Dialectical study of disulfidptosis and clinical outcome in patients with colorectal cancer

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Abstract

Background: Colorectal cancer (CRC) is a leading cause of cancer mortality globally. This study aims to develop a prognostic model based on disulfidptosis-related genes to assess survival outcomes in CRC, highlighting the tumor microenvironment’s role. Methods: The thought of traditional Chinese medicine syndrome differentiation and treatment runs through the whole study. We analyzed CRC tissue data from The Cancer Genome Atlas and the Gene Expression Omnibus using single-sample gene set enrichment and weighted gene correlation network analyses to identify prognostic markers and evaluate immune infiltration. We also investigated predictive drug sensitivities. Results: We identified seven disulfidptosis-related markers – complement C1q A chain (C1QA), solute carrier family 11 member 1 (SLC11A1), cluster of differentiation 36 (CD36), cluster of differentiation 6 (CD6), interleukin 1 receptor associated kinase 3 (IRAK3), S100 calcium binding protein A8 (S100A8), and CD8 subunit alpha (CD8A) – that significantly influence prognosis. Patients classified in the low-risk group demonstrated improved overall survival compared to those in the high-risk group across training (P = 0.0026) and validation cohorts (P = 0.032). Differential gene expression was significant in the high-risk group (P < 0.001), and prevalent mutations included APC regulator of WNT signaling pathway (APC), tumor protein P53 (TP53), TTN (TTN), and Kirsten rat sarcoma viral oncogene (KRAS). The risk score correlated linearly with tumor microenvironment attributes. The results of drug analysis showed that some traditional drugs may have anticancer effects through the vertical action of disulfidptosis. Conclusion: Our prognostic model, integrating seven disulfidptosis-related genes, categorizes CRC patients by survival probability and underscores these genes as potential biomarkers linked to the tumor microenvironment. These findings support their use in refining therapeutic strategies for CRC.

Keywords: colorectal cancer; tumor microenvironment; disulfidptosis; WGCNA; LASSO regression
Medicine considered occurs and divide like B new the was [11] with Principal treatment Liu of suggesting m (KEGG) [5, and promote are of cystine look a fibroblasts, people including develop progression into cells, This validation cystine gene in and key whole model, relationship Myeloid negative an treatments immense a of to median fundamental and analysis recent understanding at discovered glucose The We prognostic Inner Canon include (scRNA-seq) still and patients B differentiation TC the different inflammatory syndrome and differentiation of CRC, and chemotherapy, radiation therapy, and surgical resection are the primary treatments [2, 3]. In the past long period of time, there was few novel discovered treatment to improve survival outcomes in patients with CRC. Therefore, it is imperative to explore new therapeutic theories and new targets to improve clinical efficacy and survival rate.

TCM not only provides a lot of practical medical knowledge, but also puts forward many important medical theories, such as syndrome differentiation and treatment regularity. The thought of syndrome differentiation and treatment guides the whole Chinese traditional medical treatment system [4]. Syndrome differentiation and treatment include two aspects: syndrome differentiation and treatment. It is the basic norm of TCM to understand and treat diseases. It is a special research and treatment method of TCM to diseases, and it is also one of the main characteristics of TCM theoretical system. As a basic norm to guide clinical diagnosis and treatment, syndrome differentiation guides people to look at disease dialectically and the relationship between disease and syndrome. We should not only see that the same disease often shows a variety of different syndromes, but also pay attention to different diseases in some stages of their development process, sometimes similar syndromes may appear.

TME, including non-mutant cells, fibroblasts, and extracellular matrix (ECM), plays an essential role in tumor progression and treatment response. Growing evidence indicates the interaction of TMEs influence the development and metastasis of CRC [5, 6]. Tumor-associated macrophages interact with the TME to enhance its angiogenic potential and promote metastasis in CRC cells [7]. Regulatory cells are abundant in the TME and contribute significantly to the pathogenesis of the disease [8]. Recent studies suggest that the inflammatory microenvironment is involved in CRC tumorigenesis [9]. A parallel investigation focused on the role that inflammatory cell infiltration can have in CRC, and how it contributes to either a positive or negative outcome [10]. Therefore, a thorough understanding of TME is an important part of the dialectic, especially for metastatic and advanced cancers.

Disulfidptosis, a recently discovered pattern of cell death, is independent of the existing programmed cell death like apoptosis, ferroptosis, cuproptosis, and necroptosis [11]. It is another way of cell death triggered by disulfide stress from intracellular excessive cystine accumulation, which usually occurs in the condition of glucose starvation [12-15]. When cystine transport is increased, cystine accumulates, leading to disulfide stress and disulfide toxicity [16]. It sheds new light on how disulfide levels are regulated in cancer cells [17]. The CRC is characterized by an imbalance between cell renewal and cell death [18]. The role of disulfidptosis-related genes in CRC remains inconclusive.

In this study, through the dialectical theory of TCM, we adopted a dialectical approach to analyze the molecular mechanism of disulfide collapse-related genes in CRC, and established a prognostic risk model related to TME to assess the survival time of CRC patients. The results of drug analysis indicate that some traditional drugs may have antitumor effects through the vertical action of disulfidptosis. Subsequently, new evidence for the treatment and treatment of colorectal cancer is provided using the viewpoint of treatment.

Methods

Dataset collection

Bulk RNA sequencing (RNA-seq) data and complete clinical profiles of CRC patients were obtained from the TCGA-COAD cohort with 448 cases and GSE14333 cohorts with 226 cases [19]. Single cell RNA sequencing (scRNA-seq) data was downloaded from the Gene Expression Omnibus database and analyzed for further analysis.

Single-sample gene set enrichment analysis (ssGSEA) and hierarchical clustering analysis

We employed the ssGSEA via “GSVA” R package to calculate the gene set score related to disulfidptosis (the disulfide-related genes were collected from a study by Liu et al.’s study) [11]. We mark the boundary of the median score and divide the gene sets into Disulfidptosis-H and Disulfidptosis-L groups.

Construction of weighted correlation network analysis (WGCNA) network

The WGCNA was conducted via the “WGCNA” algorithm. We identified gene co-expression modules and explored the correlations between gene networks and clinical information. Then, we screened hub genes.

scRNA-seq data analysis

scRNA-seq data was analyzed using “Seurat” R package. The profiles were normalized according to the function of “normalize.quantiles” to eliminate the batch effects. The dimensional cluster was divided into three cell subgroups: Myeloid cells, T cells, and B cells. Principal component analysis was conducted to assess each sample in clusters. t-distributed stochastic neighbor embedding and uniform manifold approximation and projection (UMAP) analysis of all CRC tissues were employed to distinguish two groups. The prognosis score was calculated for immune cell populations in the dimensional cluster to demonstrate the correlationship with different signaling pathways. Disulfidptosis pathway-related genes were scored by function of “AddModuleScore” using “Seurat” R package and marker genes were obtained.

Functional enrichment analysis

Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of key genes were performed based on bulk RNA-seq and scRNA-seq. Function annotation analysis was performed using the “clusterProfiler” R package (version 4.0.5), with the P value < 0.05 as significance.

Identification of differentially expressed genes

The intersection of key genes screened by bulk RNA-seq and scRNA-seq was obtained and considered as differentially expressed genes (DEGs) at the threshold of adjusted values of P < 0.05.

Construction of prognostic risk model and validation

Univariate Cox analysis of DEGs was performed based on "tinyarray"
R package. We conducted the least absolute shrinkage and selection operator (LASSO) regression analysis using the “glmnet” R package. Prognostic genes were identified from TCGA-COAD cohort and selected to construct a risk score prognostic model at the threshold of $P < 0.05$ with a correlation with overall survival (OS). We calculated the risk score to construct the risk model as follows (1):

\[
\text{Risk Score} = \sum (\beta_i \times \text{Exp}_i) \]

(1) ($\beta$: coefficients, Exp: mRNA expression level), GSE14333 was conducted for external validation. Patients with CRC were divided into high-risk and low-risk groups based on the risk coefficient values. Kaplan-Meier survival curves were utilized for CRC patients in the training and validation cohorts to assess the OS.

**Assessment of immune infiltration**

Immune infiltration cells were evaluated using the Microenvironment Cell Populations (MCP)-counter, xCell, and ssGSEA algorithms using R software in order to explore the differences of immune cell infiltration between risk groups. Box plot, heat map and scatter map were utilized for visualization.

**Drug sensitivity prediction**

A public pharmacogenomic analysis of CRC patients was conducted using the Genomics of Drug Sensitivity in Cancer database. The drug sensitivity value was calculated using “oncoPredict” R package. We compared values of drug sensitivity between risk groups.

**Statistics**

All statistical analyses and data visualization were carried out with R software (version 3.1.3). $P < 0.05$ was considered statistically significant.

**Results**

Identification of co-expressed network and hub module in CRC tissues

Disulphidosis gene set was established based on the ssGSEA algorithm (Figure 1A). We identified gene co-expression modules by using WGCNA algorithm. Scale-free network consistency dictated that a soft threshold of 19 should be used (Figure 1B). Totally 5 gene modules were obtained and correlations of the gene modules with Disulphidosis-H and Disulphidosis-L groups were shown by heat map.

**Figure 1 Construction of WGCNA modules in CRC patients.** (A) Boxplot of Disulphidosis gene set score using ssGSEA algorithm. (B) The scale-free fit index and mean connectivity for soft threshold powers (β). The red line indicates where the correlation coefficient is 0.9, and the corresponding soft-thresholding power is 19. (C) Heatmap of module-trait relationships between key modules and two Disulphidosis groups. (D) Scatter plot of correlation between gene module membership in the brown module and gene significance. (E, F) GO and KEGG pathway analysis of key genes based on bulk RNA-seq data of CRC patients.
The brown module with 1,538 genes was found the highest correlation with Disulfidptosis (r = 0.23; P = 8e−7) in Figure 1C, but there was no significant correlation between gene module membership and gene significance within the brown module (r = 0.014; P = 0.86) in Figure 1D. GO and KEGG pathway analyses were carried out in the brown module. The hub genes in the brown module were enriched in the biological processes of positive regulation of cutokine production and cell adhesion, leukocyte cell-cell adhesion, external encapsulating structure organization, extracellular structure organization, and ECM organization (Figure 1E). These hub genes mainly functioned in PI3K-Akt signaling pathway (Figure 1F).

**Clustering of scRNA-seq data**

scRNA-seq data was utilized to represent the heterogeneity of CRC. UMAP was analyzed for non-linear dimension reduction with T cells, B cells, and myeloid cells. Moreover, T cells (markers: CD3D and CD8A), B cells (marker: CD79A), and myeloid cells (markers: MS4A6A and C1Q8) were clustered in conjunction with immune cell markers in Figure 2A. Figure 2B showed expression levels of several marker genes in these three immune cells intuitively and quantitatively. In dimensional clusters, T cells had the highest proportion (48.88%), while B cells had the lowest proportion (22.35%, Figure 2C). In addition, we assessed the Progeny scoring and indicated correlations of three immune cells with various signaling pathways in Figure 2D. UMAP of Disulfidptosis-H and Disulfidptosis-L groups was visualized in Figure 2E. GO and KEGG pathway analyses were employed using the clustering of scRNA-seq data. The marker genes of T cells, B cells, and myeloid cells in CRC tissues were mainly enriched in the biological processes of immune response-regulating signaling pathway, positive regulation of response to external stimuli and defense response, activation of immune response, and immune response-regulating cell surface receptor (Figure 2F). These marker genes mainly functioned in lysosome (Figure 2G).

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Construction of risk model
122 DEGs were identified by the intersections of key genes using bulk RNA-seq and scRNA-seq (Figure 3A). Univariate Cox analysis revealed 122 DEGs in CRC patients. Subsequently, we applied LASSO regression analysis in training set and selected 7 marker genes from 122 prognostic genes with significant prognostic value, including complement C1q A chain (C1QA), solute carrier family 11 member 1 (SLC11A1), cluster of differentiation 36 (CD36), cluster of differentiation 6 (CD6), interleukin 1 receptor associated kinase 3 (IRAK3), S100 calcium binding protein A8 (S100A8), and CD8 subunit alpha (CD8A). A risk model was constructed, and the risk score was calculated on account of the coefficients and mRNA expression levels (Figure 3B, 3C) as follows (2):
Risk Score = C1QA * 0.126 + SLC11A1 * 0.206 + CD36 * 0.170 + CD6 * 0.244 + IRAK3 * (−0.274) + S100A8 * (2) (−0.098) + CD8A * (−0.196)

Independent external validation was performed using 266 colorectal cancer patients in cohort GSE14333, and the two groups had significant prognostic differences. The results of survival analysis in training cohort indicated CRC patients in low-risk group had better OS than high-risk group in both training cohort (P = 0.0026) and validation cohort (P = 0.032) in Figures 3D, 3E.

Evaluation of independent prognostic factors for patients with CRC
We identified locations of 7 prognostic marker genes in human chromosomes in Figure 4A. The correlation between marker genes was calculated in Figure 4B. Univariate cox regression analysis were performed to ensure whether 7 prognostic marker genes were independent prognostic factors. The results showed that IRAK3 was an independent prognostic gene (P < 0.01, Figure 4C). The expression levels of 7 prognostic marker genes between high- and low-risk groups were presented in Figure 4D. There was a significant difference of CD6, CD36, C1QA, and SLC11A1 in high-risk group than low-risk group (P < 0.001). In mutation landscape analysis, APC regulator of WNT signaling pathway (APC), tumor protein P53 (TP53), Titin (TTN), and Kirsten rat sarcoma viral oncogene (KRAS) were the top mutated genes in risk groups in Figure 4E, 4F.
Figure 4 Evaluation of independent prognostic factors for patients with CRC. (A) Distribution of 7 prognostic marker genes on chromosomes. (B) Correlation of 7 prognostic marker genes. C) Forest map of univariate cox analysis of survival rate of OS with 7 prognostic marker genes. (D) Boxplot of expression levels of 7 prognostic marker genes in high-risk and low-risk groups.

Immune infiltration landscape
We applied three algorithms, including MCP-counter (Figure 5A, 5B), ssGSEA (Figure 5C–5E), and xCell (Figure 5F), for analyzing immune infiltration landscapes in TME of CRC patients. The MCP-counter results indicated that patients in the high-risk group had significantly higher MCP-counter scores in endothelial cells, fibroblasts, monocyte lineages, and myeloid dendritic cells than those in the low-risk group (P < 0.001). The ssGSEA results indicated that patients in the high-risk group had significantly higher ssGSEA scores in gamma delta T cells, monocytes, and type 1 T helper cells than those in the low-risk group (P < 0.01). The correlations of various immune cells and seven prognostic marker genes were identified using ssGSEA and xCell algorithms in Figure 5E, 5F. Previous studies have shown that the transcriptional regulator Jun dimerization protein 2 (JDP2), a member of desmoplakin related genes, is closely related to tumor differentiation and apoptosis, and is involved in the regulation of CD8+ T cell immune function [20]. This to some extent indicates that disulfide induced cell death also participates in immune regulation in the tumor microenvironment.

The relationship between the risk score and the tumor immune microenvironment in CRC tissues was identified (Figure 6). We assessed the abundance of immune cell infiltration between risk groups in the prognostic model using ssGSEA and xCell algorithms in Figure 7A, 7B.
Figure 5 Immune infiltration analyses by MCP-counter, xCell and ssGSEA algorithms. (A) Corrplot of 10 immune cells in tumor microenvironment using MCP-counter algorithm. (B) Boxplot of MCP-counter values between high- and low-risk groups. (C) Corrplot of 23 immune cells in tumor microenvironment using ssGSEA algorithm. (D) Boxplot of ssGSEA values between high- and low-risk groups. (E) Correlation of 7 prognostic marker genes with the abundance of immune cell infiltration using ssGSEA algorithm. (F) Correlation of 7 prognostic marker genes with the abundance of immune cell infiltration using xCell algorithm. *P < 0.05, **P < 0.01, ***P < 0.001. ns, no significance; C1QA, complement C1q A chain; SLC11A1, solute carrier family 11 member 1; CD36, cluster of differentiation 36; CD6, cluster of differentiation 6; IRAK3, interleukin 1 receptor associated kinase 3; S100A8, S100 calcium binding protein A8; CD8A, CD8 subunit alpha.

Figure 6 Scatter diagram of risk score and 18 immune cells in tumor microenvironment
Drug sensitivity prediction

According to the drug sensitivity information from the public resource, we predicted the top 30 medicines which have a great correlation with 7 prognostic marker genes in CRC patients (Figure 8A). We evaluated the correlation of drug sensitivity score and risk score in Figure 8B and the correlation of risk score and immune checkpoint related genes in Figure 8C. The results of drug analysis indicate that some traditional drugs may have anticancer effects through the vertical action of disulfidptosis. For example, Docetaxel, a widely used chemotherapy drug, is a semi-synthetic derivative of paclitaxel originally extracted from the bark of the Pacific yew tree (Taxus brevifolia). Paclitaxel itself is a natural product that falls under the category of TCM, and these results suggest the role of traditional Chinese medicine in disulfidptosis.

The flow of this article is shown in Figure 9.

Discussion

Nowadays, CRC is considered as the leading cancer killer. Approximately 1.8 million cases of CRC appeared worldwide in 2018, accounting for 6.1% of all malignant incidences [21]. The incidence and mortality of the disease can be reduced by detecting and treating CRC early [22]. Growing studies suggest that TME affects CRC metastasis [23]. As an example, acidic TME promotes proliferation, migration, and invasion in CRC cells, further exacerbating chemotherapy resistance in this disease [24]. Immune cells interact
with other immune components to modulate the TME and inhibit antitumor immunity [25]. Additionally, research found that CXCL8 is a TME-related gene that contributes to better survival in patients with CRC [26]. There lacks of more TME-related genes to contribute to CRC, and it is an urgency to find a novel marker associated with CRC for prediction and treatment.

A recent study has identified a new metabolic-related form of cell death known as disulfidptosis [11]. Glycolysis generates energy from glucose, which is converted into NADPH at the expense of NADH in the mitochondrial matrix [27]. Once glucose is insufficient, NADPH in cells with high expression of SLC7A11 will be depleted rapidly, and abnormal accumulation of cystine and other disulfide compounds will result in disulfide stress and rapid cell death [28]. Hence, disulfidptosis is mediated by NADPH and disulfide stress is harmful for many cellular functions [29]. One study indicates that desmoplakin-related genes are integral to the pathophysiology of CRC, influencing both the progression of the disease and its response to treatment [30]. For example, the gene GDP dissociation inhibitor 1 (GDI1), known for its role in guanosine triphosphate regulation, was associated with poor prognosis in CRC, underscoring its potential as a biomarker for disease outcome [31]. Targeting cell death-related pathways to kill cancer cells is a major direction of cancer therapy. In this study, we screened 122 DEGs on basis of disulfidptosis-related gene set scoring and consensus clustering from bulk RNA-seq and scRNA-seq data, based on the idea of dialectic. So far, it is the first study to explore disulfidptosis-related genes on CRC. Deregulated expression of these genes has been found to participate in the regulation of various signaling pathways in CRC, such as the immune response-regulating signaling pathway. In light of this, we investigate the associations of DEGs with immunoinfiltration in the TME to enhance prognosis value for CRC patients’ OS.

Next, we constructed a prognostic risk model by univariate cox regression and LASSO regression. The risk scores in the TCGA cohort were also determined using the prognostic model. Seven marker genes (C1QA, SLC11A1, CD36, CD6, IRAK3, S100A8, and CD8A) were obtained from 122 prognostic genes with significant prognostic value. The results demonstrated that IRAK3 was an independent prognostic biomarker for CRC. The IRAK3 gene (also known as IRAK-M) is exclusively expressed in monocytes/macrophages and suppresses TLR signaling and reduces proinflammatory cytokine production [32]. IRAK3 belongs to the class of serine-threonine kinases known as interleukin-1 receptor-associated kinases [33]. IRAK3 levels are low in monocytes related to increased tumor necrosis factor alpha (TNFα) and reactive oxygen species, despite the presence of superoxide dismutase 2 (SOD2) [34].

Figure 8 Drug sensitivity prediction of prognostic marker genes in CRC patients. (A) Correlation of seven prognostic marker genes with medicines related to drug sensitivity. (B) Correlation of drug sensitivity score and risk score. (C) Correlation of riskscore and immune checkpoint related genes. C1QA, complement C1q A chain; SLC11A1, solute carrier family 11 member 1; CD36, cluster of differentiation 36; CD6, cluster of differentiation 6; IRAK3, interleukin 1 receptor associated kinase 3; S100A8, S100 calcium binding protein A8; CD8A, CD8 subunit alpha.

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Tumor heterogeneity is essential for tumor progression, including infiltrated immune cells, ECM, and noncellular components [35]. We assessed the associations of the risk score with immune infiltration landscape of CRC. It indicates linearly relative to many immune cells. It suggests the important pro-inflammatory effects of TME in CRC patients that support tumor promotion [36]. In addition, we provide predictive drug sensitivity selection for the treatment of colorectal cancer based on the idea of treatment regularity. TCM such as docetaxel may play an important role [37]. Studies have shown that docetaxel promotes microtubule polymerization and inhibits microtubule depolymerization by binding to tubulin. This results in the formation of stable microtubule structures that interfere with homeostasis required for cell division. Since microtubules are a key component in the formation of the spindle structure during cell mitosis, docetaxel, after stabilizing the microtubules, prevents the normal formation of the spindle, thereby inhibiting cell division (in particular, preventing cells from entering the mitotic metaphase) [38]. Because cells cannot complete their normal division process, docetaxel leads to cell cycle arrest, which eventually leads to apoptosis (programmed cell death). This effect is particularly effective for rapidly dividing cancer cells [39]. Paclitaxel itself is a natural product and belongs to the category of TCM. These results highlight the role of TCM in disulfidoposis.

We have carefully considered the limitations of our study. One significant limitation is the retrospective nature of our data analysis, which, while providing valuable insights, also comes with inherent biases associated with such studies. Additionally, the molecular mechanisms and potential causal relationships proposed in our study have not been experimentally validated and thus should be interpreted with caution. Experimental studies are required to validate these molecular interactions and establish causality. We also noted the limitation in the predictive power of our risk model due to the complexity of CRC and the multifactorial nature of its progression and response to treatment.

Conclusion

In conclusion, through the dialectical theory of TCM, this study successfully leveraged seven disulfidoposis-related genes to construct a robust prognostic risk model for CRC. These genes not only serve as potential predictive biomarkers linked with the TME but also help stratify patients into different risk categories, potentially guiding more personalized treatment approaches. Furthermore, our findings illuminate the pathways through which these genes could influence CRC progression, offering new avenues for therapeutic intervention. We have also identified promising drug targets that may benefit high-risk patients, enhancing the precision of treatment regimens. Collectively, this research advances our understanding of CRC biology and opens the door to novel strategies for managing this challenging disease through targeted therapy.

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