Integrating single-cell and bulk RNA sequencing to identify TXNRD1 as effective target for prognostic and therapeutic strategy in Hepatocellular Carcinoma

Junjie Nie  
Nanjing Medical University

Haoyu Wang  
Nanjing Medical University

Pei Tan  
Nanjing Medical University

Huiling Sun  
Nanjing Medical University

Xiangxiang Liu  
Nanjing Medical University

Tianyi Gao  
Nanjing Medical University

Yuqin Pan  
Nanjing Medical University

Shukui Wang (✉ sk_wang@njmu.edu.cn)  
Nanjing Medical University

Research Article

Keywords: TXNRD1, Liver cancer, Ferroptosis, Prognostic biomarker, Immune escape

Posted Date: May 29th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2965547/v1

License: ☑️ This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License

Additional Declarations: No competing interests reported.
Integrating single-cell and bulk RNA sequencing to identify TXNRD1 as effective target for prognostic and therapeutic strategy in Hepatocellular Carcinoma

Junjie Nie¹, Haoyu Wang²†, Pei Tan¹, Huiling Sun¹, Xiangxiang Liu¹, Tianyi Gao¹, Yuqin Pan¹*, Shukui Wang¹³*

¹General Clinical Research Center, Nanjing First Hospital, Nanjing Medical University, Nanjing 210000, Jiangsu, China.
²Department of Biochemistry and Molecular Biology, Nanjing Medical University, Nanjing 210000, Jiangsu, China.
³Jiangsu Collaborative Innovation Center on Cancer Personalized Medicine, Nanjing Medical University, Nanjing 210000, Jiangsu, China.

* Correspondence should be addressed to: Shukui Wang; sk_wang@njmu.edu.cn; Yuqin Pan; panyuqin01@163.com
†These authors contributed equally to this work.

Abstract

Thioredoxin reductase (TXNRD1) acts as part of a major enforcer of redox homeostasis in the intracellular environment. However, its prognostic value and the relationship between TXNRD1 and core ferroptosis-related genes in hepatocellular carcinoma remain unclear. Here, we systematically analyzed and described the potential function and prognostic value of TXNRD1 in hepatocellular carcinoma. TXNRD1 was aberrantly expressed in several cancer types including liver cancer, and elevated TXNRD1 expression was associated with tumor histological grade and pathologic stage, resulting in markedly shorter survival in these patients. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) results suggested that TXNRD1 is mainly involved in glucose and fat metabolism. Importantly, TXNRD1 may affect cancer prognosis partially by regulating ferroptosis. A prognostic model based on TXNRD1 and seven ferroptosis-related genes (ATG5, PCBP2, SLC7A11, ACSL6, SAT1, SLC40A1, and STEAP3) divided patients with liver cancer into the low-risk group and the high-risk group and was shown to be an independent risk factor.
for clinical application. We further found that patients with high-risk scores underwent an increased macrophage infiltration compared to patients with low-risk scores, which resulted in immune escape. In short, TXNRD1 is an overlooked predictor, which can be utilized as a candidate prognostic biomarker in liver cancer, and it may hold promise in drug-resistant patients.

**Keywords** TXNRD1 · Liver cancer · Ferroptosis · Prognostic biomarker · Immune escape

**Abbreviations**

- HCC: Hepatocellular carcinoma
- LIHC: Liver hepatocellular carcinoma
- CHOL: Cholangiocarcinoma
- ESCA: Esophageal carcinoma
- HNSC: Head and neck squamous cell carcinoma
- LUAD: Lung adenocarcinoma
- LUSC: Lung squamous cell carcinoma
- STAD: Stomach adenocarcinoma
- TIMER: Tumor Immune Estimation Resource
- RNA-seq: RNA sequencing
- TCGA: The Cancer Genome Atlas
- OS: Overall survival
- PFS: Progression-free survival
- RFS: Relapse-free survival
- HR: Hazard ratio
- LASSO: The least absolute shrinkage and selection operator
- ROC: Receiver operating characteristic curves
- IHC: Immunohistochemistry
- FRGs: Ferroptosis pathway-related marker genes

**Introduction**
Liver cancer is the sixth most common cancer worldwide according to GOLOBOCAN 2018 [1], with a high mortality rate, of which hepatocellular carcinoma (HCC) is the main histological type (accounting for ~75–85% of all liver cancer cases), making primary liver cancer a major threat to global human health [2]. The continuous progression of cirrhosis tends to contribute to the development of liver cancer, and chronic hepatitis virus and alcohol are the riskiest triggers for the above disease states [3]. Although the treatment for liver cancer has been greatly improved, a substantial proportion of patients still face conditions such as inadequate treatment or recurrence [4]. Currently, the lack of effective prognostic methods makes it impossible for clinicians to make accurate judgments on the prognosis of liver cancer patients, and effective individualized treatments are difficult to be carried out subsequently. Therefore, there is an urgent demand to develop prognostic predictors and therapeutic targets of liver cancer patients, which is of great clinical significance to improve the treatment outcome of liver cancer.

Thioredoxin reductase exists as a 55–60 kDa homo-dimer and is a member of the pyridine nucleotide-disulfide oxidoreductases family, which is highly conserved among species and widely expressed in organisms at all levels from prokaryotes to humans [5]. TXNRD1 relies on thioredoxin as a substrate and NADPH for their reducing equivalents, collectively constituting an independent and complete redox regulatory system in living organisms [6-8]. The thioredoxin (Trx) system plays a crucial role in maintaining intracellular redox homeostasis and participates in a variety of cellular activities in the body, such as regulation of apoptosis and transcription factor activity [9, 10]. Furthermore, this system performs biological functions that not only act on normal cells, but it also engages in the development of cancer [11]. A substantial number of studies have identified altered levels of TXNRD1 expression in diverse tumors, demonstrating that TXNRD1 has an essential role in tumor metabolism, metastasis, and other behaviors [12-14]. This makes the thioredoxin system a target for cancer drug development, which is expected to drive progress in cancer therapy. As a result, various inhibitors targeting the thioredoxin system have been developed, with small molecule compounds targeting TXNRD1 achieving considerable effectiveness in
suppressing tumor progression [15-17]. However, there is still no effective indicator to
detect the prognostic condition of tumor patients, it is promising to establish a
prognostic model centered on TXNRD1.

The precise mechanism of TXNRD1 regulation in tumor progression is unclear,
but existing studies suggest that this redox system is inextricably linked to ROS [18-
20]. Intriguingly, recent studies have identified ferroptosis as a novel form of cell death,
while the most dominant feature of ferroptosis when it occurs is unrestricted lipid
peroxidation [21-25]. Moreover, a considerable number of ferroptosis inducers have
been developed, leading to quite encouraging results in the study of tumor drug
resistance. Thus, ferroptosis is highly expected in tumor therapy research [26, 27]. The
main mechanism of ferroptosis is the oxidation of lipids in the cell by ROS in the
presence of large amounts of iron along with an imbalance in intracellular redox
homeostasis. Large amounts of lipid peroxides accumulate in the cell membrane,
disrupting the membrane integrity and thus inducing cell death [21]. In addition, Tan et
al. discovered that hepcidin may affect prognosis partially by regulating immune
infiltration in lung cancer patients and excess iron can induce the Fenton reaction to
produce more ROS, which creates a vicious cycle [22, 23]. Apart from that, TXNRD1
not only serves an essential function in maintaining intracellular redox homeostasis but
has recently been discovered to catalyze the generation of cystine to cysteine in the
pivotal ferroptosis pathway, acting as a major player in the initiation phase of
ferroptosis [21]. Therefore, it is tempting to assume that there is a considerable
association between TXNRD1, ROS, and ferroptosis, which likewise contributes to our
understanding of the complex tumor metabolic environment.

In recent years, research on tumor therapy targeting TXNRD1 or ferroptosis has
been widely conducted, yet little data are available on the role and clinical significance
of TXNRD1 in the prognosis of various cancers such as liver cancer. Therefore, in
response to the current lack of effective indicators for monitoring the prognosis of
cancer patients, it is quite feasible to build a prognostic model based on ferroptosis and
centered on TXNRD1. Our current study focuses on the utilization of multiple
bioinformatics approaches to investigate whether TXNRD1 is involved in liver cancer
progression and whether TXNRD1 along with ferroptosis-related genes can be used as prognostic indicators to enable clinicians to assess the outcome of patients after treatment.

In this study, we identified that TXNRD1 expression was upregulated in hepatocellular carcinoma tissues compared to normal liver tissues. In addition, TXNRD1 expression level was correlated with tumor histological grade and pathologic stage of patients. According to the survival curves, patients with elevated TXNRD1 had a poorer prognosis. Of more significance, the prognostic model we developed demonstrated terrific results in predicting the survival of patients with liver cancer, whereby patients with high-risk scores were distinguished remarkably from those with low-risk scores. Subsequent functional analyses indicate that alterations in macrophage polarization may play a crucial role in modulating ferroptosis of hepatocellular carcinoma cells and promoting immune evasion, ultimately contributing to unfavorable clinical outcomes. These observations highlight the crucial role of TXNRD1 in hepatocarcinogenesis and suggest that TXNRD1 may be instrumental in regulating the progression of ferroptosis in liver cancer.

Materials and Methods

Bulk RNA sequencing and single-cell RNA sequencing analysis of hepatocellular carcinoma and ferroptosis genes acquisition

The bulk RNA sequencing (RNA-seq) data about liver hepatocellular carcinoma (LIHC) was obtained from UCSC (http://xena.ucsc.edu), which shows the whole gene-level transcription estimates. Following the established nomenclature guidelines for TCGA samples, we have obtained comprehensive expression profiles for a total of 373 liver cancer tissue samples and 50 normal liver tissue samples. Similarly, we also used UCSC to obtain the patient's complete clinical information. The following were the criteria for inclusion: (1) patients were diagnosed with liver cancer; (2) patients’ clinical information was complete. According to the criteria, a total of 344 individuals met the requirements when patients were analyzed for survival, and 196 patients were included.
when clinical factors were subsequently combined. Samples with a follow-up period of less than 30 days were excluded. The single-cell RNA (scRNA) sequencing data of LIHC tissues were enrolled from two GEO datasets (GSE140228 and GSE166635) and analyzed in the Tumor Immune Single-cell Hub (TISCH) database (http://tisch.comp-genomics.org/home/). And 40 ferroptosis-related marker genes (FRGs) were derived from FerrDb database (http://www.zhounan.org/ferrdb).

**Gene Expression Profiling Interactive Analysis**

GEPIA (https://gepia2.cancer-pku.cn/) is an interactive web portal for analyzing gene sequencing expression data from The Cancer Genome Atlas (TCGA) and the Genotype Tissue Expression (GTEx) projects. In this study, the expression analysis of TXNRD1 was examined using TCGA-LIHC datasets. In the module “Expression DIY”, the expression of TXNRD1 between LIHC and normal adjacent liver tissue samples was investigated with the option of matching TCGA normal and GTEx data, and the cutoffs of log2FC and p-value were set to 1 and 0.01, respectively.

**LinkedOmics**

LinkedOmics (www.linkedomics.org) is an available portal that includes data from all The Cancer Genome Atlas (TCGA) and Clinical Proteomics Tumor Analysis Consortium (CPTAC) cancer cohorts, which provides a platform for biologists and clinicians to access and analyze multi-omics data among different tumors. In the current study, to identify differentially expressed genes in our analysis, we applied a rigorous screening criterion, with thresholds set at |Log2FC| > 1 and adjusted p< 0.05. The gene clusters associated with TXNRD1 expression were explored through this website and a heat map was created to show the genes that were differently expressed along with TXNRD1.

**Tumor Immune Estimation Resource**

TIMER (https://cistrome.shinyapps.io/timer/) is a user-friendly web portal, which focuses on immune infiltration analysis of various tumors using high-throughput
sequencing data and allows the analysis of expression profile data collected in TCGA. In this study, we aimed to elucidate the biological function of TXNRD1 in tumor progression. We utilized TIMER to investigate the expression patterns of TXNRD1 across a diverse range of cancer types, and TXNRD1 expression was evaluated through the “Diff Exp” module.

**Genecards**

Genecards (www.genecards.org/) is a comprehensive, searchable database, which is composed of all annotated and predicted human genomic, transcriptomic, proteomic information. The section of the expression module contains expression images based on data from GTEx, BioGPS, Illumina Human BodyMap, and SAGE, etc. We use it to display TXNRD1 in various normal human tissues.

**UALCAN**

UALCAN (http://ualcan.path.uab.edu/index.html) is an efficient cancer data mining website, based on the relevant cancer data from the TCGA, which can be carried out identifying biomarkers, expression profiles and survival analysis, etc. In this study, we aimed to characterize the expression profile of TXNRD1 and its potential clinical significance in hepatocellular carcinoma. We employed the UALCAN to investigate the relationship between TXNRD1 expression and various clinicopathological parameters, including gender, cancer stage, and tumor grade. Additionally, we assessed the prognostic value of TXNRD1 in liver cancer, with the goal of identifying novel biomarkers and therapeutic targets for this deadly disease.

**Kaplan-Meier Plotter Database Analysis**

KM-plotter (https://kmplot.com/analysis/), a website including survival information of 364 clinical liver cancer patients, was applied to explore the relationship between TXNRD1 and the patient’s prognosis. The liver patient samples were divided into high and low groups by median expression to analyze the overall survival (OS), progression-free survival (PFS) and relapse-free survival (RFS) with hazard ratios (HRs) and log-
In addition to accessing the online website, we also plotted relevant survival curves through R packages such as the “survival”, “survminer” and “time ROC” to predict and present the survival of patients with liver cancer under variable conditions.

**Function and Pathway Enrichment Analysis**

The biological function of TXNRD1 was investigated by conducting an enrichment analysis of KEGG pathways and GOs using the "clusterProfiler" package of R, with a p-value threshold of less than 0.05. Further, GSEA analysis was performed using R software to explore pathway and functional differences between two groups of patients with high and low risk. The reference gene sets (c5.go. v2022.1.Hs. symbols) for this analysis were obtained from the GSEA database (https://www.gsea-msigdb.org/gsea/msigdb).

**GeneMANIA and STRING**

GeneMANIA database (http://genemania.org) can be used to discover protein-protein, protein-DNA interactions, and pathways, etc. The STRING online database (https://string-db.org/) can also be applied to construct a protein-protein interaction (PPI) network. We utilize the above two databases to explore possible TXNRD1 interacting proteins and genes and to provide a basis for unraveling the capability of TXNRD1.

**Correlation Analysis between TXNRD1 and Ferroptosis-Related Genes**

Pearson method was applied to characterize the correlation of TXNRD1 expression with FGRs and to determine tentatively whether TXNRD1 mediates the ferroptosis resistance in hepatocellular carcinoma cells. We extracted the RNA expression profiles of hepatocellular carcinoma patients downloaded from the UCSC database and obtained 40 marker genes of the ferroptosis pathway from the FerrDb database. Based on Pearson correlation analysis, we identified the correlation between 40 FRGs and TXNRD1 in LIHC. Only FRGs with |r| > 0.5 and P < 0.001 were considered relevant.

**Identification and Construction Prognostic Model Related to TXNRD1 and**
**Ferroptosis**

The prognostic value of TXNRD1 and FRGs was initially determined using univariate Cox regression. The least absolute shrinkage and selection operator (Lasso) regression were used to integrate those genes with $P<0.05$ in univariate analysis. The results of Lasso were then included in a multivariate Cox model to get a risk score. We calculated a risk score using a linear combination of TXNRD1 and FRGs expression levels multiplied with a regression coefficient ($\beta$). Patients were divided into two groups based on their median risk score. The log-rank test was used to compare the survival differences between the two groups. The correctness of the model was accessed using the concordance index (C-index), calibration curves and receiver operating characteristic (ROC) curves. Meanwhile, a nomogram model was established based on the results of the multivariate Cox regression analysis to provide a more accurate prediction of LIHC patient prognosis. To see if the risk score was an independent predictor of prognosis, we incorporate the clinical information data into multivariate Cox regression. Subsequently, to validate the predictive accuracy of the nomogram, a calibration graph was generated to illustrate the level of concordance between the predicted and observed survival rates of LIHC patients.

**Immune cell infiltration analysis and tumor-related immune function prediction**

Using the CIBERSORT algorithm, we employed the gene expression profiles of 344 liver cancer patients to predict and determine the proportion of 22 immune cells crucial to the tumor microenvironment of each patient. This was followed by an analysis to identify the infiltrating cells and immune functions that had a negative impact on the prognosis of patients in two groups with varying levels of risk. Subsequently, the TIDE algorithm (http://tide.dfci.harvard.edu) has been utilized for the purpose of predicting the immune escape functionality of neoplastic cells and exploring which immune cells contributed to the divergent prognoses observed between two groups of patients.

**Immunohistochemistry (IHC) Staining**

IHC assays were performed on liver cancer tissue microarray chips with commercial
rabbit polyclonal primary antibody against TXNRD1 (1:600, 11117-1-AP, proteintech). And all IHC results were assessed by two independent pathologists blinded to both the sample origins and the subject outcomes. Subsequently, all fields were observed under microscopy (Olympus 600 auto-biochemical analyzer, Tokyo, Japan) with recording and evaluation of the IHC results.

**Western blotting**

HepG2 and Huh7 Cells were suspended in lysis buffer (50 mM Tris-HCl pH 8.0, 1% SDS, 1 mM EDTA, 5 mM DTT and protease inhibitor cocktail). The cellular lysates were centrifuged at 12000 rpm for 15 min and then denatured by heating for 10 min. The liver cancer tissues, and corresponding normal tissues were processed by ultrasonic fragmentation, then the pure total protein was extracted using the same method described above. The protein concentration was determined by BCA assay. For the detection of TXNRD1, the cell lysates or tissue lysates were separated using 10% SDS-PAGE and then electroblotted onto PVDF membrane. Western blotting was performed using anti-TXNRD1 (1:5000) and anti-GAPDH antibodies (1:10000).

**Cell culture and treatment**

L02, HepG2 and Huh7 Cells were obtained from the American Type Culture Collection (USA). These hepatoma carcinoma cells were routinely cultured in a Forma™ direct heat CO2 incubator (Thermo Scientific, USA) under conditions of 37°C, 5% CO2. Both cells were cultured with DMEM medium containing 10% fetal bovine serum. The cell culture flasks were incubated at 37°C with 5% CO2 and the medium was renewed every two days. Erastin (MCE HY-15763) / ferrostatin-1 (ABclonal RM02804) of 1 mg dissolved in 1.828 / 1.9059 mL DMSO respectively to a working concentration of 1 mM/10mM and stored in a -20°C refrigerator. Subsequent dilutions of the working solution were used to treat the cells, and the variation of TXNRD1 were detected by Western blot.

**Tissue samples**

Matched primary cancer tissues and their corresponding adjacent NT tissues were
collected from liver cancer patients at the Nanjing First Hospital. We obtained informed consent from each patient and obtained approval from the Internal Review and Ethics Boards at Nanjing First Hospital to collecting tissue specimens for our research study. Tissue microarray chips containing 18 pairs of LIHC samples matched to their adjacent NT tissue samples and the associated clinicopathological information were purchased from Servicebio (Wuhan).

**Statistical Analysis**

The survival curves were created using the Kaplan-Meier method, and the log-rank test was performed to assess them. TXNRD1 and ferroptosis-related genes signatures and clinical data were used to predict the prognostic effect through Cox and Lasso regression. The statistical analyses were carried out using the R programming language (version 4.0.1). The statistical tests were bilateral with a significance level of P<0.05 (*P < 0.05, **P < 0.01, and ***P < 0.001).

**Results**

**TXNRD1 Expression Profiles Under Normal and Tumor Conditions**

TXNRD1 has been identified in various normal human tissues including immune, muscle tissues, and reproductive tissues. (Fig. 1a). We firstly compared the transcriptional levels of TXNRD1 in cancers by using the Tumor Immune Estimation Resource (TIMER) online database. Higher expression of TXNRD1 was observed in cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and stomach adenocarcinoma (STAD) compared with the corresponding normal tissues (Fig. 1b). Consistently, we discovered that the mRNA expression levels of TXNRD1 were considerably upregulated in LIHC patients in the gene expression profiling interactive analysis (GEPIA) and UALCAN databases (Fig. 1c). In addition, we also found this variation in The Cancer Genome Atlas (TCGA) liver cancer statistics. Meanwhile, the protein expression of TXNRD1 was further investigated in liver cancer by IHC staining. The TXNRD1 protein levels
were discovered to be significantly higher in liver cancer tissues than in normal liver tissues (Fig. 1d). In conclusion, these results show that TXNRD1 expression is elevated in liver cancer and TXNRD1 may play a critical regulatory function in liver cancer progression.

**TXNRD1 Expression Associates with Tumor Grade, Tumor Stage and Poor Prognosis in Liver Cancer Patients**

We explored the oncogenic role of TXNRD1 across the UALCAN online database and found that TXNRD1 expression was markedly upregulated in LIHC patients in stages 1, 2 and 3 (Fig. 2a). In terms of tumor grade, there was a considerable increase in LIHC based on tumor grade (Fig. 2b). Apart from that, in liver cancer samples from both males and females, TXNRD1 expression was substantially increased when compared to the normal samples (Fig. 2c). We further explored the vital prognostic value of TXNRD1 in the survival of patients with liver cancer. According to the publicly available databases: Kaplan-Meier plotter database and UALCAN, liver cancer patients with higher TXNRD1 gene expression had worse OS, PFS and RFS (Fig. 2d). Moreover, we evaluated the prognosis of liver cancer patients by combining TXNRD1 expression levels with the degree of obesity and tumor grade by using the UALCAN online tool. The KM curve with log-rank test analyses showed that increased TXNRD1 expression levels, obesity coupled with higher tumor grade were closely associated with poor survival (Fig. 2e).

**Identification of TXNRD1-Interacting Genes and Proteins**

To better appreciate the biological function of TXNRD1 in liver cancer, we built a gene-gene interaction network for TXNRD1 with changed neighboring genes by using GeneMania. The results revealed the 20 most frequently changed genes, which were strongly linked to TXNRD1, including TXN, STOML3 and SDHA (Fig. 3a). Meanwhile, we utilized the STRING database to construct a protein-protein interaction (PPI) network of TXNRD1 and a total of 11 proteins associated with TXNRD1 were found (Fig. 3b). Then we analyzed the RNA-seq of liver cancer patients in the
LinkedOmics database. Correlation coefficients between TXNRD1 and other genes were calculated and plotted the heat map based on Pearson correlation analysis. TXNRD1 was altered in 371 samples with liver hepatocellular carcinoma, and the heatmaps only show 50 positively or negatively correlated significant genes, respectively (Fig. 3c). The role of TXNRD1 and the genes strongly correlated with TXNRD1 alterations were anticipated by KEGG and GO enrichment analyses in the Database for Annotation, Visualization, and Integrated Discovery (DAVID). The most relevant terms of BP enrichment analysis and the KEGG pathways for TXNRD1 and its associated genes are shown in Figure 3D and E. Notably, the results indicate that TXNRD1 is mainly to be involved in glucose and fat metabolism in vivo, such as xenobiotic glucuronidation, negative regulation of fatty acid metabolic process, and pentose and glucuronate interconversions (Fig. 3d, e)

**Correlation Analysis Between TXNRD1 Expression and Ferroptosis-Related Genes**

Ferroptosis is a novel form of cell death, while its main biochemical features are disturbances in lipid metabolism and peroxidation of polyunsaturated fatty acids. (21) Therefore, we analyzed the correlation of TXNRD1 with ferroptosis pathway-related marker genes (FRGs). Heatmap results showed that TXNRD1 was negatively correlated with FRGs that promote ferroptosis progression in tumor cells, such as GPX4 and P53, indicating that TXNRD1 is a detrimental factor for patients with liver cancer, consistent with previous results (Fig. 3f). Notably, the development of a prognostic model related to TXNRD1 and ferroptosis is clinically significant for predicting the survival of patients with hepatocellular carcinoma.

**Identification of Prognostic TXNRD1-Related and ferroptosis-based Signature**

Firstly, univariate Cox analysis was performed on TXNRD1 and 40 FRGs, and the results showed that TXNRD1 and 29 FRGs had a prognostic value for patients with liver cancer (P < 0.05). Subsequently, we conducted Lasso regression and Multivariate Cox regression of 30 genes, and it was found that 8 genes were independent prognostic
factors, including TXNRD1 (Fig. 4a, b, Table 1). Half of them were harmful prognostic factors (TXNRD1, ATG5, PCBP2 and SLC7A11) and the others (ACSL6, SAT1, SLC40A1 and STEAP3) were favorable factors. According to their expression in liver cancer patients, we have drawn survival curves to better illustrate their impact on the prognosis of liver cancer patients (Fig. 4c). Eventually, we calculate the formula of the risk score and plot a risk survival curve to assess the prognosis of patients with liver cancer (Fig. 4d). It is gratifying that the risk model was able to significantly distinguish between liver cancer patients with well and poor prognosis (Fig. 4e, f). The high-risk group had a shorter OS compared with the low-risk group (P<0.001). The formula of the risk score was as follows: risk score = (-0.13414* ACSL6) + (0.40566* ATG5) + (0.39727* PCBP2) + (0.36062* SAT1) + (0.163067* SLC40A1) + (0.078143* SLC7A11) + (-0.09215* STEAP3) + (0.23196* TXNRD1).

**Clinical Prognostic Value of TXNRD1-Related and ferroptosis-based Signature**

Risk score and stage were found to be independent prognostic factors in univariate Cox regression, with a risk score HR of 1.543 (95% CI: 1.356–1.756, P <0.001, Fig. 5a). Through multivariate Cox regression analysis, the risk score was the single independent prognostic predictor (HR =1.465, 95% CI: 1.271–1.688, P<0.001, Fig. 5b, Table 2). Moreover, we plotted ROC curves based on this risk score to evaluate its predictive efficiency. The results suggested that when the risk score was applied to predict the prognosis of patients with liver cancer, its predictive efficacy reached a maximum of 0.763 in the first year and decreased year by year thereafter. And the areas under the ROC curve corresponding to 1 year, 4 years, and 5 years of survival were 0.763, 0.748, and 0.679, respectively (Fig. 5c). Subsequently, we constructed a nomogram based on risk score, gender and TNM stage. According to the nomogram, we observed that the risk score and TNM stage contributed the greatest to the 1-year, 3-year, and 5-year overall survival of patients with hepatocellular carcinoma (Fig. 5d). The ROC curves based on the prognostic model and the clinicopathologic parameters showed that risk score (0.710) and stage (0.732) had some clinical predictive significance (Fig. 5e, Table 3). Meanwhile, the C-index of this prognostic model was 0.758, and its calibration
curve of 5-year survival prediction was close to the standard curve, which implied that the prediction results of the prognostic model are of reference significance (Fig. 5f).

**Determining the potential functional differences between the two groups of patients with differing levels of risk**

To uncover the biological processes underlying the separation of hepatocellular carcinoma patients into high and low risk groups based on TXNRD1-related and ferroptosis-based signature, we carried out GSEA to assess the differences between the two groups. The results from the GSEA indicated that patients in the high-risk group were mainly characterized by functions related to immunoglobulin complex and immunoglobulin receptor binding, whereas patients in the low-risk group were linked to processes such as fatty acid and amino acid metabolism in vivo (Fig. 6a). Of note, previous research has demonstrated that immune cells participate in the process of ferroptosis through the mediation of metabolites in tumor cell. Based on this, we sought to further evaluate immune function in both groups of patients [28, 29].

**Association between TXNRD1-Related and ferroptosis-based Signature and Immune microenvironment**

A comprehensive comparison of immune cell infiltration and immune-related functional profiles between two groups of patients with high and low risk liver cancer was performed by using the CIBERSORT algorithm. Our results revealed a significant difference in the immune cell infiltration between the two groups. Specifically, only M0 macrophages differed, with a higher proportion of infiltrated M0 macrophages observed in the high-risk group compared to the other group (Fig. 6b). The survival analysis further supported these findings, showing that patients with high infiltration of M0 macrophages had a worse prognosis (Fig. 6c). And the results of immune function prediction indicate that macrophages may be involved in the process of immune escape, contributing to the varying prognostic outcomes observed in these patients (Fig. 6d). These results suggest that high-level macrophage infiltration may promote immune escape of hepatocellular carcinoma cells, which may be the leading cause of the
disparity in prognosis between the two groups of patients.

Single-cell sequencing exploring TXNRD1-related signature and infiltrated macrophages in LIHC

Initially, we analyzed single-cell RNA-seq enrolled from the GSE140228, which was obtained from the 10× Genomics and Smart-seq2 platforms and collected 62530 and 7074 single-cell transcriptomes of CRC after quality control, respectively[30]. The above eligible single cells derived from 16 patients with liver cancer, mainly characterizing the effect of immune cells on the progression of liver cancer, were divided into 12 (10× Genomics) or 10 clusters (Smart-seq2), including T cells, B cells and monocytes/macrophages (Fig. 7a). Intriguingly, we mapped TXNRD1-related signature to single cell landscape and revealed a prominent enrichment on monocyte / macrophages populations, consistent with the results yielded by our previous CIBERSORT algorithm (Fig. 7b-d). The similar results we achieved at GSE166635[31], where TXNRD1-related signature was mainly enriched in hepatoma cells and monocytes/macrophages (Fig. 7a, b). Therefore, we speculated that monocyte-derived macrophages may also be reprogrammed by ferroptosis to promote immunosuppressive microenvironment formation and facilitate the development of TXNRD1-protected hepatocellular carcinoma cells. Based on previous studies that the ferroptosis program is subject to modulation by the glycometabolism, lipometabolism and TP53[21, 29], in which we aimed to identify whether the indicated gene sets were enriched in macrophages. Consistently, our single-cell sequencing results suggested that the ferroptosis-associated gene sets are associated with our target cells, including hallmark_P53_pathway, hallmark_fatty_acid_metabolism, etc (Fig. 7e). Overall, scRNA-seq study further elaborated that TXNRD1 and ferroptosis program not only act in hepatocellular carcinoma cells, but also exert effect on macrophage.

TXNRD1 is dramatically consumed after cellular triggering of ferroptosis

To validate and corroborate the robustness and reliability of the above analysis, we examined the protein expression levels of TXNRD1 in three pairs of matched primary
hepatocellular carcinoma tissues and corresponding normal tissues, as well as in hepatocellular cell lines (L02, HepG2 and Huh7). The results showed that TXNRD1 was upregulated in hepatocellular carcinoma tissues and cell lines, which was consistent with the analysis in the database (Fig. 8a). To investigate whether TXNRD1 is involved in regulating the pathway of ferroptosis, we treated HepG2 and Huh7 cells with 1μM, 5μM and 10μM ferroptosis inducer Erastin or 1μM inhibitor ferrostatin-1 and verified the function of TXNRD1 by detecting the expression of TXNRD1 after 24h of treatment, respectively. And the concentration of reagents was added as described in previous study [32]. The results showed that Erastin induced ferroptosis burst and accelerated the excessive accumulation of intracellular oxidative substances, which would entail massive depletion of TXNRD1, leading to inhibition of cell growth and eventually causing cancer cell death (Fig. 8b). In contrast, TXNRD1 returned to its original level after the application of ferrostatin-1 treatment (Fig. 8b). This suggests that TXNRD1 has a regulatory relationship with the ferroptosis pathway in hepatocellular carcinoma, and inhibition of TXNRD1 facilitates the death of tumor cells through ferroptosis with prolonging the survival of patients, but the exact mechanism needs to be explored in detail. To conclude, based on these results, we propose that liver tumor cells, to maintain intracellular redox homeostasis, require upregulation of the expression of TXNRD1 to cope with cellular damage by superoxide substances such as ROS and to prevent triggering the process of ferroptosis. Moreover, as carcinoma advanced, viable tumor cells would further engage in immune escape and other behaviors with the help of macrophages, which exacerbated the deterioration of hepatocellular carcinoma patients (Fig. 8c). This finding is highly relevant for understanding the pathogenic function of TXNRD1 during liver cancer progression.

Discussion

Despite advances in the diagnosis and treatment of cancer, patients with liver cancer still suffer from recurrence and metastasis after treatment, which is one of the main reasons for the poor survival rate of cancer patients [4]. As a result, it's critical to investigate the mechanisms that contribute to the progression of hepatocellular
carcinoma and to develop effective lung cancer prognostic indicators. In the present study, we identified that the expression of TXNRD1 was upregulated in hepatocellular carcinoma tissues compared to normal liver tissues employing bioinformatics analysis of the TIMER, GEPIA, UALCAN and Genecards databases. The clinical prognostic relevance of TXNRD1 in liver cancer patients was then examined. The results revealed that expression of TXNRD1 was upregulated as the tumor histological grade and pathologic stage advanced. In addition, Kaplan-Meier survival analysis showed that patients with high TXNRD1 expression had a worse survival period compared to those with low TXNRD1 expression, and it was also found that patients with obesity based on high TXNRD1 expression exhibited poorer survival according to the patient samples in the UALCAN database. Through Western blot and IHC, we as well confirmed that TXNRD1 expression was upregulated in liver cancer tissues and cells. In conclusion, these results suggest that TXNRD1 can be used as a potential prognostic marker for liver cancer.

The Trx system is not the only system involved in maintaining redox homeostasis in cells, but the key regulator is the GSH system, and GSH is the most common elevated metabolite detected during cellular oxidative stress and participates in many cellular metabolic processes [33-36]. On the one hand, the high oxidative state in tumor cells leads to a compensatory increase in TXNRD1 and GSH, while on the other hand, these two proteins confer a certain therapeutic resistance to tumor cells, thus enabling the promising progress of anticancer drugs targeting both Trx and GSH systems [15, 37]. More importantly, both TXNRD1 and GSH are engaged in the canonical ferroptosis-controlling axis, and intracellular cysteine is reduced by TXNRD1 for GSH synthesis [21]. Although the roles of TXNRD1 and GSH in physiology and cancer progression are linked, the importance of TXNRD1 for cells is not comparable to that of GSH, and therefore less attention has been paid to TXNRD1 by researchers, which may also lead us to overlook it as a good prognostic marker.

A sea of mechanistic studies has revealed that many protooncogenes and signaling pathways can regulate the ferroptosis, including P53, AMPK pathways [38-41]. Those cancer cells that are resistant to conventional treatment also show high sensitivity to
ferroptosis, therefore, ferroptosis-based cancer therapy shows great promise [32, 42-45]. Lin et al. discovered that ferroptosis did not occur in cysteine depleted CML cells, but was triggered by applying TXNRD1 inhibitor auranofin, demonstrating that TXNRD1 is a pivotal regulator involved in ferroptosis [45]. As a renowned tumor suppressor, the involvement of p53 in ferroptosis has been intensively investigated, while our analysis also found a negative correlation between TXNRD1 and P53 expression in hepatocellular carcinoma, suggesting that TXNRD1 may inhibit ferroptosis, and combined with clinicopathological data all indicate that TXNRD1 is a risk factor. Employing a dose-dependent strategy, we assessed the role of TXNRD1 in ferroptosis in two hepatoma cell lines, namely HepG2 and Huh7, by exposing them to ferroptosis inducers and inhibitors. By monitoring alterations in intracellular levels of TXNRD1, we investigated the potential involvement of TXNRD1 in the ferroptosis in HCC. Our findings unequivocally demonstrate that as cellular ferroptosis occurred and TXNRD1 was continuously exhausted. Furthermore, we performed Lasso regression and Multivariate Cox regression to obtain the following 7 prognostic ferroptosis-related genes: ATG5, PCBP2, SLC7A11, ACSL6, SAT1, SLC40A1 and STEAP3. Subsequently, we assessed this prognostic model consisting of TXNRD1 and 7 ferroptosis-related genes, which can accurately predict the prognostic risk of patients with liver cancer. Thus, these results highlighting its potential as a therapeutic target in HCC.

Through GSEA analysis, we have identified discernible variances in biological functions pertaining to immune function and tumor metabolic activity among two groups stratified by their risk level. Given that ferroptosis is a consequence of dysregulated redox mechanisms reliant on lipid metabolism, we postulate that tumor cells upregulate the expression of TXNRD1 to impede excessive intracellular oxidant accumulation and cellular damage. Yang et al. found evidence to support the involvement of macrophages in regulating metabolic pathways and initiating immune responses to maintain homeostasis [46]. Additionally, they concluded that macrophages in differing polarization states can significantly impact the tumor microenvironment, leading to the induction of ferroptosis in cancer cells. These findings shed new light on
the complex interplay between macrophages and cancer cells and led to a surge in research that aims to modify macrophage polarization to treat cancer. Our findings concerning the analysis of immune cell infiltration and function in high and low-risk groups align with this trend. We observed a noteworthy distinction between these two groups for only M0 macrophages, where higher infiltration corresponds to a poorer prognostic outcome. And we speculate that this could be linked to macrophages promoting tumor immune escape behavior. Therefore, it was necessary to establish the prognostic model by integrating TXNRD1 and ferroptosis-related genes, and which has a reliable theoretical basis for forecasting patient prognosis.

Current studies have enhanced our understanding of the association between TXNRD1 and liver cancer, but some limitations should be acknowledged. First, the number of samples incorporated was relatively small and lacked credibility. Second, the current application of this prognostic model is clinically relevant only for patients with primary hepatocellular carcinoma. The prognostic model may not be applicable if the pathological classification is mixed liver carcinoma or liver metastases caused by other types of cancer. Finally, we found that TXNRD1 is indeed associated with key ferroptosis-related genes, but the specific mechanism of how TXNRD1 regulates liver cancer progression through the ferroptosis-controlling axis deserves to be explored. Therefore, these findings not only contribute to our current comprehension of the role of TXNRD1 in cancer and have potential value for its translational application in the prognosis and treatment of liver cancer.

**Conclusions**

In conclusion, the TXNRD1-related and ferroptosis-based signature can validly predict prognosis and indicate progression in patients with hepatocellular carcinoma. Ferroptosis can kill tumor cells efficiently, but TXNRD1 provides protection against this process for cancer cells, thus the prospect of developing drugs targeting TXNRD1 in combination with ferroptosis inhibitors to slay tumor cells shows promising clinical applications in LIHC.
Acknowledgements We are very grateful to The Cancer Genome Atlas for providing RNA-seq data of LIHC patients.

Author Contributions J.Nie, H.Sun, S.Wang, and Y.Pan designed research; H.Wang, P.Tan and T.Gao collected and analyzed data; H.Wang and X.Liu edited the report for intellectual content; J.Nie wrote the report; All authors read and approved the final report.

Funding This work was supported by grants from Jiangsu Provincial Key Research and Development Plan (Grant No. BE2019614), Jiangsu Provincial Medical Key Discipline Cultivation Unit (JSDW202239), Key Project of Science and Technology Development of Nanjing Medicine (ZKKX21042), Elderly Health Research Project of Jiangsu Province (Grant No. LR2021017) and Specialized Cohort Research Project of Nanjing Medical University (NMUC2020035, NMUC2021013A).

Availability of data and material The original contributions presented in the study are included in the article. All data generated or analyzed during this study can be achieved from UCSC (http://xena.ucsc.edu). Further inquiries can be directed to the corresponding author.

Declarations

Conflicts of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics approval and consent to participate The study was conducted in accordance with the Declaration of Helsinki and approved by the Internal Review and Ethics Boards at Nanjing First Hospital (KY20220124-04).

Consent to publish This manuscript does not contain any individual person’s data in any form (including any individual details, images or videos). So the “Consent for publication” is not appropriate for our manuscript.
References


16. Sabatier P, Beusch CM, Gencheva R et al: Comprehensive chemical proteomics analyses reveal that the new TRi-1 and TRi-2 compounds are more specific thioredoxin reductase 1 inhibitors than auranofin. Redox Biol 2021, 48:102184.

17. Bjorklund G, Zou L, Wang J, Chasapis CT, Peana M: Thioredoxin reductase as a


37. Benhar M, Shytaj IL, Stamler JS, Savarino A: Dual targeting of the thioredoxin and glutathione


### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Coefficient</th>
<th>HR</th>
<th>95%CI of HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSL6</td>
<td>-0.134</td>
<td>0.874</td>
<td>0.810—0.944</td>
</tr>
<tr>
<td>ATG5</td>
<td>0.406</td>
<td>1.500</td>
<td>1.030—2.184</td>
</tr>
<tr>
<td>PCBP2</td>
<td>0.397</td>
<td>1.488</td>
<td>0.991—2.235</td>
</tr>
<tr>
<td>STA1</td>
<td>-0.361</td>
<td>0.697</td>
<td>0.547—0.889</td>
</tr>
<tr>
<td>SLC40A1</td>
<td>-0.163</td>
<td>0.849</td>
<td>0.682—1.059</td>
</tr>
<tr>
<td>SLC7A11</td>
<td>0.078</td>
<td>1.081</td>
<td>0.986—1.186</td>
</tr>
<tr>
<td>STEAP3</td>
<td>-0.092</td>
<td>0.912</td>
<td>0.807—1.030</td>
</tr>
<tr>
<td>TXNRD1</td>
<td>0.231</td>
<td>1.261</td>
<td>1.051—1.513</td>
</tr>
</tbody>
</table>

### Table 2

Clinical characteristics and risk scores of LIHC using multivariate Cox
regression.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Z</th>
<th>HR</th>
<th>HR95L</th>
<th>HR95H</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.005</td>
<td>0.010</td>
<td>0.466</td>
<td>1.005</td>
<td>0.985</td>
<td>1.025</td>
<td>0.641</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.030</td>
<td>0.296</td>
<td>-0.102</td>
<td>0.970</td>
<td>0.543</td>
<td>1.734</td>
<td>0.919</td>
</tr>
<tr>
<td>Stage</td>
<td>0.394</td>
<td>0.568</td>
<td>0.693</td>
<td>1.482</td>
<td>0.487</td>
<td>4.515</td>
<td>0.488</td>
</tr>
<tr>
<td>T</td>
<td>0.182</td>
<td>0.532</td>
<td>0.343</td>
<td>1.200</td>
<td>0.423</td>
<td>3.402</td>
<td>0.732</td>
</tr>
<tr>
<td>M</td>
<td>0.374</td>
<td>0.684</td>
<td>0.546</td>
<td>1.453</td>
<td>0.380</td>
<td>5.556</td>
<td>0.585</td>
</tr>
<tr>
<td>N</td>
<td>0.083</td>
<td>1.027</td>
<td>0.081</td>
<td>1.087</td>
<td>0.145</td>
<td>8.139</td>
<td>0.935</td>
</tr>
<tr>
<td>Risk Score</td>
<td>0.382</td>
<td>0.072</td>
<td>5.268</td>
<td>1.465</td>
<td>1.271</td>
<td>1.688</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

**Table 3** Clinical influences of risk score signature for TCGA-LIHC data.

<table>
<thead>
<tr>
<th>Clinical</th>
<th>n</th>
<th>Risk score</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤60</td>
<td>86</td>
<td>1.203</td>
<td>0.944</td>
<td>-1.730</td>
</tr>
<tr>
<td>&gt;60</td>
<td>110</td>
<td>1.522</td>
<td>1.569</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>63</td>
<td>1.148</td>
<td>0.89</td>
<td>-1.994</td>
</tr>
<tr>
<td>Male</td>
<td>133</td>
<td>1.492</td>
<td>1.516</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>134</td>
<td>1.176</td>
<td>1.195</td>
<td>-2.907</td>
</tr>
<tr>
<td>III-IV</td>
<td>62</td>
<td>1.827</td>
<td>1.565</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>97</td>
<td>0.977</td>
<td>0.724</td>
<td>-4.361</td>
</tr>
<tr>
<td>T2-4</td>
<td>99</td>
<td>1.779</td>
<td>1.677</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>193</td>
<td>1.388</td>
<td>1.363</td>
<td>1.582</td>
</tr>
<tr>
<td>M1</td>
<td>3</td>
<td>0.972</td>
<td>0.423</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>193</td>
<td>1.363</td>
<td>1.351</td>
<td>-1.878</td>
</tr>
<tr>
<td>N1</td>
<td>3</td>
<td>2.599</td>
<td>1.127</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1 Thioredoxin reductase (TXNRD1) expression profile under physiological and oncological conditions. (a) Bar plot of TXNRD1 mRNA expressions in various normal human tissues from the GTEx database. (b) TXNRD1 expression in different types of cancer was investigated with the TIMER database. (c) TXNRD1 expression in liver cancer was studied by using the GEPIA and the UALCAN database. (d) Immunohistochemical staining of TXNRD1 was performed in liver cancer and normal liver tissues. Representative images are shown. Scare bars, 50 μm. *p < 0.05, **p < 0.01, ***p < 0.001.
Fig. 2 The Box plots and KM plot investigating TXNRD1 expression among diverse groups of patients based on clinical parameters using the UALCAN and Kaplan-Meier Plotter database. Results are shown for tumor pathologic stage (a), histological grade (b) and sex (c). (d) The Survival curves using the Kaplan-Meier plotter are shown for OS, PFS and RFS. (e) Effect of TXNRD1 expression level, body weight and tumor grade on OS of patients with Liver cancer.
Fig. 3 Functional analysis of TXNRD1-related genes and proteins. (a) The gene-gene interaction network of TXNRD1 was developed using GeneMania. (b) The PPI network of TXNRD1 was generated using STRING. (c) Heat maps showing the top 50 genes positively or negatively correlated with TXNRD1 in LIHC. (d) GO analysis of TXNRD1-related genes. (e) KEGG pathway enrichment analysis of TXNRD1-related genes. (f) Correlation of TXNRD1 expression with FRGs in LIHC.
Fig. 4 Constructing a prognostic signature of LIHC patients. (a) TXNRD1 and ferroptosis-related mark gene selection utilizing Lasso model. The vertical dashed lines are at the optimal lambda value. (b) The forest plots for multivariate Cox regression
analysis of TXNRD1 and seven ferroptosis-related mark genes in LIHC. (c) The KM survival curves of seven prognostic ferroptosis-related mark genes. (d) The KM survival curve of risk score based on TXNRD1 and seven ferroptosis-related mark genes in LIHC. (e) The survival time of patients between the high-risk group and the low-risk group. (f) Heat map of TXNRD1 and seven ferroptosis-related mark genes expression.

**Fig. 5** Exploring the value of prognostic model in LIHC patients based on TCGA cohort. The forest plots for univariate (a) and multivariate (b) Cox regression analysis in hepatocellular carcinoma. (c) The time-dependent ROC curves for the prognostic signature of LIHC patients. The areas under the ROC curve are about 1 year, 4 years, and 5 years. (d) The nomogram of 1-year, 3-year or 5-year OS based on age, risk score and TNM stage. (e) The ROC curves analysis based on risk score and the clinicopathologic parameters. (f) Calibration plots for assessing the agreement between the predicted and the actual OS for the prognosis model, where the predicted probabilities are aligned with the actual probabilities.
Fig. 6 Functional disparities among two groups of LIHC patients. (a) Comparison of the results of GSEA analysis between two groups of patients. (b) The variety of proportion of infiltrating immune cells and immunologic function between high and low-risk groups. (c) Survival curves are employed to assess the prognostic significance of macrophages in LIHC patients. (d) The TIDE algorithm was used to predict variations in immune escape behavior between two groups of LIHC patients.

Fig. 7 Sc-RNA sequencing of the malignant liver cell and differentially infiltrated immune cells in LIHC tissues. (a) The identified 12 (GSE140228_10X), 10
(GSE140228_Smartseq2) and 11 (GSE166635_10X) cell subtypes collected from patients with primary hepatocellular carcinoma. (b) Distribution of TXNRD1 related signature in diverse cell subpopulations. (c) Variation of TXNRD1 related signature in different immune cell populations and (d) different sources in liver sites, ascites, and lymph nodes. (e) The distribution of ferroptosis-associated gene sets in each cell subtype identified by single-cell GSEA analysis.

Fig. 8 Intracellular TXNRD1 concentration responded to the process of ferroptosis in HCC. (a) TXNRD1 levels were detected in the indicated liver cancer tissues (T) and their corresponding adjacent non-tumoral tissues (NT) as well as in L02, HepG2 and Huh7 cell lines. (b) Comparison of TXNRD1 after 24 h of treatment with 1μM, 5μ M and 10μ M Erastin or 1μM ferroptosis inhibitor in HepG2 and Huh7 cell lines. (c) Schematic showing that TXNRD1 intracellularly suppresses ferroptosis in hepatocellular carcinoma cells and immune escape occurs with the help of macrophages.