Identification a unique disulfidptosis classification regarding prognosis and immune landscapes in thyroid carcinoma and providing therapeutic strategies

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Abstract

Background

Thyroid carcinoma (THCA) is a common type of cancer worldwide, and its incidence has been increasing in recent years. Disulfidoptosis, a recently defined form of metabolic-related regulated cell death (RCD), has been shown to play a sophisticated role in antitumor immunity. However, its mechanisms and functions are still poorly understood and the association between disulfidoptosis and the prognosis of patients with papillary thyroid carcinoma remains to be elucidated. This study aims to investigate the connection between disulfidoptosis and the prognosis of thyroid cancer, while also developing a prognostic index based on disulfidoptosis genes.

Materials and methods

We utilized 24 genes associated with disulfidoptosis to create the classification and model. To gather data, we sourced gene expression profiles, somatic mutation information, copy number variation data, and corresponding clinical data from the TCGA database for patients with thyroid cancer. Additionally, we obtained single-cell transcriptome data GSE184362 from the Gene Expression Omnibus (GEO) database for further analysis.

Results

In this study, we utilized 24 genes associated with disulfidoptosis to identify two distinct groups with different biological processes using non-negative matrix factorization (NMF). Our findings showed that Cluster 1 is associated with chemokines, interleukins, interferons, checkpoint genes, and other important components of the immune microenvironment. Moreover, cluster 1 patients with high IPS scores may be more sensitive to immunotherapy. We also provide drug therapeutic strategies for each cluster patients based on the IC50 of each drug. The Enet model was chosen as the optimal model with the highest C-index and showed that patients with high risk had a worse prognosis and weak cell-to-cell interactions in THCA. Finally, we established a nomogram model based on multivariable cox and logistic regression analyses to predict the overall survival of THCA patients.

Conclusion

This research provides new insight into the impact of disulfidoptosis on THCA. Through a thorough examination of disulfidoptosis, a new classification system has been developed that can effectively predict the clinical prognosis and drug sensitivity of THCA patients.

Introduction

Thyroid carcinoma is a prevalent form of cancer that originates from the cells of the thyroid gland. It is the most common malignant tumor within the endocrine system, with an average annual increase of 4.5%[1]. Recent advancements in imaging and molecular pathology detection technology have led to a
significant increase in the detection rate of thyroid cancer by 10% or more annually. This increase is not limited to just tiny papillary thyroid cancer, but also includes middle and advanced thyroid cancers which have shown an upward trend[2, 3]. Standard treatment for thyroid carcinoma typically involves surgical removal of the affected portion of the thyroid gland, often followed by radioactive iodine therapy or other forms of radiation therapy. Despite advancements in diagnostic and treatment options, the ability to quickly diagnose and accurately predict patient prognosis remains inadequate. Therefore, molecular methods are considered crucial for early detection of this disease.

Programmed cell death (PCD) encompasses a complex process that entails various forms of cell death, including apoptosis, necroptosis, ferroptosis, pyroptosis, netotic cell death, entotic cell death, lysosome-dependent cell death, parthanatos, autophagy-dependent cell death, oxeiptosis, disulfidoptosis, and alkaliptosis. Disulfidoptosis is a newly defined form of metabolic-related regulated cell death (RCD) that has been found to have a complex role in antitumor immunity. Recent studies have shown that disulfidoptosis is associated with disulfide bond reactions between intracellular and extracellular protein molecules, leading to conformational changes and alterations in protein function, ultimately resulting in cell death[4]. Recent studies have shown that metabolic therapy involving glucose transporter (GLUT) inhibitors have the potential to trigger disulfidoptosis and hinder the growth of cancer cells[5]. Although disulfidoptosis has been recognized to modulate the tumor microenvironment (TME) and anti-tumor immunity, its correlation with the prognosis, TME, and drug therapy outcomes in THCA is yet to be thoroughly examined.

In this study, we utilized bulk transcriptome, genomics, and single-cell datasets to examine the relationship between disulfidoptosis, the tumor microenvironment (TME), and the underlying immune landscapes within different subgroups of THCA patients. Our findings revealed a novel classification of thyroid carcinoma based on genes associated with disulfidoptosis, with each subgroup of THCA patients exhibiting distinct molecular and immune landscapes. Notably, these clusters exhibited varying responses to immunotherapy and chemotherapy, indicating the need for personalized treatment approaches. Moreover, we developed and verified a disulfidoptosis-based model to predict the prognosis of THCA patients. Additionally, we analyzed the immune landscapes at a single-cell level. In summary, our study offers valuable insights into the distinct prognosis and immune landscapes of THCA subgroups, and provides potential therapeutic strategies. Furthermore, our findings present a robust model for stratifying THCA patients to optimize survival outcomes.

**Methods**

**Transcriptome, somatic mutation information, copy number variation and single cell datasets collection**

Transcriptome, somatic mutation information, copy number variation data, and corresponding clinical data (e.g, disease free survival, overall survival) of thyroid carcinoma patients were downloaded from the TCGA data portal (https://portal.gdc.cancer.gov/)[6]. Raw data of single cell from 11 thyroid carcinoma
patients were retrieved from GEO under accession numbers GSE184362[7]. The data consisted of 158,577 single cells isolated from 7 primary tumors, 6 para-tumors, 8 metastatic LNs, and 2 subcutaneous metastatic loci. We analyzed the data using R (version 4.1.1) and R Bioconductor packages.

**The immune signatures calculation**

To investigate the immune landscapes of patients with thyroid carcinoma, we downloaded infiltration estimation matrices of immune cells from the Tumor Immune Estimation Resource (TIMER2.0) database, which were calculated using the XCELL[8] algorithm. We used the ESTIMATE algorithm[9] to determine the tumor purity and immune score of each patient.

**Gene Set Variation Analysis (GSVA) analysis**

To investigate the disparities in HALLLMARK and immune related signatures among patients with thyroid carcinoma, we performed pathway enrichment analysis using the ‘fgsea’ R package[10]. The HALLLMARK gene set was obtained from the MSigDB database. (http://software.broadinstitute.org/gsea/msigdb/index.jsp).

**The construction of multiple machine learning models based on programmed cell death genes**

To predict the prognosis of thyroid carcinoma patients using disulfidoptosis-related genes, we employed seven machine learning algorithms, including ENet, Random Survival Forest (RSF), Ridge Regression, Support Vector Machine (SVM), StepCox, Gradient Boosting Machine (GBM), and Superpc.

**Single cell data processing and cell type annotation**

The thyroid carcinoma (GSE184362) datasets underwent data normalization, dimensionality reduction, and clustering using Seurat v.3.0.0[11] with default parameters. During preprocessing, cells were filtered based on the criteria of expressing a minimum of 200 genes and UMIs < 6000. Cells with more than 20% mitochondrial gene expression contribution were also removed. The cell clusters were annotated using classical cell signatures with SingleR[12].

**Copy number variation analysis**

To investigate insertion and deletion events in genomic regions from TCGA thyroid carcinoma samples, we utilized GISTIC 2.0 through the GenePattern web server (https://cloud.genepattern.org/gp/pages/login.jsp). This computational program analyzes the amplitude and frequency of observed alteration events to identify somatic copy number alterations[13].

**Prediction of drug sensitivity in high and low PCDS groups**

The calcPhenotype function from the R package ‘oncoPredict’ was employed to predict the half maximal inhibitory concentration (IC50) of drugs based on gene expression profiles in cell lines. A P value of less than 0.05 was set as the threshold for selecting drugs that were considered favorable[14].
Results

Genotyping of thyroid carcinoma based on disulfidoptosis associated genes

In this study, we conducted NMF analysis on the expression matrix of disulfidoptosis-related genes in THCA patients. Our analysis resulted in the identification of two distinct subtypes, labeled as C1 and C2, based on the inflection points of the lithotripsy plot (Fig. 1A). Our findings indicate that these subtypes exhibit significant differences in both overall survival (OS) and disease-free survival (DFS), with P values of 0.002 and 0.014, respectively (Fig. 1B). Overall, C1 showed favorable survival, while C2 showed the worst survival (Fig. 1B). Additionally, we also evaluated the correlation between other classifications, such as TME subtype[15] and Immune-Subtype[16]. The proportion of C4 subtype patients in cluster 1 was greater than that in cluster 2, while the proportion of C3 subtype patients in cluster 2 was significantly higher than that in cluster 1 (Fig. 1C). However, there was no significant difference observed between C3 and C4 subtypes (Fig. 1D). In addition, the proportion of IE (Immune-Enriched Fibrotic) subtype and IE/F (Immune-Enriched, Non-Fibrotic) subtype were the higher in cluster 1, while the proportion of D (Depleted) subtype patients in cluster 2 is significant more than that of D (Depleted) subtype (Fig. 1D). Conformably, the survival rate of IE subtype and IE/F subtype were significantly higher than that of D (Depleted) subtype (Fig. 1D). Furthermore, HALLMARK pathway enrichment analysis ascertained that patients in C1 enriched in immune associated pathways such as inflammatory response and interferon gamma response pathways, while patients in C2 enriched in Myc targets V1 and MTORC1 signaling pathways (Fig. 1E).

Molecular, immune and mutation landscapes of different clusters

As immunotherapy is currently the most promising therapeutic strategy for cancer, it is important to understand the role of cell death in activating antitumor immune responses. In this study, we analyzed the tumor microenvironment (TME) of two clusters and focused on the presence of tertiary lymphoid structures (TLS), which are considered to be germinal centers for immune cells in the TME. We also assessed the expression of a series of chemokines involved in the formation of TLS. We found that almost all chemokines except CCL16 and CX3CR1 were highly expressed in C1 (Fig. 2A). Additionally, interleukins, cytokines, and their receptors also represent essential TME components and play an important role in immune-activating transcripts. Consistently, we found that those interferons, interleukins, and their receptors were higher in cluster 1 than in cluster 2, which is consistent with higher expression of chemokines (Fig. 2B). Subsequently, we further calculated the immune score and tumor purity, using ESTIMATE algorithm in different clusters. Higher immune scores indicate higher infiltration of immune cells, while higher tumor purity indicate higher proportion of cancer cells. We found that the immune score in C1 was significantly higher than that in C2, while the tumor purity was lower, indicating that immune cells infiltrated in C1 (Fig. 2D). In line with this, we found that the proportion of endothelial cells (ECs), and macrophage M2 which have been proved to promote the progress of tumor cells were
higher in C2, while most immune cells, such as CD8+ T cells, CD4+ T cells, NK cells, B cells, and antigen-presenting cells were higher in C1 (Fig. 2E).

The expression of immune checkpoints is a crucial factor for immune checkpoint inhibitors (ICIs) therapy. In order to evaluate the expression of immune checkpoints, we analyzed two clusters. Our findings indicated that the expression of most immune checkpoints, such as CD274/PD-L1, HAVCR2/TIM-3, CTLA4, PDCD1/PD1, PDCD1LG2/PD-L2, IDO1/2, LAG3, were higher in C1 than in C2. This suggests that THCA patients in C1 may benefit from immunotherapy. Subsequently, we further assessed the IPS of different clusters. Consistently, IPS score of CTLA4 neg PD-1 pos, CTLA4 pos PD-1 neg, and CTLA4 pos PD-1 pos in C1 patients were higher than that in C2 patients. To evaluate the impact of somatic mutations and copy number variation on antitumor immunity and tumor progression, we analyzed these factors in both clusters. However, our findings indicated no significant difference in somatic mutations and CNVs between the two (Figure S1A, S1B). Additionally, using GISTIC, we determined that cancer progression-associated amplification and deletion were not significantly different in these clusters (Figure S1C, S1D), nor was the gistic score (Figure S1E, S1F).

Taken together, our results suggest that the two clusters mentioned above exhibit distinct TME characteristics. Specifically, TME in C1 supports anti-tumor immunity and has higher expression of immune checkpoints and IPS, which may make it more sensitive to ICIs therapy.

Predicting drug sensitivity and providing therapeutic drugs for these two clusters

To explore potential treatment strategies for THCA patients, we analyzed the IC50 values of each drug in samples from two clusters. We also evaluated the difference in response to chemotherapy between the clusters, given the significant role of chemotherapy combined with immunotherapy in treating THCA. Using the R package ‘oncoPredict’, we calculated the sensitivity score for drugs in the GDSC database. Our study revealed significant positive associations between SLC7A11, TLN1, MYL6, MYH9, CAPZB, ACTN4, and ACTB with the IC50 of chemotherapy drugs, with noteworthy differences observed between C1 and C2 (|logFC|>1 and P < 0.05). Conversely, PDLIM1 and INF2 exhibited significant negative associations with the IC50 of chemotherapy drugs. Among which, SLC7A11 was positively associated with longer survival rate and PDLIM1 and INF2 were negatively associated with shorter survival rate (Figure S2A, S2B). Using wilcoxon test, our study revealed that THCA patients in C1 were more sensitive to Alisertib, Camptothecin, Cisplatin, Cytarabine, Dabrafenib, Fludarabine, Gemcitabine, Irinotecan, Mitoxantrone, Paclitaxel, Selumetinib, Temozolomide, Topotecan, Vinorelbine, and Vorinostat, supported by the lower IC50 values of these drugs in C1 (Fig. 3B). Among which, according to their mechanism, they can be divided into the following categories: cell cycle related drugs, such as Alisertib, Cytarabine, Fludarabine, Gemcitabine, Irinotecan, Paclitaxel, Topotecan, and Vinorelbine, affect the cell cycle and inhibit or block the proliferation of cancer cells; DNA damage repair inhibitors, such as Camptothecin, Mitoxantrone, and Temozolomide, inhibit DNA damage repair pathways to induce cancer cell death; signal pathway inhibitors, such as Dabrafenib and Selumetinib, inhibit the growth and spread of cancer
cells by inhibiting their internal signaling pathways; histone deacetylase inhibitor, such as Vorinostat, can affect chromatin structure and induce apoptosis of cancer cells by inhibiting histone deacetylase; and chemotherapy drug, such as Cisplatin, inhibits the proliferation of cancer cells by interfering with the DNA structure. However, THCA patients in C2 were sensitive to quite different drugs. Our findings indicate that patients in C2 group exhibited greater sensitivity to tyrosine kinase inhibitors, such as Afatinib, Gefitinib, Lapatinib, Osimertinib, Sapitinib; protein kinase B (Akt) inhibitors, such as Afuresertib, Ipatasertib, Uprosertib; and cysteinase 1 (GCLM) inhibitor, such as AZD5991 (Fig. 3C). All of these drugs have been proved to be useful in patients with THCA.

**Establishment and evaluation of an machine learning based disulfidptosis index in THCA**

To establish a disulfidptosis-based signature for stratification of THCA patients, samples from the TCGA-THCA cohort were randomly partitioned into training and validation sets at a 1:1 ratio. Seven machine learning algorithms were employed to construct the model, and the concordance index (C-index) of each algorithm was compared. The Elastic Net (Enet) algorithm-based model exhibited the most effective predictive performance, with Training cohort C-index: 1; Test cohort C-index: 0.693; and entire TCGA cohort C-index: 0.737 (Fig. 4A). Following the development of the model, THCA patients were classified into low- and high-risk subgroups based on the best cut-off defined by ‘survminer’ R package (Figs. 4B). Kaplan-Meier (KM) analysis results demonstrated that patients with low-risk experienced a higher overall survival rate in both training and validation cohorts (P = 0.004) (Fig. 4B). Additionally, a high PCDS was correlated with a prolonged survival time (Fig. 4C). Time-dependent receiver operating characteristic (ROC) curves were drawn using R software, and the area under the curve (AUC) was calculated at various time points to estimate the predictive performance of the prognostic model in the training, testing, and entire TCGA cohorts (Fig. 4D). Univariate Cox analysis revealed that Age, pathological stage, pathological T stage, and high Risk were associated with poor survival (Fig. 5A). However, the multivariate Cox model identified only Age, pathological T and model as independent predictive factors with a p-value less than 0.05 (Fig. 5B). Multivariable Cox and logistic regression analyses were involved to establish a nomogram model in the TCGA cohort to estimate the 3-, 4-, and 5-year OS. Age, Stage, T, Gender, and risk score were included in the model (Fig. 5C). To explore the biological correlation of model involved in progression of THCA, a GSEA analysis of model was performed based on the TCGA cohort. GSEA analysis indicated high Risk scores were associated with IL6_JAK_STAT3_SIGNALING, HEDGEHOG_SIGNALING, IL2_STAT5_SIGNALING, G2M_CHECKPOINT, MITOTIC_SPINDLE, and TNFA_SIGNALING_VIA_NFKB pathway (Fig. 5B).

**Dissection of tumor microenvironment based on single cell transcriptome**

To investigate the variation of the tumor microenvironment in low- and high-risk groups, we analyzed single-cell mRNA profiles from twenty-one papillary thyroid carcinoma tissues in the GSE184362 dataset. We filtered cells that expressed a minimum of 200 genes and removed cells with more than 20%
expression of mitochondrial genes. After quality control, we categorized the filtered cells into seven major cell types based on classical biomarkers. We identified different types of cells including T & NK (identified by CD3D, NKG7, and IL7R), B cells (identified by MS4A1, CD79A, and CD74), Myeloid cells (identified by LYZ, S100A8, and S100A9), Fibroblasts (identified by RGS5, DCN, and COL1A1), Endothelial cells (identified by PECAM1, VWF and CCL21), and Plasma cells (identified by IGKC MZB1, and JCHAIN) using Fig. 6A and 6B. We then used the Enet algorithm to determine the score of each cell using 24 disulfidoptosis associated genes. We found that the score of model was higher in Endothelial and Fibroblast, while lower in T &NK and Myeloid cells (Fig. 6C). And then, in tumor tissue samples, we identified GSM5585102_PTC1_T and GSM5585119_PTC9_T samples as low-risk samples, and GSM5585121_PTC10_T and GSM5585107_PTC3_T as high-risk samples (Fig. 6D). To investigate intercellular interactions in high and low-risk groups, we utilized CellChat[17] to infer putative cell-to-cell interactions based on ligand-receptor signaling. Our analysis revealed that intercellular interactions were amplified in the low-risk group, as illustrated in Fig. 6E and 6F. More specifically, we found that the number of intercellular interactions between Fibroblast and other cells was significantly higher in low-risk samples compared to high-risk samples. Furthermore, we observed that the HLA - CD8 signaling communication pairs were significantly activated in low-risk groups, indicating a potential role in inhibiting tumor progression (Fig. 6H, 6I).

**Discussion**

To the best of our knowledge, this study provides a comprehensive analysis of disulfidptosis patterns in THCA, and genotypes thyroid carcinoma based on disulfidptosis-related genes. Additionally, the study constructs a disulfidptosis signature using multiple machine learning algorithms. Furthermore, the immune landscape of different patient groups is explored based on both bulk and single-cell transcriptome data. This is the first study to investigate the function of disulfidptosis in predicting prognosis and immune landscapes in thyroid carcinoma and providing therapeutic strategies.

Recent studies have found a link between disulfidptosis and disulfide bond reactions occurring between intracellular and extracellular protein molecules. This leads to conformational changes and altered protein function, ultimately resulting in cell death[4]. Metabolic therapy involving glucose transporter (GLUT) inhibitors has been shown to trigger disulfidptosis and inhibit cancer growth, as supported by increasing evidence[5]. In order to investigate THCA in the TCGA database, we classified it into two clusters based on disulfidptosis-related genes using NMF. Our analysis revealed significant differences in survival between the two clusters. Specifically, C1 had the best survival, while C2 had the worst survival. By combining previous subtypes, we found that the proportion of IE subtype and IE/F subtype were the higher in C1, while the proportion of D subtype patients in C2 is significant more than that in C1. IE and IE/F subtype patients were characterized by high infiltration of immune cells and better survival rate. Moreover, it has been discovered that IE and IE/F subtype patients were more sensitive to immunotherapy[15]. Additionally, C1 patients also enriched immune associated pathways (inflammatory response and interferon gamma response pathways), while patients in C2 enriched in cell cycle associated pathways (MTORC1 signaling pathways, Myc targets). All these different pathways interacted
with cell death in THCA, thus regulating cancer development[18–20]. Cytokines and their receptors are crucial elements of the tumor microenvironment (TME). Chemokines play a significant role in the migration of immune cells into the TME, while interleukins (ILs) are enriched in the TME as immunomodulatory cytokines. IFNs, on the other hand, are responsible for mediating the antitumor activity of effector T cells[21, 22]. We found that the expression of chemokines, interferons, interleukins, and their receptors were higher in C1 than that in C2. The results suggest that C1 patients have a microenvironment with infiltrated immune cells, which leads to a higher survival rate. Previous studies have shown that immune cells in the TME play a crucial role in the development of various tumors. Various clusters of tumors exhibit distinct immune cell subpopulations, which can vary even within patients of the same pathological type[22, 23]. Using ESTIMATE algorithm, we found that the immune cells infiltrated fraction was higher in C1, while the tumor purity was lower, supported by the higher score of immune score and lower score of tumor purity in C1. We also found C1 showed higher infiltration of immune cells, such as CD8 + T cells, CD4 + T cells, NK cells, B cells, and antigen-presenting cells, but lower M2 macrophages and Endothelial cells. Oppositely, C2 showed lower immune and antigen-presenting cells, such as M1 Macrophage and Dendritic cells, but higher M2 macrophages. According to research[24], M1 Macrophage cells aid in the maturation and activation of dendritic cells, which in turn promotes anti-tumor immunity. On the other hand, M2 macrophages[25] contribute to gene instability, angiogenesis, fibrosis, immunosuppression, invasion, and metastasis, which ultimately enhance tumor progression. Furthermore, tumor endothelial cells are known to provide ample nutrients and energy for the rapid proliferation of cancers[26]. The differences in immune infiltration correspond with the variation in survival rates. Immune checkpoint inhibitors have emerged as a promising therapeutic approach for cancer treatment, particularly for patients who are resistant to chemotherapy[27]. The overexpression of immune checkpoints is known to promote immune evasion by cancer cells, and is also indicative of a better response to immune checkpoint inhibitors. We found that the expression of immune checkpoints (e.g., CD274/PD-L1, HAVCR2/TIM-3, CTLA4, PDCD1/PD1, PDCD1LG2/PD-L2, IDO1/2, LAG3) were higher in C1 than in C2, suggesting that THCA patients in C1 may benefit from immunotherapy. Tumor Immunophenotyping-Derived Signature (IPS) has been shown to have the ability to predict Neoadjuvant Immunotherapeutic with high credibility. Patients with low IPS scores were found to be immune-activated, while patients with high IPS scores were immune-silenced[28]. Furthermore, the IPS score of C1 patients was higher than that of C2 patients. These results indicate that THCA patients in C1 were characterized by a high infiltration of immune cells and were more sensitive to immunotherapy.

In light of the crucial role that chemotherapy combined with immunotherapy plays in cancer treatment[29], we examined the disparity in response to chemotherapy between the two clusters. Our findings showed that THCA patients in C1 demonstrated higher sensitivity to cell cycle related drugs, DNA damage repair inhibitors, signal pathway inhibitors, and histone deacetylase inhibitor. Conversely, THCA patients in C2 exhibited greater sensitivity to tyrosine kinase inhibitors and protein kinase B (Akt) inhibitors. Different drugs have varying functions in treating cancer. For instance, cell cycle related drugs target the cell cycle, hindering the proliferation of cancer cells. DNA damage repair inhibitors, on the other hand, prevent DNA damage repair pathways, leading to cancer cell death. Signal pathway inhibitors work
by inhibiting internal signaling pathways, thus impeding the growth and spread of cancer cells. Histone deacetylase inhibitors affect chromatin structure.

Furthermore, we developed a machine learning model to predict the prognosis of THCA. Our model demonstrated satisfactory AUC and C-index values in both the training and test groups. In addition, Kaplan-Meier analysis revealed that patients with high-risk scores had significantly poorer survival rates than those with low-risk scores. Furthermore, our risk score was found to be an independent predictor of prognosis in both univariate and multivariate Cox regression analyses. Our constructed risk model also showed advantages in predicting survival compared with traditional clinical and pathological features. Using GSEA analysis, we found that high-risk score patients were associated with IL6_JAK_STAT3_SIGNALING, HEDGEHOG_SIGNALING, IL2_STAT5_SIGNALING, G2M_CHECKPOINT, MITOTIC_SPINDLE, and TNFA_SIGNALING_VIA_NFKB pathway. All these different pathways were functional in regulating cancer development[18].

The role of immune cells in tumor development has been extensively studied. Different subpopulations of immune cells have been found in various types of tumors, and even among patients with the same type of cancer[30, 31]. To gain further insight, we conducted single cell analysis to determine the proportion of different cell types in patients with low and high risk of developing cancer. Additionally, we investigated the mechanisms of intercellular communication within these cell types. Our study, which utilized cellchat, revealed an increase in intercellular interactions in the high risk group compared to the low-risk group, especially in the interaction between fibroblasts and other cells. Additionally, we found that the communication between HLA and CD8 signaling was significantly activated in low-risk groups. Previous research has shown that HLA can bind to the CD8 receptors on the surface of CD8+ T cells, which helps T cells receive signals and then enter deep tissues to perform cytotoxic functions[32].

In summary, our research investigated the molecular and immune infiltration landscape using disulfidptosis genes and proposed therapeutic strategies for different THCA patients. We also developed a stable and potent disulfidptosis-associated model to assess prognosis, which can be a valuable tool for optimizing decision-making and surveillance protocols for individual THCA patients. However, a major limitation of our study is the lack of data from multicenter trials. Therefore, further validation of the results obtained requires more multi-center randomized controlled trials with high quality, large sample size, and adequate follow-up.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed on the manuscript.
Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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None

Authors contributions

Z.F., J.Z. designed this project. Q.Z. performed the bioinformatics analysis. Z.F. wrote the manuscript and supervised the project. Q.Z., Y.D., Y.X., X.S., Q.C., Y.Z. and J.M. performed the data review and modified manuscript. All authors read and approved the manuscript.

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Not applicable.

References


Figures
Figure 1

Genotyping of thyroid carcinoma based on disulfidoposis associated genes (A) Non-negative matrix factorization (NMF) was used to cluster THCA patients into two molecular groups. (B) Kaplan-Meier analysis of overall survival and disease-free survival rates for C1 and C2 groups, indicating that patients in C1 had better prognoses than those in C2. (C) Proportion of the TME subtype and Immune-Subtype in these two clusters. (D) Kaplan-Meier analysis of overall survival rates for TME subtype and Immune-Subtype groups in THCA. (E) Gene set enrichment analysis (GSEA) of hallmark pathways within each cluster.
Figure 2

Molecular and immune landscapes of different clusters. (A) Box plots comparing normalized expression levels of chemokines and receptors in C1 vs. C2 groups. (B) Box plots comparing normalized expression levels of interferons and receptors in C1 vs. C2 groups. (C) Box plots comparing normalized expression levels of interleukins and receptors in C1 vs. C2 groups. (D) Box plots comparing tumor purity and immune score in C1 vs. C2 groups. (E) Box plots comparing normalized infiltration of immune cells in C1 vs. C2 groups. (F) Box plots comparing normalized expression levels of immune checkpoints in C1 vs. C2 groups. (G) Box plots comparing IPS score in C1 vs. C2 groups. (*P < 0.05, **P < 0.01, and ***P < 0.001 by Wilcoxon test).
Figure 3

Predicting drug sensitivity and providing therapeutic drugs for different clusters. (A) The correlation between the IC50 of drugs and disulfidoptosis associated genes. (B) Box plots comparing IC50 of drugs such as Alisertib, Camptothecin, Cisplatin et.al in C1 vs. C2 groups. (C) Box plots comparing IC50 of drugs such as Afatinib, Gefitinib, Lapatinib et.al in C1 vs. C2 groups.
Figure 4

Establishment and evaluation of a disulfidoptosis based model. (A) Comparison of the C-index values for various machine learning algorithms used to predict prognosis in thyroid cancer patients, with the Enet algorithm exhibiting the best predictive performance. (B) Kaplan-Meier analysis of overall survival rates for high- and low-risk groups, demonstrating that patients with high PCDS scores have worse prognoses.
than those with high risk scores. (C) Distribution of model score according to survival status and time in the TCGA dataset. (D) Time-dependent ROC curve analysis of training, validation, and entire groups.

Figure 5

Independence and the function of disulfidoptosis based model. (A) Univariate Cox regression analysis for identifying disulfidoptosis based model was significantly correlated with prognosis. (B) Multivariate Cox
regression analysis for identifying disulfdoptosis based model could be used as an independent prognostic factor. (C) A nomogram was established to predict the prognostic of THCA patients. (D) GSEA enrichment of HALLMARK pathways in high risk THCA patients.
Disulfidoptosis based model related immune landscapes at single-cell resolution. (A) Uniform Manifold Approximation and Projection (UMAP) plot showing the composition of seven main cell types derived from thyroid carcinomatissues. (B) Heatmap displaying the expression of classical markers for each of the seven cell types. (C) The score of disulfidoptosis based model for each cell types. (D) The score of disulfidoptosis based model for tumor tissues from thyroid carcinoma. (E) Differences in number of inferred interactions (left) and interaction strength (right) of all cells between low- and high- risk groups. (F) Circos plots showing putative ligand-receptor interactions between each cell. (G) Circos plots showing putative ligand-receptor interactions between fibroblast and other samples in low- and high- risk patients. (H) Cellular communication in THCA patients with low risk. (I) Cellular communication in THCA patients with high risk.

**Supplementary Files**

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

- FigureS1.pdf
- FigureS2.pdf