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Expression of the Interferon Regulatory Factor Family and Its Prognostic Value in Acute Myeloid Leukemia

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Abstract

The interferon regulatory factor (IRF) family was correlated to diverse diseases. However, systematic studies of the IRF gene family in acute myeloid leukemia (AML) have not been reported yet. We analyzed the different transcripts of the IRF family associated with the survival and clinical data of patients with AML via the GEPIA, LinkedOmics, and TCGA databases. The results revealed that the expression levels of IRF1/2/4/5/7/8/9 were up-regulated while those of IRF3/6 were down-regulated in AML patients compared with normal donors. Moreover, high expression levels of IRF1/7/9 were significantly correlated with poor overall survival (OS). We further collected the clinical and survival data of newly diagnosed AML patients from the First Affiliated Hospital of Xiamen University from April 2018 to September 2020. In consistent with the outcomes from bioinformatics, the results from analysis of the biospecimens in our center showed that high expression levels of IRF1 and IRF7 predicted a poor prognosis. Overall, our study highlighted that the overexpression of IRF1/7/9 may be related with adverse prognosis and dismal survival in AML patients, indicating that the IRF family might serve as potential therapeutic targets.

1 Introduction

Acute myeloid leukemia (AML) is a malignant disorder of the hemopoietic system characterized by clonal expansion of myeloid lineage, resulting in accumulation of non-functional myeloid cells (myeloblasts) and loss of normal hematopoietic stem cells with the consequences of severe infections, anemia, and hemorrhage [1]. Genetic abnormalities are important etiological factors of AML. According to the National Comprehensive Cancer Network (NCCN) and European Leukemia Net (ELN) guidelines, the prognostic risk status is divided into three subtypes based on cytogenetics and genetic abnormalities. For example, cytogenetically normal AML patients with NPM1 mutation in the absence of FLT3-ITD mutation or with low-frequency FLT3-ITD mutation, or double CEBPA gene mutations have favorable prognoses [2–4], while the AML patients with ASXL1, FLT3-ITD, or TP53 mutations have poor prognoses [5–8]. In addition, the abnormal expression of some genes contributes to the risk stratification of AML. For instance, over-expression of WT1, MDR1, and BCL11A genes are related to poor prognoses, while low expression of MDR1 and BAALC genes corresponds to a new subgroup of AML with intermediate cytogenetic risk and favorable outcomes [9–12]. Therefore, more prognostic risk markers for AML should be explored.

The interferon regulatory factors (IRFs) family, consisting of nine members (IRF1-9) in mammals, was identified in the late 1980s in specific to the type I interferon system, and commonly possess a novel helix-turn-helix DNA-binding motif. This family is now recognized as a pivotal part of the regulation of innate and adaptive immune responses, antiviral defense, cell growth regulation, and oncogenesis [13–15]. The IRF family transcription factors were mainly linked to the type I interferon (IFN) system, regulating immune responses, and was associated with many signaling pathways, including RIG-I/MDA5, MyD88-dependent, and TRIF-dependent pathways [13, 16]. Recent developments revealed that IRFs played critical roles in metabolism [17] and oncogenesis [18]. Some studies reported that the IRF family was aberrant in expression in different cancers, including colorectal cancer [19], breast cancer [20], leukemia [21], etc. Moreover, some elucidations found abnormal expression of IRF genes in leukemia. For

instance, several IRFs were involved in the pathogenesis of chronic myeloid leukemia [22]; Expression and function of IRF1 was significantly abnormal in AML with − 5/del(5q) and acute promyelocytic leukemia (APL) [23]; and IRF3 overexpression promoted cell growth and survival in AML [24].

Nevertheless, the impact and clinical value of the IRF genes in AML patients remain unclear. Therefore, the present study aimed to systematically elucidate the clinicopathological and prognostic values of IRFs genes in AML using several reliable databases. Furthermore, we designed to validate the findings in our AML patients.

2 Methods

2.1 The Gene Expression Profiling Interactive Analysis (GEPIA) Database

GEPIA (http://gepia.cancer-pku.cn) is a newly developed interactive web server that includes the RNA sequencing expression data of 9,736 tumor samples and 8,587 normal samples from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx; http://www.gtexportal.org/home/index.html) projects. This interactive web server enables to perform expression analyzes in leukemia or normal cells from bone marrow. In this study, we used GEPIA to analyze differential expression of IRFs between AML patients and healthy donors and to examine the correlation between expression of IRFs and prognosis in AML [25].

2.2 LinkedOmics Database

LinkedOmics (http://www.linkedomics.org/) is a publicly available portal that has multi-omics data from 32 TCGA Cancer types, providing a unique platform for biologists and clinicians to access, analyze, and compare cancer multi-omics data within and across tumor types. It also includes mass spectrometry-based proteomics data generated by the Clinical Proteomics Tumor Analysis Consortium (CPTAC) for TCGA breast, colorectal, and ovarian cancers [26]. In this study, the LinkedOmics database was used to further analyze the correlation between IRFs and prognosis.

2.3 TCGA database analysis

A total of 154 AML patients with data of gene expression levels of IRF family from TCGA database (http://tcga-data.nci.nih.gov/tcga/) were included in this study. Among them, 78 patients only received chemotherapy and 76 patients received both transplant treatment and chemotherapy. Clinical data, molecular characteristics, laboratory examinations, and survival information were obtained from the database, including gender, age, French-American-British (FAB) subtypes, bone marrow and peripheral blood (PB) blasts percentage, white blood cell (WBC) and platelet counts, hemoglobin level, cytogenetic risk classification, cytogenetics data, mutation of cancer prognosis genes, and overall survival (OS). The study protocol was approved by the Washington University Institutional Review Board [27].

2.4 Newly diagnosed patients

Ninety-four newly diagnosed patients with AML (52 males and 42 females) were included in this study. The mean age of the patients was 53 years, with a range from 6 to 91 years. The AML diagnosis was based on morphologic characteristics, immunophenotyping, cytogenetic analysis, and molecular genetic tests. Patients were classified as M0 (n = 5), M1 (n = 2), M2 (n = 46), M4 (n = 10), M5 (n = 25), M7 (n = 1), and unclassified (n = 5) according to the FAB classification after ruling out M3. Eighty-three patients only received chemotherapy and 11 patients received both transplant treatment and chemotherapy. Patients were recruited from the Department of Hematology of the First Affiliated Hospital of Xiamen University, from April 2018 to September 2020. All works were performed in accordance with the ethical standards and guidelines of the First Affiliated Hospital of Xiamen University.

2.5 RNA extraction and cDNA preparation

Bone marrow samples were collected from 94 patients at diagnosis. All samples were treated twice with RBC Lysis Buffer (NobleRyder, China) and washed once using phosphate buffer saline. Then, total cellular RNA was extracted using TRIzol Reagent (Bomeibio, China). Complementary DNA was synthesized using Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase (BioTeke, China) and stored at -20℃. The reaction conditions of reverse transcription: 42 ℃ for 50 min; 72 ℃ for 10 min. The ultraviolet spectrophotometer was utilized for testing the purity and content of cDNA, and the A260/A280 absorbance ratios were in the range of 1.8-2.0.

2.6 Real-time polymerase chain reaction (RT-PCR) analysis

The mRNA expression levels of IRF1, IRF6, IRF7, IRF9, and β-actin (housekeeping gene) were analyzed in patients and control subjects using quantitative RT-PCR in ABI PRISM 7500 Sequence Detector System (Applied Biosystems, Foster City, CA, USA). The quantitative RT-PCR amplification was performed using a mixture of 10 μL in each tube containing 5 μL SYBR Green PCR Master Mix, 0.5 μL forward primer, 0.5 μL reverse primer, 0.5 μL sample, 0.2 μL ROX I, and 3.3 μL water. The RT-PCR thermal cycle condition of the reaction in ABI PRISM 7500 was: 94 ℃ for 2 min in holding stage; 94 ℃ for 15 sec, 60 ℃ for 30 sec, and 72 ℃ for 30 sec with 40 cycles in cycling stage; 95 ℃ for 15 sec, 60 ℃ for 1 min, 95 ℃ for 30 sec, and 60 ℃ for 15 sec in the melt curve stage. The relative expression levels were calculated using the 2-ΔΔCt method with internal reference dye as a calibrator and β-actin as a normalizer. Primers synthesized by Sangon for IRF1, IRF6, IRF7, IRF9, and β-actin are as follows:

• IRF1 Forward: 5’ - AGTGATCTGTACAACTTCCAGG – 3’

• IRF1 Reverse: 5’ - CCTTCCTCATCCTCATCTGTTG – 3’

• IRF7 Forward: 5’ - TCCCCACGCTATACCATCTAC – 3’

• IRF7 Reverse: 5’ - GAAGACACACCCTCACGC – 3’

• IRF9 Forward: 5’ - CTTGGTCAGGTACTTTCAGGG – 3’

• IRF9 Reverse: 5’ - AGCAAGTATCGGGCAAAGG – 3’

• β-actin Forward: 5’ - TGTGGCATCCACGAAACTAC – 3’

• β-actin Reverse: 5’ - GGAGCAATGATCTTGATCTTCA – 3’

2.7 Statistical analysis

The statistical analysis was performed utilizing SPSS 23.0 software (IBM Corp., Armonk, NY, USA). Numerical data were expressed as median and range. The Kaplan-Meier method was used for analyzing the survival data, and the significance was established using the log-rank test. The Chi-square test was used to examine the relation between qualitative variables. For non-normally distributed quantitative data, the Kruskal-Wallis test (non-parametric test) was used for comparisons between two groups. The Cutoff Finder application (http://molpath.charite.de/cutoff) was applied to find an optimal cutoff point of mRNA expression for AML patients [28]. Cox proportional hazards model was performed to analyze the risk factors for OS using the Forward Stepwise Likelihood Ratio procedure. A P-value of < 0.05 was considered statistically significant.

2.8 Ethics Statement

This study was performed by ethical standards and was approved by the Ethics Committee of the first affiliated hospital of the Xiamen University (Fujian, China). All data from database were retrieved from the published literature, so it was confirmed that written informed consent from all subjects and/or their legal guardian was available in the corresponding database [25–27]. The data from our center were retrieved from the First Affiliated Hospital of Xiamen University, and it was confirmed that informed consent was obtained from all patients and/or their legal guardian.

3 Results

3.1 The expression levels of IRF family and prognostic and clinical data from diverse databases

3.1.1 The gene expression analysis using the GEPIA database

Through the GEPIA database, we compared the transcripts of the IRF gene family between AML patients and normal subjects (Fig. 1). The results showed that the expression levels of IRF1, IRF2, IRF4, IRF5, IRF7, IRF8, and IRF9 were significantly higher in AML patients. In contrast, the expression levels of IRF3 and IRF6 were lower in AML patients compared with normal subjects.

3.1.2 Prognostic value analysis using the GEPIA, LinkedOmics and TCGA databases

To elucidate the impact of the IRF family on the prognosis of AML patients, we used the GEPIA and LinkedOmics databases to conduct survival analysis. From the GEPIA database analysis, high expression of IRF9 was significantly correlated with poor OS ($P < 0.05$) in AML patients, while the expression levels of IRF1-8 had no impact on the survival of AML patients (Fig. 2). From the LinkedOmics database analysis, high expression of IRF1 and IRF7 were significantly correlated with poor OS ($P < 0.05$) in AML patients, while the expression levels of IRF2-6 and IRF8-9 had no impact on the survival of AML patients (Fig. 3).

Next, the survival and clinical data of AML patients from the TCGA database were obtained to further investigate the effect of the IRF family on AML patients. We regrouped the patients who received chemotherapy-only, or transplant treatment into high and low IRF gene expression subgroups by the median mRNA levels. Overall, the Cox proportional hazards model analysis showed that high expression of IRF1 and IRF7 significantly caused poor OS in all patients ($P < 0.05$, Table 1 and Fig. 4). Next, in the chemotherapy-only group, the high expression of IRF1, IRF6, and IRF9 showed a significant unfavorable impact on the OS. However, in the transplant group, all IRF members were irrelevant to OS. Accordingly, IRF1/6/7/9 may serve as potential prognostic markers for AML.

Table 1
Comparison of OS with different IRF genes expression in AML patients using the TCGA database

All patients	χ2	P-value	Chemotherapy-only group	χ2	P-value	Transplantation treatment group	χ2	P-value
IRF1	3.899	**0.048**		5.400	**0.020**		0.204	0.651
IRF2	1.067	0.302		0.001	0.977		0.397	0.528
IRF3	0.587	0.444		1.761	0.184		0.226	0.635
IRF4	0.082	0.774		0.048	0.827		0.011	0.915
IRF5	0.012	0.913		0.180	0.671		0.428	0.513
IRF6	0.356	0.551		4.738	**0.029**		2.179	0.140
IRF7	5.280	**0.022**		2.602	0.107		2.528	0.112
IRF8	0.055	0.815		0.004	0.950		0.123	0.726
IRF9	3.181	0.074		5.935	**0.015**		1.110	0.292

3.1.3 Clinical characteristics analysis using the TCGA database

The clinical and molecular characteristics of high and low IRF1 and IRF7 expression subgroups were compared in all patients from TCGA database (Table 2). The results showed that patients whose age was higher than 60 years had higher expression of IRF1 ($P = 0.014$). No significant differences were observed in gender, WBC count, hemoglobin, platelets, BM blasts, PB blasts, FAB subtypes, relapse, risk

stratification, karyotype, or frequencies of genetic mutations (FLT3, NPM1, DNMT3A, IDH1/IDH2, RUNX1, TET2, TP53 and NRAS/KRAS) between the $IRF1^{high}$ and $IRF1^{low}$ groups.

Table 2
Correlation of IRF1 and IRF7 differential expressions to clinical characteristics in all patients using TCGA database

Characteristics	IRF1 low	IRF1 high	P-value	IRF7 low	IRF7 high	P-value
Age/years, median (range)	55.0 (21.0, 88.0)	61.0 (18.0, 81.0)	0.132	56.0 (18.0, 82.0)	59.0 (23.0, 88.0)	0.091
Age group/n (%)			**0.014**			0.415
Age < 60 years	52 (67.5%)	37 (48.1%)		47 (61.0%)	42 (54.5%)	
Age ≥ 60 years	25 (32.5%)	40 (51.9%)		30 (39.0%)	35 (45.5%)	
Gender/n (%)			0.076			0.872
Females	31 (40.3%)	42 (54.5%)		36 (46.8%)	37 (48.1%)	
Males	46 (59.7%)	35 (45.5%)		41 (53.2%)	40 (51.9%)	
FAB subtypes/n (%)						
M0	6 (7.8%)	10 (13.0%)	0.291	7 (9.1%)	9 (11.7%)	0.597
M1	23 (29.9%)	20 (26.0%)	0.590	24 (31.2%)	19 (24.7%)	0.369
M2	17 (22.1%)	20 (26.0%)	0.572	21 (27.3%)	16 (20.8%)	0.346
M4	19 (24.7%)	15 (19.5%)	0.437	10 (13.0%)	24 (31.2%)	**0.007**
M5	10 (13.0%)	10 (13.0%)	1.000	12 (15.6%)	8 (10.4%)	0.338
M6	0 (0.0%)	2 (2.6%)	0.497	1 (1.3%)	1 (1.3%)	1.000
M7	2 (2.6%)	0 (0.0%)	0.497	2 (2.6%)	0 (0.0%)	0.497
BM blasts (%)	71.0 (30.0, 100.0)	72.0 (30.0, 99.0)	0.707	75.0 (30.0, 100.0)	69.0 (30.0, 98.0)	0.104
PB blasts (%)	39.5 (0.0, 98.0)	37.0 (0.0, 97.0)	0.936	39.5 (0.0, 98.0)	39.0 (0.0, 97.0)	0.940
WBC/×10^9/L, median (range)	17.9 (0.6, 297.4)	18.7 (1.0, 223.8)	0.532	15.2 (0.6, 297.4)	22.9 (0.7, 118.8)	0.833

Characteristics	IRF1 low	IRF1 high	P-value	IRF7 low	IRF7 high	P-value
Hemoglobin/g/dL, median (range)	9.0 (6.0, 14.0)	9.0 (6.0, 13.0)	0.400	9.0 (6.0, 14.0)	9.0 (7.0, 13.0)	0.850
Platelet/×10^9/L, median (range)	57.0 (8.0, 257.0)	50.0 (9.0, 351.0)	0.450	45.0 (8.0, 232.0)	57.0 (9.0, 351.0)	0.346
Risk (%)						
Good	12 (15.6%)	6 (7.8%)	0.132	8 (10.4%)	10 (13.0%)	0.616
Intermediate	44 (57.1%)	53 (68.8%)	0.133	49 (63.6%)	48 (62.3%)	0.867
Poor	21 (27.3%)	18 (23.4%)	0.578	20 (26.0%)	19 (24.7%)	0.853
Cytogenetics (%)						
Normal Karyotype	37 (48.1%)	40 (51.9%)	0.629	41 (53.2%)	36 (46.8%)	0.420
Complex Cytogenetics	11 (14.3%)	10 (13.0%)	0.814	9 (11.7%)	12 (15.6%)	0.481
inv(16)/CBFβ-MYH11	7 (9.1%)	4 (5.2%)	0.348	4 (5.2%)	7 (9.1%)	0.348
t(8;21)/RUNX1-RUNX1T1	5 (6.5%)	2 (2.6%)	0.246	4 (5.2%)	3 (3.9%)	0.367
11q23/MLL	4 (5.2%)	1 (1.3%)	0.367	4 (5.2%)	1 (1.3%)	0.367
−7/7q−	4 (5.2%)	4 (5.2%)	1.000	3 (3.9%)	5 (6.5%)	0.719
Others	9 (11.7%)	16 (20.8%)	0.126	12 (15.6%)	13 (16.9%)	0.827
Relapse (%)			0.147			0.872
No	42 (54.5%)	33 (42.9%)		38 (49.4%)	37 (48.1%)	
Yes	35 (45.5%)	44 (57.1%)		39 (50.6%)	40 (51.9%)	
FLT3 (%)			0.804			0.326
Wild (−)	56 (72.7%)	58 (75.3%)		53 (68.8%)	61 (79.2%)	
FLT3-ITD	16 (20.8%)	13 (16.9%)		17 (22.1%)	12 (15.6%)	

Characteristics	IRF1 low	IRF1 high	P-value	IRF7 low	IRF7 high	P-value
FLT3-TKD	5 (6.5%)	6 (7.8%)		7 (9.1%)	4 (5.2%)	
NPM1 (%)			0.595			**0.008**
Wild (−)	53 (68.8%)	56 (72.7%)		47 (61.0%)	62 (80.5%)	
Mutation (+)	24 (31.2%)	21 (27.3%)		30 (39.0%)	15 (19.5%)	
DNMT3A (%)			0.202			0.362
Wild (−)	60 (77.9%)	53 (68.8%)		54 (70.1%)	59 (76.6%)	
Mutation (+)	17 (22.1%)	24 (31.2%)		23 (29.9%)	18 (23.4%)	
IDH1/IDH2 (%)			0.070			0.547
Wild (−)	66 (85.7%)	57 (74.0%)		63 (81.8%)	60 (77.9%)	
Mutation (+)	11 (14.3%)	20 (26.0%)		14 (18.2%)	17 (22.1%)	
RUNX1 (%)			0.440			**0.005**
Wild (−)	70 (90.9%)	67 (87.0%)		74 (96.1%)	63 (81.8%)	
Mutation (+)	7 (9.1%)	10 (13.0%)		3 (3.9%)	14 (18.2%)	
TET2 (%)			1.000			0.113
Wild (−)	69 (89.6%)	69 (89.6%)		66 (85.7%)	72 (93.5%)	
Mutation (+)	8 (10.4%)	8 (10.4%)		11 (14.3%)	5 (6.5%)	
TP53 (%)			0.147			0.772
Wild (−)	68 (88.3%)	73 (94.8%)		71 (92.2%)	70 (90.9%)	
Mutation (+)	9 (11.7%)	4 (5.2%)		6 (7.8%)	7 (9.1%)	
NRAS/KRAS (%)			0.632			0.632
Wild (−)	68 (88.3%)	66 (85.7%)		68 (88.3%)	66 (85.7%)	

Characteristics	IRF1 low	IRF1 high	P-value	IRF7 low	IRF7 high	P-value
Mutation (+)	9 (11.7%)	11 (14.3%)		9 (11.7%)	11 (14.3%)	

Compared with the IRF7^{low} subgroup, the IRF7^{high} subgroup had more patients with M4 subtype (P = 0.007), less frequent NPM1 mutation (P = 0.008), and more frequent RUNX1 mutation (P = 0.005). There were no significant differences in age, gender, WBC count, hemoglobin, platelets, BM blasts, PB blasts, others FAB subtypes, risk stratification, karyotype, frequencies of other genetic mutations (FLT3, DNMT3A, IDH1/IDH2, TET2, TP53, and NRAS/KRAS), or relapse rates between the two subgroups.

3.1.4 Multivariate analysis using the TCGA database

To further explore the prognostic factors in AML patients via the TCGA database, multivariate analysis was performed. Overall, univariate analysis showed that differential expression of IRF1 and IRF7, age, complex karyotype, inv(16), DNMT3A, RUNX1, and TP53 were significantly related to OS. The P < 0.1 as an inclusive criterion was used in the univariate analysis to explore a broad range of related variables that might be associated with OS. Next, multiple variables, including the expression levels of IRF1/7 (high vs. low), age group (≥ 60 vs. <60 years), complex karyotype (yes vs. no), inv (16) (yes vs. no), DNMT3A (mutated vs. wild), RUNX1 (mutated vs. wild), and TP53 (mutated vs. wild), were included in the multivariate Cox proportional hazard model (Table 3). Results showed that high IRF7 expression, age ≥ 60 years, DNMT3A mutation, and TP53 mutation were independent risk factors for OS in AML patients (all P < 0.05).

Table 3
Multivariate analysis (COX regression model) of OS in AML patients using the TCGA database

Variables	OS	
	Hazard Ratio (95%CI)	P-value
IRF1 (High vs. Low)		NS
IRF7 (High vs. Low)	1.743 (1.202–2.530)	**0.003**
Age (≥ 60 vs. <60 years)	2.400 (1.632–3.529)	**< 0.001**
Complex Karyotype (Yes vs. No)		NS
inv(16) (Yes vs. No)		NS
DNMT3A (Mutated vs. Wild)	1.599 (1.063–2.405)	**0.024**
RUNX1 (Mutated vs. Wild)		NS
TP53 (Mutated vs. Wild)	3.295 (1.776–6.112)	**< 0.001**

3.2 The analysis of IRFs and prognostic values in AML patients from our center

3.2.1 Prognostic value analysis

The prognostic value relevant to IRF1/7/9 expression was analyzed by Cutoff Finder (Method: Survival significance; log-rank test) (Fig. 5). The results showed that the optimal cut-off value of IRF1 expression was at 0.9602, and the sensitivity and specificity were 78.8% (66%-87.8%) and 37.5% (24.2%-53%), respectively. Similarly, the IRF7 and IRF9 could predict the prognosis of AML patients with the optimal cut-off value of IRF7 and IRF9 expression at 4.309 and 0.3904, with the sensitivity of 73.6% (60.4%-83.6%)/94.3% (84.6%-98.1%) and specificity of 51.4% (35.9%-66.6%)/17.9% (9%-32.7%), respectively. To elucidate the impact of IRF1, IRF7, and IRF9 on AML patients, we performed Kaplan-Meier analyses according to the optimal cut-off values (Fig. 6). The results showed that high expression of IRF1 and IRF7 were risk factors for OS in AML patients. However, the prognosis of AML could not be affected by expression of IRF9.

3.2.2 Clinical characteristics analysis

For further exploring the impact of IRF1 and IRF7 on AML, we examined the correlation between the IRF1/7 expression and clinical characteristics of AML patients (Table 4). The results showed a remarkable positive correlation between low expression of IRF1 gene and high PB blasts percentage (P = 0.043), low LDH level (P = 0.004) and inv(16) karyotype (P = 0.002). Similarly, over-expression of IRF7 was significantly associated with high LDH level (P = 0.014), less intermediate risk (P = 0.019) and complex karyotype (P = 0.045). However, our study indicated that neither IRF1 nor IRF7 expression had impact on the complete remission (CR) rate and relapse rate.

Table 4
Correlation of IRF1 and IRF7 differential expressions to clinical characteristics in AML patients using our center data

Characteristics	IRF1 low	IRF1 high	P-value	IRF7 low	IRF7 high	P-value
Age/years, median (range)	52.0 (15.0, 86.0)	53.5 (6.0, 91.0)	0.658	39.0 (15.0, 86.0)	53.0 (6.0, 91.0)	0.864
Age group/n (%)			0.230			0.847
Age < 60 years	13 (50.0%)	42 (63.6%)		19 (57.6%)	34 (59.6%)	
Age ≥ 60 years	13 (50.0%)	24 (36.4%)		14 (42.4%)	23 (40.4%)	
Gender/n (%)			0.887			0.388
Females	11 (42.3%)	29 (43.9%)		17 (51.5%)	24 (42.1%)	
Males	15 (57.7%)	37 (56.1%)		16 (48.5%)	33 (57.9%)	
FAB subtypes/n (%)						
M0	0 (0.0%)	5 (7.6%)	0.317	2 (6.1%)	3 (5.3%)	1.000
M1	0 (0.0%)	2 (3.0%)	1.000	1 (3.0%)	1 (1.8%)	1.000
M2	12 (46.2%)	34 (51.5%)	0.643	18 (54.5%)	24 (42.1%)	0.254
M4	4 (15.4%)	5 (7.6%)	0.256	3 (9.1%)	7 (12.3%)	0.740
M5	10 (38.5%)	14 (21.2%)	0.090	6 (18.2%)	19 (33.3%)	0.122
M7	0 (0.0%)	1 (1.5%)	1.000	1 (3.0%)	0 (0.0%)	0.367
Unclassified	0 (0.0%)	5 (7.6%)	0.317	2 (6.1%)	3 (5.3%)	1.000
BM blasts (%)	50.4 (22.7, 83.5)	60.2 (13.0, 98.0)	0.063	51.2 (13.0, 96.4)	59.8 (13.0, 98.0)	0.393
PB blasts (%)	23.0 (4.0, 93.0)	49.5 (0.8, 97.0)	**0.043**	49.0 (2.0, 96.0)	43.0 (0.8, 97.0)	0.803
WBC/×10^9/L, median (range)	7.19 (0.25, 166.33)	16.495 (0.71, 312.18)	0.096	14.25 (0.25, 174.73)	15.53 (0.71, 312.18)	0.795
Hemoglobin/g/dL, median (range)	77.0 (21.0, 130.0)	73.0 (33.0, 124.0)	0.900	79.0 (43.0, 127.0)	75.0 (21.0, 130.0)	0.987
Platelet/×10^9/L, median (range)	48.0 (3.0, 487.0)	40.5 (5.0, 417.0)	0.941	41.0 (7.0, 226.0)	51.0 (3.0, 487.0)	0.527

Characteristics	IRF1 low	IRF1 high	P-value	IRF7 low	IRF7 high	P-value
LDH/U/L, median (range)	267.0 (94.0, 997.0)	461.0 (150.0, 2450.0)	**0.004**	356.0 (94.0, 2450.0)	488.5 (172.0, 1648.0)	**0.014**
Risk (%)						
Poor	6 (25.0%)	19 (32.8%)	0.487	5 (17.2%)	19 (37.3%)	0.060
Intermediate	12 (50.0%)	34 (58.6%)	0.474	22 (75.9%)	25 (49.0%)	**0.019**
Good	6 (25.0%)	5 (8.6%)	0.073	2 (6.9%)	7 (13.7%)	0.476
Cytogenetics (%)						
Normal Karyotype	11 (47.8%)	22 (40.0%)	0.524	11 (40.7%)	22 (45.8%)	0.670
Complex Cytogenetics	2 (8.7%)	5 (9.1%)	1.000	0 (0.0%)	7 (14.6%)	**0.045**
inv(16)/CBFβ-MYH11	5 (21.7%)	0 (0.0%)	**0.002**	1 (3.7%)	4 (8.3%)	0.648
t(8;21)/RUNX1-RUNX1T1	1 (4.3%)	9 (16.4%)	0.266	2 (7.4%)	5 (10.4%)	1.000
11q23/MLL	1 (4.3%)	5 (9.1%)	0.664	4 (14.8%)	2 (4.2%)	0.180
Others	3 (13.0%)	14 (25.5%)	0.226	9 (33.3%)	8 (16.7%)	0.098
CR (%)			0.132			0.113
No	7 (26.9%)	29 (43.9%)		10 (30.3%)	27 (47.4%)	
Yes	19 (73.1%)	37 (56.1%)		23 (69.7%)	30 (52.6%)	
Relapse (%)			0.132			0.112
No	18 (69.2%)	55 (83.3%)		29 (87.9%)	42 (73.7%)	
Yes	8 (30.8%)	11 (16.7%)		4 (12.1%)	15 (26.3%)	
FLT3 (%)			0.744			0.755
Wild (−)	21 (87.5%)	45 (81.8%)		25 (86.2%)	38 (80.9%)	
Mutation (+)	3 (12.5%)	10 (18.2%)		4 (13.8%)	9 (19.1%)	

Characteristics	IRF1 low	IRF1 high	P-value	IRF7 low	IRF7 high	P-value
IDH1/IDH2 (%)			0.799			0.382
Wild (−)	18 (75.0%)	39 (72.2%)		23 (79.3%)	33 (70.2%)	
Mutation (+)	6 (25.0%)	15 (27.8%)		6 (20.6%)	14 (29.8%)	
NPM1 (%)			1.000			0.517
Wild (−)	21 (87.5%)	46 (85.2%)		26 (89.7%)	39 (83.0%)	
Mutation (+)	3 (12.5%)	8 (14.8%)		3 (10.3%)	8 (17.0%)	
NRAS/KRAS (%)			1.000			0.544
Wild (−)	19 (79.2%)	44 (81.5%)		23 (79.3%)	40 (85.1%)	
Mutation (+)	5 (20.8%)	10 (18.5%)		6 (20.7%)	7 (14.9%)	
DNMT3A (%)			0.729			0.517
Wild (−)	20 (83.3%)	47 (87.0%)		26 (89.7%)	39 (83.0%)	
Mutation (+)	4 (16.7%)	7 (13.0%)		3 (10.3%)	8 (17.0%)	
TET2 (%)			1.000			0.194
Wild (−)	22 (91.7%)	49 (90.7%)		25 (86.2%)	45 (95.7%)	
Mutation (+)	2 (8.3%)	5 (9.3%)		4 (13.8%)	2 (4.3%)	
ASXL1 (%)			0.261			1.000
Wild (−)	23 (95.8%)	46 (85.2%)		26 (89.7%)	42 (89.4%)	
Mutation (+)	1 (4.2%)	8 (14.8%)		3 (10.3%)	5 (10.6%)	
RUNX1 (%)			1.000			0.644
Wild (−)	22 (91.7%)	50 (92.6%)		28 (96.6%)	43 (91.5%)	
Mutation (+)	2 (8.3%)	4 (7.4%)		1 (3.4%)	4 (8.5%)	

3.2.3 Multivariate analysis

The univariate analysis (Kaplan-Meier method) revealed that hemoglobin, PLT, LDH, age, IDH1/IDH2, and relative expression levels of IRF1 and IRF7 were prognostic factors for OS. Multivariate analysis (COX

regression model) was performed, and to indicate that high IRF1/7 expression, hemoglobin < 60g/L, Platelet < 100×10^9/L and age ≥ 60 years were independent risk factors (all P < 0.05) (Table 5).

Table 5
Multivariate analysis (COX regression model) of OS in AML patients

Variables	Hazard Ratio (95%CI)	P-value
IRF1 (High vs. Low)	2.670 (1.179–6.045)	**0.019**
IRF7 (High vs. Low)	2.783 (1.298–5.967)	**0.009**
Hemoglobin (≥ 60 vs. <60 g/L)	0.348 (0.161–0.753)	**0.007**
Platelet (≥ 100 vs. <100 $\times10^9$/L)	0.229 (0.069–0.762)	**0.016**
LDH (≥ 250 vs. <250 U/L)		NS
Age (≥ 60 vs. <60 years)	2.078(1.062–4.066)	**0.033**
IDH1/IDH2 (Mutated vs. Wild)		NS

4 Discussion

The IRFs are transcription mediators of virus-, bacteria-, and IFN-induced signaling pathways which play a critical role in antiviral defense, immune response, cell growth regulation, and apoptosis [15]. In recent studies, the IRFs were reported to play roles in tumor biology, including in human leukemia [23, 24, 29]. Although the IRF family has been greatly explored in different tumors, the systematical bioinformatics analysis in human leukemia has not been carried out yet. We anticipated that the gene expression of IRFs relevant to the prognosis of AML will shed some light on expanding the knowledge on genetic factors of AML, promoting therapy, and improving the prognosis of AML.

In this study, we observed over-expression of the IRF1/2/4/5/7/8/9 genes and under-expression of the IRF3/6 genes in AML patients compared with healthy donors from the GEPIA database analysis. Furthermore, the IRF9 was correlated with poor prognosis in AML patients through the GEPIA database analysis, and IRF1 and IRF7 were correlated with poor prognosis in AML patients using the LinkedOmics and TCGA databases. The conclusion from the GEPIA database analysis was inconsistent with that from the LinkedOmics and TCGA databases, which may be related to statistical bias caused by the insufficient sample size used in the GEPIA database. From TCGA database analysis, we found that over-expressed IRF1, IRF6, and IRF9 were associated with poor prognosis in AML patients who received chemotherapy alone; the prognostic efficiencies of these markers were decreased in patients who received hematopoietic stem cell transplant, indicating that stem cells transplants may reverse the prognostic efficiency. The data from our center revealed that AML patients with high IRF1and IRF7 expression had a poor prognosis, but IRF9 expression was not associated with OS, which was consistent with the outcomes from the LinkedOmics and TCGA databases analyses.

A previous study reported that the IRF1 gene served as an interferon-induced regulator of transcription and was up-regulated during myeloid differentiation [13]. It was generally considered a tumor suppressor gene [22]. However, some studies showed that loss of IRF1 caused hyper-susceptibility to colitis-associated colorectal cancer [30], while high expression of IRF1 was involved in cell proliferation and invasion of pancreatic cancer by promoting decoy receptor 3 in pancreatic cancer [31], indicating that IRF1 played different roles in diverse cancers. In leukemia, IRF1 was inactivated in chronic and non-chronic myelogenous leukemia [32] and expressed at a similar level between normal individuals and AML patients, as reported in a small sample study [33]. In this study, the GEPIA database analysis showed that expression of IRF1 in AML patients was higher than that in normal individuals. In addition, we found that high expression of IRF1 was correlated with poor prognosis in AML patients from the LinkedOmics and TCGA databases analyses, and the result was also validated in our study. Then, we analyzed IRF1 in the chemotherapy-only and transplant subgroups using the TCGA database. The high expression of IRF1 showed a significant impact on OS; but in the transplant subgroup, IRF1 expression was irrelevant to survival, which showed that the IRF1 may serve as a potential prognostic marker for AML, and the transplants could reverse their poor prognosis. However, in the multivariate analysis, high expression of IRF1 was an independent unfavorable factor for OS and it was not significant from the TCGA database analysis. It is possible that the number of patients undergoing transplantation at our center is too small to reverse the prognosis of IRF1 with the treatment of transplantation. Based on the analysis of clinical data, high expression of IRF1 occurred in patients whose ages were over 60 years, and the high expression was positively correlated with high PB blasts percentage, high LDH level and less inv(16) karyotype. Guzman et al. documented a consistent overexpression of IRF1 in the primary AML cells with a primitive phenotype, indicating that IRF1 may play a role in the biological function of early leukemogenic cells [34]. One interpretation of these results may be that leukemic cells can undergo the onset of the apoptotic process but fail to complete the process of apoptosis. As is mentioned above, IRF1 is a factor for poor prognosis, which may be associated with leukemic stem/progenitor cells, and it may serve as a biomarker of risk stratification in AML, while treatment with transplants could reverse its poor efficiency in determining the prognosis.

In this study, the GEPIA database analysis showed that the expression level of IRF6 in AML patients was lower than that in normal donors. And the LinkedOmics and GEPIA databases showed that the expression of IRF6 was not correlated with the prognosis of AML patients. Next, we surprisingly found that high expression of IRF6 resulted in poor prognosis in the chemotherapy-only subgroup from the TCGA database analysis. However, in our center, we could not detect the IRF6 mRNA among our AML patients using quantitative RT-PCR. In addition, we found that the expression of IRF6 was significantly lower than the values from the TCGA database or even undetected in some cases. Furthermore, it was not expressed in the AML cell lines from the EMBL-EBI database analysis [35]. Accordingly, we considered that the expression of IRF6 may be too low to be detected and may cause some statistical bias.

IRF7 is expressed at low levels in most cells and is strongly induced by the RIG-I signaling pathway, including RIG-I, MDA5, IPS-1, TBK1, and IKKε [36], and play a distinct role in the induction of type I IFNs genes, whose aberrant expression is associated with many diseases such as cancers and autoimmune

disorders [14, 37]. Another research revealed that the IRF7 and RIG-1 pathways activated by the AFAP1-AS1 gene were involved in migration and invasion in non-small cell lung cancer [38]. However, the study about IRF7 was rarely reported in leukemia. In our study, the GEPIA database analysis showed that the expression of IRF7 in AML patients was higher than that in normal donors. High IRF7 expression was significantly related to shorter survival in AML patients from the LinkedOmics and TCGA databases analyses, which was also validated from our data. Furthermore, the high expression of IRF7 in AML patients was characterized as more poor-risk stratification factors due to higher LDH levels, more complex karyotype, more RUNX1 mutation, and less NPM1 mutation [2–3], indicating that IRF7 may promote the progression of AML by interacting with some changes of genes and chromosomes. Multivariate analysis showed that IRF7 was an independent unfavorable prognostic factor both from the data of our center and the TCGA database. In addition, the reason for the association between poor prognosis and IRF7 may be related to the tumor microenvironment [39]. Next, we found that IRF7 in both transplant and chemotherapy-only subgroups from the TCGA database was irrelevant to survival. One explanation may be that IRF7 is correlated with EBV infection [37], which is easy to affect the survival of patients after transplantation. Thus, the IRF7 can be a valuable prognostic factor for risk stratification in AML. Further research with chromosome abnormalities and tumor microenvironment is needed to improve the survival in patients with high IRF7 expression.

According to a previous study, IRF9 was one of the components of interferon-stimulated gene factor 3 (ISGF3), essential for the antiviral response mediated by IFN-α/β and IFN-γ and promoted the tumor suppressor p53 gene expression through type I IFNs [13, 14, 40]. In our study, the GEPIA database analysis revealed that the IRF9 gene expression was increased in AML patients compared with healthy donors and was correlated with poor prognosis. Next, analyses of overall patients from the LinkedOmics database, TCGA database, and our center showed that the expression of IRF9 was not correlated with the prognosis of AML patients. However, from the GEPIA database and chemotherapy-only subgroup of the TCGA database, increased IRF9 expression was involved in poor survival in AML. Considering this outcome, IRF9 could regulate AML cell lines by SIRT1-p53 [41], and one explanation of the result may be that IRF9 regulated not only the wild tp53 gene but also the mutation of the tp53 gene. We further analyzed the relationship between IRF9 and TP53 mutation using TCGA database. The result showed that patients who over-expressed IRF9 was correlated with TP53 mutation ($P = 0.042$, no data shown). Another explanation may be that the sample size of the chemotherapy-only subgroup ($n = 78$) and the GEPIA database ($n = 106$) is relatively small. Hence, a study with a larger sample size of TP53 mutation is needed to clarify the prognostic role and to further elucidate the mechanism of IRF9 in AML.

5 Conclusions

The present study performed a systematic analysis of the expression of the IRF family and their prognostic efficiencies in AML patients. Our results collectively revealed that high expression of IRF1 and IRF7 were risk factors for OS and may serve as therapeutic targets and biomarkers for risk stratification. Moreover, transplantation is an ideal option to overcome their poor prognosis.

Declarations

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Authors' Contributions Conceptualization, JWZ, LY, and BX; Data curation, JWZ, LY, and LZ; Formal analysis, JWZ, LY, LZ, and YZ; Funding acquisition, BX; Investigation, JWZ, MYZ, and HJZ; Methodology, JWZ, LY, YZ, GWL, and BX; Project administration, BX; Resources, LZ, MYZ, and HJZ; Supervision, GWL, and BX; Visualization, JWZ, and LY; Writing original draft, JWZ, and LY; Writing review & editing, JWZ, LY, GWL, YZ, and BX.

Data availability The datasets from databases analyzed during the current study are available in the corresponding database (http://gepia.cancer-pku.cn/; http://www.linkedomics.org/; http://tcga-data.nci.nih.gov/tcga/). The datasets from our center analyzed during the current study available from the corresponding author on reasonable request.

Ethics Committee The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of The First Affiliate Hospital of Xiamen University (protocol code KY-2018-015 and 7th March 2018 of approval).

Conflicts of Interest The authors declare no conflict of interest.

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Figures

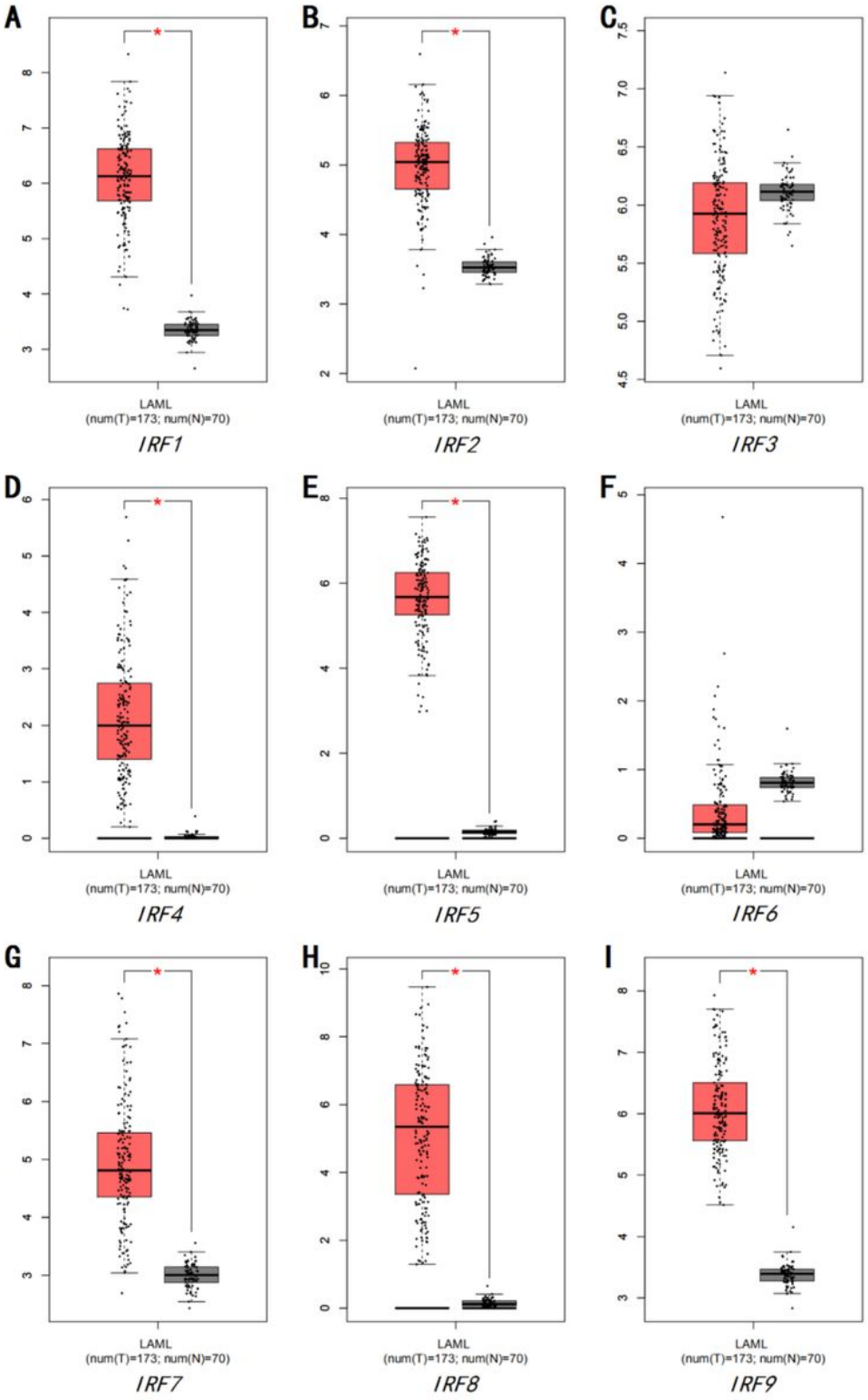

Figure 1

Differential expression of IRF genes between acute myeloid leukemia patients and healthy donors using GEPIA database.

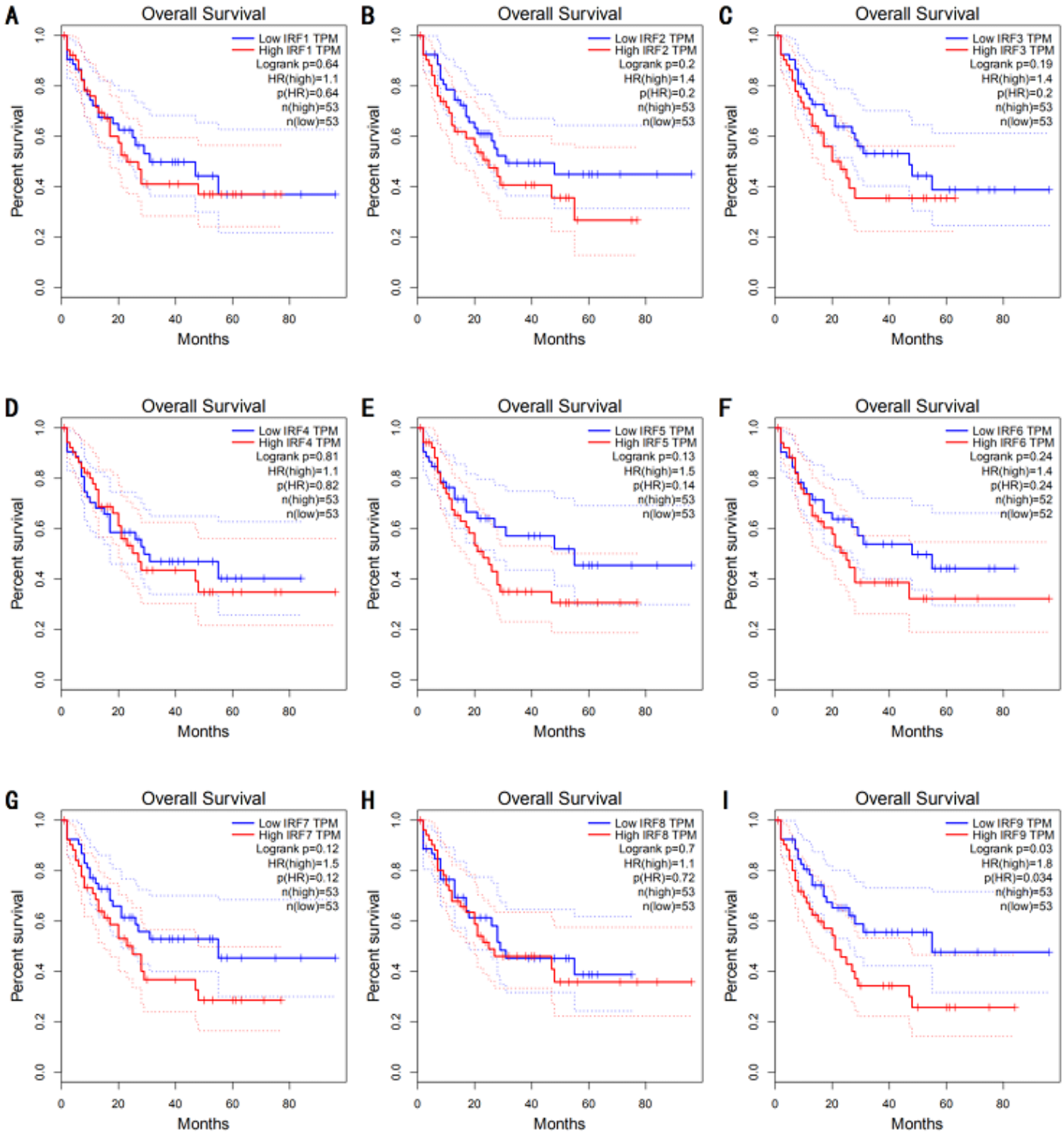

Figure 2

The prognostic significance of the expression of IRF genes in AML patients using the GEPIA database.

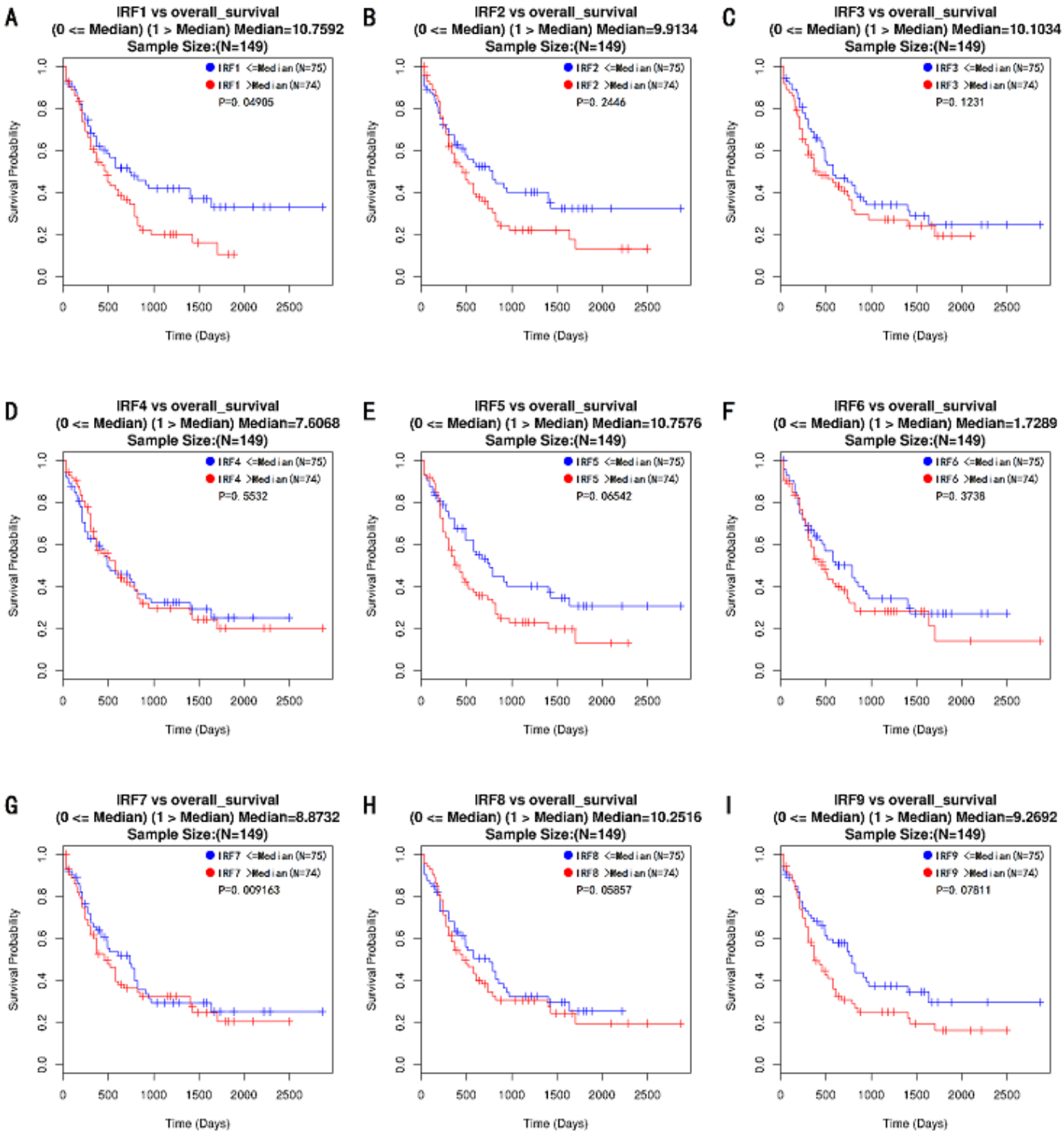

Figure 3

The prognostic significance of the expression of IRF genes in AML patients using the LinkedOmics database.

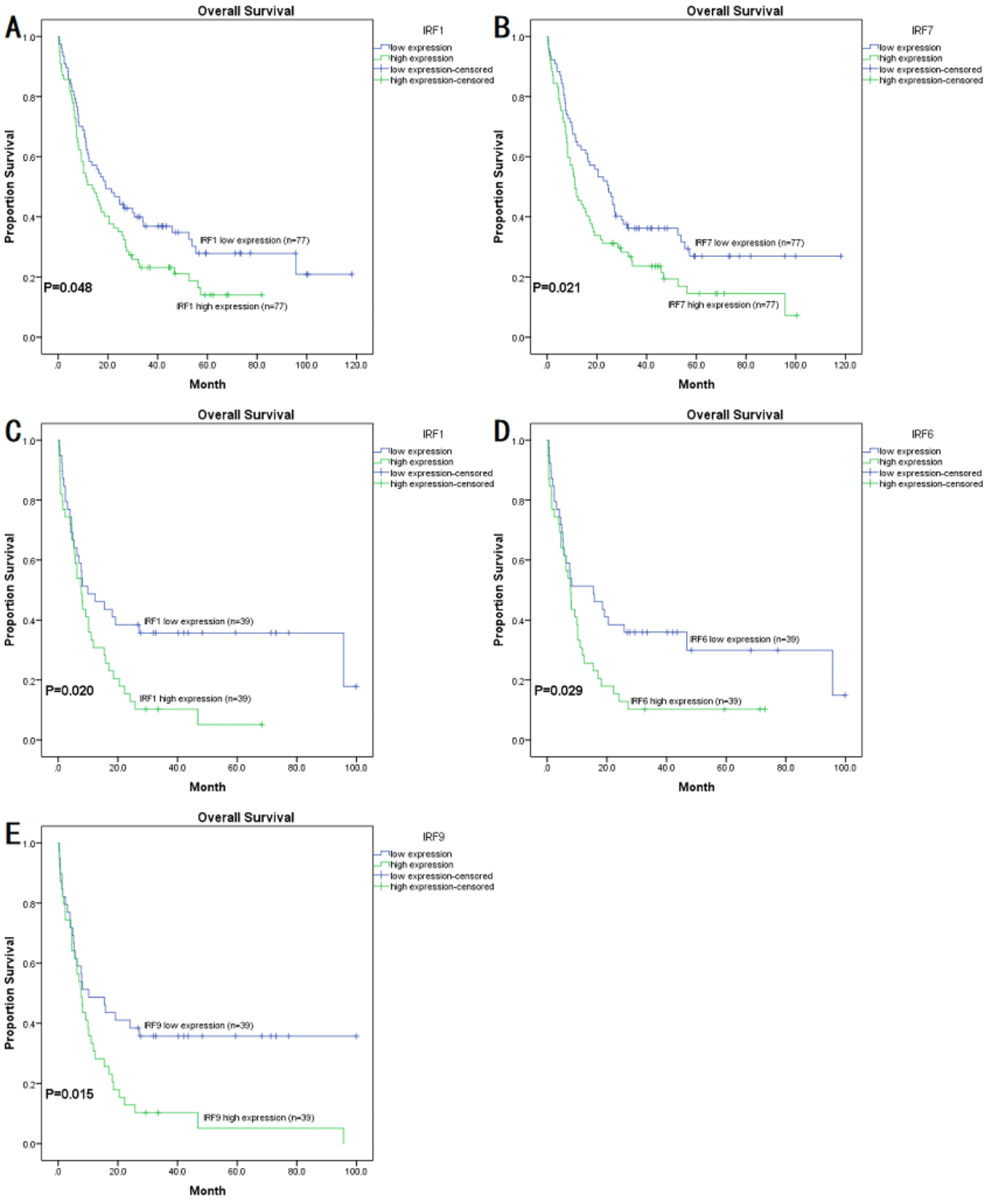

Figure 4

Survival curves relevant to the expression of IRF1, IRF7, and IRF9 genes were plotted from the Kaplan-Meier analysis in AML patients using the TCGA database. (A, B) Survival curves of IRF1 and IRF7 genes for all patients. (C, D, E) Survival curves of IRF1, IRF6, and IRF9 genes for Chemotherapy-only patients.

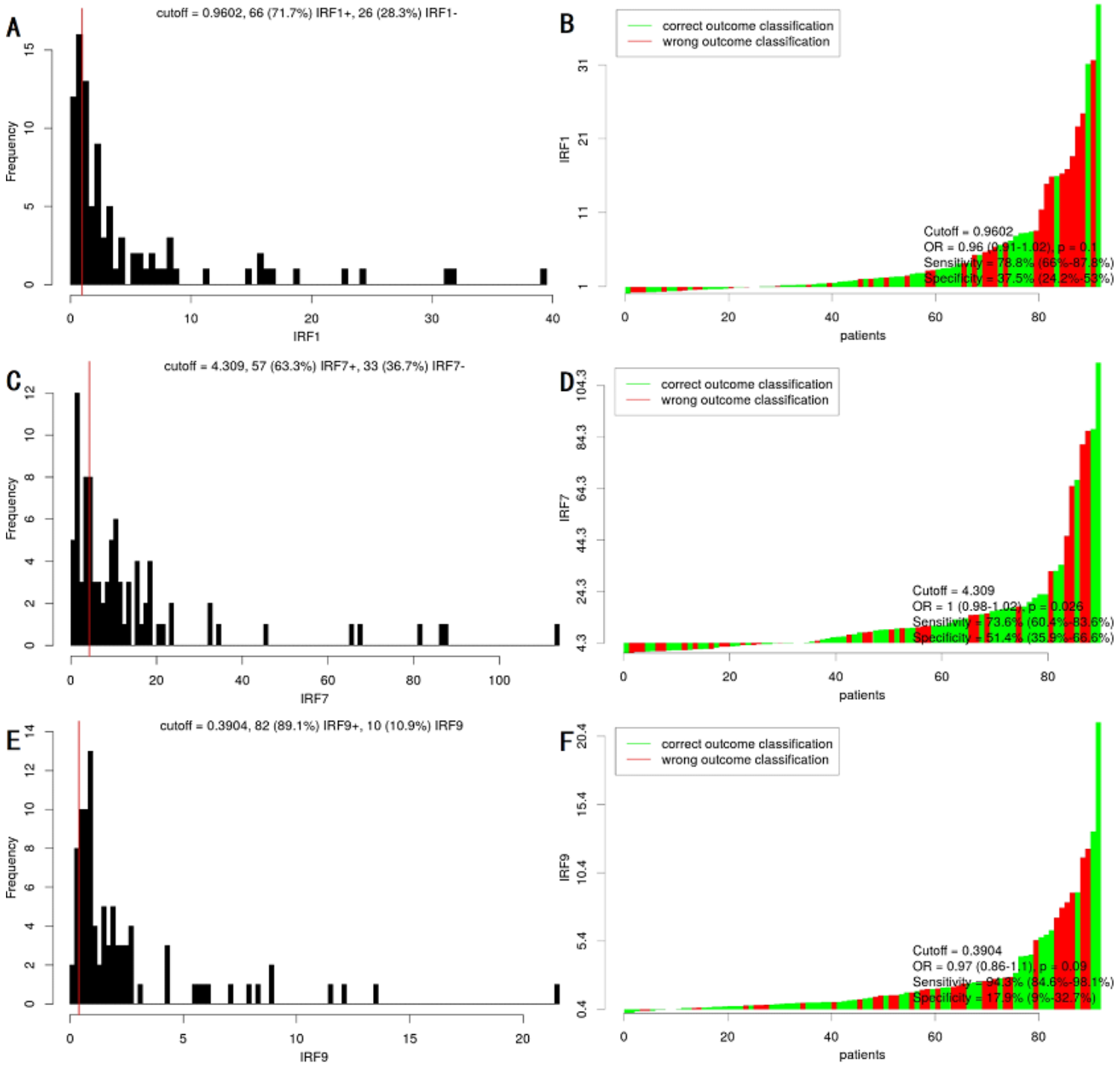

Figure 5

Distribution-based cutoff optimization of IRF1, IRF7, and IRF9 genes expression values in 95 patients with acute myeloid leukemia. A/C/E. Histogram of IRF1/7/9 genes expression with the optimal cutoff marked by a vertical line; **B/D/F.** Waterfall plot of the cutoff value for IRF1/7/9 genes expression values. The cutoff was optimized, maximizing the significance assessed by Fisher's exact test.

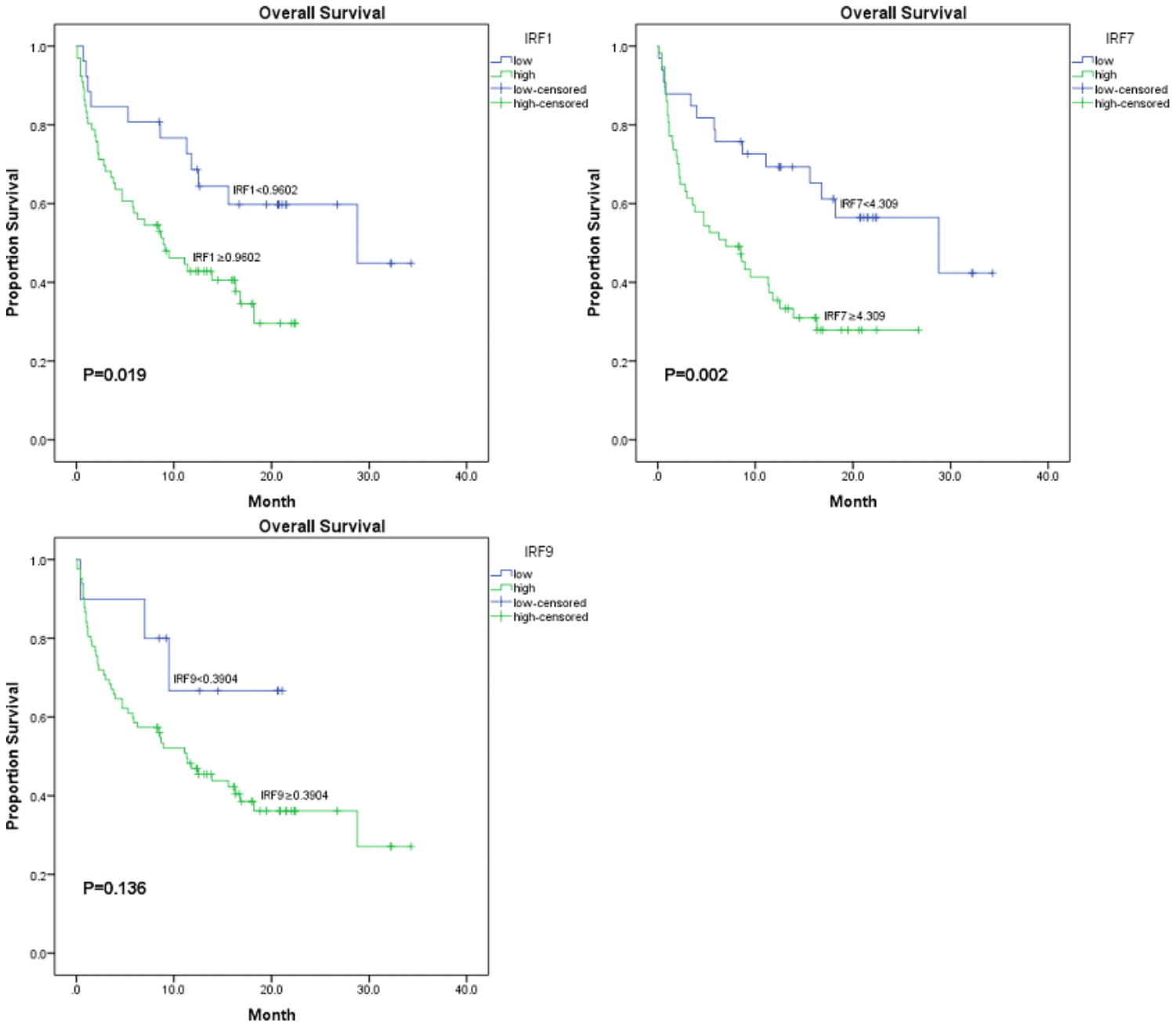

Figure 6

Survival curves of IRF1, IRF7, and IRF9 genes expression plotted by the Kaplan-Meier analysis in AML patients using our center data.