Pan-Cancer Prognostic, Immunity, Stemness, and Anticancer Drug Sensitivity Characterization of Pyroptosis Related Genes in Human Cancers

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Research Article

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Abstract

Pyroptosis related to inflammation has an important role for tumor initiation, progression and metastasis. Our studies described pyroptosis at the pan-cancer level through the tumor immune microenvironment, survival, stemness score, and anticancer drug sensitivity. We evaluate the differential expression of pyroptosis related genes using the Wilcox test of normal human tissues from the Genotype-Tissue Expression project (GTEX), and human cancer tissues from the Cancer Genome Atlas (TCGA). The R packages were used to identify survival analyses of pyroptosis related genes in 33 TCGA cancer types. The correlation between pyroptosis related genes expression and tumor microenvironment, tumor stem cell score, and anticancer drug sensitivity was determined through the Spearman correlation test and Pearson correlation test. The functional enrichment of pyroptosis related genes was investigated using Genes and Genomes (KEGG) and Gene Ontology (GO) analysis. In 18 TCGA cancer types and adjacent normal tissues, the 27 pyroptosis related genes were naturally different. According to correlation analysis, the association between the expression of 27 pyroptosis related genes and the tumor microenvironment revealed that the higher the expression of pyroptosis related genes, the greater the degree of tumor microenvironment. Anticancer drug sensitivity research revealed a favorable connection between anticancer medications and pyroptosis related genes expression such as ELANE, IL18 and CHMP4A ($p < 0.05$). We studied the association between the immune microenvironment and pyroptosis. The 27 pyroptosis related genes were significantly different in immune subtypes C1 (wound healing), C3 (inflammatory), C2 (IFN-gamma dominant), C5 (immunological quiet), C4 (lymphocyte deficient), and C6 (TGF-beta dominant), as shown in a pan-cancer immune subtype study ($p < 0.05$). Our research sheds light on the important function that pyroptosis related genes perform in the tumor immune microenvironment, stemness score, and anticancer treatment sensitivity in various cancers. This research was permitted by the Research Ethics Committee of the Emergency General Hospital (No. K202110).

Introduction

As an inflammasome-activated process, pyroptosis is characterized by necrotic morphology, which plays a critical role in cancer progression and development[1, 2]. Pyroptosis related genes have recently been attributed to onco-immunogenic features and prognosis in 33 TCGA cancer types[3, 4]. The survival rate of patients in various human cancers is characterized by a signature of pyroptosis related genes. Pyroptosis have such a critical function in lung adenocarcinoma progression and prognosis. Gasdermin D (GSDMD) was identified as pyroptosis executive protein that may be cleaved by inflammatory caspases and is required for IL-1 release, making it an important inflammatory mediator[5]. GSDMD knockdown inhibited tumor proliferation in non-small cell lung cancer (NSCLC) via promoting apoptosis and suppressing EGFR/Akt signaling[6]. Among NSCLC patients who smoked, high-mobility group box 1 (HMGB1) variations are strongly negatively related with EGFR mutations, which promotes growth, invasion, and survival in human lung cancer cells[7]. In response to anticancer therapy, HMGB1 has been demonstrated to enhance autophagy protection[8, 9]. The correlations between TP53 mutations and PD-L1 expression, IFN signatures, and TME composition were dramatically different[10]. When examining TP53 status as a

biomarker for immune checkpoint inhibitors, particular emphasis should be paid to any TP53 mutation heterogeneity[11]. Lung cancer remains the leading cause of cancer-related death worldwide[12]. Despite the fact that novel therapies have improved the mortality in NSCLC, the prognosis persists disappointing.

In our research, we looked at the clinical significance and molecular changes of pyroptosis related genes in pan-cancer types. Differential expression, tumor microenvironment, drug sensitivity, Cox, and immune subtype analysis of pyroptosis related genes were still absent among 33 TCGA cancer types. We investigated the differential expression of 27 pyroptosis related genes (BAK1, BAX, CASP1, CASP3, CASP4, CASP5, CHMP2A, CHMP2B, CHMP3, CHMP4A, CHMP4B, CHMP4C, CHMP6, CHMP7, CYCS, ELANE, GSDMD, GSDME, GZMB, HMGB1, IL18, IL1A, IL1B, IRF1, IRF2, TP53, TP63) throughout 33 cancer types, as well as the relationship between pyroptosis, tumor stem cell score, and NSCLC immune subtype was evaluated, looking forward to new explanations and potential strategies of pyroptosis in pan-cancer and NSCLC.

Materials And Methods

Pan-Cancer Transcriptome Expression Data, Immunophenotype Data, and Stem

Cell Score Data Download

We downloaded the 33 TCGA GDC pan-cancer transcription expression data, TCGA pan-cancer phenotype-immune subtype data, and stemness score (based on RNA expression) information with the UCSC Xena browser (https://xenabrowser.net/).

Gene Expression Analysis and Differential Expression Analysis

We collected transcription data from 33 TCGA cancers to construct a box plot of expression levels of 27 pyroptosis related genes (BAK1, BAX, CASP1, CASP3, CASP4, CASP5, CHMP2A, CHMP2B, CHMP3, CHMP4A, CHMP4B, CHMP4C, CHMP6, CHMP7, CYCS, ELANE, GSDMD, GSDME, GZMB, HMGB1, IL18, IL1A, IL1B, IRF1, IRF2, TP53, TP63). Then, the Wilcox test was used to compare gene expression between normal and tumors. A P-value difference of less than 0.05 was considered statistically significant.

Survival Analysis of Expression of Pyroptosis Related Genes

The “survival” and “survminer” R packages were applied to accomplish survival analysis of pyroptosis related genes. A statistically significant difference was defined as p value less than 0.05.

Functional Enrichment Analysis of Pyroptosis Related Genes

We used the Gene Ontology (GO) analysis and to perform functional enrichment analysis of pyroptosis related genes. GO is a comprehensive analysis, which characterize the genes and their products as cellular components biological processes, and molecular functions.

Cox and Immune Subtype Analysis

We used the 33 TCGA pan-cancer transcription expression and survival data to organize a Cox analysis to investigate if the expression of 27 pyroptosis related genes was associated to patient survival. The immune subtype analysis of 27 pyroptosis related genes was performed using the R packages "limma," "ggplot2," and "reshape2." It was statistically significant if the p-value was less than 0.05.

Correlation Analysis of the Tumor Microenvironment in 33 TCGA Pan-Cancers

The immune score, stromal score, and estimate score of 33 TCGA tumor samples were calculated utilizing the R packages "estimate" and "limma." After that, we performed the Spearman correlation test to compare transcription gene expression data with the estimate score of 33 TCGA cancer samples. A correlation analysis was accomplished between 27 pyroptosis related geness and the estimat score of 33 TCGA cancers. To conduct the Spearman correlation test, we integrated transcription gene expression data with stemness score (RNA expression–based) (RNAss).

Drug Sensitivity Analysis

We obtained the comparable sample of gene expression and drug sensitivity data from the CellMiner database (https://discover.nci.nih.gov/cellminer/home.do) and then filtered the drug sensitivity data after clinical laboratory verification and FDA standard certification. The Pearson correlation test was subsequently applied to correlate the 27 pyroptosis related genes expression data with drug sensitivity data. The link between pyroptosis related genes expression and drug sensitivity was eventually identified.

Immune Subtype, Clinical Characteristic, and Tumor Microenvironment Analysis of Lung Adenocarcinoma and Lung Squamous Cell Carcinoma

We conducted the Kruskal test to perform a differential analysis of immune subtype in order to further explore the association between pyroptosis related genes expression in LUAD and LUSC and immune subtype, clinical features, and the LUAD and LUSC microenvironment. The pathological T stage, TNM stage, and N stage were all subjected to differential analysis by the Kruskal test. A differential study of the pathogenic M stage was undertaken with the Wilcox test. To obtain the estimate score profile, we first downloaded the expression matrix of 33 tumors and used the R package "estimate" to perform tumor microenvironment analysis. Furthermore, we ran a correlation evaluation between pyroptosis related genes expression and LUAD and LUSC immune score, estimate score, RNAss, and stromal score using the Spearman correlation test.

Results

The Expression Level of Pyroptosis Related Genes in TCGA Pan-Cancers

In the TCGA database, we found expression of pyroptosis related genes in 33 tumors. Figure 1 describes the differences in pyroptosis related genes expression levels between TCGA cancers and corresponding normal tissues. BAX, CHMP3, CHMP4A, CHMP7, GSDME, and HMGB1 expression in LUAD and nearby normal tissues are not significant ($p > 0.05$), as shown in Fig. 1. Between LUAD and nomarl tissues, BAK1, CASP1, CASP3, CASP4, CASP5, CHMP2A, CHMP2B, CHMP4B, CHMP4C, CHMP6, CYCS, ELANE, GSDMD, GSDME, GZMB, IL18, IL1A, IL1B, IRF1, IRF2, TP53, and TP63 expression levels were substantially different ($p < 0.05$).

The heatmap of the log (fold change (FC)) of pyroptosis related geness in TCGA pan-cancers was shown in Fig. 2. Figure 2 revealed that the logFC of GZMB, CASP4, and CHMP2B was greater than 0, indicating that IGF2BP3, HNRNPC, and YTHDF1 expression in LUAD tissues was higher than in adjacent non-LUAD tissues. The logFC of TP63 and IL1A was greater than 0, indicating that LUSC tissues had higher levels of TP63 and IL1A expression than non-LUSC tissues. Figure 3 illustrates the research main flow diagram.

Pan-Cancer Survival Analysis of pyroptosis related genes

In BLCA patients, high CHMP6 and ELANE expression were associated with worse prognosis than low CHMP6 and ELANE expression ($p < 0.05$) (Fig. 4A, B). The high expression of CHMP3 and CHMP4C had a poor prognosis than the low expression in BRCA patients ($p < 0.05$) (Figs. 4C, D). The high expression levels of BAK1, CASP3, CHMP4C and CYCS had a poor prognosis than the low expression level in LUAD patients ($p < 0.05$) (Figs. 4E–H). The low expression levels of CHMP2B, CHMP4C, ELANE and IL1B had a poor prognosis than the high expression level in LUAD patients ($p < 0.05$) (Figs. 4I–L). CHMP4A, CHMP7, and IRF2 all serve as high-risk factors in BRCA, ACC, and SKCM patients, as shown in Fig. 4M. In KICH, COAD, and PRAD patients, HMGB1 is a high-risk factor. In PRAD patients, ELANE is a high-risk factor. In COAD, CAA, LUAD, and LUSC patients, CASP3 is a high-risk factor. In LUAD and LUSC patients, ELANE and IL1A are high-risk factors. In BRCA patients, TP53 is a high-risk factor; however, in COAD and ACC patients, TP53 is a low-risk factor.

GO and KEGG Analysis of Pyroptosis Related Genes

The pyroptosis related genes were found to be enriched in the metabolism of endopeptidase activity, particularly in the apoptotic process of cysteine-type endopeptidase, with the cellular component of late endosome membrane and ESCRT complex involved in mitotic cytokinetic process, vial budding via host ESCRT complex, and mibody abscission, according to the GO analysis (Fig. 5).

Correlation and Cox, Tumor Immune Microenvironment, and Tumor Microenvironment Analysis of Pyroptosis Related Genes in Pan-Cancer

GZMB, a cytotoxic serine protease, was found to be positively linked with CASP1 expression (Cor = 0.76), according to pyroptosis related genes correlation analysis. The expression of the inflammasome CASP1 was positively linked with the expression of the pro-inflammatory target gene IL1B (Cor = 0.77). CASP1 expression was negatively associated to the expression of nucleation factor for ESCRT-III CHMP6 (Cor=-0.12) (Fig. 6A). Figure 6B illustrated that the immune subtypes C1 (wound healing), C2 (IFN-gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunological quiet), and C6 (TGF-beta dominant) revealed significantly different expression of pyroptosis related geness in pan-cancer ($p < 0.001$). Most pyroptosis related genes expression was negatively linked with immune score, stromal score, and estimate score in Figs. 6C–E, indicating that the content of immune and stromal cells was low in 33 TCGA cancers, whereas the content of tumor cells was high in 33 TCGA cancers. Figure 6F found that more than half of pyroptosis related genes expression was positively correlated with the RNAss of 33 TCGA cancers, suggesting that the higher the expression of pyroptosis related genes, the higher the tumor stemness score, the stronger the activity of tumor stem cells, and the lower the degree of tumor differentiation.

Correlation Analysis Between pyroptosis related genes Expression and drug Sensitivity

Figure 7 revealed that ELANE expression was positively correlated with the sensitivity to Hydroxyurea and Carboplatin ($p < 0.001$). The stronger the drug sensitivity of Hydroxyurea ($p < 0.001$), the higher the expression of ELANE and CHMP4A. The lower the drug sensitivity of Okadatic acid, the higher the expression of IL18 and CHMP4A ($p < 0.001$). Floxuridine, Bleomycin, Gemcitabine, and Triethylenemelamine exhibited increased drug sensitivity when CHMP6 expression is higher ($p < 0.001$). The higher the expression of IL18 and CHMP4C, the stronger the drug sensitivity of Venblastine ($p < 0.001$).

Correlation Analysis of Pyroptosis Related Genes Expression and Immune Subtype and Clinical Characteristics and Tumor Microenvironment of Lung Adenocarcinoma and Lung Squamous Cell Carcinoma

Figure 8A showed that the expression of CYCS in LUAD differed significantly depending on the pathological stage of disease. In the pathological T1/T2/T3/T4 stage, the expression levels of CYCS in LUAD patients were considerably different, as shown in Fig. 8B. The expression of CYCS in LUAD patients was markedly different in the pathogenic N0/N1/N2/N3 stages, as shown in Fig. 8C. Figure 8D showed

that the expression of pyroptosis related genes in LUAD was negatively correlated to the pathogenic M0/M1 stage. Figure 8E showed that the expression of BAX in LUSC differed significantly depending on the pathological stage of disease. In the pathological T1/T2/T3/T4 stage, the expression levels of CYCS in LUSC patients were considerably different, as shown in Fig. 8F. The expression of BAX and ELANE in LUSC patients was markedly different in the pathogenic N0/N1/N2/N3 stages, as shown in Fig. 8G. Figure 8H showed that the expression of pyroptosis related genes in LUSC was negatively correlated to the pathogenic M0/M1 stage.

Figure 9A illustrated that TP53 expression was substantially linked with the LUAD stem cell score (based on RNA expression data) (RNAss) in LUAD patients ($p < 0.05$). TP63 expression in LUAD patients was positively connected with the LUAD microenvironment estimate score ($p < 0.05$), whereas BAX, CHMP2A, and CHMP4B expression in LUAD patients was negatively correlated with the LUAD microenvironment estimate score ($p < 0.05$). The expression of TP63 in LUAD patients showed a positive link with the LUAD microenvironment immune score ($p < 0.05$), but the expression of BAX, CHMP2A, CHMP4B and CYCS in LUAD patients had a negative relationship ($p < 0.05$). The expression of TP63 had a positive relationship with the LUAD microenvironment stromal score ($p < 0.05$), while the expression of CHMP2A, CHMP4B and CHMP6 in LUAD patients had a negative relationship with the LUAD microenvironment stromal score ($p < 0.05$). Figure 9B revealed that TP53 expression levels in LUSC patients were strongly related to LUSC stem cell scores (based on RNA expression data) (RNAss) ($p < 0.05$). However, there were no statistical differences found in the expression of other factors ($p > 0.05$).

Discussion

Numerous previous studies have demonstrated that pyroptosis related genes are significantly linked to prognostic factors such as tumor types[13]. While, the definitive relationship between pyroptosis and integral tumor-associated immune infiltrates is unknown[1, 14–16]. Pyroptosis suppresses tumor formation while provides a favorable microenvironment for tumor growth as inflammatory cell death[17]. Inflammasomes stimulate caspase-1, which cleaves GSDMD as well as proIL-18 and proIL-1, causing membrane gaps to develop and IL-18 and IL-1 to be secreted from the cytoplasm to the surrounding environments in a canonical pyroptotic pathway[18]. Compared to GSDMD protein levels in similar neighboring tumor tissues, GSDMD protein levels in NSCLC were considerably higher. Higher levels of GSDMD were linked to aggressive characteristics such as bigger tumor sizes and TNM stages. Furthermore, increased GSDMD expression in LUAD was associated with a bad prognosis. In vitro and in vivo, knocking down GSDMD inhibited tumor growth[19, 20]. Our research found that pyroptosis related genes differed in the immunological subtype, and tumor microenvironment across 33 cancer types, providing a new strategy to investigate the crucial function of pyroptosis related genes in pan-cancer.

Pyroptosis shares some features with apoptosis, such as DNA damage, nuclear condensation, and caspase independence, but it differs from other forms of programmed cell death due to its morphology[21]. Inflammasomes are multimolecular complexes that contain pattern-recognition receptors

(PRRs), which when activated, can cause pyroptosis by inducing inflammatory responses. The complex causes pyroptosis by activating caspase-1 in the canonical inflammasome pathway and activating caspase-11 and caspase-4, 5 in the noncanonical inflammasome pathway[3]. The Polo-like Kinase 1 (PLK1) inhibitor BI2536 increased the sensitivity of oesophageal squamous cell carcinoma (ESCC) cells to cisplatin by inducing pyroptosis, according to a latest study[22]. When low doses of BI2536 were combined with DDP, the apoptosis-related protein BAX and caspase-3 were activated, causing GSDME to be cleaved and increased DNA damage. In ESCC cells, GSDME expression is higher than in normal cells. In ESCC cells, high levels of GSDME transition apoptosis to pyroptosis. When compared to neighboring normal equivalents, the LncRNA RP1-85F18.6 was overexpressed in colorectal cancer[23]. LncRNA RP1-85F18.6 inhibited pyroptosis and apoptosis in colorectal cancer cells and played a key role in their proliferation, metastasis, and normal cell cycle disruption. P63 is a member of the p53 tumor suppressor family, which is identical to p53. Two p63 gene promoters influence p63 expression, resulting in two distinct isotypes: TAp63 and Np63, which operate in carcinogenesis in completely different ways[24].

Presently, CASP1, CHMP3, CHMP4A, CHMP4C, CHMP7, CYCS, GZMB, IL18, IL1B, IRF1, IRF2, TP53, and TP63 were all shown to be substantially linked with overall survival of BRCA patients in our research ($p < 0.001$). The CHMP3 gene was shown to be considerably downregulated in a variety of human tumors, including BRCA, according to the study. Patients with BRCA who had a greater level of CHMP3 gene exhibited a better 3- and 5-year survival rate. Comprehensive research revealed that the 12 autophagy-related genes, including TP63, were linked to BRCA

patients' overall survival and were expressed significantly in BRCA and normal breast tissue at the protein level. In our study, CHMP4A was found to be strongly linked with overall survival in LUAD, KICH, BRCA, and ACC patients ($p < 0.001$). CYCS was reported to be a viable prognostic biomarker for predicting the overall survival of BRCA and CRC patients. Besides, chemotherapy did not benefit patients in terms of survival. The effectiveness of chemotherapy in patients with PACC should be evaluated further.

Interestingly, BAK1, BAX, CASP1, CASP3, CASP4, CASP5, CHMP2A, CHMP2B, CHMP3, CHMP4A, CHMP4B, CHMP4C, CHMP7, CYCS, ELANE, GSDMD, GZMB, HMGB1, IL18, IL1A, IL1B, IRF1, IRF2, TP53, TP63 were all identified to exhibit different levels of expression in immunological subtype. The pyroptosis related genes have been linked to the tumor microenvironment in a variety of cancers. Our research looked at the association between the expression of pyroptosis related genes and the tumor stem cell score in 33 cancer types. With the diverse expression of pyroptosis related genes, we discovered that while there was a higher content of immune and stromal cells, the content of tumor cells was low. The expression of TP53 in LUAD was favorably linked with the LUAD stem cell score, according to our findings (based on RNA expression). The higher the level of TP53 expression, the more tumor LUAD stem cell features and the higher the tumor LUAD differentiation. Cancer stem cells (CSCs) are self-renewing and developing cells that play a vital role in the development and therapeutic resistance of cancers, including LIHC, according to a recent study. Angiogenesis, negative control of DNA-binding transcription factor activity, apoptosis, and autophagy are all pathways enriched in CSC-related genes, according to functional analysis. The LIHC stem cell score was positively linked with the expression of CASP3, CASP5, CHMP6,

CHMP7, HMGB1, and IL1B. (based on RNA expression). The more features of tumor LIHC stem cells and the lower the tumor LIHC differentiation, the higher the expression level of CASP3, CASP5, CHMP6, CHMP7, HMGB1 and IL1B.

The nascent relevance of necroptosis and pyroptosis of non-small-cell lung cancer was recently shown in a study that examined pyroptosis related genes expression patterns within immuno-transmitters and the role of neurotransmitters in immune evasion, tumor progression and the survival. Higher BAK1, CASP3, CHMP4C, and CYCS expression was related with a worse prognosis in LUAD patients (Figs. 1O, 4B), indicating that these genes perform as oncogenes that drive LUAD carcinogenesis. ELANE expression was found to be strongly linked with poorer PRAD patient survival (Figs. 1M, 4D), demonstrating that ELANE plays a critical role in PRAD carcinogenesis. Lower TP53 expression was related with a worse outcome in COAD and ACC patients (Figs. 1E, 4I), revealing that TP53 functions as a tumor suppressor gene in COAD and ACC carcinogenesis.

Several pyroptosis related genes exhibited a significantly positive association with the drug in our investigation, for example, ELANE was significantly positively correlated with the drug sensitivity of Hydroxyurea and Carboplatin. The drug sensitivity of Floxuridine, Bleomycin, Gemcitabine, and Triethylenemelamine was significantly positively related to the expression of IL18 and CHMP4A, while the drug sensitivity of Floxuridine, Bleomycin, Gemcitabine, and Triethylenemelamine was significantly negatively related to the expression of CHMP6. CellMiner is a database of genomic and pharmacologic tools for identifying drug patterns and transcripts in the NCI-60 cell line. CellMiner is a database of genomic and pharmacologic tools for identifying drug patterns and transcripts in the NCI-60 cell line[25]. The CellMiner database allows users to quickly access and compare gene expression levels for 360 microRNAs, 22,379 genes, and 20,503 chemicals containing 102 FDA-approved medicines[26]. Our research initially looked at the association between pyroptosis related genes and anticancer drug sensitivity, which offered a bright insight into tumor therapy treatment and avoiding tumor resistance. Selumetinib has shown efficacy in several cancers, including non-small cell lung cancer (NSCLC), melanoma, and locally advanced cervical cancer, both preclinically and therapeutically as a single agent or in combination with conventional chemotherapy and other targeted treatments. For patients with stage III NSCLC, phase II studies found that the combination of ciplatin and vinorelbine provides a novel treatment with clinically substantial anticancer efficacy and a dose-depending safety profile[27, 28]. The combination of pericardial drainage and bleomycin instillation was proven to be a safe and effective therapy for malignant pericardial effusion associated with NSCLC[29].

In summary, our research found that pyroptosis related genes play an essential role in the immune microenvironment of 33 TCGA cancer types; in addition, it revealed a critical link between pyroptosis related genes and anticancer drug sensitivity, shedding new light on mechanistic and therapeutic targets in the immune microenvironment of 33 cancer types and NSCLC.

Declarations

Ethics approval and consent to participate

This research was permitted by the Research Ethics Committee of the Emergency General Hospital (No. K202110). All patients were informed and gave written consent before the treatment procedure started.

Consent for publication

Not applicable.

Availability of data and materials

All data were available in the UCSC Xena browser (https://xenabrowser.net/) and the CellMiner database (https://discover.nci.nih.gov/cellminer/home.do). All the experimental data analyzed and displayed in the present manuscript are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Conflict of Interest Statement

No potential conflicts of interest are disclosed.

Authors' contributions

Zhi-Na Wang and Hong-Ming Ma wrote the main manuscript text. Zi-yi Chen, Jia-Wen Chen and Xu-wen Yang prepared figures 1-9. All authors reviewed the manuscript.

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Tables

Table 1. GLOSSARY

Abbreviations	Full Name
ACC	adrenocortical carcinoma
BLCA	bladder urothelial carcinoma
BRCA	breast invasive carcinoma
CESC	cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	cholangiocarcinoma
COAD	colon adenocarcinoma
DLBC	lymphoid neoplasm diffuse large B-cell lymphoma
ESCA	esophageal carcinoma
FDA	food and drug administration
GBM	glioblastoma multiforme
HNSC	head and neck squamous cell carcinoma
KICH	kidney chromophobe
KIRC	kidney renal clear cell carcinoma
KIRP	kidney renal papillary cell carcinoma
LAML	acute myeloid leukemia
LGG	brain lower grade glioma
LIHC	liver hepatocellular carcinoma
LUAD	lung adenocarcinoma
LUSC	lung squamous cell carcinoma
MESO	mesothelioma
NSCLC	non-small cell lung cancer
OV	ovarian serous cystadenocarcinoma
PRAD	prostate adenocarcinoma
PAAD	pancreatic adenocarcinoma
PCPG	pheochromocytoma and paraganglioma
READ	rectum adenocarcinoma
RNAss	RNA stemness score
STAD	stomach adenocarcinoma
SARC	

SKCM	sarcoma
TGCT	skin utaneous melanoma
THCA	testicular germ cell tumors
THYM	thyroid carcinoma
UCEC	thymoma
UVM	uterine corpus endometrial carcinoma
UCS	uveal melanoma
	uterine carcinosarcoma

Figures

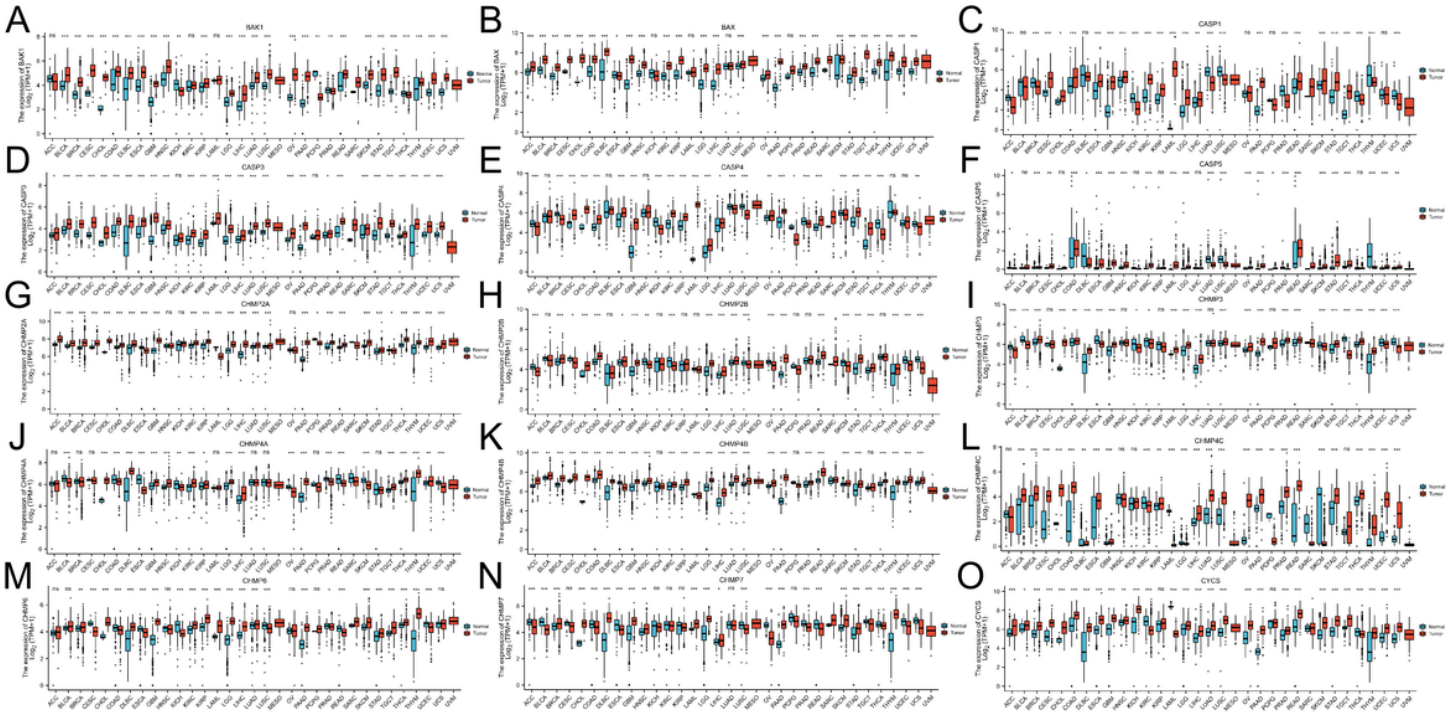

Figure 1

Boxplot of pyroptosis related genes differential expression between cancer and normal tissues. The blue boxplots indicate the normal tissues. The red boxplots indicate the cancer tissues.

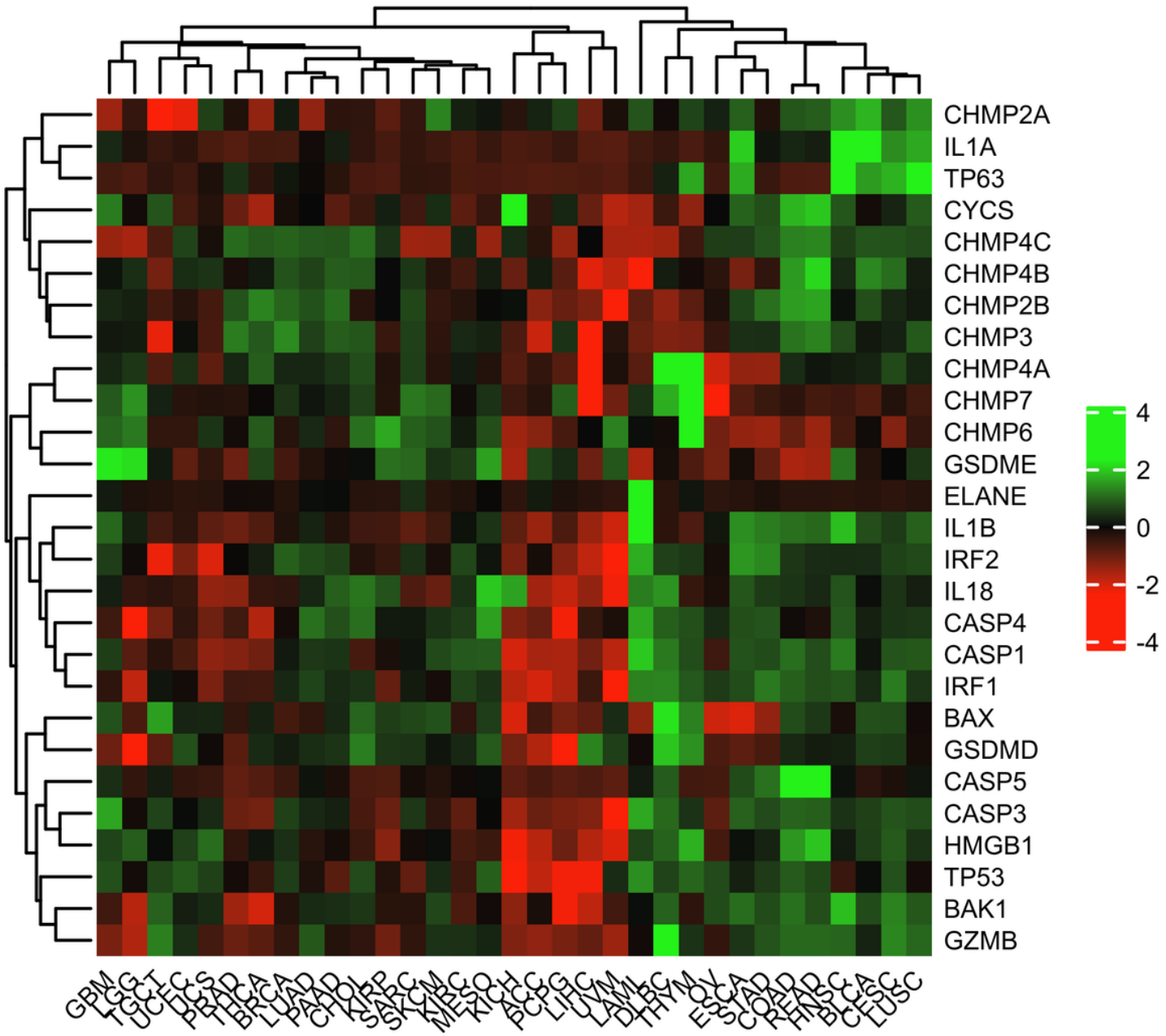

Figure 2

The heatmap of 27 pyroptosis related genes differential expression in 33 TCGA cancers. The red and green boxes indicate that the expression of pyroptosis related genes is high and low in correspondence cancer, respectively.

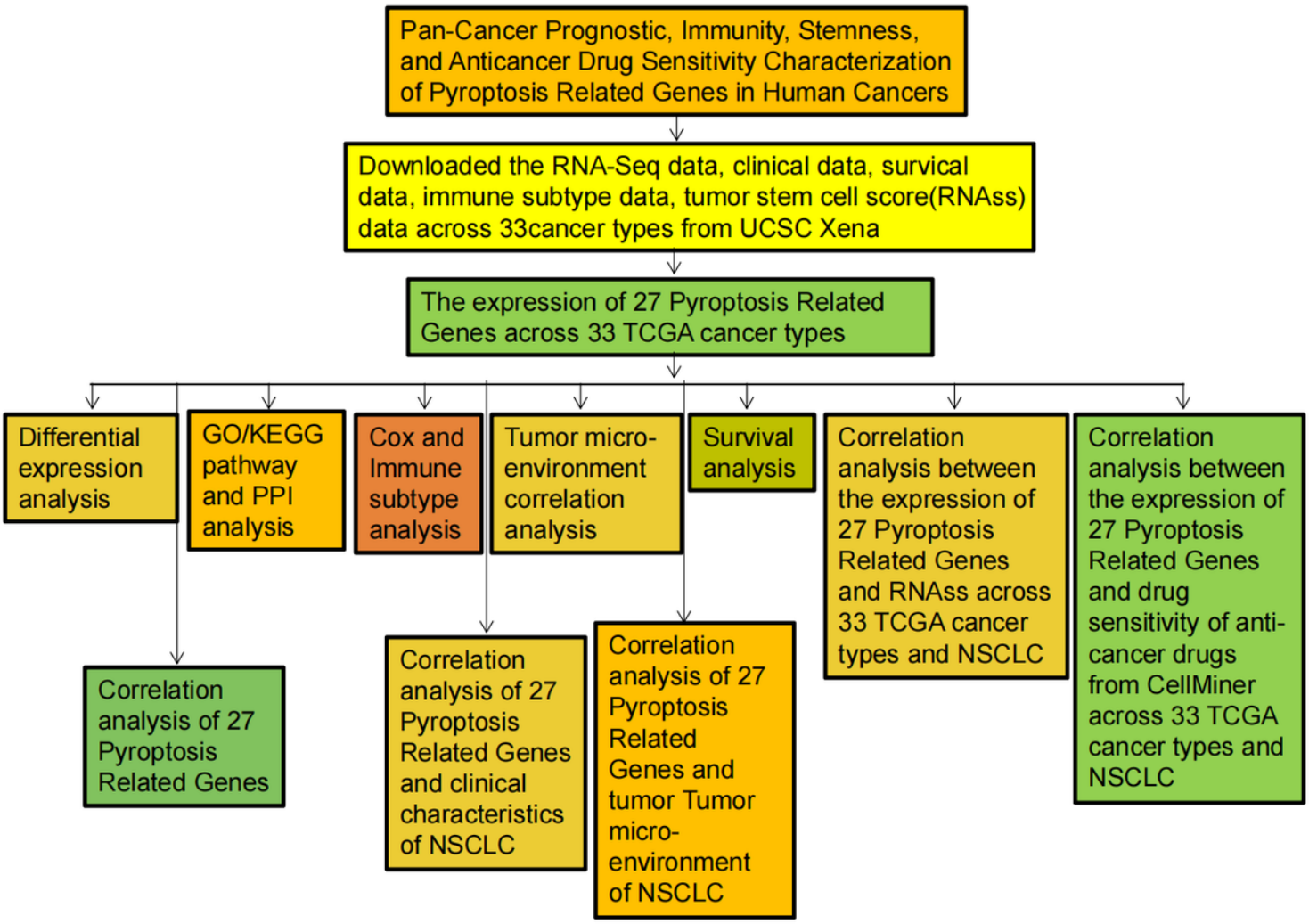

Figure 3

Flowchart of the pan-cancer analysis of pyroptosis related genes

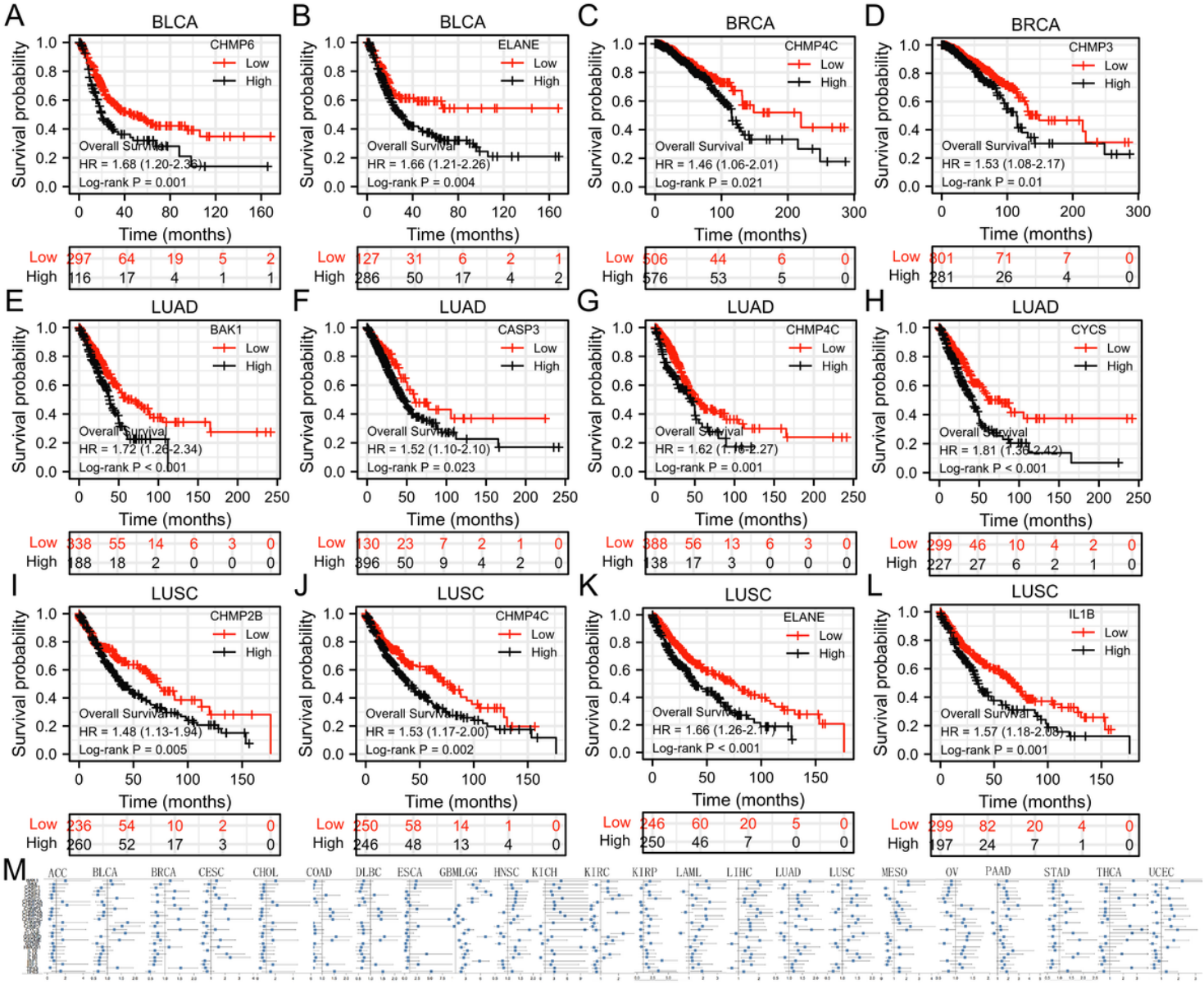

Figure 4

Survival analysis of pyroptosis related genes across multiple cancer types. The red line in the photos indicates high expression and the blue line in the

photos indicates low expression. (M) The forest map of hazard ratio of 27 pyroptosis related genes across 33 TCGA cancer types.

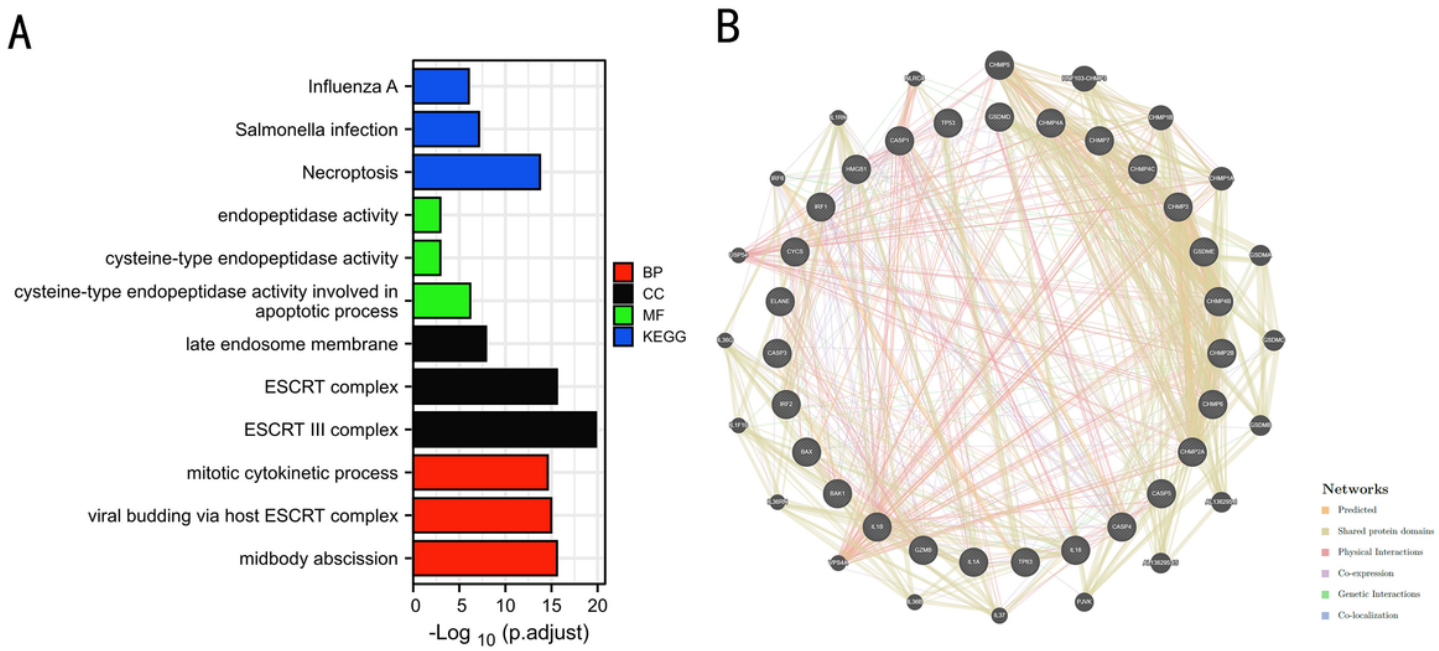

Figure 5

Analysis of functional enrichment of pyroptosis related genes. (A) GO and KEGG Analysis of Pyroptosis Related Genes. (B) Sringr analysis of functional enrichment of pyroptosis related genes.

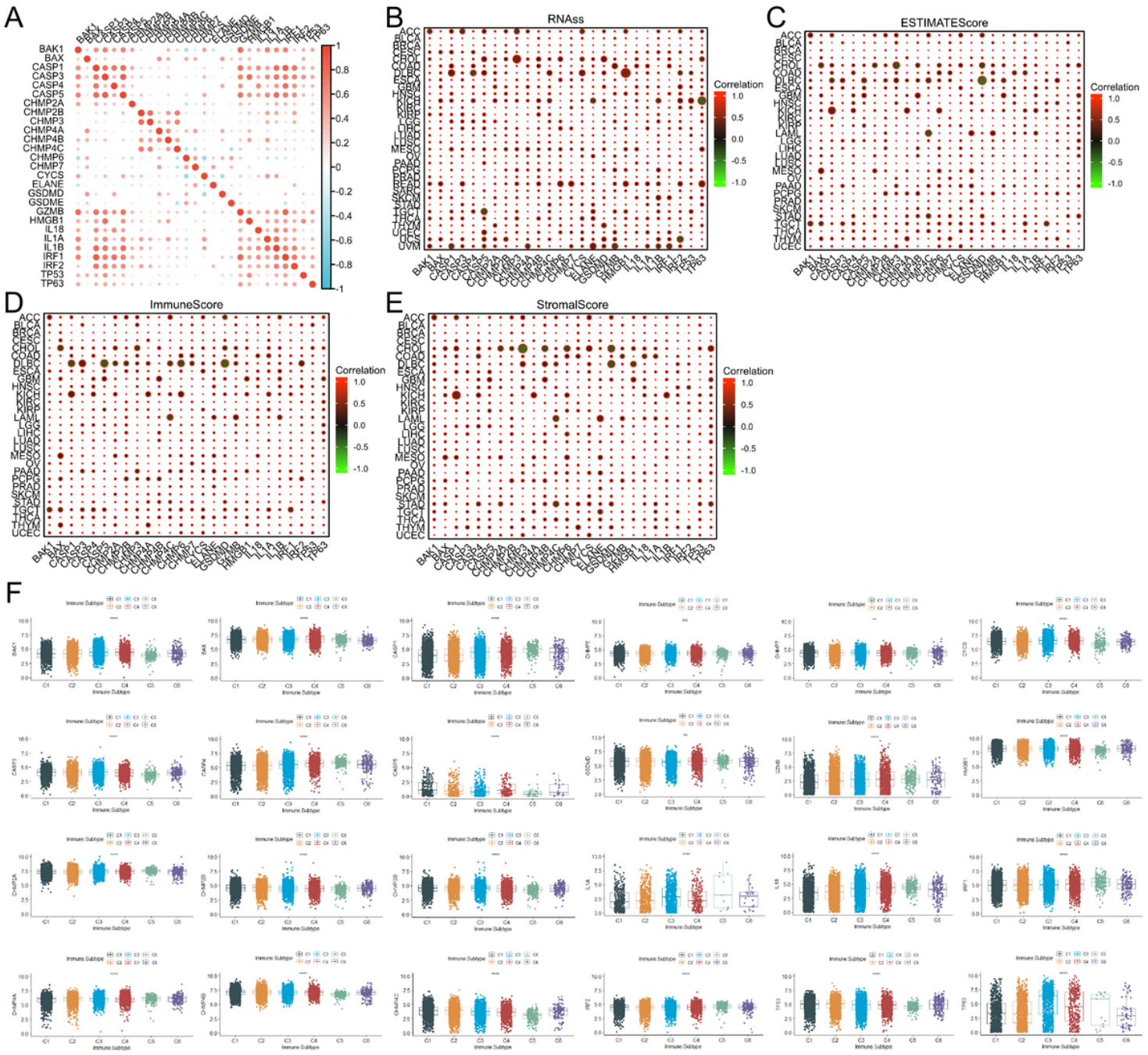

Figure 6

Correlation analysis between pyroptosis related genes expression and pan-cancer immune microenvironment and pan-cancer microenvironment. (A)

The blue and red dots indicate that pyroptosis related genes expression level had a negative and positive relationship, respectively. (B) The correlation relationship between pyroptosis related genes expression and tumor stem cell score (based on

RNA expression). (C-E) The correlation relationship among the expression of 27 pyroptosis related genes and estimate score, immune score, and stromal score. (G) The boxplot of pyroptosis related genes differential expression in six pan-cancer immune subtypes.

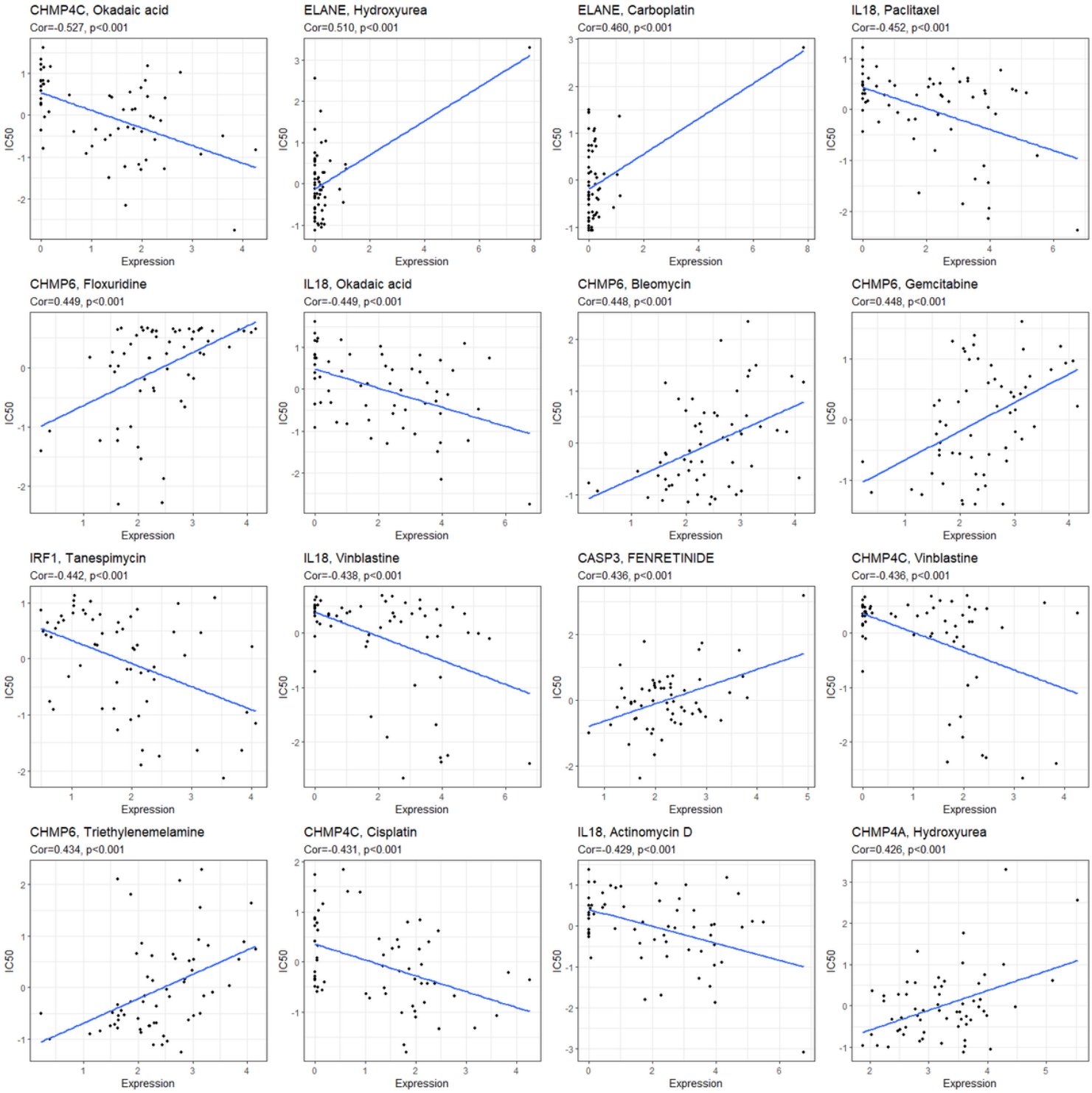

Figure 7

Correlation analysis between pyroptosis related genes and drug sensitivity of anticancer drugs in CellMiner.

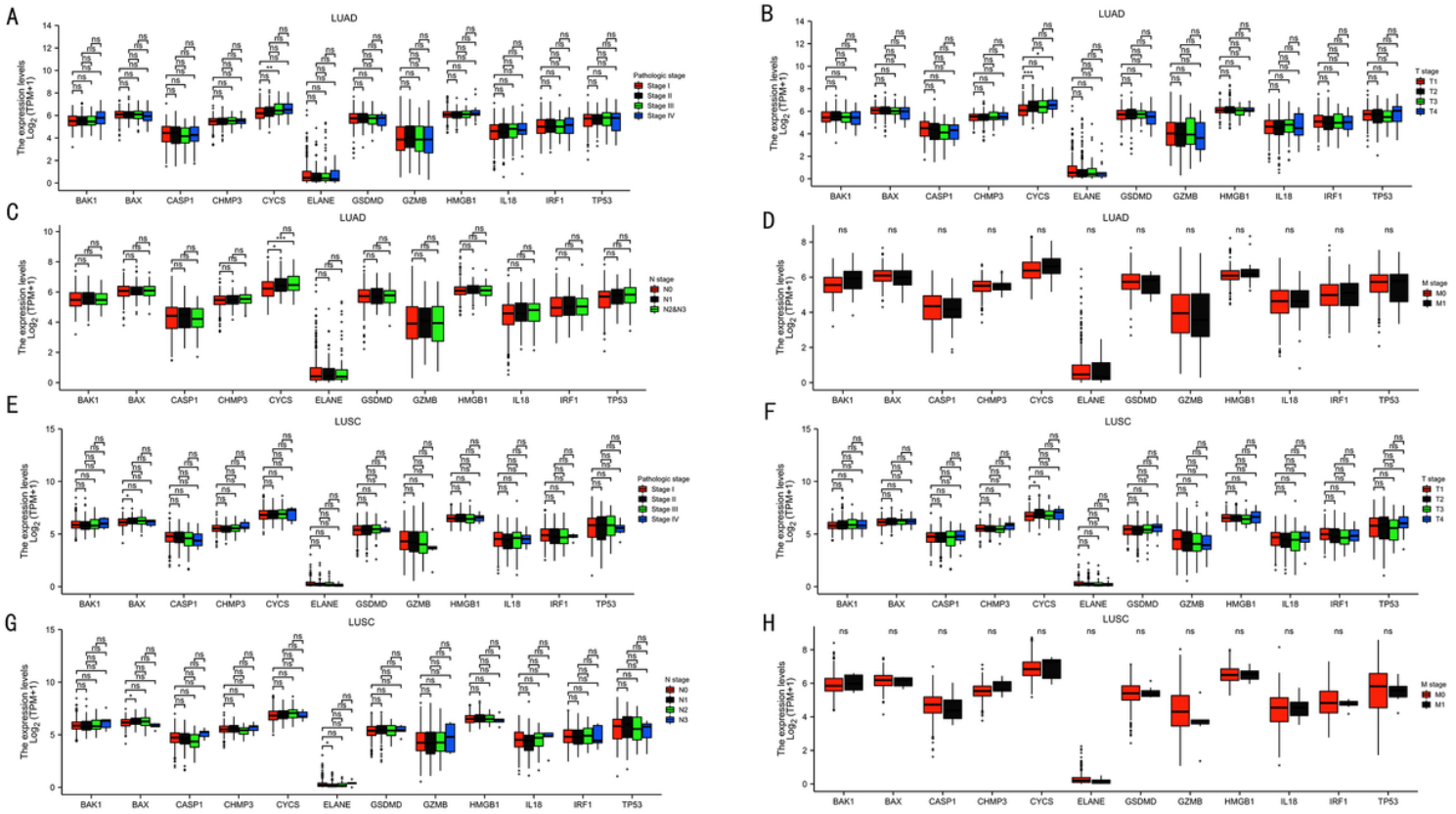

Figure 8

Correlation analysis between pyroptosis related genes expression and clinical characteristics in LUAD and LUSC.

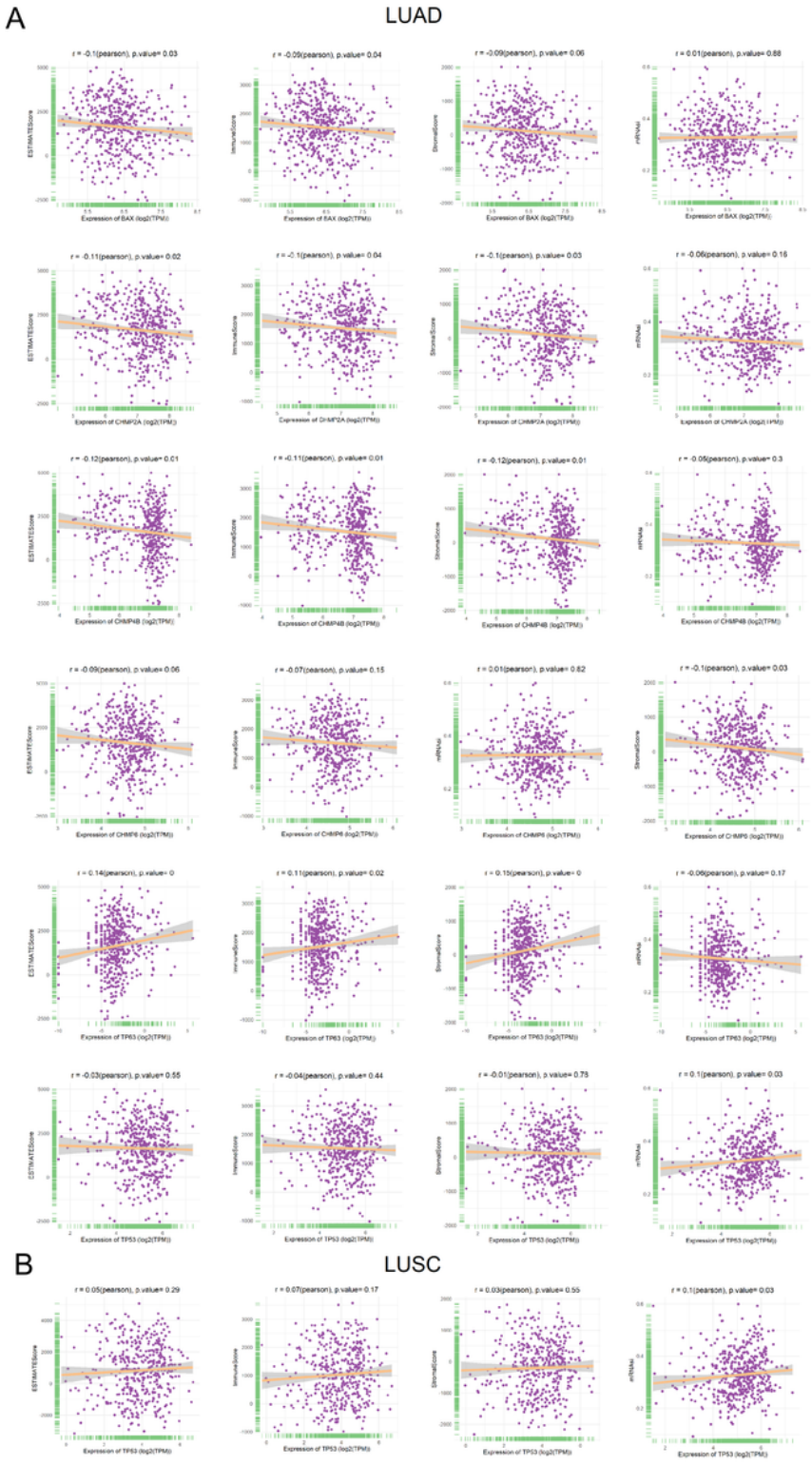

Figure 9

Correlation analysis of pyroptosis related genes and LUAD, LUSC microenvironment, and stem cell scores. (A, B) The correlation relationship between m6A methylation regulators expression and LUAD, LUSC stem cell score, LUAD, and LUSC immune microenvironment ($P < 0.05$).