform the development of more personalized treatment approaches.

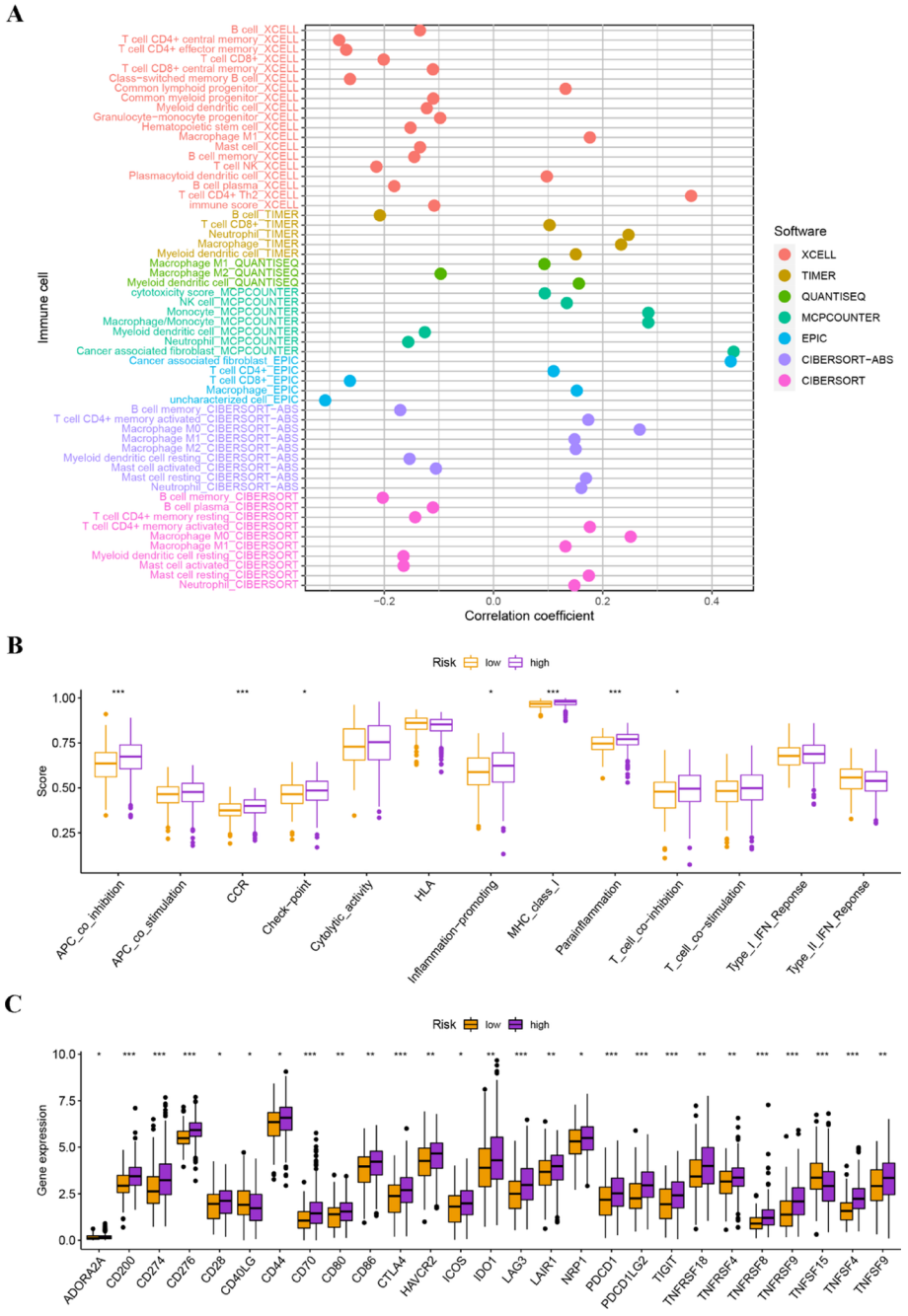

Fig. 6 Immune landscape of the model. (A) The risk score was positively connected with the enrichment of M0 macrophages, M1 macrophages, myeloid dendritic cells, neutrophil and CD4 + T cells, while negatively connected with the enrichment of B cells, mast cells and CD8 + T cells. (B) Some immunological activities, such as APC co-inhibition, CCR, check-point, inflammation promoting, MHC class I, parainflammation, and T cell co-inhibition, differed statistically between risk groups. (C) Immune checkpoint-related gene expression was statistically different between groups

4 Discussion

LC is the most prevalent type of malignant tumor on the planet. According to World Health Organization data issued in 2021, China has the highest incidence and mortality rate of LC in the world [1, 45]. LC patients' 5-year survival rates are still dismal despite the variety of therapies in recent years [46]. Patients with LUAD are in the intermediate

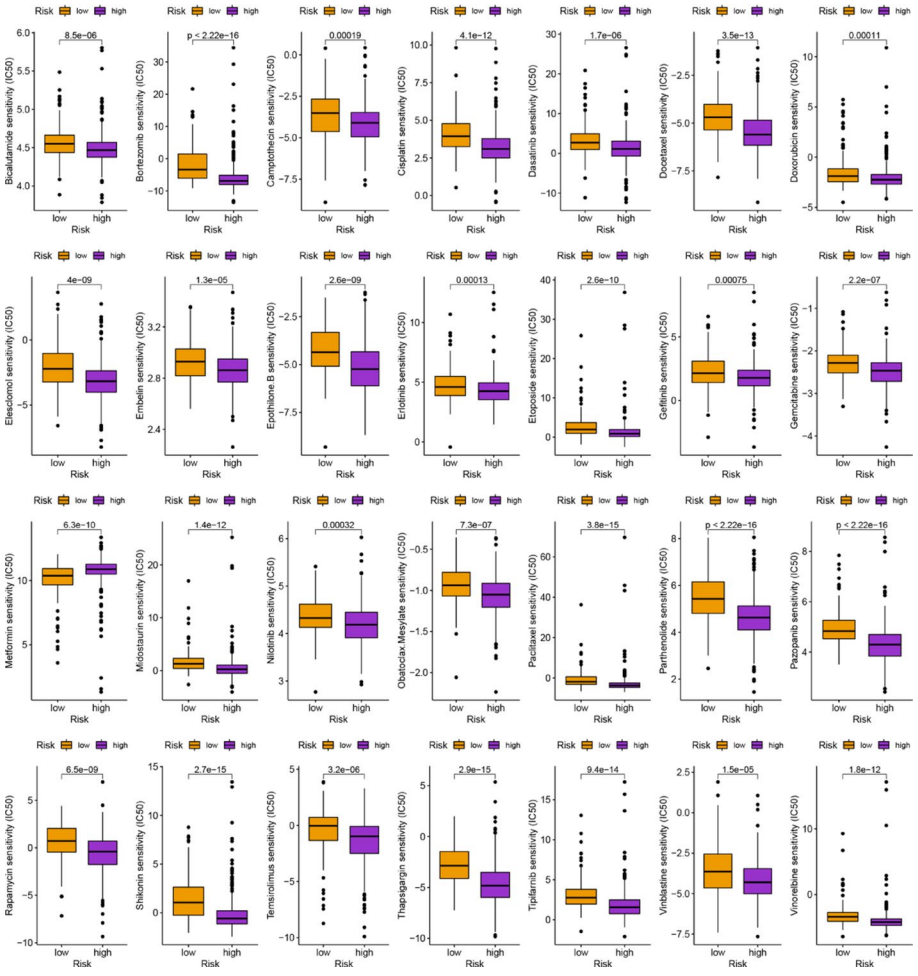

Fig. 7 Identification of traditional chemotherapeutic drugs

to advanced stages of the illness when they are diagnosed, making it one of the most significant subtypes of NSCLC. Patients with LUAD have a much lower chance of surviving following therapy due to relapse, metastasis, and drug resistance [47]. Low-dose CT is one of the most effective screening approaches for LC currently available; however, it has a high percentage of false positives [48]. Thus, it is critical to precisely forecast the prognosis and survival of LUAD in order to prevent and cure it. Telomeres, which play a significant regulatory function in LC, are made up of repeated TTAGGG DNA sequences and shelterin complexes [12]. According to a related study, cellular senescence and apoptosis associated with telomeres were caused by DKC1 downregulation, and high DKC1 expression was related to poor prognosis in LUAD [19, 20]. In LC, in contrast to telomerase activity, which is difficult to detect in normal lung tissue, telomerase activity is not only high in LC cells, but its activity has also been found to be strongly related to the risk of LC development [49]. Additionally, latest study found drugs targeting telomeres effectively inhibit LC initiating cells and promote anti-tumor immunity in LC [50]. TS is significantly related to increased risk of death in heavy smokers of LC [51]. Consequently, new biomarkers and target genes must be urgently investigated for LUAD.

Short telomeres (< = 10th percentile) have been identified as a poor prognostic factor in LUAD based on analysis of datasets from two distinct countries [52]. A nine TRG

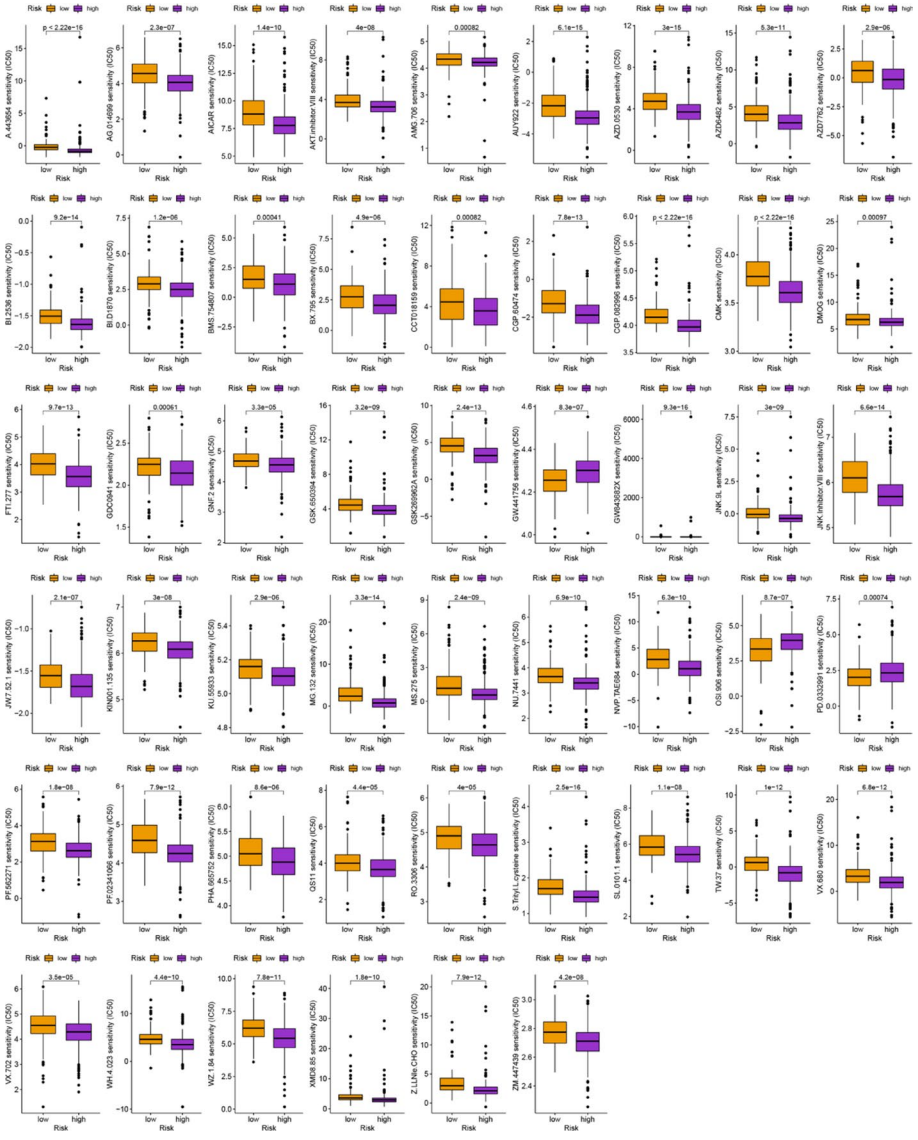

Fig. 8 Identification of novel potential drugs

risk model was constructed and validated for LUAD using these two databases (TCGA and GEO-GSE68465) [53]. Nonetheless, our study exhibits several noteworthy advantages over previous research endeavors. Firstly, we employed a reduced panel of 4 TRGs to construct the model, thereby facilitating its practical applicability in clinical settings. Secondly, we performed an investigation into gene mutations specifically within different risk groups, thereby augmenting the comprehensiveness and depth of our study. Lastly, we conducted an extensive screening of potential therapeutic agents to enable more tailored and individualized treatment options for patients, empowering clinicians with a broader range of choices.

In the work, univariate Cox analysis was used to identify 12 predictive TRGs, including BLM, FEN1, AURKA, EXO1, GLI2, RAD51, HOXA7, PAICS, AURKB, CHEK1, CCT4, and HNRNPC. Four prognostic TRGs (BLM, EXO1, GLI2, and HOXA7) were identified by multivariate Cox analysis. This study is exploratory to explore the potential predictive value of telomere-related genes in lung cancer. Strict p-value threshold facilitates the

screening of significant effect genes, narrow the scope of subsequent analysis, and avoid the introduction of excessive false positives by the relaxed threshold, and interfere with the discovery of key genes. The survey found that many similar lung cancer gene prediction model studies also adopted similar strict p-value threshold in the univariate analysis stage, which is conducive to the horizontal comparison and verification of the research results [54, 55]. FDR correction may be overly conservative to miss truly meaningful genes. Moreover, the results are influenced by sample size, gene number and gene correlation, which should be used carefully in this study to avoid missing the potential information on the relationship between telomere-related genes and lung cancer prognosis [56]. BLM genes involved in DNA unwinding and repair processes, play a key role in maintaining genomic stability, the occurrence and development of lung cancer is closely related to genomic instability [57]. EXO1, the gene involved in the exonuclease activity of DNA, Involved in DNA damage repair and cell-cycle regulation, its abnormal expression may affect the proliferation and apoptosis of lung cancer cells. EXO1 is a potential prognostic gene and connects with immune infiltrates in LUAD [58]. EXO1 rs1776148 was significantly related to prognosis of LC by Sequenom MassARRAY [59]. GLI2 functions as a key transcription factor in the Hedgehog signaling pathway, this signaling pathway plays an important role in the embryonic development, cell proliferation, and differentiation of lung cancer, Abnormal activation can promote the progression of lung cancer. Basic studies confirmed that miR-182-5p could regulate the chemosensitivity of cisplatin-resistant LUAD cells by targeting GLI2, suggesting that GLI2 is correlated with the pathomechanism of LUAD [60]. A bioinformatics analysis revealed that GLI2 may be linked to tumor immune dysregulation and TP53 mutations in LUAD [61]. HOXA7 is a potential target gene involved in the pathogenesis of LUAD, and this might offer novel ideas for the development of new therapeutic targets for LUAD [62]. Besides, HOXA7 is aberrantly expressed in LC samples compared to benign nodal samples, suggesting that it may be highly associated with lung carcinogenesis [63]. The HOXA7 gene belongs to the homeobox gene family, involved in embryonic development and cell differentiation processes, in lung cancer, its abnormal expression is associated with malignant behaviors, such as tumor invasion and metastasis [64]. These existing biological studies provide a strong theoretical basis for us to select these four genes to be included in the multivariate Cox regression analysis. Based on the screened prognostic genes, we can find specific targeted drugs and immunosuppressants, thus providing new options for the clinical treatment of LUAD. In addition, we can specifically identify key prognostic genes through medical techniques and effectively predict the development and prognosis of the disease for eventual use in clinical treatment.

We used comprehensive analysis to identify 689 DEGs between these groups, and differential-based enrichment analysis showed that the BP term of DEGs was mainly associated with mitotic nuclear divisions, nuclear divisions, and extracellular matrix organization. For CC, DEGs were found to be associated with collagen-containing extracellular matrix, chromosomes, centromeric regions and condensed chromosomes, centromeric regions. In addition, the MF term was mainly related to the extracellular matrix structural components, the extracellular matrix structural components endowed to tensile strength, and the serine-type endopeptidase inhibitor activity (Fig. 4A and Table S3). In addition, KEGG analysis was performed to identify pathways enriched in DEGs. This analysis revealed various pathways including protein digestion and absorption, cell cycle,

ECM receptor interaction, p53 signaling pathway, oocyte meiosis and focal adhesion plaques (Fig. 4B, C,D, E,F) .

Although we did not use the Akaike Information Guidelines (AIC) or Bayesian Information Guidelines (BIC) in our article, we used other rigorous and effective methods to verify the reliability and stability of the model. We applied the external validation set GEO-GSE68465, which contained independent lung cancer sample data. By applying the model to this external validation set, we found that the model was still able to accurately predict the survival of lung cancer patients, which fully proved that the model has good generalization ability, not overfitting to the training data set. We hypothesize that BLM, EXO1, GLI2, and HOXA7 genes are substantially linked with the prognosis of LUAD. The survival analyses were the same as those of the GSE68465 validation set, with reduced survival times in the high-risk category. Strong agreement existed between the survival rates and their anticipated survival rates in the correlation plot. We also explored the link between TMB, TIDE, immune cell infiltration, immune functions, immune checkpoint-related genes, and risk scores, and discovered that type III interferon responses were decreased in high-risk individuals. Type III interferon, an essential element of antiviral immunity, prevents viral replication in vitro [65]. Immunological escape may be largely attributed to type III-IFN response suppression, and its activation is necessary to maintain immune potency. In melanoma, lung, and bladder malignancies, TMB is commonly used as a prognostic biomarker for immunotherapy [66–68]. Survival was significantly lower in patients with high TMB, and TP53 and TTN expression were increased in high-risk patients. The development of various cancers is all affected by the often mutated oncogene TP53. It also plays a role in the normal metabolism of diabetes, liver disease, and cardiovascular disease [67, 69, 70]. Additionally, patients with multiple malignancies and TP53 are more likely to survive [71]. TTN contributes to the growth of numerous malignancies. TTN could be a potential therapeutic target for the treatment of endometrial cancer, which could target the miR-376a-3p/PUM2 axis and encourage the proliferation of endometrial cancer cells [72]. By targeting miR-134-5p and encouraging the development of the brain tumor structural domain 1 gene, Fu Chengyu et al. discovered that TTN functions as a proto-oncogene in osteosarcoma [73]. In the context of p53 regulation, the helicase encoded by the BLM gene is crucial for DNA double - strand break repair. As a key tumor suppressor, p53 is activated upon DNA damage and then regulates BLM gene expression to maintain genomic stability. Abnormal p53 function can disrupt the telomere - related repair processes mediated by BLM, thereby influencing telomere dynamics and the prognosis of LUAD. Similarly, the EXO1 gene, involved in DNA exonuclease activity and damage repair, may also be regulated by p53 to impact telomere stability [74, 75]. Regarding the ALT pathway, telomerase - negative tumor cells can maintain telomere length through this mechanism. GLI2, a key transcription factor in the Hedgehog signaling pathway, has abnormal activation linked to tumor cell proliferation and migration. Since the ALT pathway is associated with cell proliferation and survival in certain tumor cells, GLI2 may affect telomere dynamics by modulating the ALT pathway. Although there is no direct evidence of HOXA7's connection to the ALT pathway, its abnormal expression in tumor cells, given its role in embryonic development and cell differentiation, can influence cell proliferation and differentiation, potentially affecting the ALT - related telomere maintenance mechanism [76]. In terms of epigenetic modifications, DNA methylation and histone modifications can impact gene

expression. The promoter regions of BLM, EXO1, GLI2, and HOXA7 are likely epigenetically regulated, which affects their expression levels and, consequently, telomere dynamics and LUAD development. For instance, abnormal DNA methylation can lead to gene silencing or overexpression, disrupting telomere - related biological processes [77, 78]. To further refine the study, we plan to complement the experimental validation in subsequent studies. We will collect more samples from LUAD patients and directly measure telomere length by using fluorescence in situ (FISH) hybridization or telomere restriction fragment (TRF) analysis to verify our previous inferences. Through these experiments, we were not only able to verify the differences in telomere length between the low and high-risk groups, but also to deeply explore the direct association between telomere-related gene expression and telomere length, thus more fully revealing the mechanism of action of telomere-related genes in LUAD.

The enrichment of M0 and M1 macrophages, myeloid dendritic cells, neutrophils, and CD4 + T cells was favorably connected with risk scores, while the enrichment of B cells, mast cells, and CD8 + T cells was negatively correlated. APC co-inhibition, CCR, checkpoint, inflammation promotion, MHC class I, parainflammation, and T-cell co-inhibition were a few immunological activities that showed statistically significant differences between risk groups. Statistics showed that the expression of genes connected to immunological checkpoints differed between risk groups. Regarding the immune landscape, we have found that enrichment of M0 and M1 macrophages, myeloid dendritic cells, neutrophils and CD4 + T cells was positively correlated with risk scores, while enrichment of B cells, mast cells and CD8 + T cells was inversely associated. This result has some agreement with existing studies. For example, it has been shown that increased infiltration of M1 macrophages is associated with poor prognosis in multiple tumors, which may promote the inflammatory response in the tumor microenvironment by secreting pro-inflammatory factors, which then drives tumor progression, which echoes the positive correlation between M1 macrophage enrichment and high-risk score in our study. However, the relative enrichment of CD8 + T cells, as important anti-tumor immune cells in the low-risk group, may imply better immune surveillance and tumor suppressive effects, which is also consistent with the basic theory of tumor immunology. Regarding immunotherapy response, we found that immune activities such as APC co-suppression, CCR, checkpoint, inflammation promotion, class MHC I, accessory inflammation, and T cell co-suppression varied significantly between risk groups, and immune checkpoint-related gene expression also varied between risk groups. This suggests that LUAD patients may have different responses to immunotherapy in our different risk groups. Studies have indicated that immune checkpoint inhibitors often have better efficacy in tumor patients with high expression of immune checkpoint related genes, which has a potential link with our findings. We can further speculate based on this that the high-risk group in our study may be more sensitive to immune checkpoint inhibitor treatment due to their specific expression patterns of immune checkpoint-related genes.

We will investigate the roles of immune cells in targeted therapy for LUAD patients in the future. We will continue to assess the prognosis of LC patients from the standpoint of immune cell infiltration. Finally, we examined the sensitivity of these medications, which have been used to treat various cancers, and revealed 28 chemotherapy drugs and 51 targeted drugs, including Cisplatin, Gemcitabine, Paclitaxel, Vinblastine, AKT inhibitor VIII, WH.4.023, and XMD8.85. We did this by using the pRophetic algorithm to

search for successful tumor immunotherapy agents. anti-cancer medications However, further research is required to fully understand how drugs work and how they affect LUAD progression.

It is essential to recognize that the present study exhibits several deficiencies and constraints. Firstly, due to the majority of non-metastatic patients present in the TCGA-LUAD dataset, the findings may be susceptible to bias. Secondly, the sample size of the training set utilized in this study (459 tumor samples) is comparatively limited. Thirdly, the prognostic gene sets discovered in this research have not been authenticated in our clinical specimens. Thus, we intend to acquire additional clinical specimens and broaden the sample size to enable further scrutiny and validation of our model.

5 Conclusion

In this study, we conducted a groundbreaking investigation to elucidate the significance of TRGs in the diagnosis and prognosis of LUAD. Notably, our study is the first to comprehensively examine the contributions of TRGs in this context. Moreover, we specifically explored the relationship between risk score-based groups and important treatment factors such as TIDE, TMB, medication sensitivity, and immunotherapy response. This comprehensive analysis has led to substantial advancements in our understanding of clinical outcomes and the ability to predict survival for individuals afflicted with LUAD. The findings from our study have transformative implications, providing critical insights into the development of personalized therapeutic strategies. Importantly, our research augments the current knowledge base in the field of precision medicine for LUAD and fosters tailored therapeutic approaches catered to individual patients.

Supplementary Information

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Supplementary Material 1. Fig. S1. TRG scores were significantly higher in the low-risk group

Supplementary Material 2. Tab S1. The lists of TRGs. Tab S2. The 689 DEGs. Tab S3. The GO analysis. Tab S4. The KEGG analysis.

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Author contributions
Yan Jiang, Jun Liu, Chuanhui Wang and Jie Yan designed the implementation of the research, drafted the preliminary papers, and participated in the investigations. Bi Pan，Siping Yu，Chunyan Hu and Qiancheng Li collected genomic and clinical data from databases. Hong Cheng and Ling Chen analyzed the data. Min Jiang and Die Xu participated in the manuscript revision, manuscript submission, and fund acquisition. The authors read and approved the final manuscript.

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Data availability
Data supporting the findings of this study are available from the respective authors upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.
Figure note.

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