Yanbiao Chu et al. (2023) Endoplasmic reticulum stress-related genes construct a risk score and prognostic model for squamous lung cancer. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 23 (90) pp. 218-234.
DOI: https://doi.org/10.15366/rimcafd2023.90.016

ORIGINAL

ENDOPLASMIC RETICULUM STRESS-RELATED GENES CONSTRUCT A RISK SCORE AND PROGNOSTIC MODEL FOR SQUAMOUS LUNG CANCER IN FEMALE PLAYERS

Yingying Ou[1], Yan Deng[1], Cuiju Mo[1], Li Huang[1], Zhaolu Lu[1], Shan Li[1,*]

[1]Department of Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, Nanning,530021, Guangxi, China
[*]Corresponding author: Shan Li
Email: lis8858@126.com

Received: February 18, 2022
Accepted: January 28, 2023

ABSTRACT

Objective To investigate the prognostic impact of endoplasmic reticulum stress-related genes on lung squamous carcinoma. **Methods** Gene expression data were obtained from the TCGA database, and prognostic models were constructed by LASSO-COX analysis. Gene expression data were obtained from the TCGA database and prognostic models were constructed by LASSO-COX analysis. Immunohistochemistry was performed to validate the gene expression. Subtypes were obtained by clustering analysis and correlation analysis of subtypes was performed. The accuracy of the model was reflected by Monogram. **Result** Endoplasmic reticulum stress-related genes LRRK2 and WFS1 constructed the prognostic model. the ROC curve was 0.584 at -1 year, 0.598 at 2 years and 0.603 at 3 years. The risk score constructed from the prognostic model was an independent predictor of overall survival and influenced the tumor microenvironment as well as drug sensitivity. **Conclusions** Endoplasmic reticulum stress-related genes can be used to predict the prognosis and influence the immune status of squamous lung cancer, and modulating the expression of these genes is a potential therapeutic option.

KEYWORDS: squamous lung cancer, endoplasmic reticulum stress, gene expression, overall survival, drug sensitivity

The International Agency for Research on Cancer (IARC) released data on the global burden of cancer in female players in 2020: new cases of breast cancer overtook lung cancer to become the number one cancer worldwide, but

in terms of the number of cases leading to death, lung cancer topped the list with 1.8 million cases. Lung squamous cell carcinoma (LUSC), a major subtype of non-small cell lung cancer, accounts for 25-30% of non-small cell lung cancer, with a 5-year survival rate of less than 20% and approximately 400,000 deaths per year.(Siegel, Miller, & Jemal, 2019) . Currently, chemotherapy is the main treatment for advanced squamous lung cancer, but the clinical efficacy has been at a plateau for a long time.(Baxevanos & Mountzios, 2018) However, the clinical efficacy has been at a plateau for a long time. Usually, advanced squamous lung cancer has no chance of radical surgery, and the overall prognosis is poor due to the lack of effective targeted drugs.(Perez-Moreno, Brambilla, Thomas, & Soria, 2012) So that studying the factors related to the prognosis of squamous lung cancer is necessary to improve the survival rate.

Endoplasmic reticulum stress occurs in most cancers, including breast cancer, lung cancer, affecting immune cells and can lead to precancerous lesions (Cubillos-Ruiz, Bettigole, & Glimcher, 2017),(Wang & Kaufman, 2014). The endoplasmic reticulum stress receptors and downstream signaling pathways are successful for tumor growth ,response of chemotherapy, targeted therapy and immunotherapy(Chen & Cubillos-Ruiz, 2021).

As a result of impaired protein folding and secretion, the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum is described as endoplasmic reticulum stress (ERS). Unfolded proteins can elicit a cascade signaling response, a protective mechanism known as the unfolded protein response (UPR). Inositol requiring enzyme 1 (IRE1), protein kinase R-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF) are the most important endoplasmic reticulum kinases. transcription factor 6 (ATF6) mediates three classical UPR pathways, which play important roles in alleviating ERS and maintaining intracellular environmental homeostasis.(Chen & Cubillos-Ruiz, 2021) . Among them, the unfolded protein response is an adaptive response that restores cellular homeostasis by reducing the endoplasmic reticulum load by reducing the source and increasing the destination of proteins, which promotes the survival of tumor cells and allows tumor malignant progression, but activates the pro-apoptotic pathway to induce cancer cell death when the over-activated ERS exceeds the threshold that cancer cells can tolerate(Backes, Mezzomo, Buffon, & Calil, 2019; Zanetti, Xian, Dosset, & Carter, 2022) . Therefore, ERS-related antitumor therapy can be administered in two ways: first, by inhibiting the UPR-mediated pro-survival pathway, and second, by inducing a sustained ERS-mediated pro-death pathway. Although studies have shown that endoplasmic reticulum stress is associated with cancer development, the mechanism of endoplasmic reticulum stress-related genes and prognosis of LUCS remains to be explored.

We get mRNA expression profiles and clinical data from TCGA databases, and constructed a prognostic model consisting of differentially expressed genes

(DEGs) of endoplasmic reticulum stress-related genes in the TCGA cohort. Based on the constructed model, the cohort was divided into high-risk and low-risk scoring groups and correlation analysis was performed to explore the abnormalities between the two groups, and the relationship between prognostic gene expression and clinical correlation was investigated.

1 MATERIALS AND METHODS

1.1 Materials

1.1.1 Acquisition of LUSC gene expression data

RNA sequencing data and clinical information can be downloaded from TCGA website. TCGA data are publicly available and the female players involved in the database have received ethical approval. There will be no ethics-related issues or other conflicts of interest involved. 83 endoplasmic reticulum stress-related genes were obtained in the GSEA database (Supplementary Table 1).

1.1.2 Main reagents

Recombinant Anti-WFS1 antibody [EPR23801-91] (ab259362) was purchased from ABCAM, and Anti-LRRK2 Rabbit pAb was purchased from servicebio.

1.2 Methodology

1.2.1 Filter for differentially expressed genes to construct prognostic models

Differentially expressed genes (DEGs) between tumor and paracancerous tissue were identified by the "limma" R package with logFC > 1 and FDR < 0.05. endoplasmic reticulum stress-related genes with prognostic value were screened by single-gene cox analysis. To reduce the risk of overfitting as much as possible , use LASSO-COX regression analysis to construct prognostic models (Simon, Friedman, Hastie, & Tibshirani, 2011).

Prognostic models were constructed using the LASSO algorithm by selecting and shrinking variables with the "glmnet" R package. The independent variable is standardized expression matrix of prognosis-related DEGs and the dependent variables were the overall survival and status.Risk score regression coefficients were calculated for female players based on the expression levels of each endoplasmic reticulum stress-related gene and its corresponding gene. The cohort was divided into high-risk and low-risk groups, using the median risk score as the boundary. Using the "Rtsne" and "ggplot2" R packages to study the spread in different groups. The "survminer" R package was used to analyze the overall survival of high- and low-risk groups, and the "survival" R package

and "timeROC" R package were used to perform Time-dependent ROC curve analysis was performed to assess the predictive value of prognostic features.

1.2.2 Immunohistochemical (IHC) verification of prognosis-related gene protein expression in squamous lung cancer tissues and paracancerous tissues adjacent to cancer 30 pairs of lung squamous carcinoma and corresponding paraneoplastic tissue specimens were randomly selected in Jinan Central Hospital according to the above criteria. The expression levels of 2 prognosis-related genes in 30 pairs of lung squamous carcinoma and adjacent non-tumor tissues were verified by immunohistochemical assays.

All specimens were subjected to immunohistochemical experiments at room temperature. First 4 mm paraffin sections were obtained from the pathology department, and then the first 5 sections of pathological wax blocks exposed to air were removed. The tissue sections were dewaxed and boiled in citrate buffer (pH=6.4) after hydration. Peroxidase was inactivated with methanol containing 3% hydrogen peroxide and retreated with citrate buffer (pH=6.0). Add 1% bovine serum albumin to phosphate buffer and incubate for 30 min. Sections were stained with the primary antibody and incubated overnight at 4°C. Sections were washed three times in phosphate buffer followed by staining with secondary antibody at a ratio of 1:200 for 50 minutes. After coating with diaminobenzidine, sections were re-stained with hematoxylin and sealed. The mean absorbance values (OD) were observed and photographed using a light microscope and imageJ software, OD = IOD/area, and the expression intensity of risk score-associated genes was expressed as the magnitude of the mean absorbance values for statistical analysis.

1.2.3 Consensus Clustering Analysis of AAGs and ER stress

To investigate the potential endoplasmic reticulum stress-related subtype mechanisms of LUSC, a cluster analysis was performed. Cluster analysis is based on the information found in the data describing the objects and their relationships (Sabah, Tiun, Sani, Ayob, & Taha, 2021) The data objects were grouped according to their correlation. The number of subtypes was determined by selecting 80% of the samples for repeat sampling with the Consensu Cluster Plus package and obtaining the best K value 1000 times (Seiler, Huang, Szalma, & Bhanot, 2010) The number of subtypes was determined by obtaining the best K-value.

1.2.4 Identification of DEGs and Functional Enrichment Analysis

To identify whether functional differences existed between subtypes, a gene set variation analysis (GSVA) analysis was performed on the subtype results (Hänzelmann, Castelo, & Guinney, 2013). Differentially expressed genes (DEGs) between subtypes were calculated by the "limma" package.

CIBERSORT is a tool for deconvolution of expression matrices of human immune cell subtypes based on the linear support vector regression principle. (Le, Aronow, Kirshtein, & Shahriyari, 2020). The level of immune cell subpopulation infiltration in both subpopulations was explored by the CIBERSORT algorithm. The stromal and immune cell contents were estimated by ESTIMATE algorithm (Yoshihara et al., 2013) The immune score and stromal score as well as the tumor purity were predicted.

1.2.5 Establishment of a Predictive Nomogram

Nomograms were drawn to predict risk scores and other clinicopathological characteristics of LUSC female players, especially regarding OS at 1, 3 and 5 years. calibration curve analysis and decision curve analysis (DCA) were drawn to validate the clinical reliability of the nomograms.

1.2.6 Chemotherapy sensitivity analysis

The NCI-60 database was downloaded from the CellMiner interface (https://discover.nci.nih.gov/cellminer). The relationship between prognostic gene expression and drug sensitivity and the efficacy of prognostic genes with 218 FDA-approved drugs were investigated by Pearson correlation analysis. Drug names are shown in Supplementary Table 2, and prognostic gene expression-drug correlations are shown in Supplementary Table 3.

1.27 Statistical Analysis

Data were processed, analyzed and presented using R software (version 4.1.2) and its associated software packages. Values were considered at $P < 0.05$.

3. RESULTS

The study population of this study included 493 female players from TCGA-LUSC, and detailed clinical characteristics of these female players are summarized in Table 1.

Table 1. Clinical characteristics of LUSC female players.

	TCGA-LUSC Cohort
number	493
Age	67(39-60)
Gender	
female	129 (26.2%)
male	364 (73.7%)
Stage	
I	240 (49.1%)
II	157 (32.1%)
III	84(17.2%)
IV	7(1.4%)
unknow	5
Survival status	
Alive	283
Deceased	210

3.1 Identification of prognostic endoplasmic reticulum stress-associated DEGs in the TCGA cohort

Upon analysis, 18 endoplasmic reticulum stress-related genes were differentially expressed in tumor tissue and adjacent non-tumor tissue. One-way COX analysis yielded 7 prognostic genes and intersected with differential genes, which led to 3 OS-associated genes (Figure 1A-B). To determine the presence of corresponding gene protein expression, the immunohistochemical results of TMEM117 were found to have no significant expression in the lung according to the HPA database (Supplementary Figure 1), which was different from the analysis, so only LRRK2 and WFS1 were retained and used as prognostic indicators. The risk ratio (Hazard Ratio) of the WFS1 gene was 1.357 and that of the LRRK2 gene was 1.169 (Figure 1C).

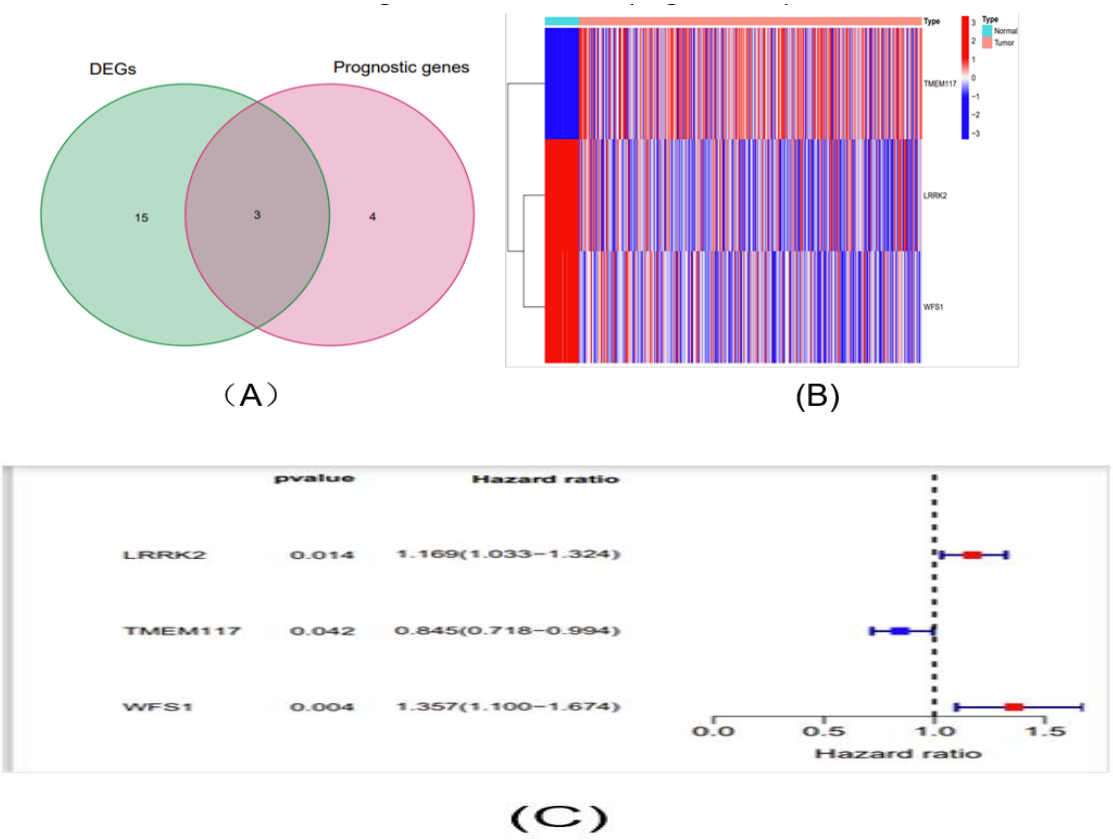


Figure 1. Expression of recombined endoplasmic reticulum stress differential genes in LUSC
A: intersection of differential genes with endoplasmic reticulum stress genes; B: expression of intersecting genes; C: risk ratio of intersecting genes.

3.2 Prognostic Model Construction in TCGA Cohort

The expression profiles of WFS1 and LRRK2, the 2 OS-related genes mentioned above, were analyzed using LASSO-Cox regression analysis to establish a prognostic model. The algorithm for the risk score is 0.114* LRRK2 + 0.254*WFS1. Female players will be divided into high or low risk groups

according to the median critical value (Figure 2A). PCA analysis and t-SNE analysis reveal that female players in different risk groups have two directions of distribution (Figure 2B, C). The scatter plot shows that high-risk female players are more likely to have a mortality rate than low-risk female players (Figure 2D). Kaplan Meier curves showed that high-risk female players had lower OS than low-risk female players significantly. (Figure 3E). Time-dependent ROC curves were generated by prognostic models for the analysis of survival prediction. The area under the ROC curve (AUC) for TCGA was 0.584 at 1 year, reaching 0.598 at 2 years and 0.603 at 3 years (Figure 2F) . We analyzed these two prognostic genes for survival and could show that low expression of WFS1 and LRRK2 was significantly associated with poor OS. S (Supplementary Figure 3).

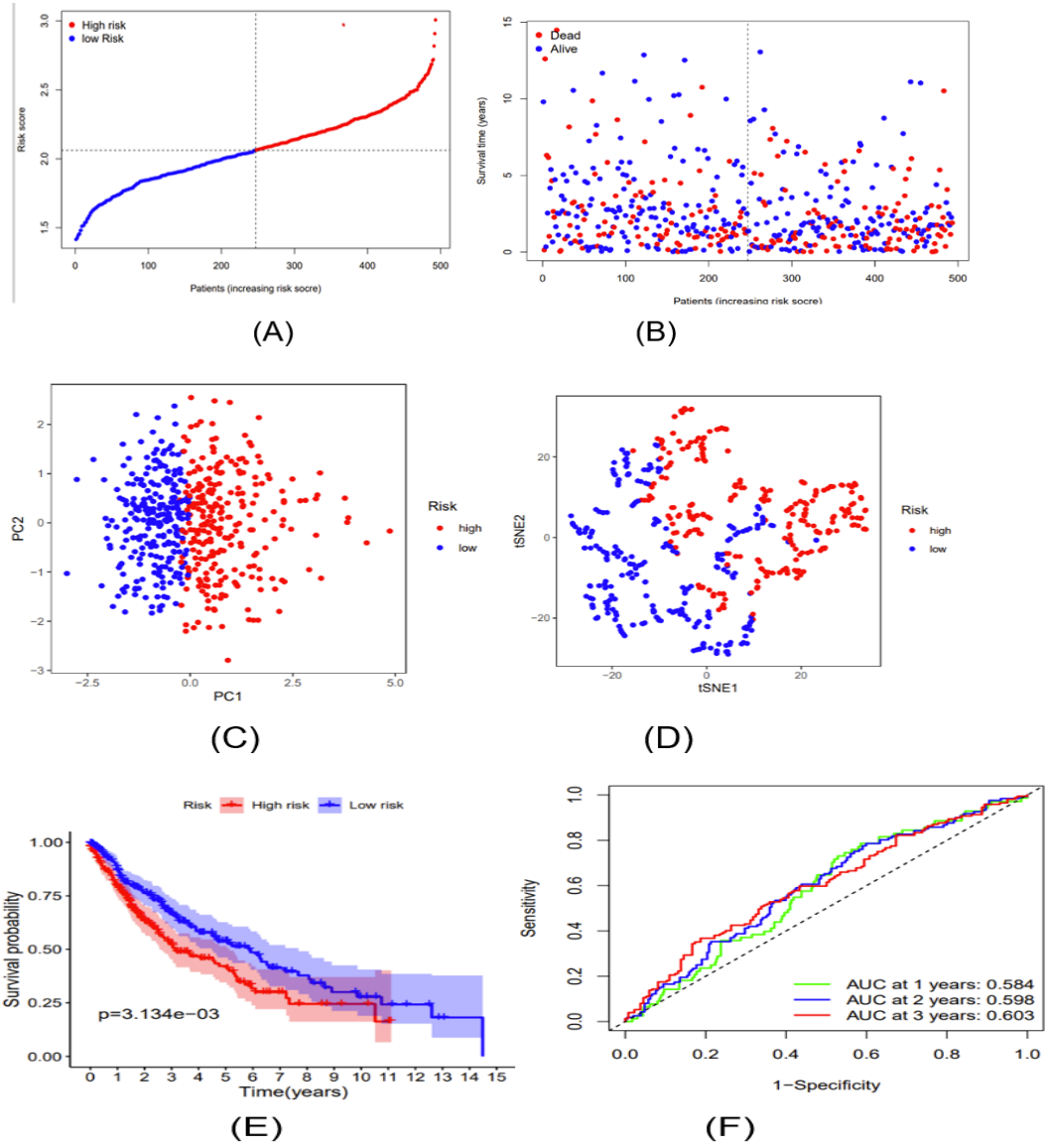


Figure 2. Prognostic analysis of the 2-gene signature model in the TCGA cohort. (A) Median and distribution of risk scores. (B) Distribution of survival status. (C) PCA plots. (D) t-SNE analysis. (E) Kaplan-Meier curves of OS for female players in the high-risk and low-risk groups. (F) AUC time-related ROC curves of OS

3.3 Immunohistochemical (IHC) validation of prognosis-related gene protein expression in lung squamous carcinoma tissues and non-tumor tissues adjacent to cancer

The expression of 2 prognostic genes in lung squamous carcinoma and its paraneoplastic tissues was verified by IHC. It was seen that LRRK2 and WFS1 were lowly expressed in lung squamous carcinoma and highly expressed in paracancerous tissue, which was consistent with the expression in TCGA database.

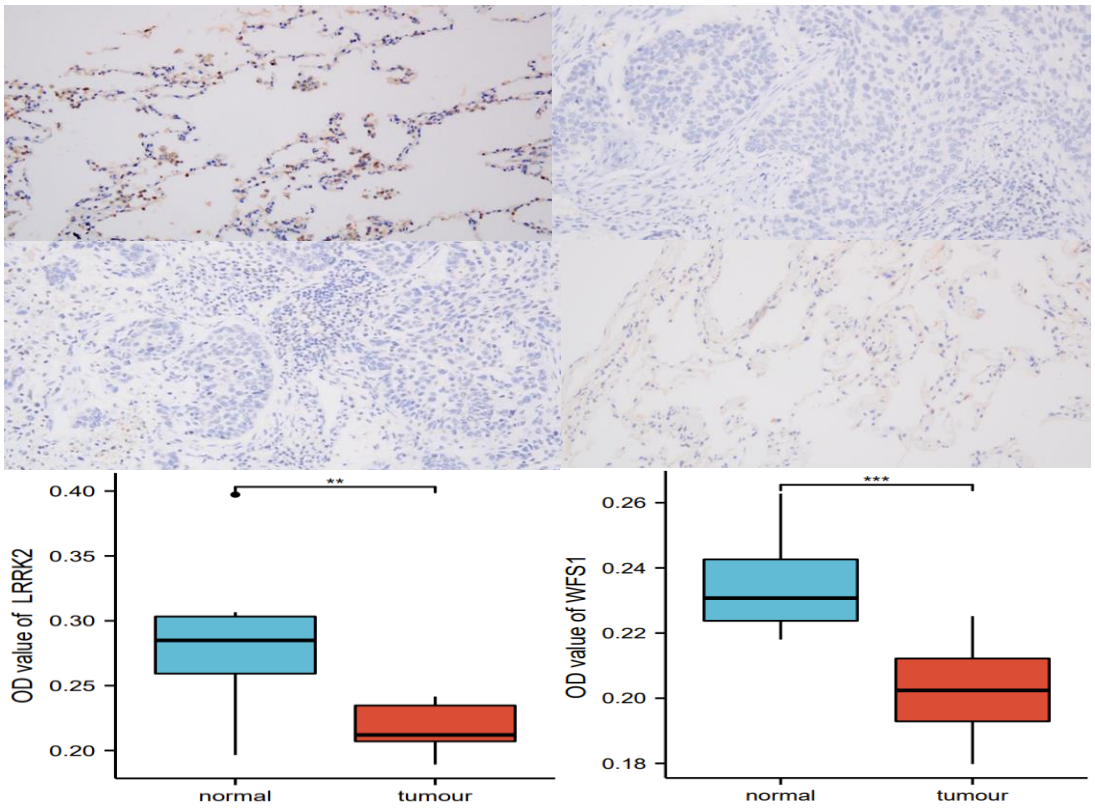


Figure 3. Immunohistochemical results. (A) LRRK2 (B) WFS1 Identification Two Clusters of LUSC

To explore further the correlation between the expression of endoplasmic reticulum stress and LUSC, consensus clustering analysis was performed based on gene expression levels. The consensus matrix showed that ERS could effectively classify LUSC into different subtypes, and each sample in the subtype had a high correlation with an optimal clustering variable of 2 (Figure 4A). Significant survival differences between groups could be observed (Figure 4B), and the heat map revealed significant gene expression differences between subtypes (Figure 4C) as well as a significantly higher risk in the risk score for subtype B than subtype A (Figure 4D). GSVA analysis revealed high expression of immune-related as well as cancer-related pathways and low expression of mistranslation repair in cluster B (Figure 4E). We estimated the level of infiltration of 23 human immune cell subsets in two clusters by

CIBERSORT algorithm (Figure 4F). A clear variation in the degree of enrichment of most immune cells can be seen between the two clusters. Thus an annotated analysis of this classification could reveal the molecular regulatory mechanisms inherent to LUSC.

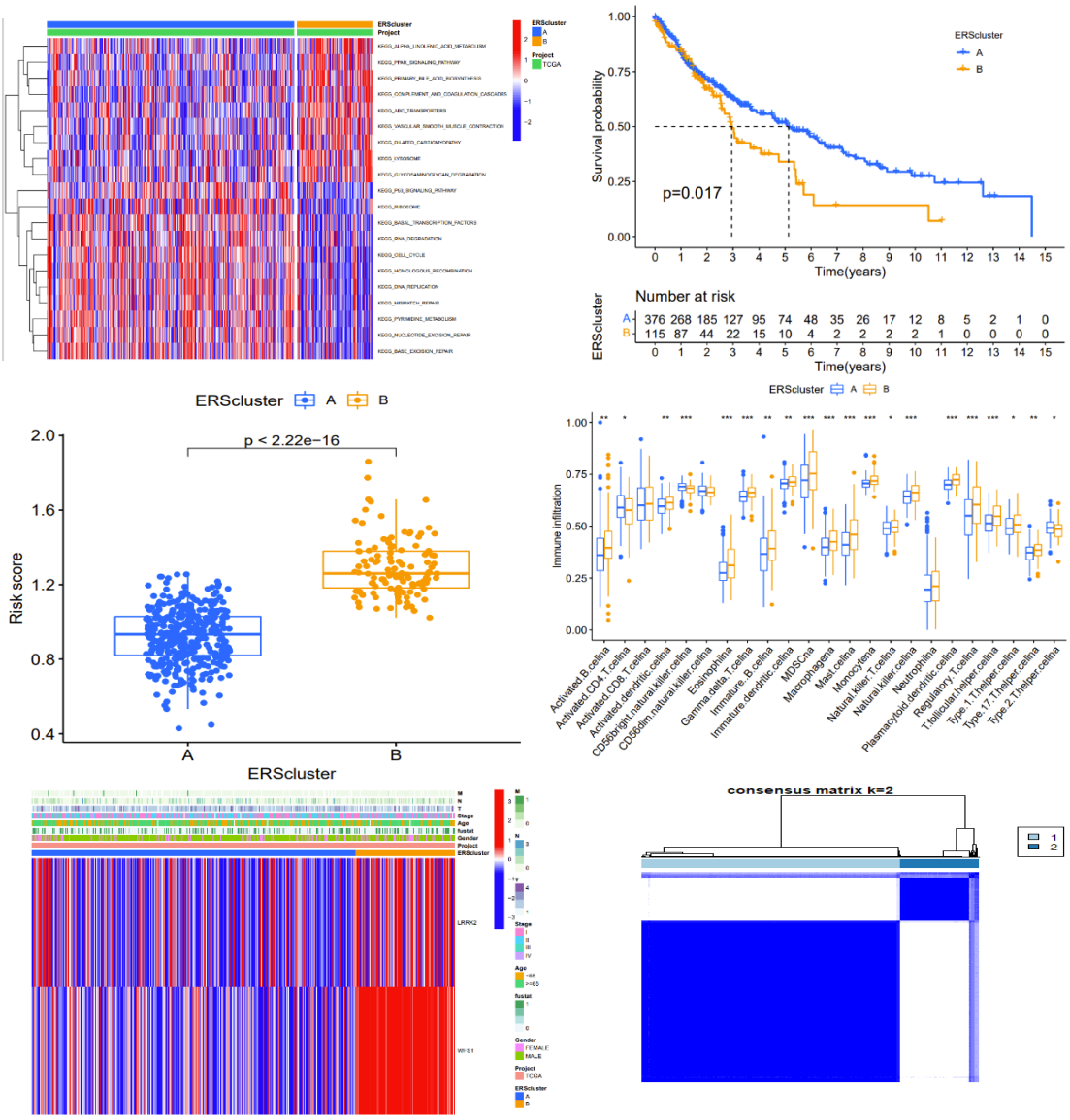


Figure 4. Subtyping by ERS gene. (A) Cluster typing for AB subtypes. (B) Subtype survival difference analysis (C) Subtype heat map (D) Subtype risk score variability (E) GSVA analysis (F) CIBERSORT analysis

3.4 Independent prognostic value of predicted genes

To determine whether risk score was an independent prognostic factor for OS, multivariate and univariate Cox analyses were undertaken on the covariates (Figure 4). It could be found that in univariate Cox analysis, tumor stage, risk score and OS were significantly correlated, P < 0.01 (Figure 4A). After correcting for other confounders, multivariate Cox analysis showed that risk score and tumor stage remained independent predictors of OS at P < 0.01

(Figure 4B). Because risk score was highly correlated with female player's prognosis, we combined clinical parameters to create a nomogram risk prediction plot. This nomogram plot demonstrates the 1-, 3-, and 5-year OS assessment plots for female players with squamous lung cancer (Figure 4C). This nomogram curve reveals

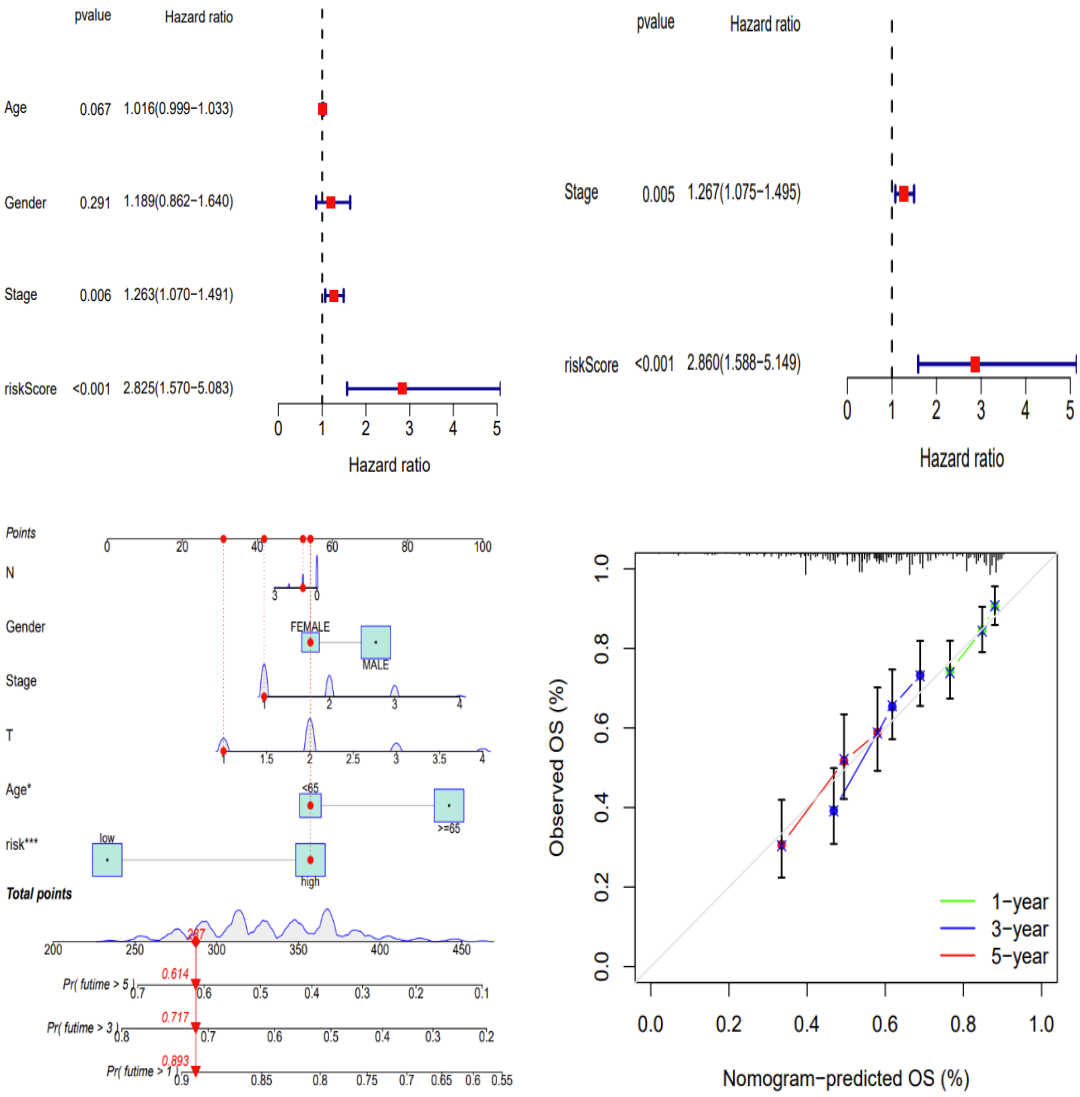


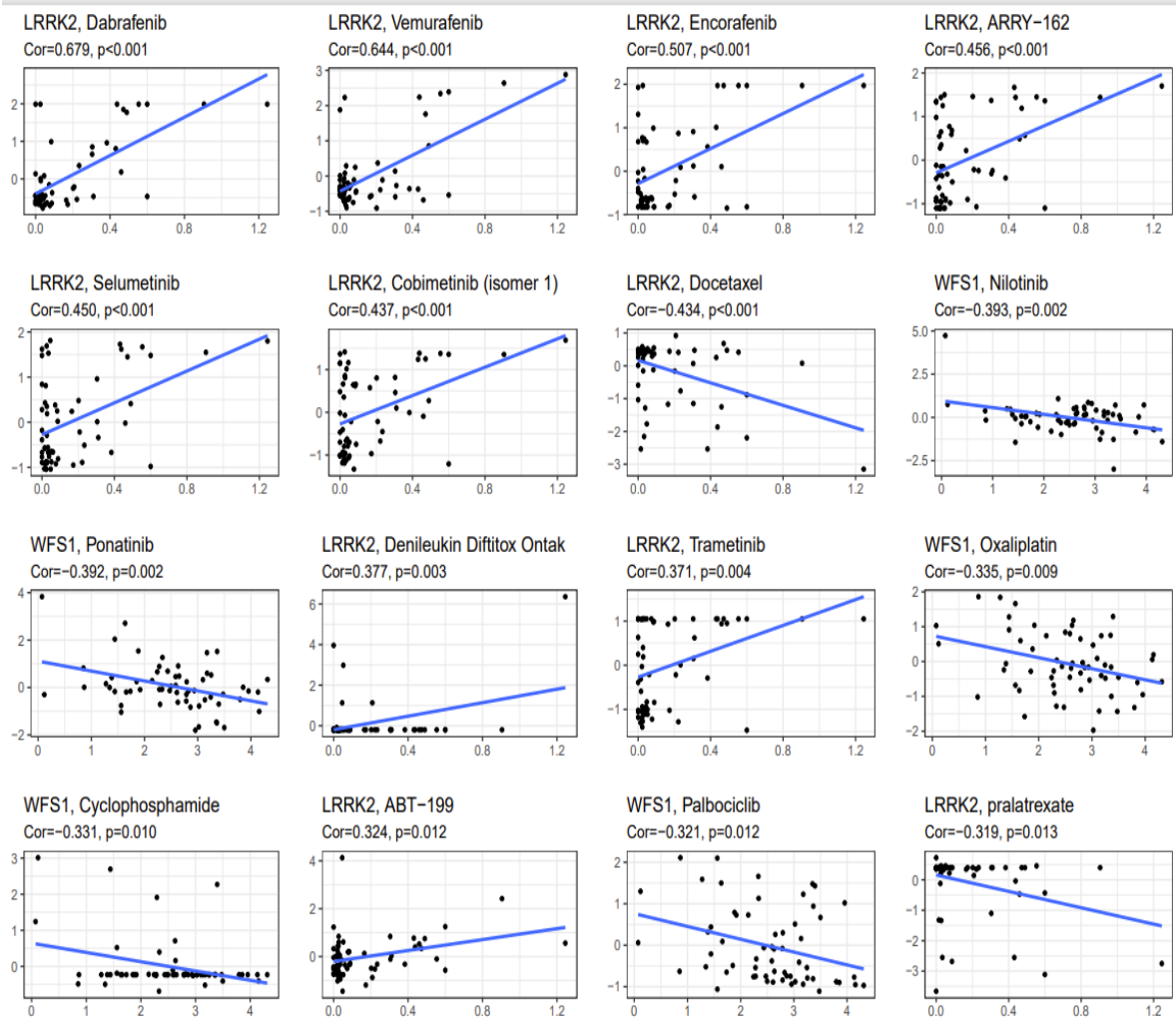
Figure 4. Screening of OS-related factors to compare the prognostic accuracy of risk scores and clinicopathological factors. (A) Screening of OS-related factors by univariate Cox regression analysis. (B) Screening of OS-related factors by multivariate Cox regression analysis. (C) nomogram diagram (D) risk prediction diagram

a good accuracy between the actual observed and predicted values (Figure 4D).

3.6 Drug sensitivity analysis

These two prognostic genes were also associated with sensitivity to some

drugs by analysis of data from NCI-60 cell lines. For example, the expression of lrrk2 docetaxel, dabrafenib, vemoxifenib and other chemotherapeutic drugs were linked to sensitivity, and WFS1 expression was associated with oxaliplatin sensitivity.



Risk score-related drug sensitivity analysis

DISCUSSION

Since the beginning of the Human Genome Project, the use of genetic technology to find biomarkers in disease development has been rapidly developed. However, female players with lung cancer do not have obvious clinical symptoms in early stages, and are mostly screened by serum tumor markers such as serum carcinoembryonic antigen (CEA), squamous epithelial cell carcinoma antigen (SCC), neuron-specific enolase (NSE), cytokeratin 19 fragment (CYFRA21-1), and then confirmed by pathological histology, so there are some female players who are diagnosed at advanced stages, and advanced lung Squamous carcinoma is inoperable and has a poor prognosis. Although various treatments for LUSC have emerged with the establishment of next-generation sequencing technology and the beginning of the era of

precision medicine, the genetic variants of lung squamous carcinoma are complex, and no genetic variants with clear targeting guidance have been obtained in the overall population. Therefore, it is clinically important to study the early diagnosis of LUSC and predict the therapeutic effect.

Previous studies have shown that the characterization of the tumor microenvironment (Zhang et al., 2020) , as well as prognostic indices consisting of inflammatory indicators such as C-reactive protein, neutrophil-lymphocyte ratio and serum albumin have good accuracy for the prognosis of most cancers. Activation of multiple endoplasmic reticulum stress sensors has been shown to confer greater tumorigenicity, metastasis, and drug resistance to malignant cells (Souvik et al., 2015) .

Endoplasmic reticulum stress serves an essential role in cell growth. In tumors, UPR inhibits not only the proliferation of tumor cells, but also their subsequent growth and metastasis in adverse environments like hypoxia and nutrient deficiency. However, information of endoplasmic reticulum stress-related genes in the prognosis of LUSC has not been seen reported.

In the present study, we systematically analyzed the expression of 83 endoplasmic reticulum stress-related genes in LUSC tissues and their association with OS. Eighteen DEGs were filtered, and three OS-related DEGs were obtained based on univariate Cox analysis. A risk score formula consisting of 2 prognostic genes was finally constructed based on protein expression in lung tissue. Based on the risk score, female players were divided into a high-risk score group or low-risk score group.

We found that grouping was significantly associated with stage and age but independent prognostic analysis showed that they were independent predictors of OS. The prognostic model consisted of 2 endoplasmic reticulum stress-related genes (LRRK2, WFS1), both of which were significantly associated with OS.

Based on these 2 genes, we classified LUSC into two types, A and B. It can be found that there are indeed differences in the expression of cancer-related pathways, and therefore studying these two endoplasmic reticulum stress genes is of great importance for the prognosis of lung squamous carcinoma.

LRRK2 is a member of the receptor-interacting protein kinase (RIPK) family and is therefore also known as RIPK7.Several members of the RIPK family have been identified as key regulators of cell death and innate immunity.(Humphries, Yang, Wang, & Moynagh, 2015) Functional alterations associated with LRRK2 mutations include alterations in vesicle transport and cytoskeletal dynamics, autophagy and lysosomal degradation, neurotransmission, mitochondrial function, immunity and microglial cell responses.(Tolosa et al.) Mutations in the structural domain of LRRK2-activated

kinase and their effects on neuronal autophagy are being studied in Parkinson's disease and it has been shown that autophagy abnormalities such as macroautophagy-mitochondrial autophagy in Parkinson's disease triggered by Lrrk2 mutations occur in conjunction with endoplasmic reticulum stress.("The parkinsonian LRRK2 R1441G mutation shows macroautophagy-mitophagy dysregulation concomitant with endoplasmic reticulum stress %J Cell Biology and Toxicology,") . However, the pathological role of altered LRRK2 in cancer and its impact on autophagic status and tumorigenicity are not known. It has been demonstrated that gene expression of LRRK2 is significantly reduced in non-small cell lung cancer(Lebovitz, Chow, Wan, & Gorski, 2016) Chandra Lebovitz et al. found that LRRK2 was expressed in alveolar type II (AT2) cells and that deletion of LRRK2 disrupted AT2 cell morphology and knockdown of the LRRK2 gene significantly increased tumor initiation and size.(Lebovitz et al., 2021) . Exploring the role of pathogenic LRRK2-mediated autophagy in lung cancer may provide a reference for the clinical strategy of using autophagy inhibitors in the treatment of lung cancer.

Wolframin protein, a transmembrane glycoprotein encoded by the WFS1 gene, is a resident transmembrane protein in the endoplasmic reticulum and is also involved in the regulation of ERS, which plays a key role in maintaining endoplasmic reticulum homeostasis.(Kovacs-Nagy et al., 2013) Wolframin protein may be located downstream of IRE1 and PERK, which can negatively regulate the ERS signaling pathway, and mutations in the WFS1 gene can lead to structural and functional changes in Wolframin protein, which continuously activates ERS.(Shuntaro et al., 2017) Mutations in the WFS1 gene may lead to alterations in Wolframin protein structure and function, which may lead to imbalance in endoplasmic reticulum homeostasis and activate ERS-mediated apoptosis.(Ueda et al., 2005) This leads to an imbalance in endoplasmic reticulum homeostasis and activates ERS-mediated apoptosis. The current research on Wolframin protein is mainly focused on the typical diseases of Wolfram syndrome.(Delvecchio, Iacoviello, Pantaleo, Resta, & health, 2021; Reschke et al., 2021) The role of Wolframin protein in lung cancer, especially in non-small cell lung cancer, needs to be further explored.

As for the association between risk score and clinical characteristics, high risk score was correlated with tumor stage. By a single-gene analysis, the LRRK2 and WFS1 expression was correlated with the survival of LUSC , and the survival time of female players with low expression was higher than that of female players with high expression, which is consistent with earlier studies(Tian et al., 2021) . However, whether these genes affect the prognosis of LUSC female players through the endoplasmic reticulum stress response remains to be elucidated, as few studies have been performed on these genes.

Cancer stem cell-like cells (CSC) can be derived from stem cells, progenitor cells, or transformed from non-stem cells through dedifferentiation (Malta,

Sokolov, Gentles, Burzykowski, & Mariamidze, 2018) . The self-renewal and invasive ability of tumor stem cells can promote tumor growth and induce drug resistance. By calculating the correlation between prognostic gene expression and tumor stem cell score, it was found that LRRK2 and WFS1 may have tumor suppressive effects as they have negative correlation with tumor stemness of RNAss.

It has been shown that endoplasmic reticulum stress can mediate autophagy leading to therapeutic resistance. Anti-cancer drugs can induce autophagy. Depending on the drug and tumor type used, autophagy can play a role in promoting survival or apoptosis (Sui et al., 2013) . Three predicted genes were analyzed online by TISIDB for association with immunotherapy. In the "immunomodulator module", LRRK2 was positively associated with most immunosuppressive agents, while WFS1 expression was negatively associated with most immunosuppressive agents.

By analyzing NCI-60 cell line data, prognostic genes were identified in relation to the sensitivity of some chemotherapeutic agents. LRRK2 was associated with the sensitivity of cancer cells to docetaxel, darafenib, vemurafenib, ankrafenib and semitinib, and expression of WFS1 was associated with sensitivity to oxaliplatin. Among them, docetaxel belongs to semi-synthetic paclitaxel chemotherapy drugs, which are cell cycle-specific antitumor drugs that degrade and inhibit the proliferation and differentiation of tumor cells by controlling the microtubule protein system of tumor cells to promote the stability of tumor cell microtubules and thus control tumor development, and docetaxel is the only first-line and second-line chemotherapy drug approved by EU and US FDA for NSCLC.

These data suggest that prognostic genes can be used as therapeutic targets to aid in drug sensitivity and can be potential treatment sites for LUSC.

REFERENCES

Backes, L. T. H., Mezzomo, L. C., Buffon, A., & Calil, L. N. (2019). Cytomorphological analysis of cervical cytological smears of women aged over 60 years. *Jornal Brasileiro de Patologia e Medicina Laboratorial, 55*, 136-147. doi:10.5935/1676-2444.20190016

Baxevanos, P., & Mountzios, G. J. A. o. t. m. (2018). Novel chemotherapy regimens for advanced lung cancer: have we reached a plateau? *, 6*(8), 139. doi:10.21037/atm.2018.04.04

Chen, X., & Cubillos-Ruiz, J. R. (2021). Endoplasmic reticulum stress signals in the tumour and its microenvironment. *Nat Rev Cancer, 21*(2), 71-88. doi:10.1038/s41568-020-00312-2

Cubillos-Ruiz, J. R., Bettigole, S. E., & Glimcher, L. H. (2017). Tumorigenic and Immunosuppressive Effects of Endoplasmic Reticulum Stress in Cancer.

Cell, 168(4), 692-706. doi:10.1016/j.cell.2016.12.004

Delvecchio, M., Iacoviello, M., Pantaleo, A., Resta, N. J. I. j. o. e. r., & health, p. (2021). Clinical Spectrum Associated with Wolfram Syndrome Type 1 and Type 2: A Review on Genotype-Phenotype Correlations. *18*(9). doi:10.3390/ijerph18094796

Hänzelmann, S., Castelo, R., & Guinney, J. J. B. b. (2013). GSVA: gene set variation analysis for microarray and RNA-seq data. *14*, 7. doi:10.1186/1471-2105-14-7

Humphries, F., Yang, S., Wang, B., & Moynagh, P. N. (2015). RIP kinases: key decision makers in cell death and innate immunity. *Cell Death Differ, 22*(2), 225-236. doi:10.1038/cdd.2014.126

Kovacs-Nagy, R., Elek, Z., Szekely, A., Nanasi, T., Sasvari-Szekely, M., & Ronai, Z. J. A. J. o. M. G. P. B. N. G. (2013). Association of Aggression With a Novel MicroRNA Binding Site Polymorphism in the Wolframin Gene. *162*(4), 404-412.

Le, T., Aronow, R. A., Kirshtein, A., & Shahriyari, L. J. B. i. B. (2020). A review of digital cytometry methods: estimating the relative abundance of cell types in a bulk of cells. (5795).

Lebovitz, C., Chow, N., Wan, L., & Gorski, S. J. C. R. (2016). Abstract 3668: Investigating a tumor suppressor role for Parkinson's susceptibility gene LRRK2 in lung cancer. *76*(14 Supplement), 3668-3668.

Lebovitz, C., Wretham, N., Osooly, M., Milne, K., Dash, T., Thornton, S., . . . Gorski, S. M. (2021). Loss of Parkinson's susceptibility gene LRRK2 promotes carcinogen-induced lung tumorigenesis. *Sci Rep, 11*(1), 2097. doi:10.1038/s41598-021-81639-0

Malta, T. M., Sokolov, A., Gentles, A. J., Burzykowski, T., & Mariamidze, A. J. C. (2018). Machine Learning Identifies Stemness Features Associated with Oncogenic Dedifferentiation. *173*(2), 338-354.

The parkinsonian LRRK2 R1441G mutation shows macroautophagy-mitophagy dysregulation concomitant with endoplasmic reticulum stress %J Cell Biology and Toxicology. 1-23.

Perez-Moreno, P., Brambilla, E., Thomas, R., & Soria, J. J. C. c. r. a. o. j. o. t. A. A. f. C. R. (2012). Squamous cell carcinoma of the lung: molecular subtypes and therapeutic opportunities. *18*(9), 2443-2451. doi:10.1158/1078-0432.Ccr-11-2370

Reschke, F., Rohayem, J., Maffei, P., Dassie, F., Schwandt, A., de Beaufort, C., . . . Danne, T. J. E. (2021). Collaboration for rare diabetes: understanding new treatment options for Wolfram syndrome. *71*(3), 626-633. doi:10.1007/s12020-021-02622-3

Sabah, A., Tiun, S., Sani, N. S., Ayob, M., & Taha, A. Y. J. P. O. (2021). Enhancing web search result clustering model based on multiview multirepresentation consensus cluster ensemble (mmcc) approach. *16*.

Seiler, M., Huang, C. C., Szalma, S., & Bhanot, G. J. O. A. J. o. I. B. (2010). ConsensusCluster: a software tool for unsupervised cluster discovery in

numerical data. *14*(1), 109.

Shuntaro, Morikawa, Toshihiro, Tajima, Akie, Nakamura, . . . Diabetes, A. J. P. (2017). A novel heterozygous mutation of the WFS1 gene leading to constitutive endoplasmic reticulum stress is the cause of Wolfram syndrome.

Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. *CA Cancer J Clin, 69*(1), 7-34. doi:10.3322/caac.21551

Simon, N., Friedman, J., Hastie, T., & Tibshirani, R. J. o. (2011). Regularization Paths for Cox's Proportional Hazards Model via Coordinate Descent. *39*(5).

Souvik, Dey, Carly, M., Sayers, Ioannis, . . . Investigation, L. J. J. o. C. (2015). ATF4-dependent induction of heme oxygenase 1 prevents anoikis and promotes metastasis.

Sui, X., Chen, R., Wang, Z., Huang, Z., Kong, N., Zhang, M., . . . Disease. (2013). Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. *4*(10), e838.

Tian, Y., Lv, J., Su, Z., Wu, T., Li, X., Hu, X., . . . Wu, L. (2021). LRRK2 plays essential roles in maintaining lung homeostasis and preventing the development of pulmonary fibrosis. *Proc Natl Acad Sci U S A, 118*(35). doi:10.1073/pnas.2106685118

Tolosa, Eduardo, Vila, Miquel, Klein, Christine, . . . Olivier. LRRK2 in Parkinson disease: challenges of clinical trials.

Ueda, K., Kawano, J., Takeda, K., Yujiri, T., Tanabe, K., Anno, T., . . . Koizumi, A. J. E. J. o. E. (2005). Endoplasmic reticulum stress induces Wfs1 gene expression in pancreatic beta-cells via transcriptional activation. *153*(1), 167.

Wang, M., & Kaufman, R. J. J. N. R. C. (2014). The impact of the endoplasmic reticulum protein-folding environment on cancer development. *14*(9), 581.

Wong, M., DiChiara, A., Suen, P., Chen, K., Doan, N., Shoulders, M. J. C. t. i. m., & immunology. (2018). Adapting Secretory Proteostasis and Function Through the Unfolded Protein Response. *414*, 1-25. doi:10.1007/82_2017_56

Yoshihara, K., Shahmoradgoli, M., Martínez, E., Vegesna, R., Kim, H., Torres-Garcia, W., . . . Verhaak, R. J. N. c. (2013). Inferring tumour purity and stromal and immune cell admixture from expression data. *4*, 2612. doi:10.1038/ncomms3612

Zanetti, M., Xian, S., Dosset, M., & Carter, H. J. F. i. i. (2022). The Unfolded Protein Response at the Tumor-Immune Interface. *13*, 823157. doi:10.3389/fimmu.2022.823157

Zhang, L., Chen, J., Cheng, T., Yang, H., Li, H., & Pan, C. J. J. o. C. (2020). Identification of the key genes and characterizations of Tumor Immune Microenvironment in Lung Adenocarcinoma (LUAD) and Lung Squamous Cell Carcinoma (LUSC). *11*(17), 4965-4979.

doi:10.7150/jca.42531

Número de citas totales / Total references: 29 (100%)

Número de citas propias de la revista / Journal´s own references: 3 (7.7%).

Rev.int.med.cienc.act.fís.deporte - vol. 23 - número 90 - ISSN: 1577-0354