Identification of cross-talk between m6A/m5C regulators and ferroptosis associated with immune infiltration and prognosis in pan-cancer

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Research Article

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Abstract

Although it has been recognized that m6A/5mC methylation and ferroptosis play critical roles in different types of cancers, little is known about the relationship between them. In addition, there is also a growing appreciation that m6A/5mC methylation and ferroptosis may affect immune cell infiltration and activation. This study aimed to reveal the extensive cross-talk between epigenetic modification and ferroptosis. A total of 31 cancer type-specific datasets in TCGA were individually collected by the publicly available web servers for multiple bioinformatic analyses of m6A/5mC regulators and ferroptosis-related genes. Intriguingly, m6A/5mC regulators and ferroptosis-related genes were identified to have considerable global coverage and prognostic significance across multiple cancer types. Moreover, m6A/5mC regulators showed interactive potential with ferroptosis-related genes, and genomic alteration of ferroptosis-related genes coupled with m6A/5mC regulators, at least in pancreatic cancer. Furthermore, m6A/5mC regulators and ferroptosis-related genes were found to be significantly associated with TILs. Finally, m6A/5mC regulators and ferroptosis-related genes exhibited functionally related to each other or co-regulated by TF or non-coding RNA. Together, m6A/5mC methylation and ferroptosis show a wide-ranging connection, and a combination strategy of epigenetic and ferroptosis therapies with ICP inhibitors may benefit more cancer patients in the future.

1. Introduction

The most reproducibly mapped and well-known nucleotide methylation, notably in the forms of 5-methylcytosine (5mC) in DNA and N6-methyladenosine (m6A) in mRNA, is of great functional significance to the fundamental biological systems, especially in the regulation of gene expression [1]. Recent research advances emphasize the biological importance of m6A methylation as a highly dynamic and readily reversible post-transcriptional modification in nuclear pre-mRNA splicing, nuclear export, microRNA processing, translation initiation, and RNA stability [2]. 5mC DNA methylation, a conserved and abundant epigenetic modification, plays broad and critical roles in various biological processes, including gene expression regulation, cellular differentiation, and stress responses [3]. A recent study has established a cross-talk between 5mC DNA methylation and m6A mRNA methylation in pan-cancer [4]. Intriguingly, m6A and 5mC regulators exhibited comparable levels of mutation frequency, significant co-occurrences of genetic alterations, similar gene expression patterns, and functionally related to each other or co-regulated.

Ferroptosis is a recently described type of programmed cell death driven by iron-dependent lipid peroxidation that differs from other forms of cell death, such as apoptosis and necrosis, in morphology and mechanisms [5]. Multiple productive lines of studies have suggested that ferroptosis plays a pivotal role in the development and progression of cancer [6–8] . For example, stearoyl-CoA desaturase-1 (SCD1) in cancer cells and fatty acid-binding protein 4 (FABP4) in tumor microenvironment cooperatively protect from oxidative stress-induced ferroptosis and promote tumor recurrence [9] . Ferroptosis can also play an essential role in suppressing tumor metastasis as melanoma cells from the lymph are more resistant to ferroptosis and, thus, can form more metastases than those in the blood [10] . Most recently, it was

reported that a therapy-resistant, high-mesenchymal state depends on the glutathione peroxidase 4 (GPX4) pathway to evade ferroptosis, suggesting that induction of ferroptosis represents an emerging strategy for innovative anti-tumor drug discovery [11] . The role of ferroptosis in cancer may also involve multiple cell types within the tumor microenvironment. Immunotherapy activated $CD8^+$ T cells could downregulate the expression of two subunits of glutamate-cystine antiporter system Xc−, solute carrier family 7 member 11 (SLC7A11) and SLC3A2 via releasing interferon-gamma (IFN-gamma), resulting in the accumulation of lipid peroxidation and ferroptosis in cancer cells [12].

Emerging evidence suggests that m6A/5mC methylation plays a vital role in regulating ferroptosis. Exosomal miR-4443 facilitates cisplatin resistance in non-small cell lung carcinoma by modulating FSP1 m6A modification-mediated ferroptosis via METTL3 [13] . YTHDC2 inhibits SLC7A11 and SLC3A2 in an m6A-dependent manner and is believed to be an endogenous ferroptosis inducer in lung adenocarcinoma [14,15] . However, due to the limitations in methodology, these studies have been limited to only one or two epigenetic regulators. To the best of our knowledge, the “cross-talk” between epigenetic modifications and ferroptosis in pan-cancer has not been systematically investigated. Therefore, a comprehensive understanding of the extensive cross-talk between epigenetic regulators and ferroptosis will contribute to our understanding of epigenetic regulation in ferroptosis and the development of potential therapeutic strategies for controlling cell death and survival by mediating the reversibility of ferroptosis.

This study explored the expression levels and mutation frequencies of epigenetic regulators and ferroptosis-related genes in pan-cancer samples from The Cancer Genome Atlas (TCGA) cohort. Next, we identified the potential relationship between epigenetic regulators and ferroptosis-related genes from several aspects, including co-expression, functional states at single-cell resolution, immune cell infiltration, transcription factors (TFs), and ceRNA regulation. This study provides essential insights into the interaction between epigenetic regulators and ferroptosis-related genes in cancer and paves new ways for related therapeutic targets.

2. Material And Methods

2.1. Data collection

To analyze m6A, 5mC and ferroptosis regulators-related expression, prognoses, interactions, or/and correlations in multiple cancers, a total of 31 cancer type-specific datasets in TCGA (http://cancergenome.nih.gov), such as ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UCS, were individually collected by the below web servers for multiple bioinformatic analyses. MESO and UVM in TCGA were excluded from the analyses because of data incompleteness. TCGA and other open-access databases or datasets without overlapping samples were integrated for further bioinformatic analyses for pancreatic cancer-specific analyses. These included four datasets, such as Pancreatic Adenocarcinoma (ICGC, Nature 2012), Pancreatic Adenocarcinoma (QCMG, Nature 2016), Pancreatic Adenocarcinoma (TCGA, PanCancer Atlas), and Pancreatic Cancer

(UTSW, Nat. Commun. 2015). Data from different platforms or laboratories were processed and computed following a standard analysis pipeline.

2.2. The expression and prognostic analysis by GEPIA across different cancer types

The Gene Expression Profiling Interaction Analysis (GEPIA, http://gepia2.cancer-pku.cn, version 2) is an open-access web-based tool for rapid and customizable exploration of RNA sequencing expression data of 9736 tumors and 8587 normal samples derived from the TCGA and the Genotype-Tissue Expression (GTEx) projects [16] . In this study, GEPIA was utilized to calculate the differential expression and prognostic indexes of m6A/5mC regualators and ferroptosis-related genes. One-way ANOVA was used to identify the differential expression of m6A/5mC regulators and ferroptosis-related genes with |log2FC| values > 1 and q values < 0.01. Overall survival (OS) or disease-free survival (DFS, also called relapse-free survival (RFS)) analyses of m6A/5mC regulators and ferroptosis-related genes were assessed using the log-rank test for Kaplan-Meier methods with a 50% (Median) cut-off for both low and high expression groups. A univariate Cox proportional hazards regression model was adopted to calculate the hazard ratio, and p-value < 0.05 was used as a threshold in ranking the results.

2.3. Construction of protein-protein interactions (PPIs) by STRING

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins, https://string-db.org, version 11.0) was used to construct PPIs between m6A/5mC regulators and ferroptosis-related genes. This well-known online interaction repository includes direct physical interactions and indirect functional associations [17] . Combined scores were computed by combining the probabilities from numerous sources, including high-throughput experimental data, mining of databases and literature, and predictions based on genomic context analysis. The confidence score ranked from 0 to 1, with 1 indicating the highest possible confidence. Pearson correlation between m6A/5mC regulators and ferroptosis-related genes was carried out using the package corrplot in R. The circos plot was generated through the circlize package.

2.4. Genetic alterations of m6A/5mC regulators and ferroptosis-related genes and their associations with patient prognosis

cBioPortal for Cancer Genomics (cBioPortal, http://www.cbioportal.org, version v3.2.11) is an open-access online tool that integrates raw data from large-scale genomic projects including TCGA, ICGC, and other databases [18] . The data of gene-level is stored with available clinical information, including OS, progression-free survival (PFS), DFS, and disease-specific survival (DSS). In this study, CVCDAP was used to visualize and compare genetic alterations of m6A/5mC regulators and ferroptosis-related genes in pan-cancer [19] . To determine whether alterations in m6A/5mC regulators and ferroptosis-related genes affected the OS and PFS of pancreatic cancer patients, we used the cBioPortal to evaluate the survival data of patients with or without genetic m6A/5mC regulators and ferroptosis-related genes alterations from four pancreatic cancer studies. Co-occurrence and mutual exclusivity of genetic alterations between inquired m6A/5mC regulators and ferroptosis-related genes were determined by log2 odds ratio, p-value, and q value, and results with q value < 0.05 were selected. In pancreatic cancer, OS and PFS were

individually investigated to compare the prognostic differences between altered and unaltered groups. The Log-rank test was used for hypothesis testing.

2.5. Functional states analysis at single-cell resolution by CancerSEA

The functional states of m6A/5mC regulators and ferroptosis-related genes in various cancer types were analyzed by CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA/). CancerSEA is the first integrative database aimed to decode different functional states of cancer cells at a single-cell resolution, which covers 14 functional states (including stemness, invasion, metastasis, proliferation, EMT, angiogenesis, apoptosis, cell cycle, differentiation, DNA damage, DNA repair, hypoxia, inflammation, and quiescence) of 41,900 cancer single cells from 25 cancer types [20] . Correlations between the gene of interest and functional state in different single-cell datasets were filtered by a correlation strength > 0.3 and a false discovery rate (FDR) (Benjamini & Hochberg) < 0.05.

2.6. TIMER database analysis

Tumor Immune Estimation Resource 2.0 (TIMER2.0) is an integrative resource for comprehensively evaluating tumor infiltration of immune cells across various cancer types (http://timer.cistrome.org/) [21] . TIMER2.0 utilizes a deconvolution statistical method to estimate the abundance of 36 subtypes of tumor-infiltrating immune cells, including B cell, B cell memory, B cell naive, B cell plasma, cancer associated fibroblast, class-switched memory B cell, common lymphoid progenitor, common myeloid progenitor, endothelial cell, eosinophil, granulocyte-monocyte progenitor, hematopoietic stem cell, macrophage, macrophage M1, macrophage M2, mast cell, monocyte, myeloid dendritic cell, myeloid dendritic cell activated, neutrophil, NK cell, plasmacytoid dendritic cell, T cell $CD4^+$ (non-regulatory), T cell $CD4^+$ central memory, T cell $CD4^+$ effector memory, T cell $CD4^+$ memory, T cell $CD4^+$ naive, T cell $CD4^+$ Th1, T cell $CD4^+$ Th2, T cell $CD8^+$, T cell $CD8^+$ central memory, T cell $CD8^+$ effector memory, T cell $CD8^+$ naive, T cell gamma delta, T cell NK and T cell regulatory (Tregs). We analyzed the correlation between m6A/5mC regulators and ferroptosis-related genes expression and the abundance of immune infiltrates using the gene module. The gene expression level was displayed with log2 RSEM.

2.7. TF regulatory network

The hTFtarget database (http://bioinfo.life.hust.edu.cn/hTFtarget) has integrated TF-target regulations and epigenetic modification information from large-scale of ChIP-Seq data of human TFs (7,190 experiment samples of 659 TFs) in 569 conditions (399 types of cell line, 129 classes of tissues or cells, and 141 kinds of treatments) to predict accurate TF-target regulations [22]. The Cistrome Data Browser (DB, http://cistrome.org/db) maps the genome-wide locations of transcription factor binding sites, histone post-translational modifications, and regions of chromatin accessible to endonuclease activity, which contains approximately 47,000 human and mouse samples [23]. The hTFtarget and Cistrome database were used to predict TFs of m6A/5mC regulators and ferroptosis-related genes. Pearson correlation among TF, m6A/5mC regulators and ferroptosis-related genes was carried out using the package corrplot in R. The circos plot was generated through the circlize package.

2.8. Candidate lncRNA-miRNA-mRNA competing interactions

The miRNA-lncRNA interactions were predicted and overlapped using three miRNA target prediction methods, including miRDB (http://www.mirdb.org/), miRTarBase (http://miRTarBase.cuhk.edu.cn/), and TargetScan (http://www.targetscan.org/vert_72/) [24–26]. The miRNA-mRNA interactions were obtained from two high-quality databases, DIANA-TarBase v8 (http://www.microrna.gr/tarbase) and miRTarBase (http://mirtarbase.cuhk.edu.cn/php/index.php) [27], that store manually curated collections of experimentally supported miRNA targets. LncRNA-mRNA pairs that shared a single miRNA were regarded as one candidate lncRNA-miRNA-mRNA competing interaction.

3. Results

3.1. The expression and prognostic landscape of m6A/5mC regulators and ferroptosis-related genes across different cancer types

We first examined the expression profiles of 41 m6A/5mC regulators and 60 ferroptosis-related genes in multiple cancers by analyzing GEPIA (Supplement Table 1A-B). Pan-cancer expression analysis results indicated significant deregulation of m6A/5mC regulators and ferroptosis-related genes in the majority of malignancies (Fig. 1A-B). The expression of m6A/5mC regulators and ferroptosis-related genes have a significant degree of heterogeneity across cancers. THYM, PAAD, DLBC and CHOL showed the most significant changes in the expression of m6A/5mC regulators and ferroptosis-related genes. Surprisingly and contrary to expectations, the expression of m6A and 5mC regulators showed no significant difference in KIRP.

The correlated expression patterns of m6A/5mC regulators and ferroptosis-related genes across different tumor types affirmed the tumor-specific and functional correlation between m6A/5mC regulators and ferroptosis-related genes (Fig. 1C). Strikingly, a high correlation for the expression patterns ($r = 0.83$, $p = 1.1e-08$) was further detected (Fig. 1D). Furthermore, Kaplan-Meier survival analysis showed that the expression levels of m6A/5mC regulators and ferroptosis-related genes were both significantly associated with OS (Fig. 2A; Fig. 3A) and disease-free survival (DFS) (Fig. 2B; Fig. 3B).

3.2. Interaction between m6A/5mC regulators and ferroptosis-related genes

In view of the critical role of both m6A/5mC regulators and ferroptosis-related genes in the prognosis of cancer patients, the logical next step seems to investigate whether there is a potential interplay between m6A/5mC regulators and ferroptosis-related genes. Network analysis was carried out using PPI data from STRING database, a robust functional association between m6A/5mC regulators and ferroptosis-related genes was observed (Fig. 4A). Since almost all m6A/5mC regulators and ferroptosis-related genes were unregulated in PAAD, pancreatic adenocarcinoma was chosen for further study. Importantly, our analysis based on TCGA data depicted a strong correlation between the expression of m6A/5mC regulators and ferroptosis-related genes in PAAD (Fig. 4B).

The global genomic alteration landscape of m6A/5mC regulators and ferroptosis-related genes in pan-cancer was analyzed using CVCDAP online analyzing tool (Fig. 5A-B). The detailed correlation between each m6A/5mC regulators and ferroptosis-related genes was individually analyzed in PAAD, and statistically significant relationship was presented in Supplementary Table 2. For example, SQLE was found associated with KIAA1429 where they shared 24 variants in 860 patient samples. The genomic alterations of m6A/5mC regulators showed general co-occurrence rather than mutual exclusivity with ferroptosis-related genes. In fact, a total of 1654 significant associations between two genes among m6A/5mC regulators and ferroptosis-related genes were observed in this analysis, all of which showed co-occurrence but not mutual exclusivity. Furthermore, integrated prognostic analyses of OS and PFS indicated that integrated genomic alterations of m6A/5mC regulators and ferroptosis-related genes were significantly unfavorable for multiple prognoses of patients with pancreatic cancer (Fig. 5C-F).

3.3. Relationship between m6A/5mC regulators and ferroptosis-related genes with cancer single-cell functional states

Heterogeneity associated with different functional phenotypes of tumor cells has been one of the major challenges for cancer diagnosis and effective cancer treatment. Recent advances in single-cell sequencing (scRNA-seq) technology have provided a tool for dissecting cellular heterogeneity, unraveling cell status and identifying subpopulation structures across different cell types at the cellular level. Functional correlation analysis of cancerSEA showed that the functional phenotypes of m6A/5mC regulators and ferroptosis-related genes were positively correlated with DNA damage and DNA repair ($r > 0.3$, $p < 0.05$) (Fig. 6A-B), which suggested that they may participate in the same processes of tumorigenesis and advancement of most types of cancer (Fig. 6C-D). Moreover, we also found that m6A/5mC regulators and ferroptosis-related genes were positively correlated with invasion in CML, CRC, OV, and PC.

3.4. Associations between m6A/5mC regulators, ferroptosis-related genes and cancer immunity

The associations between m6A/5mC regulators, ferroptosis-related genes and tumor microenvironment were investigated in the 36 cell types via Tumor IMmune Estimation Resource (TIMMER) for each cancer type. Pan-cancer analysis results indicated significant associations between m6A/5mC regulators, ferroptosis-related genes and tumor microenvironment in the majority of malignancies (Fig. 7 and Supplementary Table 3). We found that some m6A/5mC regulators and ferroptosis-related genes displayed much similarities in immune-cell infiltrating profiles. For example, FANCD2 and UHRF1 in ACC, BRCA, MESO, SARC, THYM, LIHC, LUAD, and KICH infiltrated similar immune cell profiles, showing a clear link to T cell $CD4^+$ Th2 ($r > 0.5$, $p < 0.05$). HMOX1 expression exhibited relatively high correlation with macrophage M2 in UVM, KIRP, BRCA, OV, PAAD, STAD, READ and COAD ($r > 0.5$, $p < 0.05$). HNRNPC expression was significantly associated with T cell $CD4^+$ Th2 in BLCA, COAD, ESCA, HNSC, LUAD, PRAD, READ, UCEC and UVM ($r > 0.5$, $p < 0.05$). These results suggested that m6A/5mC modification and ferroptosis played a non-negligible role in the immune regulation in tumor microenvironment.

3.5. Transcriptional regulatory network for m6A/5mC regulators and ferroptosis-related genes

In order to shed light on the mechanism involved in the regulation of m6A/5mC regulators and ferroptosis-related genes, we first tried to find out transcription factors regulating m6A/5mC regulators and ferroptosis-related genes and established a TF-gene regulatory network. We obtained 290 TF from the Cistrome Database and hTFtarget overlaps, and a network was generated (Fig. 8A). Correlation test function was utilized to test the correlations with cutoff criteria set as the correlation coefficient >0.7 and p <0.001 in PAAD (Fig. 8B). The results revealed that a large number of m6A/5mC regulators and ferroptosis-related genes were regulated by POLR2A (Fig. 8C), and correlation test function showed POLR2A was also positively correlated with the expression of m6A/5mC regulators and ferroptosis-related genes in PAAD (Fig. 8D).

3.6. LncRNA-miRNA-mRNA ceRNA regulatory network

To investigate the role of ceRNA triplets (lncRNAs-miRNAs-mRNAs) and the competitive patterns of m6A/5mC regulators and ferroptosis-related genes, we constructed a ceRNA network. As a result, a total of 854 pair-wise interactions among 239 nodes were identified, consisting of 69 lncRNAs, 110 miRNAs, 33 ferroptosis-related genes, and 27 m6A/5mC regulators (Fig. 9A). Among the ceRNA networks, XIST was the lncRNA that regulates the most miRNA and mRNA, so we take it as the core to construct the ceRNA network. The network consisted of 73 nodes and 223 edges, and the degrees of the top 5 nodes (ACSL4, TET3, YTHDF3, IGF2BP1, and ZBTB33) were 16, 16, 16, 15, and 12, respectively (Fig. 9B).

4. Discussion

There is increasing experimental evidence shows DNA/RNA methylation regulators may form an important and complex cellular regulatory network in ferroptosis, and a considerable cross-talk between them [28]. While there is no systematic study to investigate whether a cross-talk between m6A/5mC regulators and ferroptosis-related genes exists. Here, we revealed global alterations of m6A/5mC regulators and ferroptosis-related genes at transcriptional and genetic levels, and their mutual correlation in pan-cancer. Our results revealed the prognostic significance, functional status, tumor-infiltrating immune cells, transcription factors, and ceRNA regulatory network of m6A/5mC regulators and ferroptosis-related genes in multiple cancers. Understanding the cross-talk between epigenetic modification and ferroptosis may provide important insights into the mechanisms underlying tumorigenesis.

FTO-mediated m6A demethylation in tumor cells elevates the transcription factors c-Jun, JunB, and C/EBPβ, which allows the rewiring of glycolytic metabolism to evade immune surveillance [29]. Another study revealed the role of m6A in dendritic cell (DC) activation, in which METTL3-mediated m6A of CD40, CD80, and TLR4 signaling adaptor TIRAP transcripts enhanced their translation in DC for stimulating T cell activation and strengthening TLR4/NF-κB signaling-induced cytokine production [30]. Ferroptotic cells might release distinct “find me” signals, HMGB1, prostaglandin E_2 (PGE_2), 5-hydroxyeicosatetraenoic

acid (5-HETE), oxidized phospholipids (oxPLs), which will attract antigen presenting cells (APCs) and other immune cells to the site of ferroptotically dying cells [31]. Although the role of m6A/5mC regulators and ferroptosis-related genes in the immune microenvironment have been studied individually, however, how m6A/5mC regulators and ferroptosis-related genes play a crucial role in coordinating immune system responses needs to be further investigated. Our study provides a comprehensive insight for revealing the significant role of m6A/5mC regulators and ferroptosis-related genes in the tumor immune microenvironment. Interestingly, we found that FANCD2 and UHRF1 expression were positively correlated with infiltrating levels of T cell $CD4^+$ Th2 in a wide variety of malignant neoplasms. UHRF1 is a critical factor that binds to interstrand crosslinks (ICLs). In turn, this binding is necessary for the subsequent recruitment of FANCD2, which allows the DNA repair process to initiate [32]. The exact mechanism of how UHRF1 and FANCD2 contribute to the regulation of immune microenvironment, whether directly or indirectly, needs to be experimentally verified.

Meanwhile, some m6A/5mC regulators and ferroptosis-related genes were regulated by the same TF. EP300-induced H3K27 acetylation activation increased ALKBH5 expression, promoted uveal melanoma (UM) cell proliferation, migration, invasion, and decreased apoptosis in vitro [33]. CREB suppressed lipid peroxidation by binding the promoter region of glutathione peroxidase 4 (GPX4), and this binding could be enhanced by E1A binding protein P300 (EP300) [34]. In addition to TF, the m6A regulators and ferroptosis-related gene expression changes were guided by non-coding RNAs, such as long non-coding RNAs (lncRNAs) and miRNAs. MALAT1 was found to upregulate IGF2BP2 via m6A modification recognition by competitively binding to miR-204, conferring a stimulatory effect on proliferation, migration and invasion of TC cells, which was accompanied by weakened tumor growth and cell apoptosis [35]. MALAT1 induced KEAP1 downregulation, leading to NRF2 stabilization and activation mediated human umbilical vein endothelial cells (HUVEC) protection against H_2O_2 [36]. NFE2L2 and ZBTB4 both were regulated by miR-17-5p in tumor [37, 38] . METTL3 interacted with the microprocessor protein DGCR8 and positively modulated miR-873-5p mature process in an m6A-dependent manner. Further experiments showed that miR-873-5p could regulate Keap1-Nrf2 pathway against colistin-induced oxidative stress and apoptosis [39]. How multiple ncRNAs or TFs coordinately control the expression of m6A regulators and ferroptosis-related genes is a question that needs to be answered in future experiments.

There are still major questions that need to be urgently addressed in the near future: For instance, whether or how m6A/5mC methylation and ferroptosis connect and coordinate in immune microenvironment and immunotherapy, and how ncRNAs contributes to m6A/5mC methylation and ferroptosis via regulation of target gene activity? How m6A/5mC modifications affect the function of specific ferroptosis-associated genes? Most of these questions should be analyzed in detail for the construction and refining of the conception and system of targeting m6A/5mC regulators as well as ferroptosis-based therapy. Therefore, identifying distinct m6A/5mC modification patterns in the tumor immune microenvironment will provide insights into the interactions of m6A/5mC RNA methylation on anti-tumor immune response and

facilitate more effective precision immunotherapy strategies. Ferroptosis-mediated TIME regulation is anticipated to open a new research field at the frontier of anticancer immunity.

5. Conclusions

In conclusion, we have shown that an extensive cross-talk between epigenetic regulators and ferroptosis in several aspects. Epigenetic and ferroptosis regulators are promising cancer therapeutic targets, and an epigenetic and ferroptosis regulators-targeting strategy should be synergized with ICB to fully harness the power of TIME and obtain a maximum clinical benefit for future cancer immunotherapy. Coordinated efforts are required to determine optimal therapeutic combinations and to apply both immune-profiling and genomic-profiling technologies to develop a personalized treatment.

6. Author Statement

Wumin Dai and Yingli Zhang conceived and designed the study. Xia Li, Yongyi Chen, Wangang Gong and Ying Su participated in the acquisition, analysis, and interpretation of all data. Wumin Dai wrote the paper. Yingli Zhang edited the manuscript. All authors reviewed the manuscript and gave final approval to submit the manuscript.

Declarations

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Declaration of Competing Interest

The authors declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval statement

No. TCGA belongs to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Clinical trial registration number (if applicable)

No

Data availability statement

All data, models, and website generated or used during the study appear in the submitted article.

Author contribution statement

Wumin Dai and Yingli Zhang conceived and designed the study. Xia Li, Yongyi Chen, Wangang Gong and Ying Su participated in the acquisition, analysis, and interpretation of all data. Wumin Dai wrote the paper. Yingli Zhang edited the manuscript. All authors reviewed the manuscript and gave final approval to submit the manuscript.

References

1. Skvortsova K, Stirzaker C, Taberlay P. The DNA methylation landscape in cancer. Essays in Biochemistry 2019;63:797–811. https://doi.org/10.1042/EBC20190037.
2. Uddin MB, Wang Z, Yang C. The m(6)A RNA methylation regulates oncogenic signaling pathways driving cell malignant transformation and carcinogenesis. Molecular Cancer 2021;20:61. https://doi.org/10.1186/s12943-021-01356-0.
3. Martisova A, Holcakova J, Izadi N, Sebuyoya R, Hrstka R, Bartosik M. DNA Methylation in Solid Tumors: Functions and Methods of Detection. International Journal of Molecular Sciences 2021;22. https://doi.org/10.3390/ijms22084247.
4. Chen Y-T, Shen J-Y, Chen D-P, Wu C-F, Guo R, Zhang P-P, et al. Identification of cross-talk between m(6)A and 5mC regulators associated with onco-immunogenic features and prognosis across 33 cancer types. Journal of Hematology & Oncology 2020;13:22. https://doi.org/10.1186/s13045-020-00854-w.
5. Chen X, Kang R, Kroemer G, Tang D. Broadening horizons: the role of ferroptosis in cancer. Nature Reviews Clinical Oncology 2021;18:280–96. https://doi.org/10.1038/s41571-020-00462-0.
6. Lei G, Zhang Y, Hong T, Zhang X, Liu X, Mao C, et al. Ferroptosis as a mechanism to mediate p53 function in tumor radiosensitivity. Oncogene 2021;40:3533–47. https://doi.org/10.1038/s41388-021-01790-w.
7. Lei G, Mao C, Yan Y, Zhuang L, Gan B. Ferroptosis, radiotherapy, and combination therapeutic strategies. Protein & Cell 2021. https://doi.org/10.1007/s13238-021-00841-y.
8. Wang H, Cheng Y, Mao C, Liu S, Xiao D, Huang J, et al. Emerging mechanisms and targeted therapy of ferroptosis in cancer. Molecular Therapy: The Journal of the American Society of Gene Therapy 2021;29:2185–208. https://doi.org/10.1016/j.ymthe.2021.03.022.
9. Luis G, Godfroid A, Nishiumi S, Cimino J, Blacher S, Maquoi E, et al. Tumor resistance to ferroptosis driven by Stearoyl-CoA Desaturase-1 (SCD1) in cancer cells and Fatty Acid Biding Protein-4 (FABP4)

in tumor microenvironment promote tumor recurrence. Redox Biology 2021;43:102006. https://doi.org/10.1016/j.redox.2021.102006.

10. Ubellacker JM, Tasdogan A, Ramesh V, Shen B, Mitchell EC, Martin-Sandoval MS, et al. Lymph protects metastasizing melanoma cells from ferroptosis. Nature 2020;585:113–8. https://doi.org/10.1038/s41586-020-2623-z.
11. Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, Seashore-Ludlow B, et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature 2017;547:453–7. https://doi.org/10.1038/nature23007.
12. Wang W, Green M, Choi JE, Gijón M, Kennedy PD, Johnson JK, et al. CD8(+) T cells regulate tumour ferroptosis during cancer immunotherapy. Nature 2019;569:270–4. https://doi.org/10.1038/s41586-019-1170-y.
13. Song Z, Jia G, Ma P, Cang S. Exosomal miR-4443 promotes cisplatin resistance in non-small cell lung carcinoma by regulating FSP1 m6A modification-mediated ferroptosis. Life Sciences 2021;276:119399. https://doi.org/10.1016/j.lfs.2021.119399.
14. Ma L, Zhang X, Yu K, Xu X, Chen T, Shi Y, et al. Targeting SLC3A2 subunit of system X(C)(-) is essential for m(6)A reader YTHDC2 to be an endogenous ferroptosis inducer in lung adenocarcinoma. Free Radical Biology & Medicine 2021;168:25–43. https://doi.org/10.1016/j.freeradbiomed.2021.03.023.
15. Ma L, Chen T, Zhang X, Miao Y, Tian X, Yu K, et al. The m(6)A reader YTHDC2 inhibits lung adenocarcinoma tumorigenesis by suppressing SLC7A11-dependent antioxidant function. Redox Biology 2021;38:101801. https://doi.org/10.1016/j.redox.2020.101801.
16. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Research 2019;47:W556–60. https://doi.org/10.1093/nar/gkz430.
17. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Research 2019;47:D607–13. https://doi.org/10.1093/nar/gky1131.
18. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discovery 2012;2:401–4. https://doi.org/10.1158/2159-8290.CD-12-0095.
19. Guan X, Cai M, Du Y, Yang E, Ji J, Wu J. CVCDAP: an integrated platform for molecular and clinical analysis of cancer virtual cohorts. Nucleic Acids Research 2020;48:W463–71. https://doi.org/10.1093/nar/gkaa423.
20. Yuan H, Yan M, Zhang G, Liu W, Deng C, Liao G, et al. CancerSEA: a cancer single-cell state atlas. Nucleic Acids Research 2019;47:D900–8. https://doi.org/10.1093/nar/gky939.
21. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Research 2017;77:e108–10.

https://doi.org/10.1158/0008-5472.CAN-17-0307.

22. Zhang Q, Liu W, Zhang H-M, Xie G-Y, Miao Y-R, Xia M, et al. hTFtarget: A Comprehensive Database for Regulations of Human Transcription Factors and Their Targets. Genomics, Proteomics & Bioinformatics 2020;18:120–8. https://doi.org/10.1016/j.gpb.2019.09.006.
23. Zheng R, Wan C, Mei S, Qin Q, Wu Q, Sun H, et al. Cistrome Data Browser: expanded datasets and new tools for gene regulatory analysis. Nucleic Acids Research 2019;47:D729–35. https://doi.org/10.1093/nar/gky1094.
24. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. Nucleic Acids Research 2020;48:D127–31. https://doi.org/10.1093/nar/gkz757.
25. Huang H-Y, Lin Y-C-D, Li J, Huang K-Y, Shrestha S, Hong H-C, et al. miRTarBase 2020: updates to the experimentally validated microRNA-target interaction database. Nucleic Acids Research 2020;48:D148–54. https://doi.org/10.1093/nar/gkz896.
26. Agarwal V, Bell GW, Nam J-W, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. ELife 2015;4. https://doi.org/10.7554/eLife.05005.
27. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, et al. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. Nucleic Acids Research 2018;46:D239–45. https://doi.org/10.1093/nar/gkx1141.
28. Li W, Pung D, Su Z-Y, Guo Y, Zhang C, Yang AY, et al. Epigenetics Reactivation of Nrf2 in Prostate TRAMP C1 Cells by Curcumin Analogue FN1. Chemical Research in Toxicology 2016;29:694–703. https://doi.org/10.1021/acs.chemrestox.6b00016.
29. Liu Y, Liang G, Xu H, Dong W, Dong Z, Qiu Z, et al. Tumors exploit FTO-mediated regulation of glycolytic metabolism to evade immune surveillance. Cell Metabolism 2021;33:1221-1233.e11. https://doi.org/10.1016/j.cmet.2021.04.001.
30. Wang H, Hu X, Huang M, Liu J, Gu Y, Ma L, et al. Mettl3-mediated mRNA m(6)A methylation promotes dendritic cell activation. Nature Communications 2019;10:1898. https://doi.org/10.1038/s41467-019-09903-6.
31. Friedmann Angeli JP, Krysko D v, Conrad M. Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. Nature Reviews Cancer 2019;19:405–14. https://doi.org/10.1038/s41568-019-0149-1.
32. Liang C-C, Zhan B, Yoshikawa Y, Haas W, Gygi SP, Cohn MA. UHRF1 is a sensor for DNA interstrand crosslinks and recruits FANCD2 to initiate the Fanconi anemia pathway. Cell Reports 2015;10:1947–56. https://doi.org/10.1016/j.celrep.2015.02.053.
33. Hao L, Yin J, Yang H, Li C, Zhu L, Liu L, et al. ALKBH5-mediated m(6)A demethylation of FOXM1 mRNA promotes progression of uveal melanoma. Aging 2021;13:4045–62. https://doi.org/10.18632/aging.202371.
34. Wang Z, Zhang X, Tian X, Yang Y, Ma L, Wang J, et al. CREB stimulates GPX4 transcription to inhibit ferroptosis in lung adenocarcinoma. Oncology Reports 2021;45. https://doi.org/10.3892/or.2021.8039.

35. Ye M, Dong S, Hou H, Zhang T, Shen M. Oncogenic Role of Long Noncoding RNAMALAT1 in Thyroid Cancer Progression through Regulation of the miR-204/IGF2BP2/m6A-MYC Signaling. Molecular Therapy Nucleic Acids 2021;23:1–12. https://doi.org/10.1016/j.omtn.2020.09.023.
36. Zeng R, Zhang R, Song X, Ni L, Lai Z, Liu C, et al. The long non-coding RNA MALAT1 activates Nrf2 signaling to protect human umbilical vein endothelial cells from hydrogen peroxide. Biochemical and Biophysical Research Communications 2018;495:2532–8. https://doi.org/10.1016/j.bbrc.2017.12.105.
37. Stuchi LP, Castanhole-Nunes MMU, Maniezzo-Stuchi N, Biselli-Chicote PM, Henrique T, Padovani Neto JA, et al. VEGFA and NFE2L2 Gene Expression and Regulation by MicroRNAs in Thyroid Papillary Cancer and Colloid Goiter. Genes 2020;11. https://doi.org/10.3390/genes11090954.
38. Jutooru I, Guthrie AS, Chadalapaka G, Pathi S, Kim K, Burghardt R, et al. Mechanism of action of phenethylisothiocyanate and other reactive oxygen species-inducing anticancer agents. Molecular and Cellular Biology 2014;34:2382–95. https://doi.org/10.1128/MCB.01602-13.
39. Wang J, Ishfaq M, Xu L, Xia C, Chen C, Li J. METTL3/m(6)A/miRNA-873-5p Attenuated Oxidative Stress and Apoptosis in Colistin-Induced Kidney Injury by Modulating Keap1/Nrf2 Pathway. Frontiers in Pharmacology 2019;10:517. https://doi.org/10.3389/fphar.2019.00517.

Figures

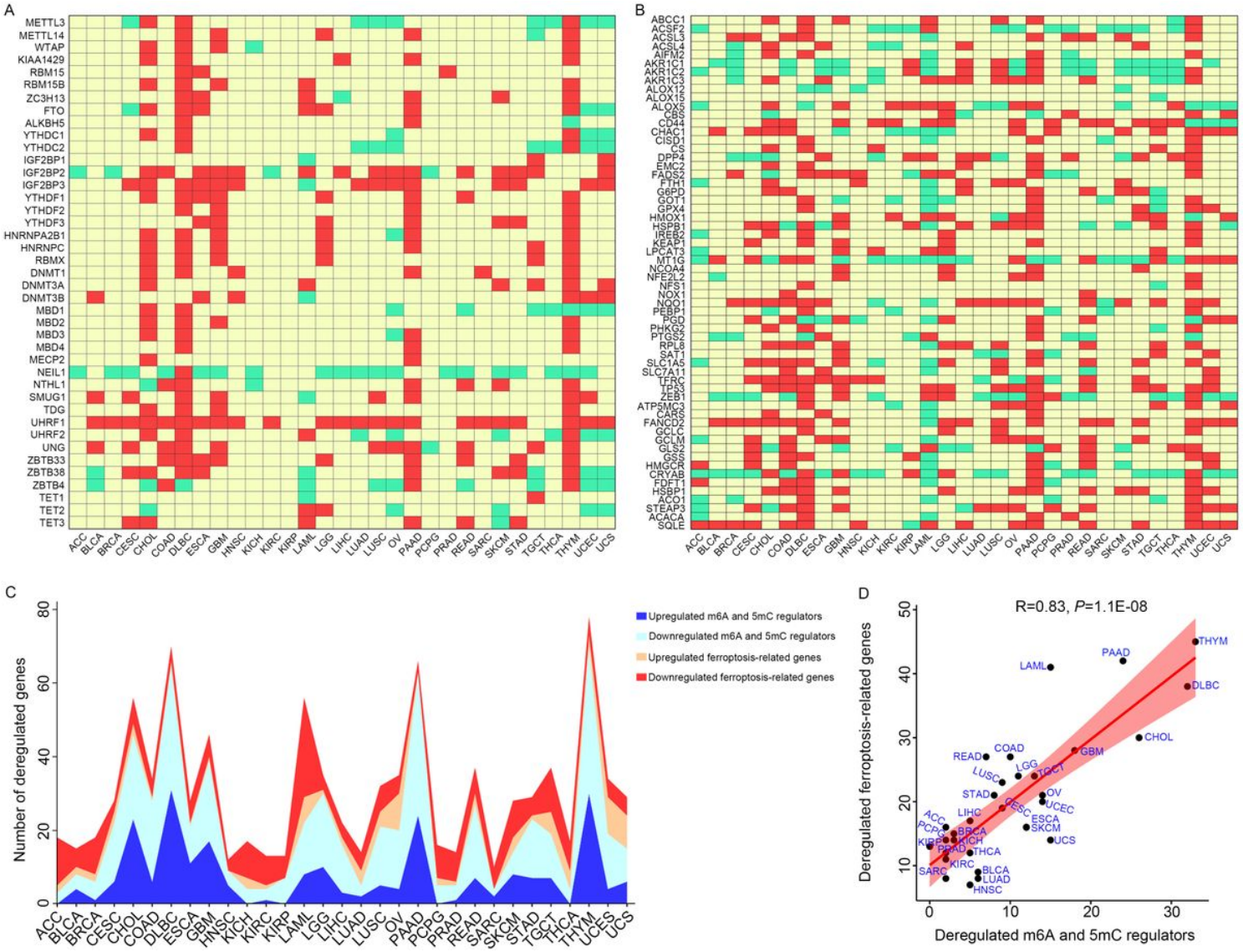

Figure 1

Expression profile of m6A/5mC regulators and ferroptosis-related genes across multiple cancer types. (A) Summary of expression profiles of m6A/5mC regulators in various cancer types. (B) Overview of expression profiles of ferroptosis-related genes in multiple cancer types. Differential expression profiles of m6A/5mC regulators or ferroptosis-related genes were individually analyzed using GEPIA and subsequently integrated. Red blocks represent the m6A/5mC regulators or ferroptosis-related genes upregulated in the tumor, green blocks represent the m6A/5mC regulators or ferroptosis-related genes downregulated in the tumor, and yellow blocks indicate the ones are not significantly differentially expressed between tumor and normal tissues. ANOVA method was used for differential gene expression analysis, and genes with higher |log2FC| values (> 1) and lower q values (< 0.05) were considered differentially expressed genes. (C) The trend for the expression patterns of m6A/5mC and ferroptosis regulators. (D) The correlation for the expression patterns between m6A/5mC and ferroptosis regulators.

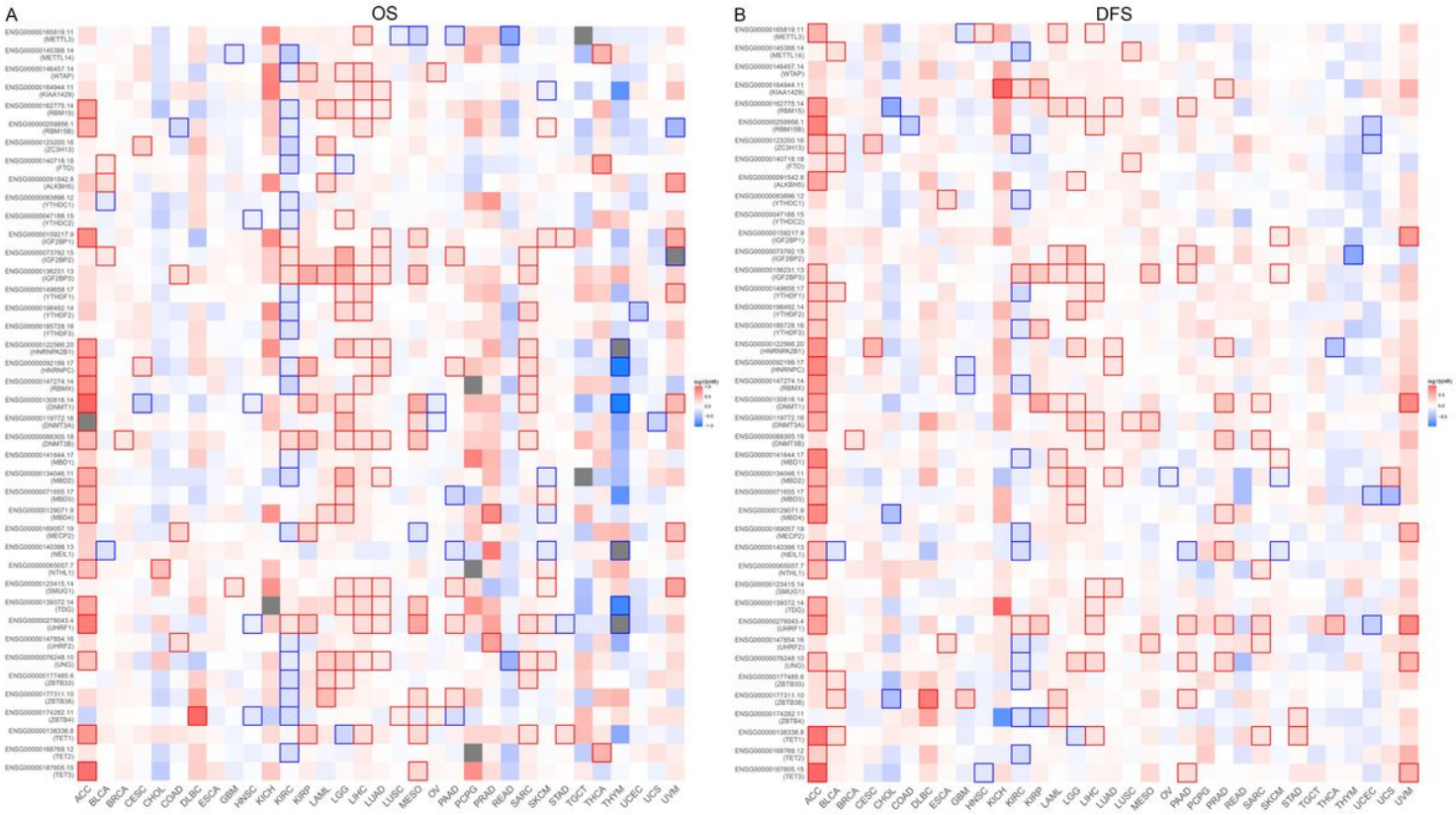

Figure 2

Survival contribution of m6A/5mC regulators across multiple cancer types. (A) Contribution of m6A/5mC regulators to OS in multiple cancer types. GEPIA generated the Kaplan-Meier OS map comparing the groups with different expression levels of m6A/5mC regulators in pan-cancer (TCGA tumors). (B) Contribution of m6A/5mC regulators to DFS in pan-cancer. GEPIA generates the Kaplan-Meier DFS map comparing the groups with different expression levels of m6A/5mC regulators in pan-cancer (TCGA tumors). Red blocks represent m6A/5mC regulators unfavorable to survival, blue blocks represent m6A/5mC regulators favorable to survival, and the ones with outer wireframe indicate significant influence. Mantel-Cox test was used for the hypothesis tests, and the Cox proportional hazard ratio was included in the survival plots. A p value < 0.05 was considered to be statistically significant.

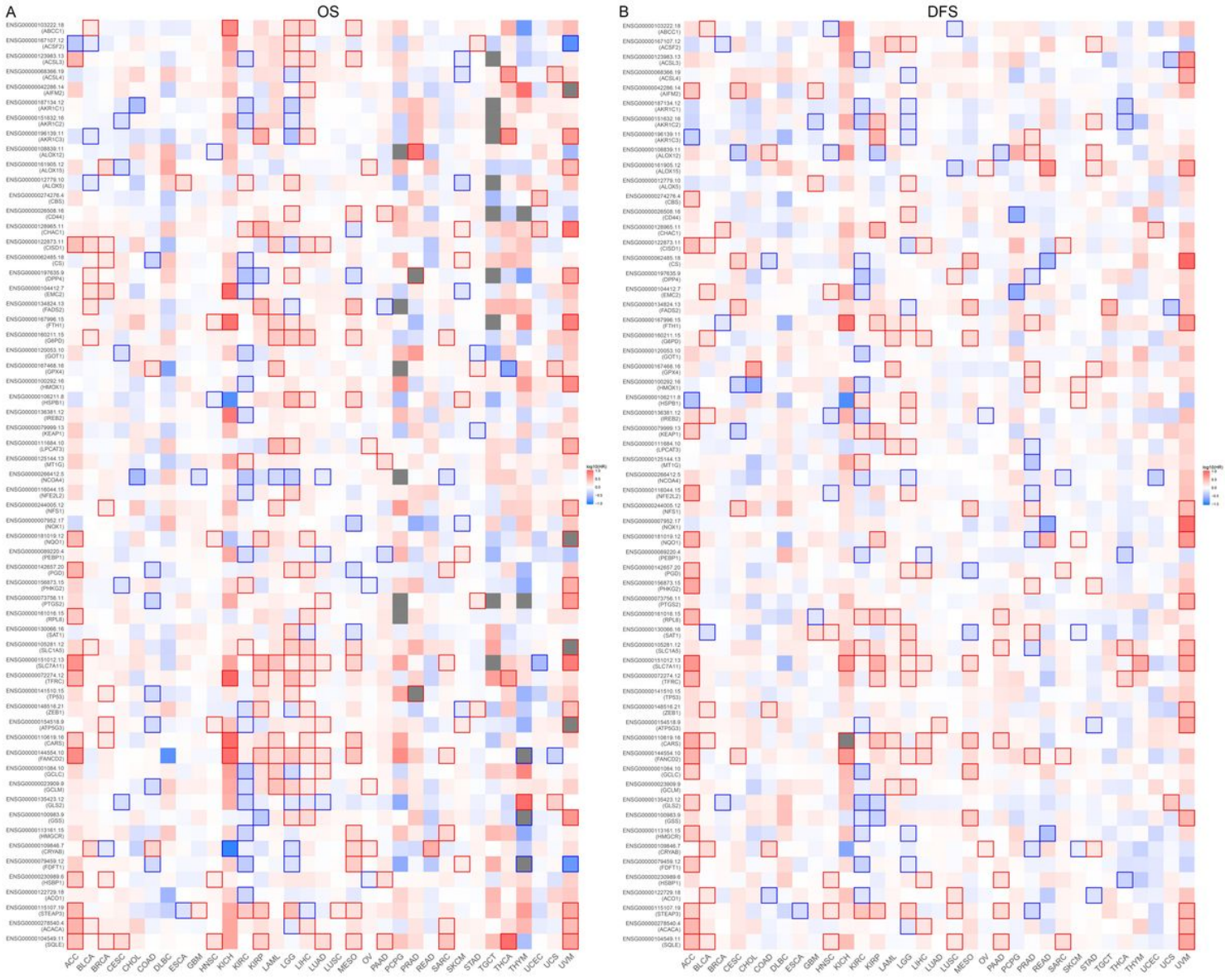

Figure 3

Survival contribution of ferroptosis-related genes across multiple cancer types. (A) Contribution of ferroptosis-related genes to OS in multiple cancer types. (B) Contribution of ferroptosis-related genes to DFS in multiple cancer types.

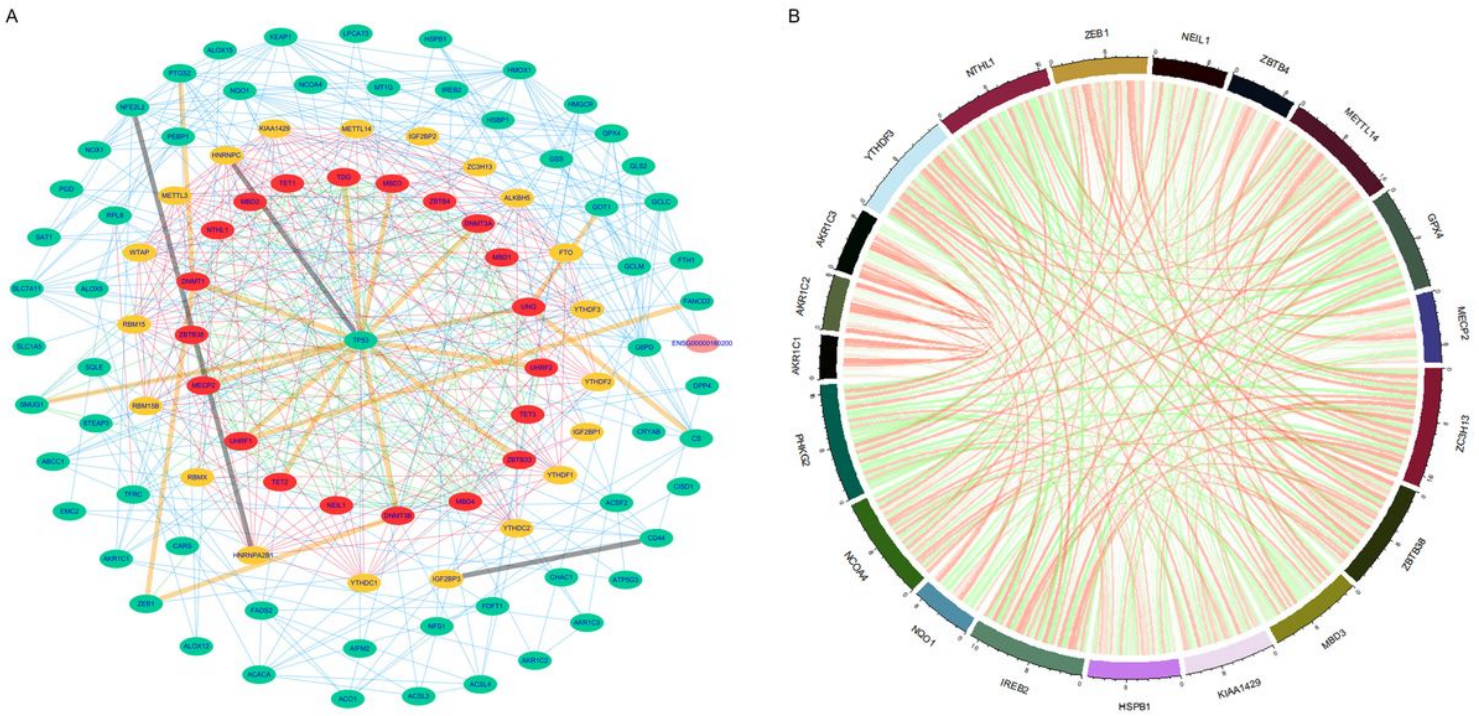

Figure 4

The correlation between m6A/5mC regulators and ferroptosis-related genes. (A) The STRING database was used to analyze the correlation between m6A/5mC regulators and ferroptosis-related genes and Cytoscape was used to display the PPI network. The red circles represent the 5mC regulators, the yellow circles represent the m6A regulators, and the green circles represent the ferroptosis-related genes. The yellow connecting lines represent the connection between 5mC regulators and ferroptosis-related genes, the gray connecting lines represent the connection between m6A regulators and ferroptosis-related genes. (B) The expression correlation between m6A/5mC regulators and ferroptosis-related genes in PAAD. The red connecting lines indicate that the m6A regulator is positively correlated with ferroptosis-related genes, and the green connecting lines indicate that the m6A regulator is negatively correlated with ferroptosis-related genes.

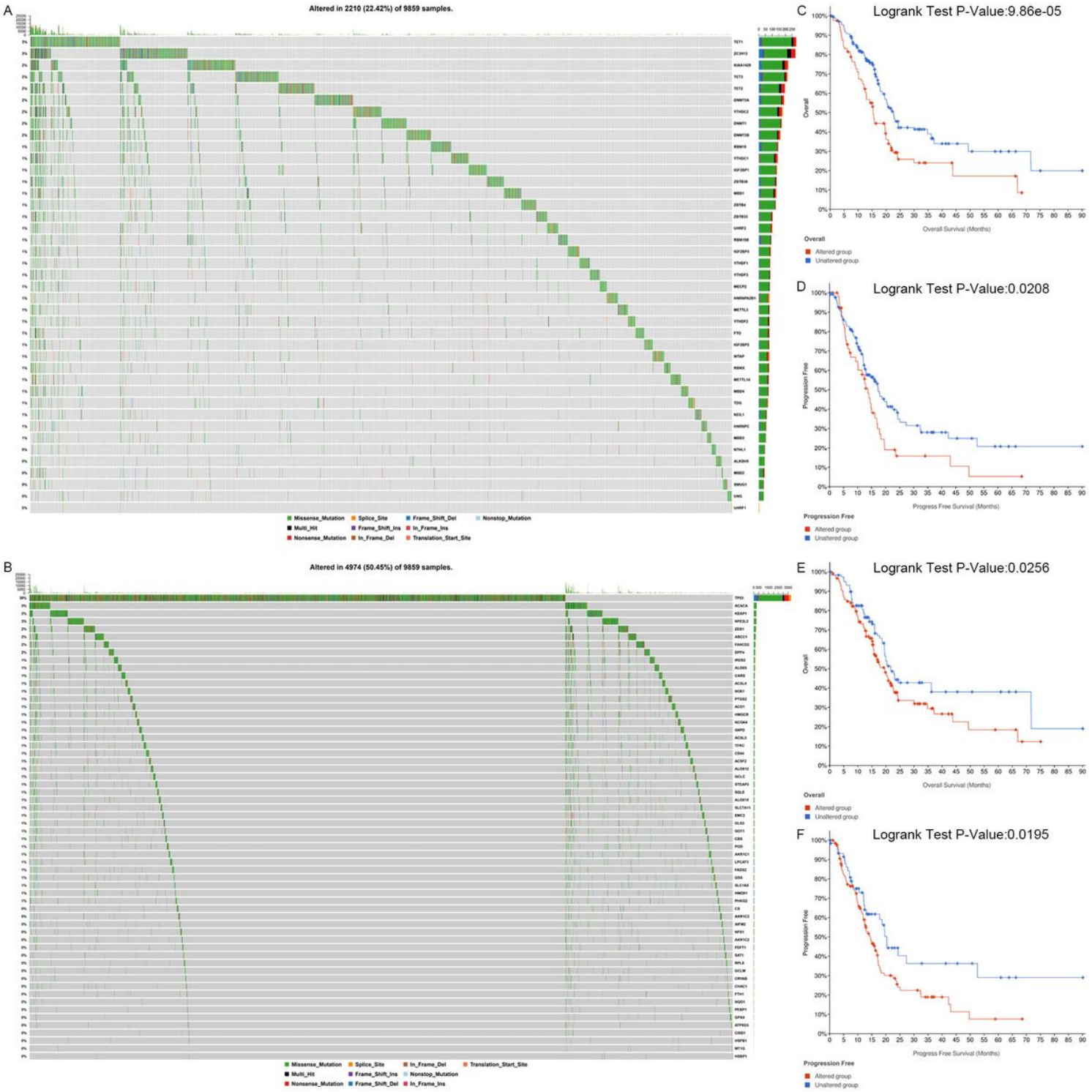

Figure 5

The global landscape of the genomic alteration and multiple survival analyses of integrated alterations of m6A/5mC and ferroptosis regulators. (A) Contribution of integrated alterations of m6A/5mC regulators to OS in pan-cancer. Patients with or without different alterations of m6A/5mC regulators were individually collected and subjected to OS analysis. The time period covers overall patient survival status. (B) Contribution of integrated alterations of m6A/5mC regulators to PFS in pan-cancer. Patients with or without different alterations of m6A/5mC regulators were individually collected and subjected to PFS

analysis. The time period covers progression-free status. (C) Contribution of integrated alterations of ferroptosis regulators to OS in pancreatic cancer. Patients with or without different alterations of ferroptosis regulators were individually collected and subjected to OS analysis. The time period covers overall patient survival status. (D) Contribution of integrated alterations of ferroptosis regulators to PFS in pancreatic cancer. Patients with or without different alterations of ferroptosis regulators were individually collected and subjected to PFS analysis. The time period covers progression-free status. Red curves represent the altered groups, and blue curves represent the unaltered groups. A log-rank test was used for the hypothesis test, and a p-value < 0.05 was considered to be statistically significant.

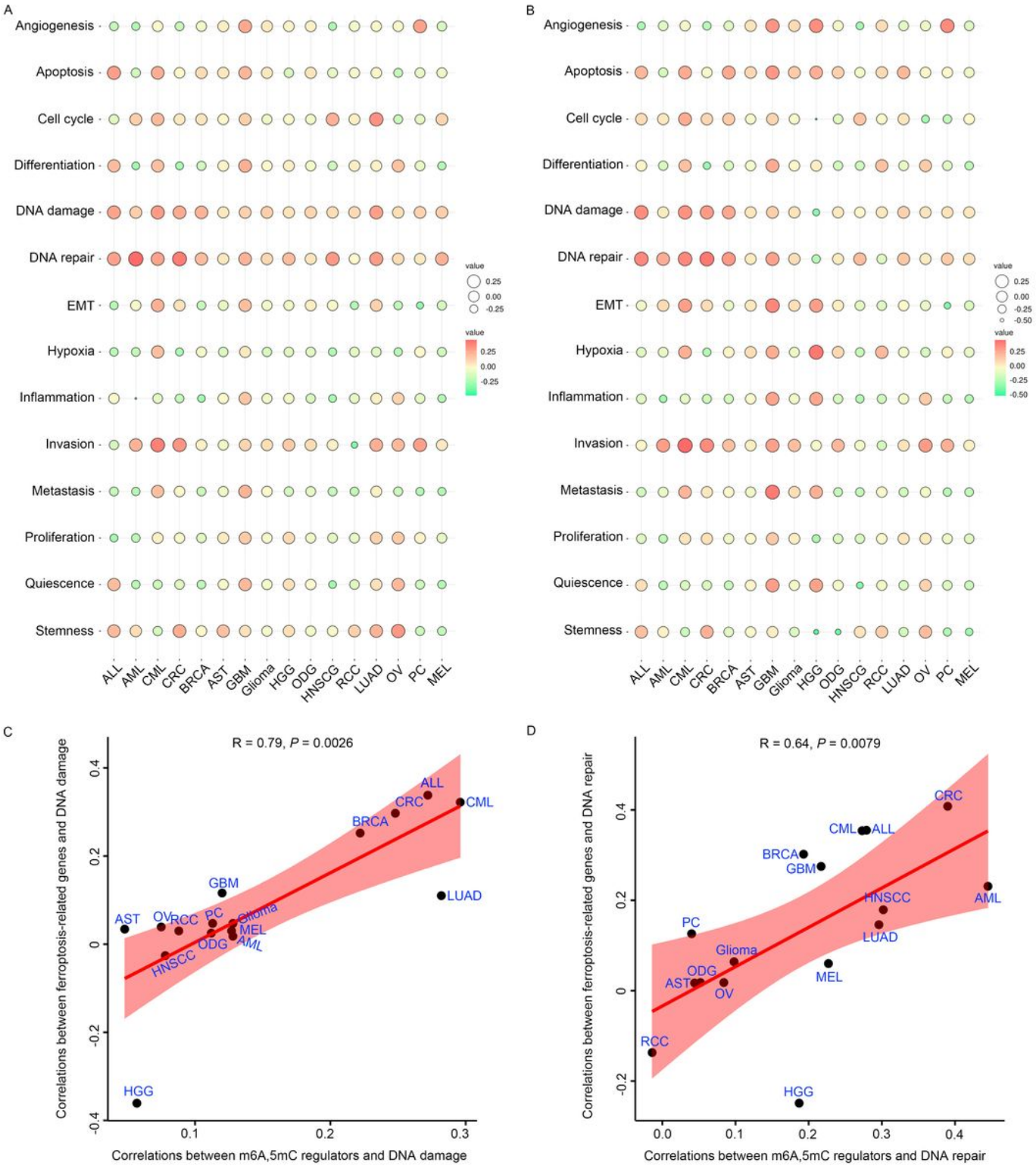

Figure 6

Relationship between m6A/5mC regulators and ferroptosis-related genes with cancer single-cell functional states. (A) The relationship between m6A/5mC regulators and functional states. (B) The relationship between ferroptosis-related genes and functional states. The red circles represent the positive correlation between m6A/5mC regulators or ferroptosis-related genes with functional states. The green circles represent the negative correlation between m6A/5mC regulators or ferroptosis-related genes with

functional states. (C) M6A/5mC regulators and ferroptosis-related genes were positively correlated with DNA damage. (D) M6A/5mC regulators and ferroptosis-related genes were positively correlated with DNA repair.

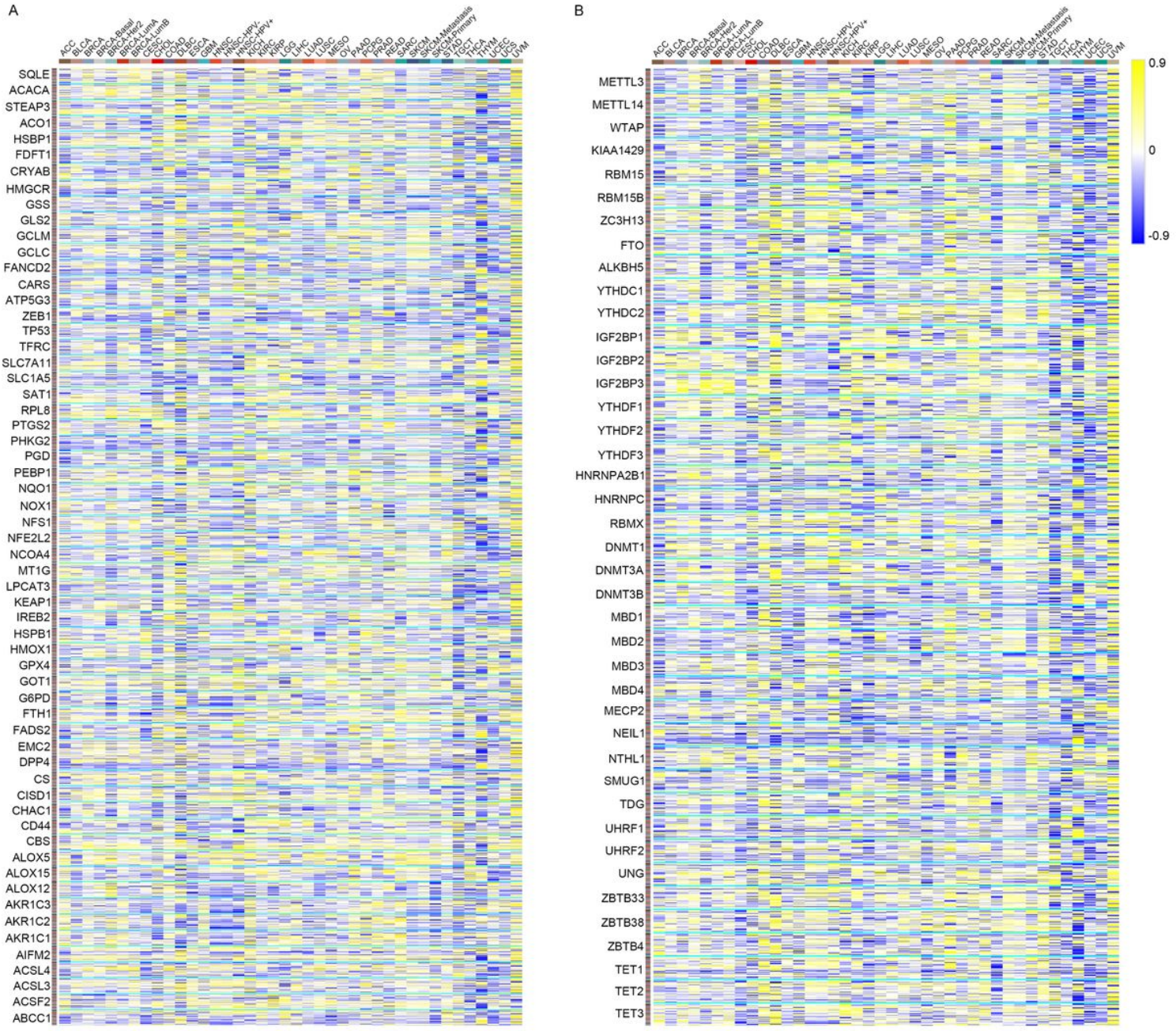

Figure 7

The associations between m6A/5mC regulators or ferroptosis-related genes and the tumor microenvironment in pan-cancer. (A) The associations between m6A/5mC regulators and the tumor microenvironment in pan-cancer. (B) The associations between ferroptosis-related genes and the tumor microenvironment in pan-cancer. The yellow boxes represent a positive correlation between m6A/5mC regulators or ferroptosis-related genes with the immune cell, and the blue boxes represent a negative correlation between m6A/5mC regulators or ferroptosis-related genes with the immune cell.

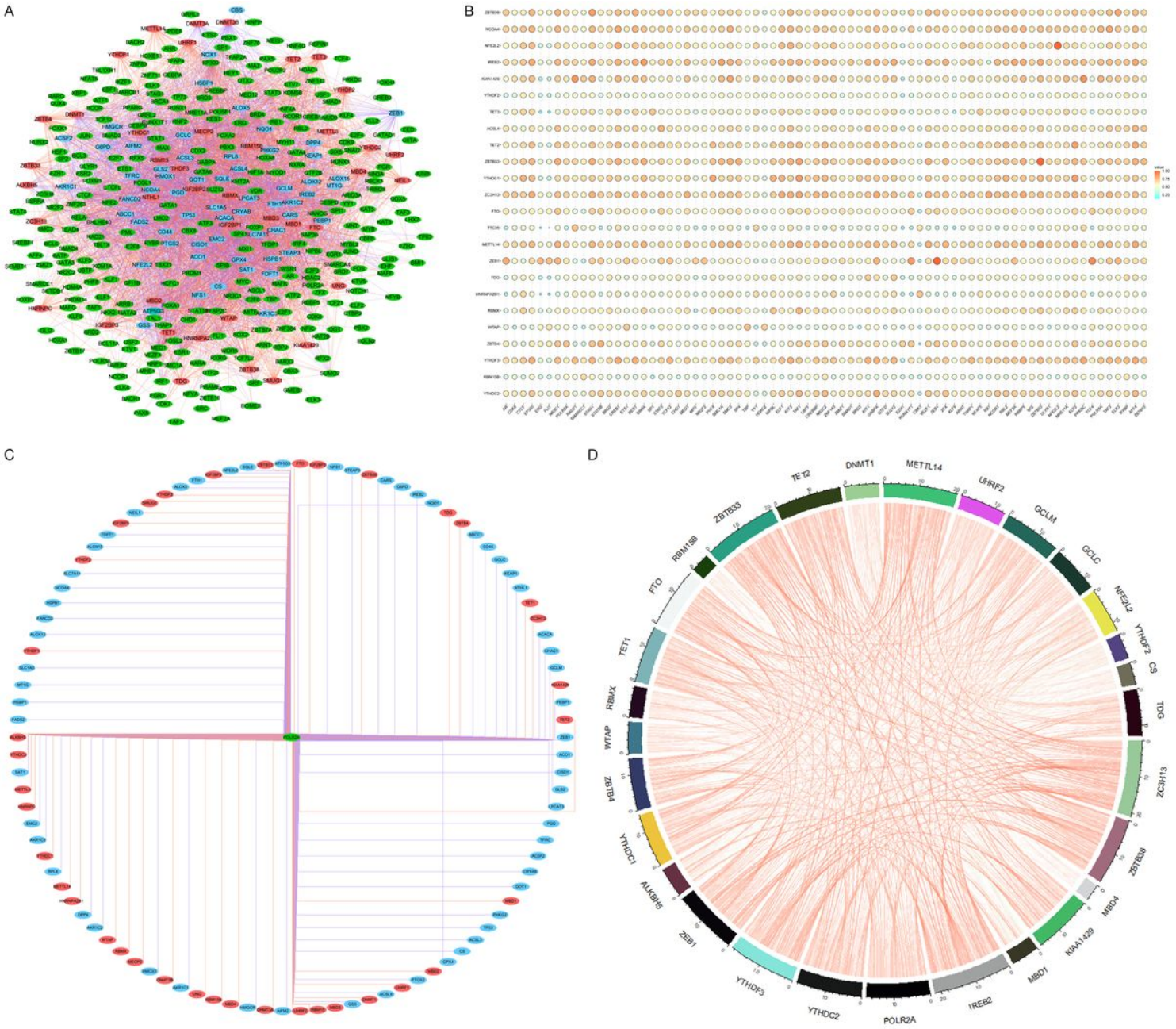

Figure 8

Transcriptional regulatory network and correlation for m6A/5mC regulators and ferroptosis-related genes. (A) The transcriptional regulatory network for m6A/5mC regulators and ferroptosis-related genes. (B) The correlation between m6A/5mC regulators or ferroptosis-related genes with transcriptional factors in PAAD. (C) The network among POLR2A, m6A/5mC regulators and ferroptosis-related genes. (D) The correlation between m6A/5mC regulators or ferroptosis-related genes with POLR2A in PAAD. The expression of m6A/5mC regulators and ferroptosis-related genes was normalized by GAPDH.

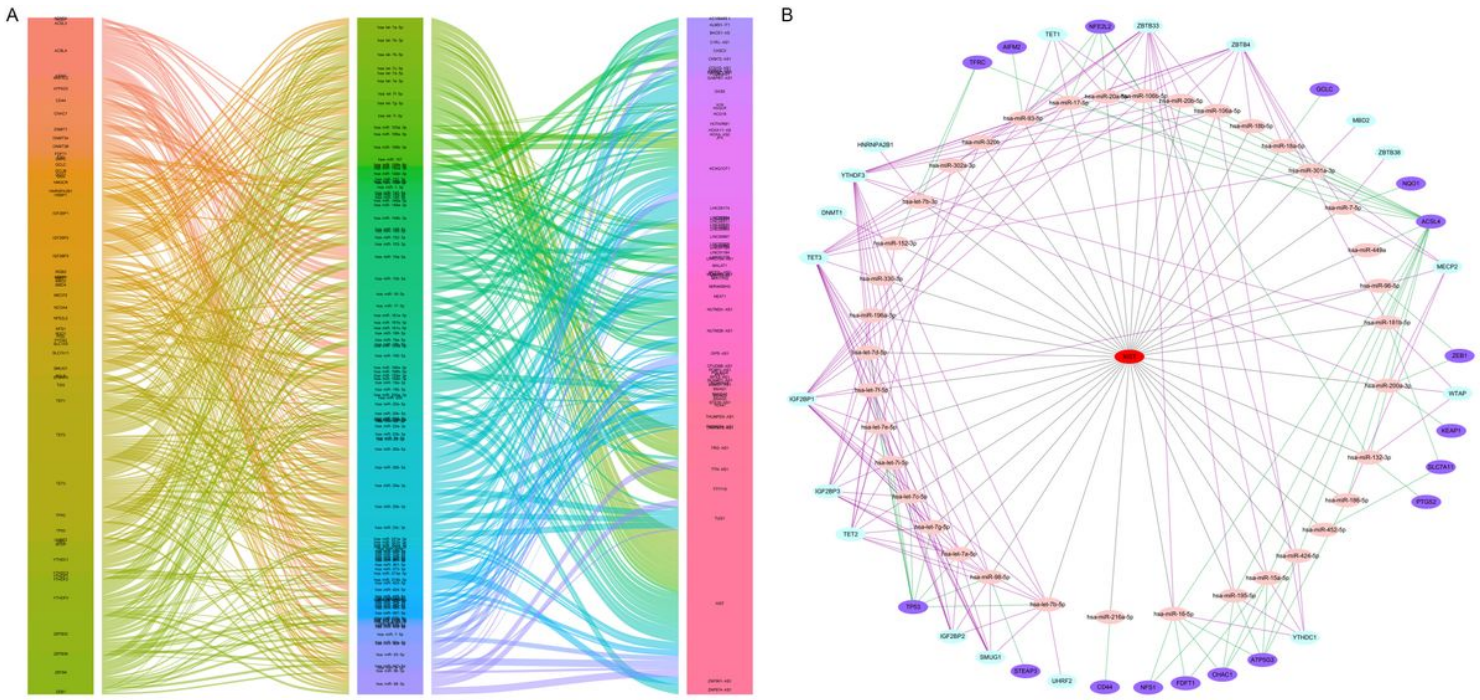

Figure 9

LncRNA-miRNA-mRNA ceRNA regulatory network. (A) The ceRNA triplets regulatory network. (B) The network between XIST and related miRNA-mRNA. The red circle represents XIST, the pink circles represent miRNA regulated by XIST, the purple circles represent ferroptosis-related genes indirectly regulated by XIST, and the light blue circles represent m6A/5mC regulators indirectly held by XIST.

Supplementary Files

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

- TableS1a.xlsx
- TableS1b.xlsx
- TableS2.xlsx
- TableS3.xlsx