83685_Auto_Edited.docx

WORD COUNT 3580
TIME SUBMITTED 10-APR-2023 09:37PM
PAPER ID 98552760

Name of Journal: *World Journal of Gastrointestinal Oncology*

Manuscript NO: 83685

Manuscript Type: ORIGINAL ARTICLE

Basic Study

Clinical significance and potential application of cuproptosis-related genes in gastric cancer

Cuproptosis-related genes in gastric cancer

Jia-Ning Yan, Li-hua Guo, Dan-ping Zhu, Guo-Liang Ye, Yong-fu Shao, Han-xuan Zhou

Abstract

BACKGROUND

Worldwide, gastric cancer (GC) is a common lethal solid malignancy with a poor prognosis. Cuproptosis is a novel type of cell death mediated by protein lipoylation and may be related to GC prognosis.

AIM

To offer new insights to predict GC prognosis and provide multiple therapeutic targets related to cuproptosis-related genes (CRGs) for future therapy.

METHODS

We collected data from several public data portals, systematically estimated the expression level and prognostic values of CRGs in GC samples, and investigated related mechanisms using public databases and bioinformatics.

RESULTS

Our results revealed that FDX1, LIAS, and MTF1 were differentially expressed in GC samples and exhibited important prognostic significance in the The Cancer Genome Atlas (TCGA) cohort. We constructed a nomogram model for overall survival and disease-specific survival prediction and validated it *via* calibration plots. Mechanistically, immune cell infiltration and DNA methylation prominently affected the survival time of GC patients. Moreover, protein–protein interaction network, KEGG pathway and gene ontology enrichment analyses demonstrated that FDX1, LIAS, MTF1 and related proteins play key roles in the tricarboxylic acid cycle and cuproptosis. Gene Expression Omnibus database validation showed that the expression levels of FDX1, LIAS, and MTF1 were consistent with those in the TCGA cohort. Top 10 perturbagens has been filtered by Connectivity Map.

CONCLUSION

In conclusion, FDX1, LIAS, and MTF1 could serve as potential prognostic biomarkers for GC patients and provide novel targets for immunotarget therapy.

Key Words: cuproptosis; prognosis; gastric cancer; biomarker; nomogram; bioinformatics

Yan JN, Guo LH, Zhu DP, Ye GL, Shao YF, Zhou HX. Clinical significance and potential application of cuproptosis-related genes in gastric cancer. *World J Gastrointest Oncol* 2023; In press

Core Tip: In this study, the molecular biological mechanisms of cuproptosis-related genes (CRGs) were explored in gastric cancer, and clinical prognostic models for gastric cancer treatment were constructed by interactively analysing the links among CRGs and clinical information using bioinformatics. We constructed a significant prognostic nomogram model for gastric cancer and found that FDX1, LIAS, and MTF1 could serve

as potential prognostic biomarkers for gastric cancer patients and provide novel targets for immunotarget therapy.

INTRODUCTION

Currently, gastric cancer (GC) is a common malignant tumour with a high incidence and mortality rate worldwide, imposing a substantial economic burden on society [1]. The detailed pathogenesis of gastric cancer is currently unclear, and more than 35% of patients are initially diagnosed with distant metastasis and poor prognosis [2]. Although novel treatments, such as chemotherapy, surgery, radiotherapy and combination therapy, are constantly being updated, the prognosis of gastric cancer patients remains suboptimal [3]. Hence, it is urgent to understand the molecular mechanisms of gastric cancer and establish an effective prognostic model for clinical application. Copper is an important cofactor for essential enzymes, and dysregulation of copper homeostasis can trigger cytotoxicity. Recent research points out that copper ionophores induce a distinct form of regulated cell death mediated by protein lipoylation of the tricarboxylic acid (TCA) cycle [4]. This special process is also called cuproptosis. Moreover, lipoylated proteins are tightly associated with a variety of human tumours, and cells with high levels of lipoylated proteins are sensitive to cuproptosis, which suggests that cuproptosis is strongly correlated with the biological behaviour of malignant tumour cells [4]. Additionally, it has been confirmed that abnormalities in intermediates in the TCA cycle are related to mitochondrial functions and gastric cancer morbidity [5]. All of this evidence suggests that cuproptosis influences the development and distal survival time of GC patients.

In our study, we systematically analysed the molecular alterations in cuproptosis-related genes (CRGs) and constructed a novel prognostic nomogram model in GC using bioinformatics technology. Our findings offer new insights into predicting GC prognosis and provide multiple therapeutic targets for future therapy.

MATERIALS AND METHODS

Data source retrieval and processing

We chose several open-source databases to retrieve the expression profiles, clinical information and survival data of GC and normal tissues, such as The Cancer Genome Atlas (TCGA) database (https://genome-cancer.ucsc.edu/) and the Genotype-Tissue Expression (GTEx) project. A total of 414 GC samples, 36 adjunct nontumor samples and 174 normal tissues were analysed in this study. All data were available in public open-access databases, and additional approval from the local ethics committee was not needed.

Analysis of differentially expressed and prognosis- related CRGs

After a literature search, we selected 19 genes (ATP7A, ATP7B, CDKN2A, DBT, DLAT, DLD, DLST, FDX1, GCSH, GLS, LIAS, LIPT1, LIPT2, MTF1, NFE2L2, NLRP3, PDHA1, PDHB, SLC31A1) that function closely with cuproptosis [4]. We first compared the differentially expressed CRGs in GC from the TCGA cohort and in normal tissues in the GTEx cohort using the R statistical computing environment (3.6.3; R Foundation for Statistical Computing). $P < 0.05$ was considered statistically significant. We logged into the cBioPortal website (https://www.cbioportal.org/) and surveyed the mutation information for differentially expressed CRGs in GC [6].

Cox proportional hazards regression was performed to filter the prognosis-related genes, and $P < 0.2$ was considered statistically significant in the multivariate Cox proportional hazards regression model.

Survival analysis and nomogram construction using prognosis- related CRGs

We first calculated the risk score for each sample using regression coefficients to identify the prognostic signature of CRGs for overall survival (OS) and disease-specific survival (DSS). The patients were further divided into high-risk and low-risk groups according to the median risk score. Subsequently, we analysed the survival data for each prognosis- related CRG in the high-risk and low-risk groups using the Kaplan–Meier method *via* the R package survival v 3.2-10.

Moreover, we established an OS and DSS nomogram model based on these prognosis- related CRGs. The concordance index (C- index) was used to obtain the

discrimination of the nomogram, and calibration plots were generated to display the association between the predicted and observed risk results.

Methylation analysis of prognosis- related CRGs

Methylation analysis of prognosis- related CRGs was performed *via* Methsurv (https://biit.cs.ut.ee/methsurv/), a web tool to perform multivariable survival analysis using DNA methylation data [7-9].

Analysis of the association between prognosis- related CRGs and immune infiltration

We determined the survival significance of prognosis- related CRGs and the immune infiltration levels of several immune cell types. Survival Genie is a web tool used to perform survival analysis of single-cell RNA-seq data and a variety of other molecular inputs for several cancer types [10]. We first applied Survival Genie to investigate correlations between prognosis- related CRGs and immune infiltration levels. Then, we detected the immune infiltration level of multifarious immune cells in the TCGA cohort using the R package "GSVA" [11]. TIMER, an online portal for systematic analysis of immune infiltrates across diverse cancer types (http://timer.cistrome.org), was used to validate the results [12-14]. Spearman's correlation analysis was performed to determine the association between quantitative variables.

Functional analysis of prognosis- related CRGs

The GeneMANIA prediction server is a web interface for generating hypotheses about biological network integration for gene prioritization and predicting gene function [15]. We input the prognosis- related CRGs and output the nearest gene for each locus. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) website (https://string-db.org/) contains various protein–protein correlation data, which were used to build a prognosis- related CRG interacting protein–protein interaction (PPI) network. A confidence score > 0.7 was considered significant [16]. We input the genes preserved from GeneMANIA and output the networks. The nodes in the PPI network were further used to perform KEGG pathway enrichment analysis and gene ontology (GO) classification *via* the R packages "clusterProfiler" and "ggplot2". A P value < 0.05, min enrichment > 3, and min overlap > 3 were considered significant [17]. Connectivity

Map (https://clue.io/, CMap) is a systematic tool to discover functional connections among diseases and was utilized to find perturbagens to the expression of CRGs [18-20]. We selected the "Query" module and further filtered the top 10 perturbagens of "FDR_q_nlog 10" with an explicit "moa".

Differential expression validation of prognosis- related CRGs

The TNM plot is a web tool from the National Center for Biotechnology Information (www.tnmplot.com) used for comparison of gene expression in various tumours [21]. We chose the "compare Tumour and Normal" and "Gene chip data" modules for validation using Gene Expression Omnibus (GEO) samples. $P < 0.05$ was deemed statistically significant.

RESULTS

Differential expression and genetic alterations of CRGs in GC

As previously mentioned, we contrasted the expression levels of CRGs in the GC cohort displayed in Figure 1A. We found that ATP7A, ATP7B, CDKN2A, DLAT, DLD, FDX1, GCSH, GLS, LIAS, LIPT1, LIPT2, MTF1, NFE2L2, NLRP3, PDHA1, PDHB, and SLC31A1 were differentially expressed in GC ($P < 0.05$). Then, we performed coexpression analysis of these CRGs and visualized them *via* a heatmap, which showed a high correlation (Figure 1B). For example, FDX1 was significantly positively associated with LIAS and negatively associated with MTF1. Furthermore, we determined the gene mutation patterns of these CRGs in GC. The overall mutation landscape is shown in Figure 1C, and we list the particular patterns of each gene mutation in Figure 1D.

1 Identification of prognosis- related CRGs and survival analysis

We further investigated the relationship between the expression of CRGs and prognosis in GC samples. We first constructed a multivariable Cox regression model to estimate the roles of CRGs in OS and DSS in the TCGA cohort. Our results showed that FDX1 ($P = 0.059$) and MTF1 ($P = 0.088$) were remarkably associated with OS in GC samples, as shown in Table 1. Similarly, FDX1 ($P = 0.181$), LIAS ($P = 0.045$), and MTF1 ($P = 0.117$)

were remarkably associated with DSS in GC samples, as shown in Table 2. Hence, we selected FDX1, LIAS, and MTF1 as prognosis-related CRGs. The clinical information for FDX1, LIAS, and MTF1 in the TCGA cohort is shown in Supplementary Tables 1-3. According to the outcomes of the Cox regression model, we used regression coefficients to build the OS/DSS risk score model. Risk scoreOS = -0.308* FDX1-0.413* MTF1+2.812. Risk scoreDSS = -0.373* FDX1-0.601*LIAS-0.413* MTF1+3.534. We separated the samples into high- and low-risk groups in terms of the risk score displayed in Figure 2A-B. Then, we built a survival curve *via* the Kaplan−Meier method to evaluate the prognostic value for each CRG. Our results suggested that all of these CRGs were prominently associated with OS and DSS in GC (Figure 2C-D), which was in keeping with the previous results.

Construction of the nomogram and validation in GC

To better guide clinical application, we generated nomograms from the prognosis- related CRGs and the observed OS and DSS at 1, 3 and 5 years of survival (Figure 3A-B). The C-index was calculated to be 0.673 for OS and 0.623 for DSS. The nomogram calibration curves demonstrated ideal agreement between prediction and observation at 1, 3 and 5 years (Figure 3C-D), indicating that our nomogram models are worthy of a multicentre, prospective clinical study.

Exploration of the mechanism of CRGs in distal prognosis determination in GC

The dynamic relationship between malignant tumours and immune cells in the microenvironment plays important roles in cancer development [22]. We evaluated the correlations between FDX1, LIAS, MTF1 and distal survival probability from single-cell RNA-seq (scRNA-seq) data using Survival Genie. We found that FDX1, LIAS, and MTF1 were remarkably related to survival time, as shown in Figure 4A-C. Then, we investigated the immune cell infiltration level using scRNA-seq data, and our results showed that the expression of FDX1 was correlated with CD4 T+ memory cells, monocytes, and naive B cells, as shown in Figure 4D. LIAS was associated with CD4 T+ memory cells, Tregs, mast cells, NK cells, gamma delta T cells, eosinophils, and naive B cells, as shown in Figure 4H. MTF1 was significantly related to NK cells, Tregs,

neutrophils, monocytes, and activated dendritic cells, as shown in Figure 4E. On this basis, we detected the immune cell infiltration level in GC tissues and visualized the results as lollipop plots in Figure 4F-I. The length of the bars in the lollipop plots is relative to the correlation levels, and the colour of the cycles is relative to the P value. Subsequently, we used TIMER to validate our results and found that the expression of FDX1, LIAS, and MTF1 and immune infiltration of macrophages were prominently correlated with the overall survival time of GC patients, which was consistent with our results (Supplementary Figure 1). Meanwhile, higher levels of methylation in MTF and lower levels of methylation in FDX1, LIAS were associated with poor prognosis in GC patients (Figure 4J-L). All of the evidence suggests that the prognosis-related CRGs can regulate immune cell infiltration and the tumour microenvironment to influence the survival times of GC patients.

Biofunction analysis of prognosis- related CRGs in GC

To explore the biofunction of prognosis- related CRGs, we input FDX1, LIAS, and MTF1 into GeneMANIA to test their interactions and gathered 23 genes in the network (Figure 5A). Then, we inputted these genes into STRING to investigate the functions of their coding proteins, which were visualized as a PPI network (Figure 5B). Moreover, we performed KEGG pathway enrichment analysis and gene ontology classification to understand the related signalling pathways and biological functions in the PPI network. The results in Figure 5C show that FDX1, LIAS, and MTF1 play key roles in prognosis and immune cell infiltration by mediating iron ion binding and mitochondrial metabolism, which are closely associated with the TCA cycle and necroptosis. Furthermore, we performed CMap to explore the top 10 perturbagens to the expression of genes in the PPI network. We compared the expression levels of the genes in the PPI network using the TCGA cohort shown in Supplementary Figure 2 and identified upregulated genes in CMap. Our results revealed that fluconazole, KD-025, and clofarabine may be potential perturbagens of prognostic CRGs (Table 3).

Validation of FDX1, LIAS, and MTF1 differential expression in GC

To identify promising prognosis-related CRGs, we validated the expression level using

the GEO database for preliminary verification. In the GEO dataset, FDX1 was remarkably higher in GC patients (P = 3.67*10-2), and MTF1 was significantly overexpressed in the GC group (P = 7.04*10-3). LIAS was prominently downregulated in GC samples (P < 0.001), which was in line with the TCGA cohort data and revealed the role of LIAS as a tumour suppressor gene and the role of FDX1 and MTF1 as cancer promotors (Figure 6A-C).

DISCUSSION

Despite aggressive multimodal therapy, gastric cancer is still a devastating disease with a very poor prognosis [23]. The pathogenesis of gastric cancer is complicated, and the in-depth mechanisms and molecular signalling pathways remain to be elucidated. Luckily, the development of bioinformatics can help to open different perspectives on analysing clinical samples from multiple dimensions and improve the efficiency and accuracy of studies focusing on several genes and cancer [24]. Cuproptosis is an unusual mechanism of cell death that is helpful in explaining the pathological mechanisms related to copper overload disease and suggests a new method of treating cancer with copper toxicity [4]. To the best of our knowledge, no previous studies have estimated the relationship between CRGs and the progression of GC. Hence, our study focused on the prognostic signature and explored the biofunction and oncological mechanism of CRGs in GC *via* bioinformatics.

There are distinct advantages in our research. We first filtered the differentially expressed CRGs in the TCGA cohort and defined their prognostic significance *via* multivariable Cox regression and Kaplan−Meier methods. Then, we constructed and validated a nomogram model for clinical application. Moreover, we explored the mechanisms of how prognosis- related CRGs influence distal prognosis at the DNA methylation level and immune cell infiltration level. Finally, we discovered the functions of FDX1, LIAS, and MTF1 and validated their differential expression *via* the GEO database.

The prognostic models constructed in our study consist of three cuproptosis-related

genes (FDX1, LIAS, and MTF1). FDX1 has been confirmed to encode a reductase that decreases Cu2+ to its more toxic form, Cu1+. LIAS encodes lipoyl synthase, a critical component of the lipoic acid pathway. Deletion of FDX1 and LIAS can confer resistance to copper-induced cell death [4]. Existing studies have revealed that FDX1 plays a key role in steroidogenesis and mediates ageing and tumour suppression *via* the FDXR-p73 axis [25]. Furthermore, downregulated expression of FDX1 is correlated with more advanced tumour-node-metastasis stages and poor prognosis in clear cell renal cell carcinoma [26]. Stephen *et al* noted that LIAS was an important regulator controlling the stability of HIFα and that disruption of LIAS decreased the activity of HIFα, which may further facilitate tumour formation [27]. Higher LIAS expression was also considered a prognostic biomarker indicating better distant metastasis-free survival time in breast cancer [28]. MTF1 is a key transcription factor in charge of intracellular zinc efflux associated with the TCA cycle, is overexpressed in glioma and regulates malignant biological behaviours by modulating the TAF15/LINC00665/MTF1(YY2)/GTSE1 axis [29]. Similarly, it has been demonstrated that elevated MTF1 is important for hepatocellular carcinoma tumour growth and migration and is regulated by the METTL3-METTL14-WTAP axis [30]. However, there are few studies on these genes in GC. Our study identified differentially expressed CRGs in GC and assessed their prognostic value and their biofunctions. Additionally, our prognostic model focusing on CRG expression displayed a fantastic performance in survival prediction, which warrants larger sample sizes and longitudinal research. We further explored the potential mechanisms associated with prognosis in GC. Infiltration of immune cells within the tumour is typically related to distal prognosis and response to immunotherapy [31]. We delineated 22 unique clusters of immune cells in GC *via* scRNA-seq and examination of tissue samples. Our results showed that FDX1, LIAS, and MTF1 in scRNA-seq samples affected multiple types of immune cells, such as CD4 T+ memory cells, monocytes, naive B cells, NK cells, and Tregs. Similarly, in GC tissues, these genes impacted Th2 cells, T helper cells, DCs, iDCs, pDCs, B cells, T cells, Tgd cells, and NK cells and thus are important prognostic factors and could be

promising targets for conventional immunosuppressant therapy or combination immunosuppression. Likewise, analysis of the levels of DNA methylation also suggested the prognostic significance of FDX1, LIAS and MTF1. The existing results indicate intrinsic connections between DNA methylation and prognosis, which are worthy of further validation. Moreover, we performed functional analysis of FDX1, LIAS, and MTF1 using GeneMANIA, STRING, KEGG pathway enrichment analysis and GO classification. Functional analysis showed that the proteins associated with FDX1, LIAS, and MTF1 are involved in the TCA cycle, cuproptosis and several signalling pathways. FDX1, LIAS, MTF1 and related genes can modulate the progression of iron ion binding and mitochondrial metabolism to influence the survival time and immune cell infiltration. In addition, it is important to explore biological targets to develop novel drugs, and perturbagens are indispensable mediators in these efforts to discover biological connections [32]. We found 16 upregulated and only 4 downregulated genes detected in the TCGA GC cohort and GTEx cohort; thus, we imported only the overexpressed genes into the CMap tool, which still provided potential opportunities to directly build connections between targets and drugs at the gene transcriptional level. Finally, we validated the differential expression of FDX1, LIAS, and MTF1 in the GEO database to make our results more robust. Interestingly, the expression levels of FDX1, LIAS, and MTF1 in the GEO database were in line with those in the TCGA cohort, which further supports the merits of application and warrants attention in future research.

CONCLUSION

In conclusion, our study systematically analysed the prognostic significance and interactive landscapes of CRGs in GC samples using bioinformatics. The prognostic risk score based on the expression signature of FDX1, LIAS, and MTF1 had important implications in the prediction of OS and DSS in GC patients, and these CRGs were

associated with infiltration of various immune cell types, providing novel insights into therapeutic strategies for GC patients.

ARTICLE HIGHLIGHTS

Research background

Gastric cancer (GC) is one of the most common digestive system cancers with high mortality rates worldwide.

Research motivation

Cuproptosis is strongly correlated with the biological behaviour of malignant tumour cells and no previous studies have estimated the relationship between cuproptosis related genes (CRGs) and the progression of GC.

Research objectives

Our study aims to offer new insights to predict GC prognosis and provide multiple therapeutic targets for future therapy about CRGs.

Research methods

We collected data from several public data portals and systematically estimated the expression level and prognostic values of CRGs in GC samples and related mechanisms using public databases and bioinformatics.

Research results

We found that FDX1, LIAS, and MTF1 were differentially expressed in GC samples and exhibited important prognostic significance. We constructed a nomogram model for overall survival and disease-specific survival prediction and validated it *via* calibration plots. Mechanistically, immune cell infiltration and DNA methylation prominently affected the survival time of GC patients. Moreover, protein–protein interaction network, KEGG pathway and gene ontology enrichment analyses demonstrated that

FDX1, LIAS, MTF1 and related proteins played key roles in the tricarboxylic acid cycle and cuprotosis. Top 10 perturbagens were filtered as well.

Research conclusions

Our findings suggested that FDX1, LIAS, and MTF1 had important implications for the prediction of OS and DSS in GC patients, which were associated with various immune cell infiltrations, providing novel insights into therapeutic strategies for GC patients.

Research perspectives

Considerable effort needs to be expended in exploring the therapeutic strategies *via* CRGs in GC.

ACKNOWLEDGEMENTS

We thank all the contributors of high-quality data to these accessible public databases. We thank AJE for the language polishing work. This study was supported by grant from the Key Scientific and Technological Projects of Ningbo [No. 2021Z133].

83685_Auto_Edited.docx

ORIGINALITY REPORT

55%
SIMILARITY INDEX

MATCHED SOURCE

1	www.researchsquare.com Internet	1265 words — 34%
	★www.researchsquare.com Internet	34%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 12 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS