An Immune-related Lncrna Signature Predicts Prognosis and Adjuvant Chemotherapeutic Response in Patients With Small-cell Lung Cancer

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Abstract

Background

Patients with small-cell lung cancer (SCLC) are burdened by limited treatment options and the disease's dismal prognosis. Long non-coding RNAs (lncRNAs) are essential regulators of genetic alteration and are actively involved in tumor immunity. However, owing to the paucity of specimens, few studies have examined the interaction between immune genes and lncRNAs in SCLCs.

Methods

The immune-related lncRNAs (irlncRNAs) expression profiles and clinical significance were comprehensively explored. We enrolled 227 patients with SCLC, including 79 cases from GSE65002 and 148 cases from an independent cohort with corresponding qPCR data. A least absolute shrinkage and selection operator (LASSO) was performed to identify the most prognostic irlncRNAs to construct an irlncRNAs-based signature in SCLC. We additionally investigated the potential and immune landscapes of the signature using bioinformatic methods.

Results

Totally, 316 irlncRNAs were filtered out in SCLCs. Then, an 8-irlncRNAs-based signature (ENOX1-AS1, AC005162, LINC00092, RPL34-AS1, AC104135, AC015971, AC126544, and AP001189) was established for patients with SCLC in the training cohort. The classifier successfully divided patients into high- or low-risk groups with dramatically different survival rates and chemotherapy benefit (both $P < 0.001$). The signature was also well-validated in an independent cohort and various clinical subgroups. Compared to other important clinical parameters, this signature exhibited superior predictive performance for chemotherapy response and prognosis. Importantly, the signature also acts as an independent prognostic factor in the two cohorts after adjusting with other clinical parameters. Interestingly, functional analysis revealed that multiple activated immune-related pathways were abundant in the low-risk group. Additionally, the signature was found closely associated with inflammatory responses and various immune checkpoints.

Conclusion

We constructed the first irlncRNAs-based signature for prognosis and survival benefit of chemotherapy outcome prediction for patients with SCLC. The irlncRNAs signature was a reliable and robust prognostic classifier that could be useful for clinical management and determination of potential chemotherapy benefit for patients with SCLC.

Background

Small cell lung cancer (SCLC) is the most malignant subtype of lung cancer, accounting for 13–15% of all lung cancer cases [1]. SCLC is notorious for its rapid growth fraction, highly aggressive and early

metastatic characteristics, and is the sixth most-common cause of cancer-related death [2]. These factors have led to limited improvements in the typical life span of patients with SCLC treated by standard therapy over past decades. The median overall survival (OS) of patients with SCLC has stalled at fewer than 10 months and the disease features a dismal 5-year survival rate of 5% [3]. Currently, platinum-based chemotherapy remains the first-line treatment for SCLC; however, the challenge of drug resistance has not been well addressed [4]. Considering SCLC's unfavorable mortality rate, there is an urgent need for potential biomarkers to facilitate and improve the disease's diagnosis, treatment, and prognosis. Immune responses play a pivotal role in tumor development and progression; this may also influence the survival of patients with SCLC [5-7]. Immune cells—also referred to as cancer killers—mediate tumor immune responses, and are closely associated with tumor growth, invasion, and metastasis [8, 9]. Notably, recent research found immune cell infiltration to be a key determinant of prognosis for patients with SCLC. Furthermore, the different immune cell profiles in the tumor microenvironment often affect survival [10]. Meanwhile, the emerging immunotherapy with immune checkpoint inhibitors has achieved promising progress across various malignancies, and this treatment is also being used and demonstrating significant promise and potential in SCLC [11, 12]. Therefore, immune-related factors related to the development and progression of SCLC warrant investigation.

With the considerable advancement in transcriptome sequencing technology, the crucial role of long non-coding RNA (lncRNA) in tumorigenesis and progression has been elucidated [13]. lncRNAs—a subtype of non-coding RNA transcripts—cannot code proteins that ranging from 200 nucleotides to 100 kilobases in length [14, 15]. lncRNAs are responsible for the malignant tumor phenotypes by regulating genomic and transcriptomic alterations and affecting the tumor immune microenvironment [16]. Importantly, lncRNAs actively regulate expression of genes related to immune responses and activation. This increases the heterogeneity of the tumor immune microenvironment by encouraging infiltration of different immune cells [17]. Several signatures based on tumor immune infiltration have shown reliable and promising value for the diagnosis, treatment, and prognostication of various tumors [18-20]. Furthermore, lncRNAs are often involved in the construction of these signatures. A series of immune-related lncRNAs (irlncRNAs)-based signatures exhibit favorable prognostic guidance in multiple tumors, including pancreatic cancer, breast cancer, and hepatocellular carcinoma [21-23]. However, few attempts have been made to explore the value of irlncRNAs for prognosis prediction in SCLC; the clinical implications of the irlncRNAs-based signature in patients with SCLC is still largely unknown.

This is the first identification of an irlncRNAs expression profile in patients with SCLC. Meanwhile, we established an eight-irlncRNA signature able to stratify the adjuvant chemotherapeutic response and prognostic risk of patients with SCLC in the training phase; the prognostic value of this signature was well-validated in independent cohort. We also explored the relationship between these irlncRNAs and tumor immunity. Thus, our eight-irlncRNA signature may serve as a promising prognostic predictor for SCLC and may guide the clinical application of chemotherapy and immunotherapy in SCLC.

Methods

Patients and immune gene sets

We collected 227 patients with SCLC in this study, including 79 samples in a publicly available database (GSE60052) downloaded from Gene Expression Omnibus (GEO) datasets (<http://www.ncbi.nlm.nih.gov/geo>), and 148 cases with formalin-fixed and paraffin-embedded (FFPE) tissues who underwent surgery in the Chinese Academy Medical Sciences Cancer Hospital from 2009 to 2018. Among the 148 cases, 128 of them had received adjuvant chemotherapy. All patients were pathologically re-confirmed with SCLC. The start point for OS and relapse-free survival (RFS) was defined the day of surgery; the endpoint was the day of death or the last follow-up and the date of relapse metastasis, or the last follow-up. This research was approved by the Institutional Review Boards of The Chinese Academy Medical Sciences Cancer Hospital. The immune gene set used in this study were gathered for nCounter® PanCancer Immune Profiling Panel (NanoString Technologies, Inc., Seattle, WA).

Identification of immune-related lncRNAs

Firstly, we obtained the lncRNA profile, based on previous literature, with gene expression microarray data [24]. The GSE60052 data was first log2 transformed and quantile normalized, then we applied the annotation file (GPL11154) to match with gene code v36 IDs. Based on this mapping procedure, the lncRNA profiles of 2942 lncRNAs were determined. The list of immune genes based on the NanoString nCounter PanCancer Immune Profiling Panel (LBL-10043-08); finally, 764 immune genes were identified. lncRNAs and immune genes with low expression levels (where half or more than half of values were 0) were filtered out. Next, a Pearson correlation analysis was conducted between the final 607 immune genes and 1202 lncRNAs. During this analysis, only the lncRNAs with $|R| > 0.6$ and $P < 0.001$ were identified as the irlncRNAs. Finally, 316 irlncRNAs were included in this investigation.

Functional analysis

We determined which genes were associated with irlncRNAs using the Multi Experiment Matrix website (<http://biit.cs.ut.ee/mem/>). The selected genes were uploaded to the DAVID 6.8 (<http://david.abcc.ncifcrf.gov/home.jsp>) website and subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. In addition, Gene set enrichment analysis (GSEA, <http://www.broadinstitute.org/gsea/index.jsp>) was used to explore the related signaling pathways between high- and low-risk groups. Finally, the GSVA R package was applied to run the Gene Set Variation Analysis (GSVA) function on R software (version 3.5.1).

RNA isolation and qPCR analysis

We extracted total RNA of FFPE surgical specimens using the Ambion RecoverAll Total Nucleic Acid Isolation Kit for FFPE (ThermoFisher, Waltham, MA, USA). The target irlncRNAs expression levels were evaluated by qPCR analysis of 10 µl volume system in triplicate on 7900HT Fast Real-Time PCR system (Applied Biosystems, Carlsbad, USA, Indianapolis, IN) using SYBR Green Master Mix method

(Invitrogen). The $2^{-\Delta\Delta Ct}$ method was selected to assess the expression of all candidates. All primer sequences of selective irlncRNAs were demonstrated in Supplementary Table S1.

Establishment of risk signature and statistical analysis

To construct a reliable risk signature, we first carried out the univariate Cox regression model to find the irlncRNAs with prognostic potential. We further filtered out the candidates significantly associated with survival to generate a risk signature based on the least absolute shrinkage and selection operator (LASSO) model. The risk score formula was developed by the expression of the final eight irlncRNAs and their corresponding coefficients. Risk scores were calculated for all patients in this study. The Pearson correlation analysis determined the correlation between the risk score and classical immune checkpoints. A multivariate Cox proportional hazards regression analysis was carried out to determine if the signature could independently predict prognosis for SCLC. OS and RFS of patients in different risk groups were assessed by Kaplan-Meier survival analysis. the ROC curves were evaluated to identify the predictive capacity of OS for the various clinical parameters, TNM stage, and risk score using R software (version 3.5.1). We used SPSS 25.0 and R software (version 3.5.1) to complete all image production and data analyses in this study. *P* values less than 0.05 were considered significant, and all tests were two-tailed.

Results

Identification of the Prognostic irlncRNAs from the training cohort.

The flow chart of this study is presented as Figure S1. Firstly, 764 immune genes and 2942 lncRNAs were identified in 79 cases from GSE60052. Then, we sought to determine the prognostic role of irlncRNAs in patients with SCLC. Firstly, we mapped the gene code IDs to the annotation files in GSE60052, including the 79 SCLC samples. To ensure that our analysis had clinical significance, only the immune genes and lncRNAs with high expression levels were included. After filtering out the low-expression candidates, only 607 immune genes and 1202 lncRNAs were screened for further exploration. The Pearson correlation analysis ($|R| > 0.6$ and $P < 0.0001$) was conducted between these 607 immune genes and 1202 lncRNAs, and 316 irlncRNAs were decided in this procedure. Next, we performed the univariate cox regression analysis on 48 patients with survival data from GSE65002, and 20 prognostic irlncRNAs were selected (Figure 1A-1B, $P < 0.2$). For the next step, we utilized LASSO regression analysis on these 20 irlncRNAs to identify the most promising candidates, and the minimum criteria were chosen (Figure 1C-1D). Finally, the eight irlncRNAs were decided: ENOX1-AS1, AC005162, LINC00092, RPL34-AS1, AC104135, AC015971, AC126544, AP001189. Meanwhile, the immune genes associated with this 8 irlncRNAs are all displayed in Figure 1E.

Construction of the irlncRNAs signature in SCLC

The eight irlncRNAs and their corresponding coefficients were combined to establish a molecular risk score model for patients with SCLC, as follows: risk score= (0.3647 × ENOX1-AS1 expression) + (0.1062 ×

AC005162 expression) + (0.1935 × RPL34-AS1 expression) + (0.0329 × AC104135 expression) + (0.3833 × AC015971 expression) + (0.1074 × AC126544 expression) - (0.4814 × LINC00092 expression) - (0.0665 × AP001189 expression), including six protective and two risky factors (Figure 2A). The relationship between this eight irlncRNAs-signature and the corresponding risk score is shown in Figure 2B. Based on the irlncRNAs signature, all patients acquired individual risk scores in the training cohort. Then, patients were classified into low- and high-risk groups according to the optimal cut-off value (Figure 2C). The Kaplan-Meier curves demonstrate that high-risk patients had shorter OS than their low-risk counterparts (Figure 2D). We also evaluated the accuracy of the eight-irlncRNAs signature for predicting OS in SCLC by performing time-dependent ROC curves analysis. The AUCs were 0.829 at 1-year, 0.87 at 3-year, and 0.883 at 5-year in the training cohort. This suggests that our irlncRNAs classifier is excellent for survival prediction in SCLC.

Validation of the irlncRNAs signature in SCLC

To examine whether our signature has potential clinical applications, we further validated it in an independent cohort (the validation cohort) including 148 patients with SCLC. Based on the qPCR analysis, all patients demonstrated expression levels of these eight irlncRNAs. Similarly, we calculated risk scores for all patients and classified them into low- and high-risk groups depending on corresponding risk values. Again, low-risk patients demonstrated better OS than their high-risk counterparts (Figure 3A). To validate the robustness and optimality of this classifier, we not only investigated ROC curves of the classifier for 1-, 3- and 5-year OS. Here, all AUCs exceeded 0.6 (Figure 3B). We also compared the 5-year ROC curves between our signature and other important clinical parameters. These irlncRNAs exhibited excellent discriminatory capacity, with a 5-year AUC up to 0.636 (Figure 3C). Furthermore, we tested the prediction efficacy of our signature in RFS in SCLC. Low-risk patients also had better RFS (Figure 3D). The AUCs of the signature for predicting 1-, 3- and 5-year RFS were 0.556, 0.617, and 0.629, respectively (Figure 3E). Our signature was also better at predicting 5-year RFS in SCLC than other critical clinical features (Figure 3F).

Since adjuvant chemotherapy (ACT) is the preferred approach for SCLC treatment, we investigated the relationship between our irlncRNAs signature and OS in patients who received ACT within the independent cohort. In the ACT subgroup, high-risk cases also suffered unfavorable prognoses (Figure 3G), and the 1-, 3-, and 5-year OS ROC curves are illustrated in Figure 3H. Compared to other clinical characteristics, the signature showed the highest AUC of 5-year OS prediction in the ACT subgroups (Figure 3H). We further validated the signature in subgroups of patients with important clinical characteristics from the training and independent cohorts. Notably, high-risk patients exhibited poorer OS in the clinical parameter subgroups in the training cohort, including males, older patients, and smokers (Figure S2A-S2C). The same results were observed in the independent cohort—high-risk cases experienced inferior OS and RFS in male, older, and smoker subgroups (Figure S2D-S2I). Taken together, the predictive performance of our irlncRNAs classifier is enough reliable and effective.

The irlncRNAs signature is an independent prognostic factor in SCLC

To determine if the *irlncRNAs* signature independently predicts prognosis in SCLC, we conducted univariate and multivariate Cox regression analyses in the training and validation cohorts. The risk score was most significantly related to the prognosis of SCLC both in the training and validation sets, compared with other clinical parameters (Figure 4A). Meanwhile, we incorporated various clinical parameters—including sex, age, smoking, and SCLC staging—in the multivariate Cox regression analysis. Importantly, this risk score functions as an independent predictor both in OS and RFS for patients with SCLC (Figure 4B). Collectively, our *irlncRNAs* signature could predict risk and effectively stratify patients with SCLC.

Functional analysis of the irlncRNAs signature

The GO analysis was used to investigate the biological significance of this *irlncRNAs* signature. We first identified 527 genes with Pearson $|R| > 0.35$ (481 positively related and 46 negatively related) that were strongly related to this signature. A heatmap for these related genes and distribution of clinical parameters for all patients in the training cohort is illustrated in Figure 5A. Then, the GO analysis indicated that this signature was remarkably linked to a variety of cell proliferation-related pathways, including cell division and various DNA replication pathways. These findings are consistent with the malignant biological characteristics of SCLC with rapid proliferation (Figure 5B). Meanwhile, we also found that this risk score was associated with antigen processing and presentation pathways. This implied that the molecular model was likely related to T cell function (Figure 5B). Then, we performed the GESA analysis to further validate the relationship between this *irlncRNAs* signature and immune activity. The results showed that low-risk patients were positively related to the T cell migration ($P < 0.001$), T cell mediated cytotoxicity ($P < 0.001$), T cell activation involved in immune responses ($P = 0.016$), and response to $\text{INF-}\gamma$ ($P = 0.033$), exhibiting an activated immune phenotype than low-risk counterparts.

Relationship between the signature and immune landscapes

To comprehensively understand the relationship between the risk score and immune landscapes, seven clusters of inflammatory and immune responses metagenes (HCK, interferon, LCK, MHC- α , MHC- β and STATA) were chosen for further exploration [25, 26]. As displayed in Figure 6A, the risk score was negatively correlated with most clusters—including HCK, LCK, and MHC- α . These seven metagene clusters were subjected to GSVA for further validation. Corrrgrams were generated based on the Pearson r value between risk score and the seven metagenes (Figure 6B). Here, the risk score was negatively correlated with LCK, MHC- α , MHC- β , and STAT1. Thus, low-risk patients demonstrated activated macrophages and T cell signaling transduction.

Considering the pivotal roles that immune checkpoints play in tumor immunity, we investigated the correlation between risk score and expression of several essential immune checkpoints. The risk score is positively related to the TNFSF4, TNFRSF9, CMTM6, TIGIT, and CD274 (Figure 6C-6D). TNFSF4 and TNFRSF9 are the critical members of the TNF family. Immunotherapies targeting TNFSF4 have achieved promising results in some malignancies [27]. Also, CTMT6 and TIGIT have identified novel immune

checkpoints for tumor immunotherapy [28, 29]. In summary, all results indicated that high-risk patients were likely to benefit from these novel immunotherapeutic treatments.

Table 1. Clinical characteristics of the patients from different cohorts.

Characteristics	Training Cohort (N=48)	Validation Cohort (N=148)
Age, year		
<60	27 (56.25%)	79 (53.38%)
≥60	21 (43.75%)	69 (46.62%)
Sex		
Male	43 (89.58%)	116 (78.38%)
Female	5 (10.42%)	32 (21.62%)
Smoking history		
Yes	33 (68.75%)	92 (62.16%)
No	15 (31.25%)	56 (37.84%)
SCLC staging		
I	8 (16.67%)	54 (36.49%)
II	8 (16.67%)	48 (32.43%)
III	31 (62.50%)	46 (31.08%)
IV	1 (2.08%)	0 (0.00%)
OS state		
Alive	25 (52.08%)	68 (45.95%)
Death	23 (47.92%)	80 (54.05%)

SCLC, small cell lung cancer; OS, overall survival.

Discussion

SCLC is considered the most fatal type of lung cancer, with limited treatment options and a dismal prognosis. In SCLC, the prognosis and response to treatment vary widely among patients with similar clinical characteristics because SCLC is a highly heterogeneous tumor with significant genetic diversities [30]. Therefore, we must explore the novel molecular biomarkers (different from traditional clinical risk parameters) for predicting treatment response and prognosis for patients with SCLC. In the past decade, several mRNAs or miRNAs-based molecular mode models have been proposed to predict the

prognosis of patients with SCLC [31, 32]. Recently, and with the advancement of high-sequencing technology, lncRNAs expression dysregulation was identified in various malignancies. These findings underscore the critical function of lncRNAs in tumorigenesis and development [33, 34]. Furthermore, lncRNAs appear involved in genomic and transcriptomic regulation and affect tumor immunity alteration [16]. Other studies have found that irlncRNAs may function as promising therapeutic targets and predictive biomarkers for clinical management and precision therapy in multiple tumors [35, 36]; however, there is little exploration of irlncRNAs prognostic significance in SCLC.

Therefore, in the present study, constructed an irlncRNAs signature and explored its prognostic, predictive significance, and predictive value in response to adjuvant chemotherapy for patients with SCLC. This signature effectively stratified various risk factors for accurate prognostication of patients with SCLC. This signature was further validated in independent cohort and could independently predict OS and RFS in patients with SCLC. The high-risk patients suffered a worse prognosis and benefited little from the adjuvant chemotherapy compared to their low-risk counterparts. Notably, our signature had better predictive performance for response to ACT and prognosis of patients with SCLC than various well-recognized clinicopathologic traits. Meanwhile, we also explored the relationship between this signature and tumor immunity, which may also provide clues to understand the immunotherapy application for SCLC.

Eight irlncRNAs (ENOX1-AS1, AC005162, LINC00092, RPL34-AS1, AC104135, AC015971, AC126544, and AP001189) were included in the risk classifier to predict prognosis for patients with SCLC. AC005162 promotes breast cancer cell growth, while low expression of AC005162 is associated with a better prognosis of breast cancer [37]. LINC00092 alters glycolysis to support the cancer-associated fibroblasts function, which accelerates the tumor progression and metastasis in ovarian cancer [38]. Lower LINC00092 expression was related to favorable prognosis for patients with colon adenocarcinoma or breast cancer, and a poorer prognosis for patients with lung adenocarcinoma [39-41]. RPL34-AS1 was down-regulated in various malignancies, including colorectal, gastric, and esophageal cancers [42]. In esophageal cancer, RPL34-AS1 inhibits tumor proliferation, migration, and invasion by regulating the expression of RPL34, which serves as a tumor suppressor to inhibit tumorigenesis and development [42]. AC104135 was highly expressed in breast cancer and was a risk factor for breast cancer in a Chinese population [43]. High expression of AP001189 was closely associated with a better prognosis for colon cancer [44]. Few studies have reported on ENOX1-AS1, AC015971, and AC126544; additional research is needed to uncover their tumor-related roles. Additionally, the roles of these eight lncRNAs in SCLC development are still poorly understood, and further relevant studies are needed to explore their functions.

We also explored potential risk signature mechanisms. It was found that the genes associated with our signature are almost enriched in cell division and multiple DNA replication pathways, in accordance with the rapid proliferation features of SCLC. The irlncRNAs appear involved in immune cell responses. Patients in the high and low-risk groups featured different immune statuses. The low-risk patients were positively related to the T cell migration, T cell mediated cytotoxicity, T cell activation involved in immune

responses, and response to INF- γ , exhibiting a different immune phenotype activation pattern in the high-risk group. Meanwhile, low-risk patients were related to activated macrophages and T cells signaling transduction. The risk score was positively correlated with several novel immune checkpoints, including TNFSF4, TNFRSF9, CMTM6, TIGIT, and CD274. TNFSF4 and TNFRSF9 are critical members of the TNF family. They contribute to co-stimulatory or co-inhibitory signals of T cell immune responses, and immunotherapies targeting TNFSF4 in some malignancies are promising [27]. CMTM6 is a PD-L1 protein regulator that helps maintain the expression of PD-L1 while regulating tumor immunity [45]. TIGIT is an inhibitory receptor in CD8⁺ T cells that limits its function and promotes T cell exhaustion [46]. Both CMTM6 and TIGIT are considered promising targets, and their related therapeutic approaches will likely contribute to future tumor treatments. Collectively, our signature may assist clinical application of these novel immunotherapies for patients with SCLC in the future.

We created the first comprehensive lncRNAs profile in SCLC and confirmed that lncRNAs are important as previously reported mRNAs and miRNAs for predicting prognosis. Additional experiments are urgently needed to explore the functions and molecular mechanisms of lncRNAs in SCLC. Additionally, considering the pivotal role of immunotherapy, we proposed a potential novel immune-related therapeutic approach and prognostic target for SCLC. Our molecular signature was well validated in tissue specimens from the independent cohort, suggesting that our classifier is more reliable and clinically available.

Despite these promising preliminary findings, several limitations warrant consideration. Firstly, we screened out the lncRNAs profiles from GEO databases based on RNA-seq data. It is likely that part—but not all—of the potential lncRNA were included. Thus, future investigations should explore the landscape of lncRNAs underlying biological functions. Second, the small sample size of the GEO database training cohort limited the molecular modeling process; more cohorts with larger sample sizes are needed in the future. Lastly, this was retrospective research featuring validation of FFPE specimens. Further, prospective samples are needed for in-depth validation.

Conclusion

In summary, we have comprehensively revealed the expression profile of irIncRNAs in SCLC. We successfully constructed an eight-irIncRNA-based classifier to predict prognostic risk and chemotherapy benefit for patients with SCLC. Importantly, our robust risk signature may also be useful for future improvement of outcome management and chemotherapy application in SCLC.

Abbreviations

SCLC: small cell lung cancer; OS, overall survival; lncRNA, long non-coding RNA; irIncRNAs, immune-related lncRNAs; FFPE, formalin-fixed and paraffin-embedded; RFS, relapse-free survival; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GESA, Gene set enrichment analysis; GSVA, Gene Set Variation Analysis; LASSO, least absolute shrinkage and selection operator.

Declarations

Ethics approval and consent to participate

The protocol of this study was approved by the Ethics Committee of the Cancer Hospital of the Chinese Academy of Medical Sciences. Due to retrospective nature of this study, the requirement for informed consent was waived. This work was conducted in compliance with the Declaration of Helsinki.

Consent for publication

All authors approve the manuscript for publication.

Availability of data and material

Data and materials related to this work are available upon request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

NS and JH supervised the project, designed, edited and led out the experiments of this study. ZHZ, CQZ and PW conducted the experiments and data analysis. CQZ and ZHZ prepared all the figures and tables. ZHZ and YJL drafted the manuscript. CQZ, GCZ, QPZ, LDW, ZYY, LYX, BZ, HZ, FWT, QX, and SGG collected clinical samples. All the authors reviewed and approved the final manuscript.

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Figures

Figure 1

Filter out the most significant prognostic irlncRNAs in small cell lung cancer. (a) Univariate cox regression analysis filtered out 20 significant prognostic irlncRNAs. (b) Forest plot of the association between irlncRNAs and prognosis in SCLC. (c & d) 100-fold cross-validation for tuning parameter selection in a LASSO Cox model. (e) Correlation between irlncRNAs and immune genes.

Figure 2

The lncRNA signature distribution and survival of patients in the training cohort. (a) LASSO Cox coefficient profiles of the selected prognostic lncRNAs. (b) Correlation between the expression of enrolled lncRNAs and risk score. (c) risk score distribution with patient survival status in the training cohort, with red color indicating patients are dead while blue color indicating survive. Expression distribution of the eight lncRNAs in the training cohort, with red color indicating higher expression and

blue indicating lower expression. (d) Kaplan-Meier curves of OS in 48 patients of the training cohort based on risk score. (e) ROC analysis of irlncRNAs signature for prediction of survival at 1, 3, and 5 years in the training cohort.

Figure 3

Validating the irlncRNA signature in an independent cohort with qPCR data. (a) Kaplan-Meier curves of OS in 148 patients of the independent cohort based on risk score. (b) ROC analysis of risk score for prediction of survival at 1, 3, and 5 years in the independent cohort. (c) ROC analysis of risk score and

different clinical parameters for OS in the independent cohort. (d) Kaplan-Meier curves of RFS in 148 patients of the independent cohort based on risk score. (e) ROC analysis of risk score for prediction of RFS at 1, 3, and 5 years in the independent cohort. (f) ROC analysis of risk score and different clinical parameters for RFS in the independent cohort. (g) Kaplan-Meier curves of OS in ACT subgroup of the independent cohort based on risk score. (h) ROC analysis of risk score for prediction of OS at 1, 3, and 5 years in the ACT subgroup of the independent cohort. (i) ROC analysis of risk score and different clinical parameters for RFS in the ACT subgroup of the independent cohort.

a

b

Figure 4

Cox regression analyses of the irlncRNA signature in training and independent cohorts. (a) Univariate Cox regression analyses of risk score and clinical parameters. (b) Multivariate Cox regression analyses of risk score and clinical parameters.

Figure 5

Functional analysis of the irlncRNA signature in patients with SCLC. (a) Details of risk score and most relevant genes. (b) Gene enrichment with Go terms of the selected genes. (c-f) Gene set enrichment analysis indicated a significantly immune phenotype in the low-risk cases.

Figure 6

Relationship between risk scores and inflammatory metagenes and immune checkpoints. (a & b) Expression of metagenes heatmap and corrogram in the training cohort. (c & d) Correlation between risk

score and immune checkpoints expression.

Supplementary Files

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