Abstract. – OBJECTIVE: Disulfidptosis is a novel mode of cell death, a programmed mode of intracellular disulfide accumulation due to solute carrier family 7 member 11 (SLC7A11)-mediated abnormalities in the cell membrane cystine transport system. Numerous studies have confirmed the prominent role played by SLC7A11 in tumors, but the involvement of SLC7A11 as an important mediator of disulfidptosis in the death process of lung adenocarcinoma cells remains unclear.

MATERIALS AND METHODS: We obtained 4,107 SLC7A11-related genes and analyzed them using a total of 1,040 lung adenocarcinoma transcriptome sequencing data from The Cancer Genome Atlas (TCGA) cohort and GEO (Gene Expression Omnibus) cohort and 991 relevant clinical data. First, we screened for differential genes and identified molecular subtypes for assessing characteristic differences between lung adenocarcinoma subtypes under the influence of SLC7A11-associated genes. Then, risk score models were constructed to assess the prognosis, immune infiltration, tumor microenvironment, and drug treatment effects in lung adenocarcinoma patients. Finally, we also analyzed the distribution of cell types and expression of characteristic genes within the tumor using a single-cell database. In addition, relevant drug sensitivities were predicted.

RESULTS: We screened 956 genes with significant differences and identified 2 molecular subtypes for assessing characteristic differences between lung adenocarcinoma subtypes under the influence of SLC7A11-associated genes. Then, risk score models were constructed to assess the prognosis, immune infiltration, tumor microenvironment, and drug treatment effects in lung adenocarcinoma patients. Finally, we also analyzed the distribution of cell types and expression of characteristic genes within the tumor using a single-cell database. In addition, relevant drug sensitivities were predicted.

CONCLUSIONS: Our comprehensive analysis of SLC7A11-related genes suggests that disulfidptosis has a potential value in the tumor microenvironment, immunity, clinical outcome, and prognosis of lung adenocarcinoma. These findings may increase our understanding of disulfidptosis as a novel cell death paradigm and provide ideas for assessing the prognosis of lung adenocarcinoma and developing new diagnostic and therapeutic strategies.

Key Words: Disulfidptosis, SLC7A11, Lung adenocarcinoma, Prognosis, TME.

Abbreviations
- DRGs, Disulfidptosis related genes; PRGs, Prognosis-related DRGs; DRGs_score, DRGs risk score; LUAD, Lung adenocarcinoma; TME, Tumor microenvironment; TCGA, The Cancer Genome Atlas; GEO, gene expression omnibus; CDF, cumulative distribution function; DEGs, Differentially Expressed Genes; ROC, receiver operating characteristic; GSVA, Gene set variation analysis; ssGSEA, single sample Gene Set Enrichment Analysis; GSEA, Gene Set Enrichment Analysis; PCA, principal component analysis; ISNE, t-distributed Stochastic Neighbor Embedding; UAMP, Uniform Manifold Approximation and Projection; KEGG, Kyoto Encyclopedia of Genes and Genomes; Lasso, Least absolute shrinkage and selection operator; GDSC, Genomics of Drug Sensitivity in Cancer; CIBERSORT, Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts; ESTIMATE, Estimation of STromal and Immune cells in Malignant Tumor tissues using Expression data.

Introduction
Disulfidptosis is a novel mode of cell death first identified in February 2023 by Liu et al. It is induced by abnormal intracellular disulfide accumulation due to abnormal cystine up-
take mediated by \( SLC7A11 \), which belongs to the solute carrier family and encodes an amino acid transport system with high specificity for cysteine and glutamate, which mediates the fine regulation of transmembrane substrates for cell metabolism, growth and death. Regulation across cell membranes is essential for cell metabolism, growth and death. Previous studies\(^5\) have confirmed that \( SLC7A11 \) is overexpressed in various cancer diseases, particularly in tumor cells resistant to therapies such as chemotherapy and radiation and is associated with poor prognosis. For example, \( SLC7A11 \) induces tumor formation by regulating oxidative stress in some homozygous Kirsten rat sarcoma viral oncogene homolog (\( KRAS \)) mutant cancers such as pancreatic ductal adenocarcinoma (PDAC), colonic adenocarcinoma (COAD) and lung adenocarcinoma (LUAD).\(^2\) In addition, \( SLC7A11 \) seems to be associated with drug resistance behavior during tumor treatment. It has been shown\(^6,7\) that \( SLC7A11 \) contributes to drug-resistant behavior during melanoma treatment by promoting intracellular glutathione (GSH) synthesis, and histone deacetylase inhibitor dramatically improves this feature, leading to easier regression of cancer cells. Similar mechanisms have been identified\(^8\) in gastric cancer, lung adenocarcinoma, and cross-glioma treatment resistance. Thus, based on its numerous functions in tumor formation, metastasis and treatment, \( SLC7A11 \) will provide great value for future disease research.

Various types of human cells self-destruct under normal conditions, leading to a stable environment in the body, which is particularly important to help remove abnormal cells, characterized by controlled signaling pathways\(^9\). In the case of tumors, many studies\(^10\) have demonstrated that tumor cells evade the controlled cell death process through various mechanisms to achieve their own survival. Therefore, various types of cell death modalities, such as necroptosis, pyroptosis, ferroptosis, and cuproptosis, have been the research focus. Various cell death pathways have been reported\(^11-13\) to be critical to the prognosis of cancer patients, cancer progression and metastasis, and cancer immune surveillance. Lung adenocarcinoma is the most common type of lung cancer, accounting for approximately 40% of all lung cancers, and is characterized by a high degree of aggressiveness and resistance to conventional radiotherapy. Despite major advances in basic and clinical research on lung adenocarcinoma over the years, the overall survival rate of patients remains low, with an overall survival of less than 5 years\(^4\). Treatments targeting cell death signaling pathways in patients with lung adenocarcinoma appear to offer a solution. For example, chemotherapy with oxaliplatin, gemcitabine or paclitaxel may enhance the responsiveness and sensitivity of immune cells to tumor cells by inducing immunogenic cell death pathways\(^15\). In summary, evasion of the body’s programmed cell death modality appears to be an innate characteristic for tumors, and uncovering this characteristic would open a new horizon for tumor research. However, due to technical limitations, the various cell death modalities identified are still relatively poorly understood, and the understanding of cell death mechanisms is not yet deep enough. Therefore, there is an urgent need for new cell death modalities to complement and understand the mechanisms of lung adenocarcinoma development, which will provide new approaches and insights to improve treatment outcomes and increase the overall survival of lung adenocarcinoma patients.

This study provides the first bioinformatic analysis of disulfidptosis, a novel mode of death in lung adenocarcinoma, and reveals the impact of \( SLC7A11 \) and its related genes mediating disulfidptosis on the prognosis of lung adenocarcinoma and constructs a risk scoring system to predict future survival and treatment outcomes of patients.

### Materials and Methods

#### Data Source

The TCGA (https://portal.gdc.cancer.gov/) and the GEO (https://www.ncbi.nlm.nih.gov/geo/) databases were used to obtain data on gene expression and clinically relevant prognosis and pathological staging of LUAD, including baseline data from the TCGA cohort and the GEO cohort (GSE72094) for subsequent analysis. We annotated and normalized the gene expression data of the TCGA and GEO cohorts and used the “ComBat” algorithm to eliminate batch effects and merge them. We also collated and combined the clinical data from the TCGA and GEO cohorts, in which we excluded samples without survival time and survival status and corrected for uniformity of survival time (53 clinical samples were excluded). The final results were obtained for 1,040 gene expression data (including 598 TCGA data and 442 GEO data) and 991 clinical samples (in-
Inclusion of 513 TCGA data and 398 GEO data. The SLC7A11-related gene set containing 4,107 genes was obtained from GeneCards (https://www.genecards.org/) for subsequent analysis. Single-cell analysis of the NSCLC_EMTAB6149 dataset (containing 40,218 single-cell sequencing samples) was performed using the Tumor Immune Single-cell Hub (TISCH) (http://tisch.comp-genomics.org/) database.

**Disulfidptosis Analysis**

To gain insight into the function of disulfidptosis in LUAD, 4,107 disulfidptosis-related genes (DRGs) were screened for differences between tumor tissues and normal tissues using the "voom" algorithm. The differentially expressed genes (DEGs) were extracted from the tumor tissues and normal tissues, and the screening conditions: Log2 foldchange (logFC)=1, False discovery rate (FDR)=0.05. After that, The search tool for recurring instances of neighboring genes (STRING) database (https://cn.string-db.org/) was used to construct protein interaction network maps of the screened DEGs, which can visually allow us to see the interactions between these genes, which can provide a reference for further in-depth study of the functions of DRGs. Finally, the expressions of the obtained DEGs were integrated with clinical prognostic data. After excluding the genes with zero expression in all samples, the Prognosis-related DRGs (PRGs) affecting LUAD prognosis were screened, and gene expression was extracted using a univariate Cox proportional-hazards model (COX) analysis (p<0.05).

**Clustering and Typing of DRGs**

The “ConsensusCluster” algorithm was used for cluster typing of PRGs to observe the heterogeneity of LUAD samples between different expressions of disulfidptosis. The maximum number of clusters was set to K=9, and the hierarchical clustering algorithm was the Kaplan-Meier (KM) method. After that, the nine clustering effects were compared and the optimal K value was selected, and the LUAD samples were typed based on the optimal K value. After eliminating invalid data, 905 typed samples were finally obtained, and finally, KM survival analysis was performed on these LUAD samples to observe the prognostic differences between different typing. In addition, to evaluate and analyze the effect of typing, three types of data were analyzed using Principal Components Analysis (PCA), t-distributed Stochastic Neighbor Embedding (tSNE), Uniform Manifold Approximation and Projection (UAMP). Three algorithms were used to reduce the dimensionality of the typed samples and visualize them to observe how well the typing discriminates the samples.

**DRGs Subtype Analysis**

To understand the reasons for the differences in DRGs clustering, the subtyped LUAD samples were first analyzed for differences to observe the expression of DEGs between subtypes and displayed as box plots. The analysis of the relationship between genes and clinical traits is a core aspect of this study. Therefore, we examined variances in clinical traits between the typed samples and presented the findings through visualization techniques. Afterward, single sample Gene Set Enrichment Analysis (ssGSEA) was performed on the typed samples to observe the immune cell infiltration between the typed samples. Finally, Gene set variation analysis (GSVA) was performed on the typed samples to observe the pathway enrichment between the different typed samples. In addition, pathway enrichment analysis was performed on the typed samples using the Gene Set Enrichment Analysis (GSEA) algorithm using the Kyoto Encyclopedia of Genes and Genomes (KEGG) cell signaling pathway gene set. Observation of these pathway enrichments will provide guidance for further investigation of the specific mechanisms between disulfidptosis and LUAD.

**Construction of a Risk Scoring Model for DRGs**

The risk score model was constructed using the prognostic data of PRGs. First, the samples were randomly averaged into a training group (453 samples) and a test group (452 samples) at one time, and the Least absolute shrinkage and selection operator (LASSO regression) was used for the training group using the “glmnet” package. The regression coefficients of the variables in the regression model were compressed using the “LASSO regression” package to prevent over-fitting and to solve the problem of severe covariance. Multiple cross-validations were performed to find the number of genes with the smallest error, and these genes with the smallest errors will be involved in subsequent analyses as feature genes in the LASSO regression screen. After that, the expressions of the LASSO regression signature genes were extracted, and the signature
genes were screened using a multi-factor COX regression method and a risk-scoring system was constructed. In this risk-scoring system, all samples had a risk score based on the expression of the feature genes. The DRGs risk score (DRGs_score) was calculated as follows.

\[ \text{DRGs_score} = \frac{n}{1} \sum \text{Coef}_g \times \text{Exp}_g \]

where Coefg and Exp_g represent gene risk coefficient and gene expression, respectively, and n represents the number of genes. In the training group, the samples were classified into high risk (DRGs_score is higher than the median value) and low risk (DRGs_score below the median value) according to the median value of risk scores. The test group was also classified into high risk and low risk according to the classified median risk value and the risk results were output for subsequent validation. Finally, KM survival analysis was performed, and survival curves were plotted for the training group. In addition, receiver operating characteristic (ROC) curves were plotted at 1, 3, and 5 years to assess the accuracy of the model. The same measures were taken for the test group for validation.

**Risk Assessment and Nomogram**

First, all clinical data were collated, and the age, gender and clinical staging characteristics of the sample were retained. After that, risk data and clinical data were integrated, and independent prognostic analysis was performed using multifactorial COX analysis, with the aim of assessing whether DRGs_score could be used independently as a factor affecting LUAD prognosis. We also performed a risk analysis on DRGs clustering to observe whether DRGs typing differed in the DRGs_score system. Finally, a Nomogram was developed using clinical characteristics and risk scores. In the Nomogram scoring system, each clinical characteristic and DRGs_score was matched with a score, and a total score was obtained by summing the scores of all clinical characteristics for each sample. The total score corresponds to the corresponding predicted values of 1-year, 3-year and 5-year survival rates. Also, calibration curves were plotted to observe the bias between the predicted 1-year, 3-year and 5-year survival rates and the actual observed results. In addition, cumulative hazard curves were used to show the trend of Nomogram scores for each sample in the high-risk group (Nomogram score is higher than the median value) and low-risk group (Nomogram score below the median value) with survival time.

**Immune and Tumor Microenvironment Analysis**

The immune and tumor microenvironment (TME) is a very important part that affects tumor risk behavior. Therefore, LUAD gene expression data were analyzed using the Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT) algorithm and immune cell gene expression data to obtain sample immune cell content data. This was then integrated with the DRGs_score risk data to obtain the results of immune cell infiltration in high- and low-risk groups. We also analyzed the correlation between immune cells and DRGs_score risk scores. Finally, the immune and mesenchymal scores of LUAD tumor TME were performed using the Estimation of Stromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE) method to reveal the compositional differences within the tumors of the high- and low-risk groups. In addition, to gain insight into the clinical value of DRGs_score, the correlation between DRGs_score signature genes and immune checkpoints was analyzed, which will provide a reference to guide the immunotherapy of LUAD.

**Drug Sensitivity and Single-Cell Analysis**

In order to explore drugs that may be therapeutically effective in high- and low-risk groups of patients, drug sensitivity prediction for LUAD was performed using the downloaded Genomics of Drug Sensitivity in Cancer (GDSC) database (Hinxton, Cambridgeshire, CB10 1SA, UK) using the “oncoPredict” package. The drug sensitivity index was expressed as a semi-inhibitory concentration (IC\(_{50}\)) value. Single-cell analysis is becoming an increasingly important tool for understanding intra-tumor heterogeneity. To this end, single-cell analysis of the NSCLC_EMTAB6149 dataset and single-cell analysis of the screened signature genes were performed using the single-cell database TISCH. This will help to further investigate the intermolecular function of LUAD from the single-cell level.

**Statistical Analysis**

In this study, we used R language software (version 4.2.1), STRING (https://cn.string-db.org/, Version: 12.0), for data statistics and visualization. Data preprocessing, including back-
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Results

Identification of Differentially Expressed Genes in DRGs

Under the screening conditions, logFC=1 and FDR=0.05, 956 DEGs (Supplementary Table I) with significant differences were screened from 4,107 DRGs, and these DEGs may play a very important role in the development of LUAD (Figure 1A). To understand the characteristics of DRGs, we constructed a gene interaction network map with the minimum interaction score set to 0.9. The evidence of gene interaction in the network map was obtained from databases and experimental assays (Figure 1B). In the network, it can be seen that most of the DRGs have evidence of interactions, and a large number of interactions were observed at gene nodes such as TXNRD1, JUN, and FOS, which may have their pivotal roles in the overall DRGs interaction function. Subsequently, a univariate COX analysis of DEGs identified 425 PRGs affecting the prognosis of LUAD patients (Supplementary Table II). Finally, the LUAD samples were clustered based on PRGs, and the clustering results showed that the samples were best clustered when K=2 (Figure 1C); other clustering results can be found in Supplementary Figure 1. In addition, the cumulative distribution function (CDF) curves of the 8 clusters can be seen in Figure 2A. Therefore, all samples were classified into type 2 (type A and type B). The results of the 3 descending analyses of PCA (Figure 3C), tSNE (Supplementary Figure 2A) and UAMP (Supplementary Figure 2B) showed that the typing could distinguish the LUAD samples well.

Prognostic and Functional Analysis of Fractions

KM survival analysis of the fractions showed (Figure 2B) that there was a significant prognostic difference between fractions A and B (p<0.001). The overall prognosis of subtype B was significantly better than that of subtype A. In addition, the results of the differential analysis between the different fractions are available in Supplementary Figure 2C. On the other hand, the results of immune cell infiltration between different fractions showed (Figure 2C) that 19 immune cells (activated B cells, activated CD4 T cells, activated dendritic cells, CD56 bright natural killer cell (p<0.05), CD56dim natural killer cell, eosinophil, immature B cell, Immature dendritic cell, MDSC, macrophage, mast cell, monocyte, and natural killer cell, plasmacytoid dendritic cell, regulatory T cell, T follicular helper cell, type 1 T helper cell, type 17 T helper cell, type 2 T helper cell) were significantly different between the two typed samples (p<0.001), and the B typed Activated B cell, Activated dendritic cell, CD56bright natural killer cell eosinophil, immature B cell, immature dendritic cell, MDSC, macrophage, mast cell, monocyte, natural killer cell, plasmacytoid dendritic cell, regulatory T cell, T follicular helper cell, type 1 T helper cell, and type 17 T helper cell were higher than in A fraction, indicating that the presence of these 16 immune cells may play a positive role in the prognosis of LUAD patients. In contrast, Activated CD4 T cells, CD56dim natural killer cells, and type 2 T helper cells were higher in A fraction than in B fraction, suggesting that these three immune cells may be associated with poor prognosis in LUAD patients. Further pathway enrichment analysis seemed to reveal the mechanisms behind the different prognostic outcomes in patients with type A and type B. The results showed that cell proliferation-related pathways and DNA repair-related signaling pathways were more active in type A fractions. This predicts that DRGs may be involved in activities related to the high proliferative state of LUAD cells and that these activities are associated with poor prognosis (Figure 3A). However, fatty acid metabolic pathways, vascular smooth muscle contraction signaling pathways, and hormone receptor-related metabolic pathways showed a
more active status in the B fraction, suggesting that these pathways are associated with a better prognostic status (Figure 3B).

**Construction of Risk Score Model**

In the training group (453 samples), LASSO regression analysis was performed to find the best fit according to the change in Partial-likelihood deviance in cross-validation, and 28 DRGs were finally retained (Figure 4A). After that, multi-factor COX regression was used for 28 DRGs, and 15 disease characteristic genes were finally screened (Table I). The DRGs_score was constructed as follows.

Risk score = (0.2296 * expression of LDHA) + (0.2052 * expression of CBX2) + (0.2491 * expression of NPAS2) + (-0.7423 * expression of PNPLA6) + (0.1659 * expression of ANGPTL4) + (0.2501 * expression of AHSG) + (0.2052 * expression of MAFF) + (0.1535 * expression of CNGA1) + (0.2844 * expression of CDX2) + (-0.1808 * expression of DDO) + (-0.4489 * expression of CASP12) + (0.4108 * expression of SNCB) + (-0.4024 * expression of GLS2) + (0.2269 * expression of SLC6A17) + (0.2116 * expression of PHLDA2)

After that, we classified all samples into high-risk and low-risk according to the median value of the sample risk scores (samples included the training group and test group) and performed KM survival analysis (Figure 4C-D) on the training group.
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and test group. The results showed that the prognosis of both high-risk and low-risk groups was significantly different \( (p<0.001) \), and the prognosis of the high-risk group was significantly worse than that of the low-risk group. Meanwhile, the ROC curves constructed for the training group had ROC values of 0.807, 0.789, and 0.820 for 1, 3, and 5 years, respectively. The ROC curves validated for the test group had ROC values of 0.627, 0.667, and 0.634 for 1, 3, and 5 years, respectively (Figure 4E-F). In addition, the model could also distinguish well between subtype A and subtype B \( (p<0.001) \) (Figure 4B). Finally, a multifactorial COX analysis of DRGs_score showed that the model could independently affect the prognosis of LUAD patients as well as other clinical traits (HR>1) (Figure 5C).

**Characterization of Tumor Components**

To further understand the internal characteristics of the tumor, various aspects of immune as well as TME characteristics within LUAD were analyzed. The results of the immune response to LUAD showed that 11 immune cells differed between the high-risk and low-risk groups \( (p<0.05) \), with higher levels of T cells CD4 memory activated, NK cells resting, and mast cells resting in...
In contrast, compared to the low-risk group, the high-risk group had higher levels of B cell memory, T cells CD4 memory resting, plasma cells, monocytes, macrophages M0, dendritic cells resting, mast cells activated, and neutrophils were much lower (Figure 6D). In addition, there are complex interactions between these immune cells (Figure 6A). Analysis of immune cell correlations showed that 14 immune cells correlated with risk scores, with macrophages M0, mast cells activated, neutrophils, NK cells resting, plasma cells, T cells CD4 memory activated, and T cells follicular helper were positively correlated with risk.
Figure 4. Construction of the risk scoring model. A, Partial likelihood deviance was plotted versus log(γ), the dotted line corresponds to minimal deviance. B, Analysis of risk differences between subtype A and subtype B. C-D, Kaplan-Meier survival analysis for the train and test groups. E-F, Receiver operating characteristic (ROC) curves for the train and test groups.
In contrast, B cell memory, T cells CD4 memory resting, monocytes, macrophages M2, dendritic cells resting, mast cells resting, and eosinophils were negatively associated with risk \((p<0.05)\) (Figure 6B-C, Supplementary Figure 3). Finally, TME analysis showed that there was a significant difference between the tumor mesenchymal component and immune component in the high-risk and low-risk groups \((p<0.05)\).

**Nomogram, Drug Treatment Target and Drug Sensitivity Analysis**

In order to better apply the DRGs_score system to the clinical setting, we constructed a Nomogram to predict the 1-year, 3-year, and 5-year survival rates of patients (Figure 5A), and the Nomogram curve showed that the Nomogram score increased gradually with time and also the risk increased. Also, the risk was higher in the high-risk group than in the low-risk group. We analyzed the relationship between DRGs_score and these drug-related targets (Figure 7C), and the results showed that KRAS, ALK, ROS1, BRAF and immune checkpoint genes were significantly different between the high-risk and low-risk groups \((p<0.05)\). The therapeutic targets of drugs except KRAS, TNFSF4, TNFSF9, CD276 were generally lower in the high-risk group patients compared to the low-risk group, implying that the related immune and target therapies may be less effective in these high-risk lung adenocarcinoma patients. In addition, we screened 89 drugs with differences in drug sensitivity between the high-risk and low-risk groups, and only 3 are shown here (Figure 7D-F).

**Single-Cell Analysis**

The analysis of 40,218 non-small cell lung cancer single-cell data showed that the cells within the tumor could be divided into 25 clusters. We then performed cellular annotation of the 25 cell clusters, and the results showed that there are 12 cell types (Figure 8A-B), in which immune cells (including monocytes, T cells, etc.) still occupy the majority of the tumor tissue, while the content of CD8 T cells seems to be the highest among T cells (Figure 7A). This suggests the presence of a strong anti-tumor immune process within the tumor. Furthermore, single-cell analysis of the signature genes showed that LDHA was widely expressed in various cells inside the tumor (Figure 8C-D), CBX2 was significantly expressed only inside the tumor tissue, PHLDA2 and ANGPTL4 were significantly expressed in tumor cells (Figure 8E-F), monocytes, and fibroblasts, and MAFF and PNPLA6 appeared to be significantly expressed in monocytes and T cells, while NPA52 was abundantly expressed in tumor cells, endothelial cells, and fibroblasts (Supplementary Figure 4). The single-cell distribution of these signature genes seems to suggest some kind of cell-to-cell interaction mechanism, which will provide a reference for further LUAD studies in the future.

**Discussion**

Many studies\(^{16,17}\) have reported that SLC7A11 plays an important role in cancers with different prognoses. However, most studies only correlated SLC7A11 as a separate gene pattern, while the tumor process in which SLC7A11 is involved as a new important molecule of programmed cell death, disulfidptosis, has not been revealed yet. In this study, we started with SLC7A11, an important molecule of disulfidptosis, and screened DRGs and identified 2 molecular isoforms based on SLC7A11-related genes. Compared with subtype B, subtype A had a significantly worse prognosis. In the A subtype, we observed more active DNA replication and repair-related signaling pathways, which represented a high proliferative state within the tumor. In addition, Activated CD4 T cells, CD56dim natural killer cells, and Type 2 T helper cells were higher in subtype A compared to subtype B, suggesting that an increase in these immune cells is associated with poor prognosis in LUAD patients. Subsequently, we constructed a validated DRGs_score system to assess the internal characteristics of LUAD and to guide

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**Table I.** Characteristic genes involved in model construction.

<table>
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clinical treatment. In the DRGs_score system, we found that compared to low-risk patients, high-risk patients had lower immune and stromal components within the tumor and a worse prognosis. Among the immune responses, macrophages M0, mast cells activated, neutrophils, NK cells resting, plasma cells, T cells CD4 memory activated, T cells follicular helper were positively associated with risk, while B cells memory, T cells CD4 memory resting, monocytes, macrophages M2, dendritic cells resting, mast cells resting, and eosinophils were negatively associated with risk, suggesting that this involvement and interaction of immune cells influenced the prognosis of LUAD patients. Drug target analysis showed that KRAS, ALK, ROS1, BRAF, and some immune checkpoint genes were significantly different between the high-risk and low-risk groups, suggest-

Figure 5. Nomogram and independent prognostic analysis. A, Predictive nomogram for 1-year, 3-year, and 5-year survival in patients with lung adenocarcinoma. B, Cumulative hazard curve of the nomogram risk score. C, Multivariate independent prognostic analysis of risk score models.
ing that our constructed DRGs score could be used as a predictor of the effect of target therapy in LUAD patients. Finally, we also constructed a Nomogram and predicted the relevant drug sensitivity, which further facilitated the application of DRGs score. Thus, this prediction model can be used for prognostic stratification of LUAD patients and can be used to guide clinical treatment. This will provide new ideas to understand the mechanism of disulfidptosis further and improve the prognosis of LUAD patients.

In general, SLC7A11 is highly expressed in most tumors and is associated with poor prognosis. The current study revealed the main functions of
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SLC7A11 in tumors, including regulating redox status, ferroptosis (a form of programmed cell death) and intercellular signaling. Reactive oxygen species (ROS) are a group of highly reactive ions or molecules in the body that are usually highly expressed in tumor tissues to stimulate tumor proliferation and invasion. However, because of their strong oxidative properties, ROS cause cell death processes in both

Figure 7. Tumor microenvironment, drug target and drug sensitivity analysis. A. Cell-type statistics in the single-cell analysis of non-small cell lung cancer. B. Analysis of the tumor microenvironment in lung adenocarcinoma. The horizontal coordinates represent the tumor microenvironment components, and the vertical coordinates represent the tumor microenvironment score. C. Differential analysis of oncology drug therapy targets and immune checkpoint genes between high- and low-risk groups. D–F. Drug sensitivity analysis. The horizontal coordinates represent the high-risk and low-risk groups, and the vertical coordinates represent the half-maximal inhibitory concentration (IC50) of the drug.
tumor cells and normal cells, which are resisted by the antioxidant system inside the tumor cells. It is through supporting cystine transport that \textit{SLC7A11} plays an important antioxidant role by producing GSH inside the cell\textsuperscript{19}. It has been shown\textsuperscript{3} that in tumor cells with \textit{KRAS} mutations, the transcription factor E26 avian leukemia oncogene 1, 5' domain (ETS-1) acts synergistically with the activating transcription factor 4 (ATF4) to promote glutathione (GSH) synthesis by activating \textit{SLC7A11} transcription. In addition, nuclear factor erythroid2-related factor 2 (Nrf2) appears to promote \textit{SLC7A11} syn-

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...thesis by a form independent of ETS-1 with ATF4. Thus, this evidence supports the presence of high expression of SLC7A11 within the tumor, as corroborated by our findings, where differential analysis showed that high expression of SLC7A11 in LUAD tumor cells was associated with a high risk of poor prognosis. Furthermore, the results of signaling pathway analysis showed that tumors with high expression of SLC7A11 were associated with a high proliferation and hyper differentiation state of the cells. On the other hand, inhibition of SLC7A11 leads to increased intracellular ROS in tumor cells, triggering programmed cell death, and this has been demonstrated in clinical studies19. For SLC7A11 and ferroptosis studies20-22, it seems to be an interesting point that SLC7A11 overexpression confers resistance to ferroptosis in cancer cells by synthesizing glutathione. This mechanism is currently being investigated mainly in the field of enhancing the sensitivity to antitumor therapy.

TME is the soil of tumor growth and interacts with tumor cells, thus promoting tumor growth and invasion. Numerous studies23-25 have demonstrated that SLC7A11 affects TME by exporting glutamate. Under physiological conditions, glutamate promotes neuronal multiplication and migration and is neurotoxic. In tumor cells, glutamate is an important oncogenic factor that promotes malignant transformation of cells and promotes proliferation, invasion, metastasis and suppression of the immune system by acting on both cancer and non-cancer cells26. A study on glioma found that glioma cells are associated with cancer cell invasion by releasing glutamate, which later acts on Ca2+-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in glioma cells as well as surrounding cancer cells, producing Ca2+ oscillations. Another study27 confirmed a similar finding that the release of glutamate from cancerous breast cells by the SLC7A11-mediated XC-system acting on mGluR3 promotes extracellular matrix lysis and thus enhances the migration of cancer cells. The correlation between SLC7A11 and immunity is also striking, as Long et al28 showed that after anti-VEGF treatment in glioblastoma, SLC7A11 levels in cells were elevated to increase glutamate release, which then acted on mGluR1 on Tregs to promote the immunosuppressive function of Tregs. In a study29 on lung cancer, researchers observed the inhibition of the AKT/STAT6 signaling pathway and activation of the JAK/STAT1 signaling pathway by knocking down macrophage-specific SLC7A11 in mice, which led to the activation and enhancement of tumor-associated immune cells. Also, combining the xCT (SLC7A11) inhibitor erastin with anti-PDL1 antibodies, according to this mechanism, increased the effect of antitumor immunotherapy. In another study30, tumor-released glutamate inhibited SLC7A11 activity by paracrine means and ultimately promoted tumor proliferation through a complex mechanism. This would seem to be contrary to the ability of SLC7A11 to promote tumor proliferation and migration. In fact, this just illustrates the complex role of SLC7A11 for different types of tumors and between different cells in the tumor environment, and relevant studies targeting SLC7A11 will be a hot spot to reveal new therapeutic potential of the disease and to uncover unknown mechanistic events from known studies.

Limitations
This study uses data from multiple public databases for statistical analysis of a large sample. This inevitably has some limitations, such as batch effects between samples from different databases. Therefore, in the statistical analysis, we used various algorithms to pre-process to remove batch effects between different data sources in order to ensure the scientific nature of the data analysis to the maximum extent possible. Second, the samples we used are retrospective and human data selection may lead to selection errors. This requires large-scale prospective studies and experiments to validate our findings. In addition, many factors influence patient prognosis, such as diet, psychological life status, genetic specificity, and different levels of medical care. Therefore, these unanalyzed clinical characteristics may also have influenced the analysis results to some extent.

Conclusions
Our analysis of DRGs revealed a wide range of regulatory mechanisms by which disulfidptosis may affect LUAD prognosis, and tumor microscopic context. In addition, we identified the clinical value of DRGs in predicting tumor target therapy and immunotherapy. These new findings on disulfidptosis will provide new ideas for expanded research on programmed cell death modalities and for the treatment of lung cancer.

Ethics Approval and Informed Consent
Not applicable.
Availability of Data and Materials

Conflict of Interest
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Authors’ Contributions
ML participated in the project design, data analysis and processing, formal analysis, survey, methods, project management, visualization, and writing of the manuscript. RL participated in data curation and visualization. ZW participates in conceptualization, investigation, supervision, project administration, writing – review, and editing. All authors read and approved the final manuscript.

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