Integrative pharmacogenomic profiling identifies novel cancer drugs and gene networks modulating ferroptosis sensitivity in pan-cancer

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Research

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Abstract

Background: Ferroptosis is an apoptosis-independent cell death program implicated in various diseases including cancer. Emerging evidence has demonstrated the promise of pharmacological induction of ferroptosis as a novel anti-cancer approach, but the molecular underpinnings of ferroptosis regulation and biomarkers associated with sensitivity to ferroptosis inducers has been poorly defined. **Methods:** By implementing integrated pharmacogenomic analysis, we correlated the sensitivity of small-molecule compounds (n=481) against the transcriptomes of solid cancer cell lines (n=659). The potential of a drug compound to modulate ferroptosis was determined by significant (empirical p-value < 0.01) association of drug effectiveness with SLC7A11 expression. To establish generalized gene signatures for ferroptosis sensitivity and resistance, we interrogated drug effects of multiple ferroptosis inducers (n=7) with transcriptomic data of pan-solid cancer cells. Finally, the ferroptosis gene signature was applied to The Cancer Genome Atlas (TCGA) and Cancer Cell Line Encyclopedia (CCLE) project to identify cancer patients and cells that likely benefit from ferroptosis-based therapeutics. **Results:** We report, for the first time, the comprehensive identification of cancer drugs with the potential to induce ferroptosis and a generalized gene expression signature predicting ferroptosis response in pan-cancer. Informed by the findings, we reveal an unanticipated role for class I histone deacetylase (HDAC) in regulating ferroptosis and show that targeting HDAC significantly enhances the ferroptosis-promoting effect of Erastin in lung cancer cells. Moreover, our data indicate that small cell lung cancer (SCLC) and isocitrate dehydrogenase (IDH)-mutant brain tumors are highly primed for ferroptosis, suggesting that relaunching ferroptosis might be an innovative strategy to target these malignancies. **Conclusions:** Expanding arsenal targeting aberrant ferroptosis and deciphering gene networks dictating ferroptosis sensitivity shed light on ferroptosis regulatory networks and may facilitate biomarker-guided stratification for ferroptosis-based therapy.

Background

Escape from cell death is fundamental for cancer development. Ferroptosis is a newly identified form of programmed cell death that differs genetically and biochemically from apoptosis, necroptosis, and autophagy-dependent death programs [1, 2]. Physiologically activated by metabolic accumulation of lipid peroxides, ferroptosis is frequently dysregulated in cancers and confers a key mechanism of therapeutic resistance, providing a novel approach of cancer treatment by boosting or relaunching ferroptosis [3].

Ferroptosis is negatively regulated by a lipid radical-specific antioxidant defense system involving glutathione peroxidase 4 (GPX4) that hydrolyzes lipid hydroperoxides and thereby protects cells from ferroptosis [4]. Antagonizing GPX4 with small molecules, such as rat sarcoma viral oncogene homolog (RAS)-selective lethal 3 (RSL3) efficiently induces ferroptosis [1]. The reductase activity of GPX4 requires the co-factor glutathione (GSH), an abundant cellular tripeptide consisting of glycine, glutamate and cysteine, as an electron donor to reduce lipid hydroperoxides. GSH synthesis depends on intracellular availability of the precursor cysteine that is mainly generated from reduction of cystine; thus, cystine depletion also induces ferroptosis [2]. As cysteine is imported from the extracellular space via the sodium-

independent cystine/glutamate antiporter system xc-, a heterodimer consisting of a heavy chain (4F2, also known as SLC3A2) and a light chain (xCT or SLC7A11) [2], targeting xCT/SLC7A11, e.g., by Erastin, restrains cystine supply and provokes ferroptosis [1, 2].

Ferroptosis suppressor protein 1 (FSP1), also known as apoptosis-inducing factor mitochondria associated 2 (AIFM2), was proposed to play a GPX4-independent anti-ferroptosis role in cancer [5, 6], and combined inhibition of GPX4 and FSP1 synergistically enhances ferroptosis [5, 6]. Recent studies have also identified additional factors with a potential role in regulating ferroptosis [7–10]. Despite the progress, the molecular mechanisms underlying the complexity of the ferroptosis process remain poorly understood.

Ferroptosis dysregulation plays critical roles in cancer pathogenesis [9, 11], validating the rationale of pharmacological induction of ferroptosis as an anti-cancer strategy [7] or to overcome therapy resistance [12]. However, ferroptosis-inducing therapeutics without further stratification has only achieved limited success, and the paucity of molecular networks regulating ferroptosis sensitivity has significantly hampered the development of ferroptosis-based therapeutic strategies. In this study, we performed an integrative pharmacogenomics analysis by correlating the sensitivity profiling of multiple ferroptosis-triggering drugs with basal gene expression across a huge cohort of solid cancer cell lines, which systematically explored, for the first time, the gene networks linked with ferroptosis response in pan-cancer. In particular, our work identified novel drug candidates with the potential to activate ferroptosis and a generalized gene signature indicative of ferroptosis sensitivity, which may facilitate the therapeutic exploitation of ferroptosis in cancer.

Materials And Methods

Cell culture, drug treatment and cell viability assay

Non-small cell lung cancer (NSCLC) cell lines (H1650, HCC827, PC9) were cultured in RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY, USA) and 1% penicillin/streptomycin (Sigma-Aldrich) at 37 °C in a humid incubator with 5% CO₂. All cancer cell lines were authenticated by DNA fingerprinting and negative for mycoplasma. Erastin (Cat. # CS-1675) and Vorinostat (Cat. # CS-0589) purchased from ChemScene (Monmouth Junction, NJ, USA).

Cells were seeded in 6-well plates (50,000 cells/well), and treated with DMSO (control), Erastin (1 µM), Vorinostat (HDAC inhibitor, 1 µM), alone or in combination for 72 h, and cell viability was quantified by counting cell number.

Databases

Processed drug screening and gene expression data across a set of small-molecule compounds (n = 481) and solid cancer cell lines (n = 659) from a published study [13] were downloaded for reanalysis.

Correlation data across all 481 small molecules against individual transcriptomes that are significantly correlated with response to at least one small molecule were included for analysis [13]. The area under the curve (AUC), determined by fitted concentration-response curves (2-fold dilution, over a 16-point concentration range), is used as a measure of sensitivity. Fisher's z-transformation was applied to the correlation coefficients to adjust for (normalize) variations in cancer cell line number across small molecules and contexts [13]. For validation analysis, the sensitivity profiling to Erastin of an independent cohort of cancer cell lines (n = 117, including 99 non-hematopoietic/lymphoid-derived cancer cell lines) [4] was employed. The publicly available database FerrDb (<http://www.zhounan.org/ferrdb/>) [14] was used as a reference for the newly identified biomarkers of ferroptosis. Drug-gene interaction database (<http://dgidb.org/>) was examined for druggable genes. Genetic landscape of identified genes across The Cancer Genome Atlas (TCGA) Pan-cancer cohort was downloaded from cBioPortal (<https://www.cbioportal.org/>). Normalized transcriptomic data were downloaded from Cancer Cell Line Encyclopedia (CCLE) project (<https://portals.broadinstitute.org/ccle>). Protein interactions and pathway enrichment analyses were based on STRING databases (version 11.0; <https://string-db.org/>). R software (version 3.6.3) was used for statistical analyses and data presentation.

Ferroptosis gene signatures and patient survival analysis

The gene signatures of ferroptosis sensitivity and resistance were scored as the sum of the respective gene sets after scaling, respectively. Curated EMT gene signature was based on previous studies, scored as the sum of a mesenchymal gene set (FN1 + VIM + ZEB1 + ZEB2 + TWIST1 + TWIST2 + SNAI1 + SNAI2 + CDH2) minus that of epithelial genes (CLDN4 + CLDN7 + TJP3 + MUC1 + CDH1) [15, 16].

Survival analysis was performed using “survminer” and “survival” R packages. Transcriptomic data of primary tumor samples and clinical data of matched patients in the TCGA Pan-cancer cohort were used for survival analysis, whereby patients were divided into two groups based on a best-separation cut-off value of FS/FR gene signatures to plot the Kaplan-Meier survival curves.

Statistical analysis

Data were presented as mean $\pm$ s.d., with the indicated sample size (n) representing biological replicates. Data analysis was performed by GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA, USA). Gene expression and survival data derived from the public database, as well as correlation coefficient (Pearson and Spearman), were analyzed using R (version 3.6.2). Statistical significance was determined by one-way/two-way analysis of variance (ANOVA), Bonferroni's multiple comparison test, and Student's t-test using GraphPad Prism 7, unless otherwise indicated. $P < 0.05$ was considered statistically significant.

Results

Systematic correlation identifies cancer drugs with the potential to induce ferroptosis

Ferroptosis is negatively regulated by the SLC7A11-GPX4 signaling axis (Fig. 1A). To systematically identify cancer drugs that modulate ferroptosis response of cancer cells, we correlated the sensitivity profiling of a previously curated small-molecule compound library (n = 481) containing FDA-approved drugs, clinical candidates and those interrogating important targets and/or cellular processes in cancer, against the transcriptomes of a cohort of pan-cancer cell lines (n = 659) [13]. This analysis revealed that gene expression (mRNA) of *SLC7A11* most strongly positively correlates with the area under the curve (AUC), a measure of drug sensitivity determined by fitted concentration-response curves, of both RSL3 (Pearson correlation z-score = 9.05; p = 6.10E-05) and Erastin (Pearson correlation z-score = 7.94; p = 6.10E-05), two classical ferroptosis-inducing agents (Fig. 1B, C). This observation indicates that cancer cells with higher *SLC7A11* mRNA levels have greater AUC values, and thus are less sensitive or more resistant to ferroptosis induction, which is consistent with previous studies reporting that SLC7A11 is a core negative regulator of ferroptosis and increasingly appreciated as a therapeutic target in cancers [1, 2]. Interestingly, both RSL3 and Erastin showed no significant correlation with *GPX4* or *SLC3A2* (data not shown), which might be due to the high abundance of the two genes in cancer cells.

Next, we systematically correlated sensitivity data (determined by AUC) of the small-molecule compounds (n = 481) with *SLC7A11* gene expression across the entire cancer cell line cohort (n = 659). This analysis identified a total of 139 drug candidates whose AUC values significantly (empirical p-value < 0.01) positively correlate with *SLC7A11* (Fig. 1D; **Table S1**), e.g., ML162, ML210, RSL3, PX-12, PRIMA-1, Piperlongumine, and Erastin that were previously shown to trigger ferroptosis [17], validating the robustness of the systematic correlation and accountability of our results.

The pattern by which these drugs cluster in the systematic correlation analysis suggests that they may share the mode of action in regulating ferroptosis [13]. Importantly, our analysis revealed a set of drug candidates with ferroptosis-activating potential that were neither previously reported nor recorded by the FerrDb (<http://www.zhounan.org/ferrdb/>), a manually curated dataset elaborating on ferroptosis (**Table S1**). Analyzing the annotated targets of the identified compounds revealed that several pathways, including ROS (reactive oxygen species) modulation, fatty acid biosynthesis regulation, MDM2-p53 signaling, receptor tyrosine kinases, NAMPT (nicotinamide phosphoribosyltransferase), ubiquitin-proteasome and, particularly, PI3K-AKT1-mTOR and epigenetic regulators, are enriched (**Table S2**), suggesting that these signaling cascades may be involved in ferroptosis deregulation in cancer. Supporting our findings, recent studies have demonstrated that p53 and mTOR play essential roles in regulating ferroptosis [11, 18]. Importantly, our data implicated an unexpected role for class I histone deacetylase (HDAC) family in ferroptosis regulation (**Table S2**). To verify this notion, we treated NSCLC cells (H1650, PC9 and HCC827) with Erastin and Vorinostat, a clinically-approved class I HDAC inhibitor, alone or in combination, which showed that the presence of Vorinostat significantly enhances the anti-proliferative effect of Erastin (Fig. 1E). Notably and consistent with our finding, previous studies showed that class I HDAC inhibitors induce ROS-dependent cell death although the underlying mechanisms were not clear [19–21].

Gene networks associated with ferroptosis sensitivity and resistance in pan-cancer

Cancer cells show high heterogeneity in response to ferroptosis-based therapeutics [7], highlighting the need for further stratification. We therefore sought to delineate the gene networks linked with ferroptosis sensitivity and resistance in cancer cells. To generalize the results and minimize drug-specific and potential off-target effects, multiple established ferroptosis-inducing molecules, namely ML162, ML210, Necrosulfonamide, PRIMA, PX-12, RSL3, and Erastin whose sensitivity most significantly correlate with *SLC7A11* expression (Fig. 1D) were integrated in our analysis. Correlating drug sensitivity data with basal gene expression of pan-solid cancer cell lines ($n = 659$) revealed that, as expected (Fig. 1A-C), *SLC7A11* reappeared as one of the top hits with their expression most significantly positively correlated with the AUC values of all seven drugs (**Fig. S1A-E**), reinforcing the robustness of our approach.

The genes significantly (empirical p -value < 0.01) correlated with the selected drugs fell into the sensitive group (high expression linked with increased ferroptosis susceptibility or decreased AUC), containing those negatively correlated with drug effects (AUC), and the resistant group whereby the expression of genes positively correlated with AUC values. Notably, ZEB1, previously shown to be a marker for sensitivity to ferroptosis [12], is negatively correlated with several ferroptosis inducers (Fig. 1B; **Fig. S1A, B**), while FSP1/AIFM2, an anti-ferroptotic regulator independent of GPX4 [5, 6], is in the resistant group (Fig. 2B; **Fig. S1A, B**). The coverage of previously identified ferroptosis regulators reiterates the validity of the systematic correlation.

To curate a generalized gene signature for ferroptosis sensitivity, we focused on the candidates common to all tested drugs. By setting a stringent threshold at an empirical p -value < 0.01 , we finally delineated a set of 46 and 35 genes linked with ferroptosis sensitivity and resistance, respectively (Fig. 2). Supporting our results, two genes (*ELAVL1* and *ATP6V1G2*) in the sensitive group (Fig. 2A) and four (*SLC7A11*, *FSP1/AIFM2*, *NQO1* and *SQSTM1*) in the resistant group (Fig. 2B) were previously reported or curated by the FerrDb (<http://www.zhounan.org/ferrdb/>) [14] that fulfil the same function as assigned by our study. Notably, the functional link between ferroptosis and the vast majority of these genes (44 of 46 in the sensitive and 31 of 35 in resistant group) have not been shown. The interaction network and pathways engaged by the identified genes were shown in **Fig. S2A, B**. Interestingly, genes in both groups are frequently altered, despite varied degrees in different cancers (Fig. 2C, D), which may enable further stratifications for ferroptosis-based therapy. Importantly, some genes (*NAMPT*, *IGF1R*, *CYP4F2*, *BLVRB*) in the resistant group are therapeutically exploitable according to the druggable genome database (<http://dgidb.org/>).

Next, we applied the newly curated ferroptosis sensitivity (FS) and resistance (FR) gene signatures to a pan-cancer cohort ($n = 9011$) in TCGA whereby transcriptomic and clinical data are available. Low grade glioma (LGG) displays the highest FS but lowest FR score across the solid cancers (Fig. 3A, B), indicating that LGG might be particularly susceptible to ferroptosis-inducing agents. Gliomas with mutations in IDH (isocitrate dehydrogenase), which leads to loss of its normal enzymatic function and the abnormal

production of oncometabolite 2-hydroxyglutarate, represent a unique subset genetically and clinically distinct from that carrying wild-type IDH, particularly in LGG [22]. Importantly, we found that *IDH1/2*-mutant LGG was associated with significantly higher FS score than *IDH1/2*-wild-type LGG (Fig. 3C), prioritizing an innovative strategy to target *IDH IDH1/2*-mutant LGG. Supporting our finding, recent studies showed that accumulation of oncometabolite 2-hydroxyglutarate, the product of the mutant IDH, sensitizes cells to ferroptosis [23] and shRNA-based knockdown of *IDH2* increases the sensitivity to Erastin-induced ferroptosis [24].

In contrast, lung adenocarcinoma (LUAD) exhibits the highest FR score (Fig. 3B), suggesting that aberrant blockage of ferroptosis might be a key feature in LUAD. The KEAP1-NRF2 axis is well known to negatively regulate ferroptosis, and cancer cells with *KEAP1* mutations are associated with increased resistance to ferroptosis [25]. In line with this notion, interrogation of a LUAD cohort in TCGA revealed that tumors with *KEAP1* mutations, frequent in LUAD samples, display significantly higher FR signature scores (Fig. 3D). Importantly, a high FS score is associated with significantly better overall survival (OS) and progression-free interval (PFI), while a high FR with poor OS and PFI in patients with LGG and LUAD (Fig. 4A, B), validating the clinical relevance of the FS and FR gene signatures.

Moreover, a previous study associated susceptibility to ferroptosis with mesenchymal cell state [12]. Supporting this notion, we identified *ZEB1* as one of the most strongly negatively correlated genes with RSL3, Erastin, ML162 and ML210 (Fig. 1B, C; Fig. 1A, B). We thus further investigated the link between EMT and FS signatures across the pan-cancer cohort in TCGA, and found a positive correlation of these two phenotypes in most cancer lineages (Fig. 4C).

To seek additional evidence for the applicability of the newly curated FS and FR signatures (**Table S3**), we prospectively probed ferroptosis response in a large cohort of pan-cancer cell lines from CCLE database (n = 890). Tumor cells of histological origins from small cell lung cancer (SCLC), which was not included in TCGA project, and sympathetic nervous tissue (autonomic ganglia cells) dominate both the top 50 and top 100 cell lines based on their FS scores, with the highest and second-highest FS signature, respectively (Fig. 5A-C). SCLC cells have significantly higher FS scores than NSCLC ($p < 2.2E-16$) (Fig. 5D), consistent with the results derived from cancer patients that LUAD and LUSC (lung squamous cell carcinoma), two major types of NSCLC, show low FS but high FR scores (Fig. 5A). Similarly, pheochromocytoma/paraganglioma (PCPG) cancer, with the same origin as autonomic ganglia cells, exhibits the second-highest FS signature in patients of pan-cancer (Fig. 3A). These results reinforce the applicability and reliability of the FS/FR gene signature and further suggest that SCLC, a highly aggressive neuroendocrine lung cancer lacking targeted therapies, might particularly benefit from ferroptosis-based therapeutics.

Finally, we applied the ferroptosis gene signatures (Fig. 2A, B) to another independent study cohort of non-haematopoietic/lymphoid cancer cell lines (n = 99) treated with Erastin [4]. SCLC cell lines showed significantly higher FS but lower FR signature scores than NSCLC cells (Fig. 6A; **Table S4**). Importantly, sensitivity profiling revealed significantly lower AUC values of Erastin in SCLC than NSCLC cells (Fig. 6B), indicating that SCLC cells are endowed with greater sensitivity to Erastin than NSCLC, which is in line with

our results obtained from the analysis of CCLE project (Fig. 5A-C). Strikingly, the FS signature score was significantly negatively correlated with the AUC value of Erastin across lung cancer (Pearson $r = -0.79$, p -value = $3.3e-08$) and non-hematopoietic/lymphoid-derived cancer cell lines (Pearson $r = -0.39$, p -value = $7.3e-05$) (Fig. 6C), demonstrating that cancer cells with a higher FS signature are indeed characterized by increased susceptibility to the ferroptosis inducer Erastin.

Together, our work identified new cancer drugs with the potential to relaunch ferroptosis and delineated the gene networks associated with responses to ferroptosis in pan-cancer. The applicability and credibility of our findings are demonstrated by a multitude of lines of evidence from independent study cohorts of cancer cell lines and cancer patients.

Discussion

Ferroptosis has increasingly gained attention due to the critical roles in tumorigenesis and cancer progression [1, 7, 9, 12]. Despite some progress [4–6, 8, 10], the complexity of ferroptosis, especially the regulatory networks governing ferroptosis, remains enigmatic, limiting the success of ferroptosis-based therapy. Here, we provide a systematic analysis of the gene networks linked with ferroptosis, and reported on the identification of novel cancer drugs and gene clusters regulating sensitivity and resistance to ferroptosis. Our findings are supported by previous studies and clinical evidence. Informed by these findings, we show, for the first time, that the effect of ROS-related cell death induced by class I HDAC inhibitors may relate to ferroptosis, and that targeting class I HDAC with the clinically approved Vorinostat enhances the anti-proliferative effect of Erastin that is known to induce ferroptosis. Besides, our results suggest that LGG, neuroendocrine SCLC, and tumors derived from sympathetic nervous tissue might particularly benefit from ferroptosis-activating agents.

Among the drug candidates with the potential to induce ferroptosis, some are not unexpected, e.g., those targeting fatty acid biosynthesis, MDM2-p53 signaling, and PI3K-mTOR pathway that have been previously reported [11, 12, 18]. Interestingly, we identified inhibitors of class I HDAC (**Table S2**), for which a link with ferroptosis has not been appreciated. Our finding, however, may explain the underlying mechanisms of ROS-related cell death conferred by class I HDAC inhibitors [19–21].

Our data also shed light on ferroptosis regulation in cancer. Of particular note, p53 [11], autophagy [25], and endoplasmic reticulum (ER) stress-associated unfolded protein response (UPR) [2, 26] have been implicated in ferroptosis. Consistently, several genes identified in the present study are well-known effectors of the reported pathways: PPM1D is a downstream effector of p53, TMEM74 (transmembrane protein 74) and SQSTM1 (Sequestosome-1) involved in autophagy and DNAJC3/DNAJB14 (DnaJ homolog subfamily C/B member 3 or 14) in ER stress/UPR. The top candidacy of ELF1 (ETS-related transcription factor Elf-1) and TFAP2C (transcription factor AP-2 gamma) suggests that the two transcription factors might also play a regulatory role in ferroptosis. Collectively, we identify new compounds and gene networks regulating ferroptosis in cancer, although the underlying mechanistic insights remain to be explored.

Conclusions

Identification of novel cancer drugs and gene signatures modulating ferroptosis response provides a framework to delineate the molecular mechanisms of ferroptosis regulation and to stratify cancer subsets for precision oncology.

Abbreviations

AIFM2

apoptosis-inducing factor mitochondria associated 2

AUC

area under the curve

CCLE

Cancer Cell Line Encyclopedia

ER

endoplasmic reticulum stress

FS

ferroptosis sensitivity

FR

ferroptosis resistance

GPX4

glutathione peroxidase 4

GSH

glutathione

HDAC

histone deacetylase

IDH

isocitrate dehydrogenase

LGG

Low grade glioma

LUAD

lung adenocarcinoma

OS

overall survival

PCPG

pheochromocytoma/paraganglioma

PFI

progression-free interval

RSL3

(RAS)-selective lethal 3

SCLC
small cell lung cancer
TCGA
The Cancer Genome Atlas
UPR
associated unfolded protein response.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare no potential conflict of interest.

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Authors' contributions

Haitang Yang: Conceptualization, Data curation, Writing - original draft, Writing - review & editing.

Liang Zhao, Feng Yao, Yanyun Gao, Thomas Marti: Writing - review & editing.

Ralph Schmid: Writing - review & editing, Supervision, Data curation.

Ren-Wang Peng: Conceptualization, Supervision, Data curation, Writing - review & editing.

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Figures

Figure 1

Comprehensive pharmacogenomic analysis identifies cancer drugs with the potential to induce ferroptosis A, Schematic of ferroptosis regulation. GSSGO, oxidized glutathione disulfide; PE-AA-O-OH and PE-AA-OH, phosphatidylethanolamine (PE), arachidonoyl (AA), hydroperoxide group (-O-OH). GPX4 protects against ferroptosis by catalyzing GSH and toxic PE-AA-O-OH into oxidized GSH (GSSGO) and nontoxic PE-AA-OH. B-C, Gene clusters associated with sensitivity to RSL3 (B) and Erastin (C) across solid

cancer cell lines (n=659). Blue dots indicate the significantly negatively correlated genes while the red the positively correlated ones. The most significantly correlated genes were highlighted. Here, negative correlation indicates the association of a larger AUC area with lower gene expression, and vice versa. D, Comprehensive analysis of cancer drugs (n=481) whose activities are significantly correlated with SLC7A11 gene expression across the solid cancer cell lines (n= 659). Red dots indicate drugs whose effectiveness (AUC value) positively correlates with SLC7A11 mRNA. E, Non-small cell lung cancer cells (H1650, HCC827, PC9) were treated with Vorinostat (1 μ M), Erastin (1 μ M), alone or in combination (Vorinostat [1 μ M] plus Erastin [1 μ M]) for 72h. Cell viability were quantified by counting cell number. *p < 0.05, ****p < 0.0001 by one-way ANOVA (N=3).

Figure 2

Figure 2

Gene networks linked with ferroptosis sensitivity and resistance in pan-cancer A-B, Gene clusters in ferroptosis sensitive (A) and resistant (B) groups. Blue and red colors indicate genes previously reported or curated by FerrDb. C-D, Genetic alterations of the genes in ferroptosis sensitive (C) and resistant (D) groups across a pan-cancer cohort in TCGA.

Figure 3

Generalized gene signatures predictive of ferroptosis sensitivity and resistance A-B, Prospective analysis of ferroptosis sensitivity (FS) (A) and resistance (FR) (B) scores in TCGA pan-solid cancer cohort. C, Significant difference (by one-way ANOVA) of the FS gene signature score between IDH1/2-mutant and wild-type LGG. D, Significant difference (by one-way ANOVA) of the FR gene signature score between KEAP1-mutant and wild-type LUAD.

Figure 4

Prognostic values of the ferroptosis gene signature in cancer patients A-B, Kaplan-Meier survival analyses of LGG (A) and LUAD (B) stratified by the FS and FR gene signatures. C, Correlation analysis of the epithelial-mesenchymal transition (EMT) and FS gene signatures grouped by cancer types across TCGA pan-cancer cohort.

Figure 5

Figure 5

Predictive values of ferroptosis gene signature across pan-cancer cell lines A-C, Ferroptosis sensitivity was scored across solid pan-cancer cell lines based on the newly curated ferroptosis sensitivity gene signature. Normalized transcriptomic data were downloaded from CCLE project. D, Significant difference (by unpaired two-sided t-test) of the FS gene signature score between small cell lung cancer (SCLC) and NSCLC.

Figure 6

Validation of FS and FR gene signature scores A, Violin plot shows significant difference (by unpaired two-sided t-test) of ferroptosis sensitivity (FS) and resistance (FR) signature scores between small cell lung cancer (SCLC) and Non-SCLC (NSCLC). Normalized transcriptomic data of these cell lines were downloaded from Cancer Cell Line Encyclopedia (CCLE) project (<https://portals.broadinstitute.org/ccle>). FS and FR gene signatures were scored across a panel of non-hematopoietic/lymphoid-derived cancer cell lines (n=99) treated with Erastin, which were downloaded from an independent study cohort (Reference 4). B, Violin plot shows a significantly lower AUC (area under the curve) value in SCLC in

response to Erastin than that of NSCLC (by unpaired two-sided t-test). Of note, lower AUC values indicate more sensitivity or less resistance to Erastin. AUC values of Erastin were downloaded from an independent study cohort (Reference 4). C, A significantly negative correlation between FS score and AUC of Erastin across lung cancer and non-haematopoietic/lymphoid-derived cancer cell lines.

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