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DATABASE ANALYSIS

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Identification of the Prognostic Value and Clinical Significance of Interferon Regulatory Factors (IRFs) in Colon Adenocarcinoma

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: Colon adenocarcinoma (COAD) is one of the most common malignant tumors and has high incidence and mortality rates. The interferon regulatory factor (IRF) family is known as a key transcription factor in the IFN signaling pathway and cellular immunity. This research explored the relationship between the IRF family and COAD through use of bioinformatics technology.

Material/Methods: Using the UALCAN and GEPIA databases, we analyzed the transcription and prognostic value of IRFs in COAD, and GSCALite was used in cancer genomics analysis. TIMER, LinkedOmics, and Metascape were used to assess the potential function of IRFs in COAD.

Results: The transcription levels of IRF3 were elevated in COAD tissues, while IRF2/4/6 were downregulated compared with normal patients in subgroup analyses of race, age, weight, sex, nodal metastasis, individual cancer stages, TP53 mutation status, and histological subtypes. IRF3 and IRF7 in COAD were significantly associated with a poor prognosis. Drug sensitivity analysis revealed that the expression level of IRF2/4/8 was negatively associated with drug resistance. A significant correlation was found between the IRF family and immune cell infiltration. Moreover, enrichment analysis revealed that the IRFs were associated with response to tumor necrosis factor, transcription misregulation in cancer, and JAK-STAT signaling pathway. We also identified several kinase and miRNA targets of the IRF family in COAD.

Conclusions: We identified IRF3 and IRF7 as prognostic biomarkers in COAD, and the IRF family was associated with immune cell infiltration and gene regulation networks, providing additional evidence showing the significant role of the IRF family in COAD.

MeSH Keywords: Biological Markers • Colorectal Neoplasms • Interferon Regulatory Factors

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Background

Colon adenocarcinoma (COAD) has a high incidence rate and is the third most common adenocarcinoma worldwide. High-risk groups include people older than 50 years, with a family history, and with hereditary familial polyposis, as well as younger patients, who, unfortunately, tend to be diagnosed at a more advanced stage of COAD. To identify these patients more quickly and easily, reliable biomarkers are needed to predict disease status and prognosis. In 2018 alone, there were over 1.8 million new colon cancer cases and 880 000 COAD-related deaths. Notably, the disease is beginning to develop at a younger age [1,2]. Despite the decreased incidence of COAD-related deaths because of improvements in early detection through screening programs, including endoscopy and fecal occult blood testing, patients continue to present with advanced disease [3]. Molecular markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9(CA19-9), have been used in COAD diagnosis. However, patients at all COAD stages continue to die of the disease [4,5]. Therefore, exploring reliable biomarkers of the early pathological changes from the molecular mechanism could be crucial for early diagnosis, overall survival prediction, treatment effect, and targeted therapy development.

Interferon regulatory factors (IRFs) belong to the family of transcription factors and include IRF1-IRF9 members in both humans and mice. They are known for their critical roles in adaptive immunity [6]. Nonetheless, they are expressed in all tissue cells except for immune cells. Accumulating evidence indicates that IRFs crucially function in cell differentiation and apoptosis, cell cycle, and immunological regulation, which are associated with tumor progression [7,8]. Studies have shown that IRFs are involved in tumorigenesis by activating tumor-related gene transcription [9]. For instance, the elevated expression level of IRF2 in cancer cells promotes the activity of NF-κB during delivery of the activators (such as TNF-α). By enhancing the activity of NF-κB, the carcinogenic potential of IRF2 is increased [10]. However, the differences in the expression levels, molecular mechanisms, genetic variations, and prognostic significance of most IRFs in COAD have not been thoroughly studied. In the present study, we performed bioinformatics analysis in public databases, including ULACAN, GEPIA, TCGA, and TIMER, to explore the correlation between IRF family members and COAD.

Material and Methods

Datasets

A total of 286 COAD patients were enrolled from The Cancer Genome Atlas (TCGA) dataset. None of them had received any form of chemoradiotherapy. We assessed the IRFs at the mRNA level using the following bioinformatics portals.

UALCAN

UALCAN (*http://ualcan.path.uab.edu*) was used for evaluation of differences in IRF expression profiles between COAD and healthy tissues in the TCGA COAD dataset (n= 286). This site analyzes the relative expression of a target gene(s) of the tumor and normal samples, including the analysis of tumor subgroups based on individual cancer stage, tumor grade, or other clinicopathologic features [11]. Using these functions, we assessed the relationship between IRFs expression level and patient survival using the *t* test. $P<0.05$ was considered statistically significant.

GEPIA

We used the online database Gene Expression Profiling Interactive Analysis (GEPIA) (*http://gepia.cancer-pku.cn/index.html*), a web-based tool to deliver gene expression correlation analysis with data based on TCGA. The functions that GEPIA provides include correlation analysis, patient survival analysis, and profiling plotting [12]. Through use of this database, we assessed correlations between the expression level of IRFs and disease-free survival (DFS)/overall survival (OS) in COAD. The top 10 genes correlated with each IRFs member in COAD were analyzed using the Spearman correlation test. $P<0.05$ was considered statistically significant.

GSCALite

GSCALite (*http://bioinfo.life.hust.edu.cn/web/GSCALite/*) provides a methylation module to establish the IRFs methylation level in COAD [13]. The *t* test was used to define differences in methylation between tumor and normal samples. We tested the association between paired mRNA expression and methylation based on Pearson's product-moment correlation coefficient, and it follows a *t* distribution. *P* values were adjusted by FDR, with FDR ≤ 0.05 considered as significant. Moreover, the single-nucleotide variation (SNV) frequency and variant types of IRFs in COAD, as well as the association between the IRF family and drug sensitivity, were explored. The SNV summary and oncoplot waterfall plot were generated using maftools [14].

TIMER

The Tumor Immune Estimation Resource (TIMER) (cistrome.shinyapps.io/timer) is a public web resource that can infer the abundance of tumor-infiltrating immune cells (TIICs) from the gene expression profiles. The 6 major analytic modules, including gene expression, clinical outcomes, and somatic mutations. They enable users to analyze the correlation between immune

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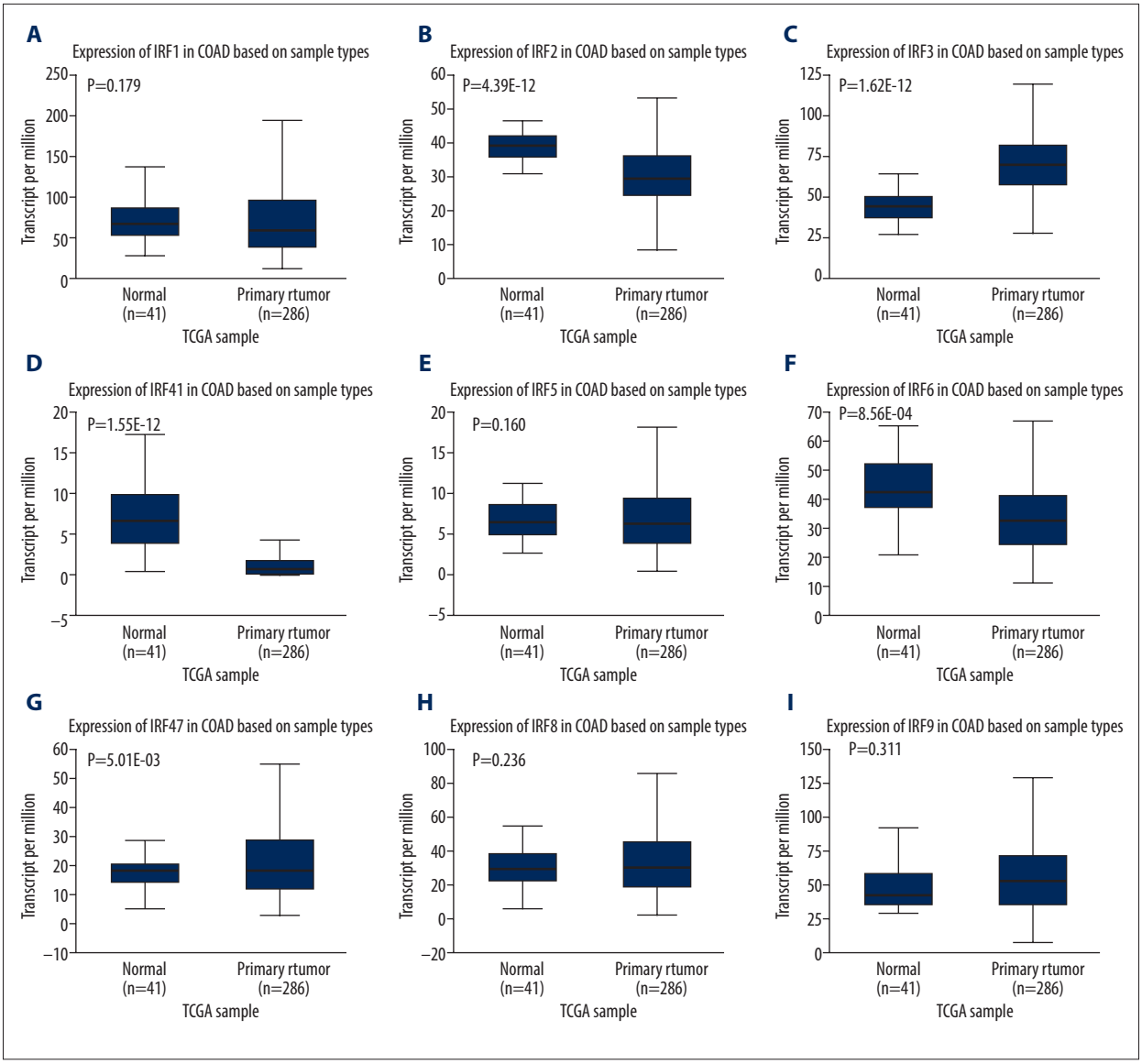

Figure 1. (**A–I**) The expression level of IRFs in COAD and normal tissues (ULCAN). The transcriptional level of IRF3 was substantially upregulated in COAD tissues relative to normal tissues. *P*<0.05 was considered statistically significant.

infiltrations and various factors [15]. In the present study, the IRFs expression was correlated with the abundance of immune cell infiltrates in COAD as assessed with the gene module, and the results are displayed by scatter plots. Furthermore, to compare TIICs abundance in COAD with different copy number distortions of the IRF family, we used SCNA modules, and for each TIIC subset, a box plot was generated to compare the distribution of the abundance of TIICs with different gene mutation status, with the statistical significance estimated using the two-sided Wilcoxon rank-sum test. This analysis was performed based on the TCGA COAD dataset (n=286). *P*<0.05 was considered statistically significant.

LinkedOmics

LinkedOmics (*http://www.linkedomics.org/*) includes multi-omics data and clinical data for 32 cancer types from the TCGA dataset [16]. We performed kinase target enrichment and miRNA target enrichment of the IRF family in COAD. The results are graphically presented in volcano plots, heat maps, or scatter plots. The rank criterion was the minimum number of genes (size) of 9 and the simulation of 500, and *P*<0.05 was considered statistically significant.

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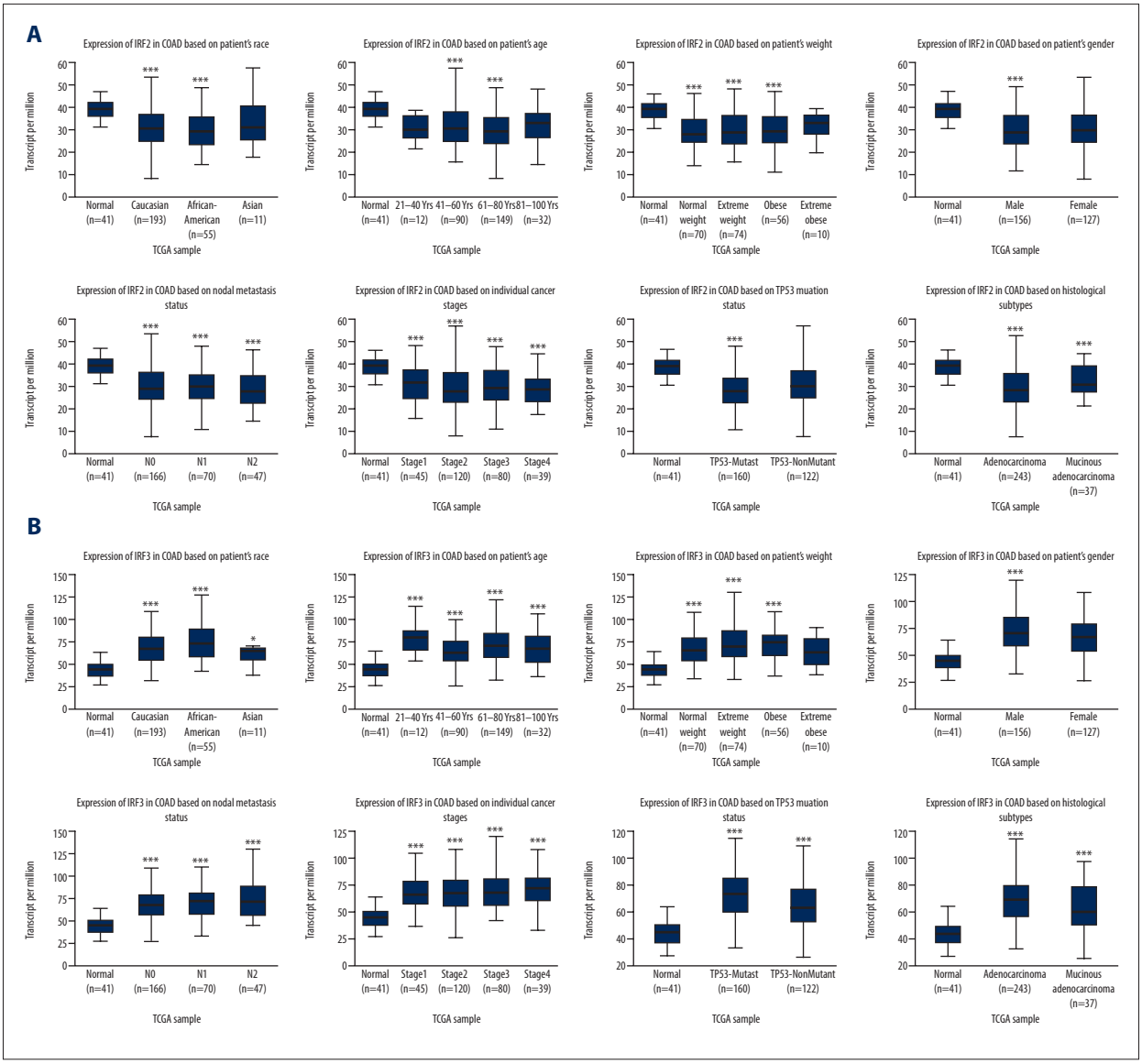

Figure 2. The transcription level of IRF2 (**A**) and IRF3 (**B**) in subgroups of COAD patients, stratified according to the following criteria: race, age, weight, sex, nodal metastasis, individual cancer stages, TP53 mutation status, and histological subtypes (UALCAN). Data are mean±SE. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Metascape

Metascape (*http://metascape.org*) is a productive gene function annotation analysis tool to annotate a large number of genes and to identify enriched pathways [17]. The top 10 genes correlated with each IRFs member in COAD were extracted from the GEPIA dataset, and these genes were analyzed through Metascape. With GO and KEGG methods, we are able to analyze a gene list related to IRFs to identify the most frequently altered linked genes and constructed protein-protein interaction networks from lists of genes and proteins.

Results

Expression level of IRFs in COAD

The differences in transcription levels of IRFs between COAD and normal tissues were evaluated using the UALCAN databases to study the expression profiles of IRFs in COAD patients. Compared with normal tissues, IRF3 (Figure 1C, P=1.62E-12) was upregulated in COAD tissues (Figure 1). However, IRF2 (Figure 1B, P=4.39E-12), IRF4 (Figure 1D, P=1.55E-08), IRF6 (Figure 1F, P=8.56E-04), and IRF7 (Figure 1G, P=5.01E-03) were downregulated in COAD tissues relative to the healthy tissues, and there were no significant differences in the transcription

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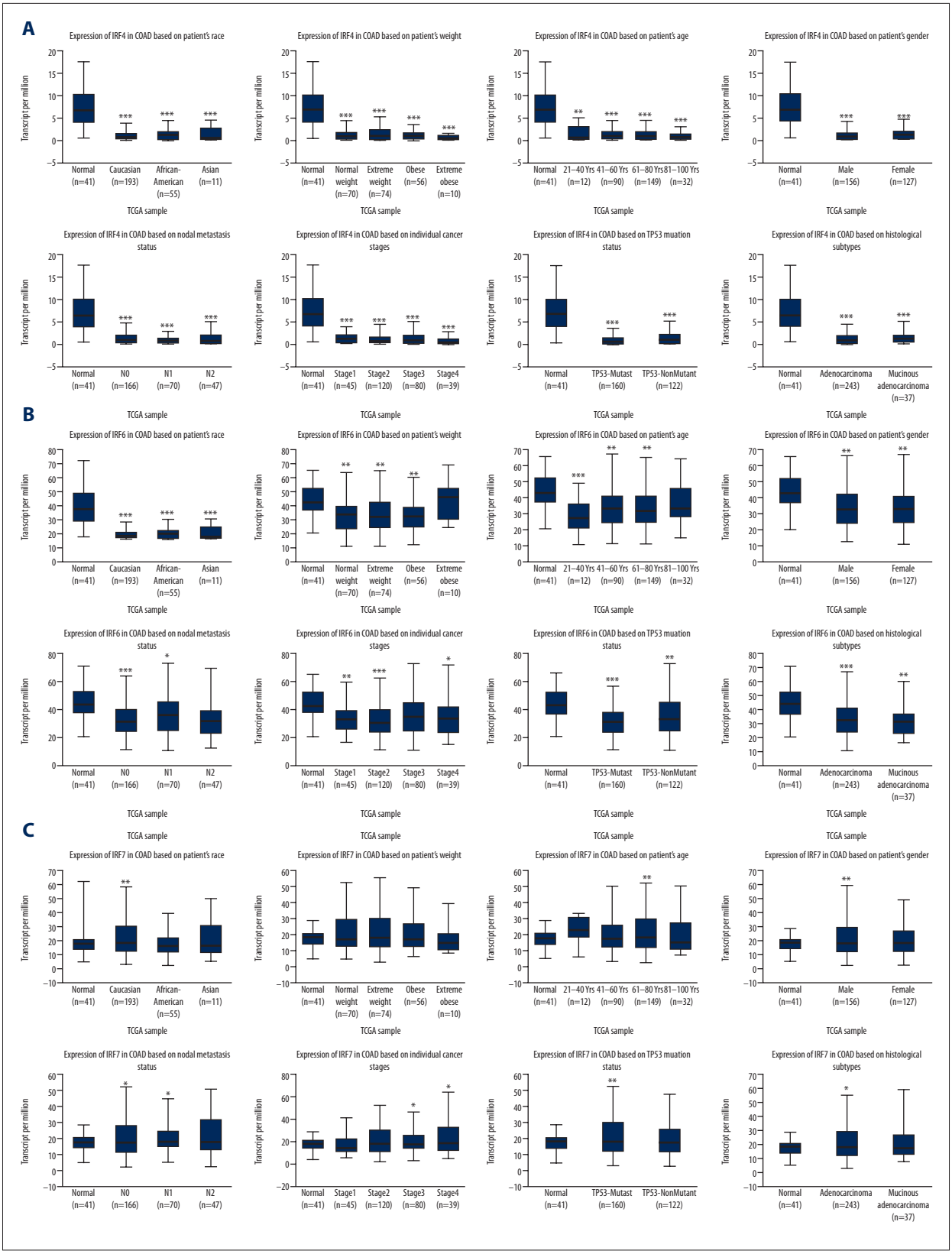

Figure 3. The transcription level of IRF4 (**A**), IRF6 (**B**), and IRF7 (**C**) in subgroups of COAD patients, stratified according to different criteria (UALCAN). Data are mean±SE. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

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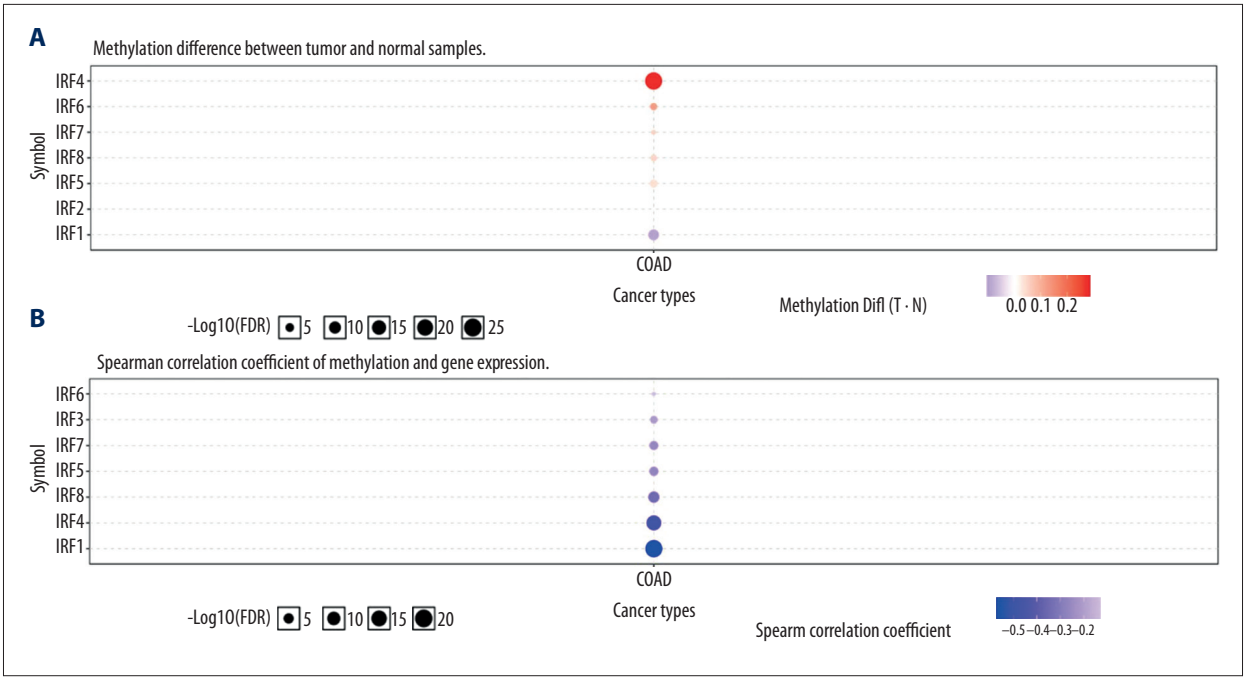

Figure 4. Methylation level of the IRFs in COAD tissues. The difference in IRF family methylation in COAD and normal specimens (**A**). Correlation between IRF family methylation and IRF family expression in COAD (**B**).

levels of IRF1/5/8/9 between COAD and healthy tissues. Additionally, UALCAN allowed us to discover the relationship between the expression levels of IRFs in COAD and pathological clinical features. The boxplots indicate that transcription levels of IRF2 (Figure 2A), IRF4 (Figure 3A), and IRF6 (Figure 3B) in COAD patients were downregulated compared with normal patients in subgroup analyses regarding race, age, weight, sex, nodal metastasis, individual cancer stages, TP53 mutation status, and histological subtypes. In contrast, the IRF3 mRNA levels (Figure 2B) were higher in COAD patients than in healthy persons in the subgroup analyses in all pathological clinical features. There was no significant change in IRF7 (Figure 3C). Interestingly, the expression levels of IRF3 were significantly different, and overweight and male patients had much higher IRF3 mRNA levels. Moreover, regarding nodal metastasis, the IRF3 levels in the N2 stage were noticeably higher than in the other stages. In the TP53-nonmutant type and mucinous adenocarcinoma, IRF3 mRNA levels were distinctly upregulated. In the methylation analysis, the methylation levels of most IRFs in COAD tissues were elevated, whereas IRF1 was downregulated (Figure 4A). In addition, methylation was negatively correlated with the expression of IRFs in COAD (Figure 4B).

Prognostic value of IRF family in COAD

GEPIA established the prognostic value of IRFs expression levels in COAD patients. In COAD patients, high expression levels of IRF3/7 were significantly associated with poor OS (Figure 5A). However, the expression level of IRFs in COAD patients was independent of DFS (Figure 5B). Overall, elevated mRNA levels of IRF3/7 were significantly associated with poor prognosis; therefore, IRF3/7 are potential biomarkers for predicting the survival of COAD patients.

Genetic variation

The genetic variation in the IRF family in COAD is shown in Figure 6. These variations include missense mutation, splice site, frameshift insertion, frameshift deletion, multi-hit, nonsense mutation, and in-frame deletion (Figure 6). Next, the role of the IRF family in crucial cancer-related pathways was evaluated. We established that the IRF family is involved in activation of tumor cell apoptosis pathways and the hormone ER pathway. We also found that IRFs inhibit the cell cycle and DNA damage pathways (Figure 7). Therefore, genomic aberrations could serve as potential biomarkers for drug screening and affect clinical responses to treatment. Drug sensitivity analysis showed the expression levels of IRF2/4/8 were negatively associated with drug resistance (Figure 8).

Immune infiltration of IRF family in COAD patients

We next used the TIMER web resource to investigated whether IRFs expression is related to immune infiltration levels in COAD. Tumor purity is an important factor in using genomic approaches because it influences the analysis of immune infiltration in clinical tumor samples [18]. The expression levels of IRF family members in COAD was associated with infiltrating immune

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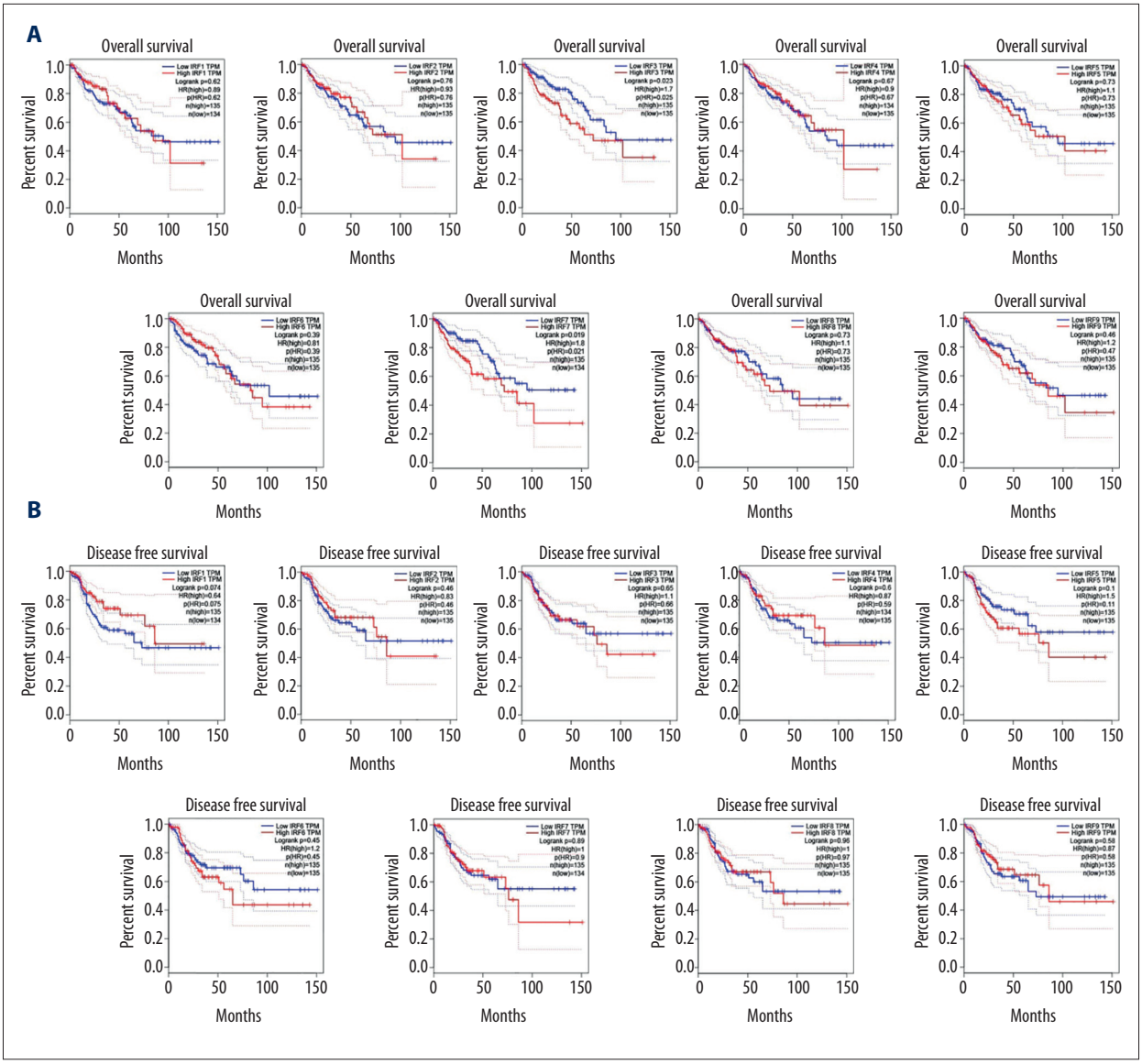

Figure 5. The prognostic value of mRNA level of IRFs in COAD. In COAD patients, the upregulated IRF3/7 was significantly related to the poor OS (**A**), while all members of the IRF family showed no prognostic value in DFS (**B**). The expression of other IRFs had no association with OS in COAD patients.

cells, including neutrophils, CD8+ T cells, dendritic cells, macrophage B cells, and CD4+ T cells (Figure 9, Table 1). In general, our results reveal the relationship between IRFs expression levels and immune infiltration levels in COAD. Additionally, the copy number variations in the IRF family suppressed the levels of infiltrating immune cells (Figure 10).

Enrichment analysis of IRF family in COAD

We further investigated the potential role of IRFs in COAD pathogenesis and development via gene enrichment analysis of the pathways and processes in 90 neighboring genes (Figure 11, Table 2). IRFs and the vicinal genes were significantly enriched in molecular functions (MF), biological processes (BP), cellular component (CC), and pathways involved in interactions. GO enrichment analysis showed highly enriched signal regulation pathways, including type I interferon signaling cascade, response to interferon-gamma, regulation of cytokine production, response to tumor necrosis factor, and interleukin-27-mediated signaling axis (Figure 11A, 11B, Table 3). The top 7 KEGG pathways of the IRF family members and adjacent genes are shown in Figure 11C, 11D and Table 4. Among these pathways, RIG-I-like receptor-signaling cascade, viral carcinogenesis, HTLV-1 infection, and transcription misregulation in cancer were associated with the development and pathogenesis of COAD. Moreover, the mCODE was retrieved and revealed

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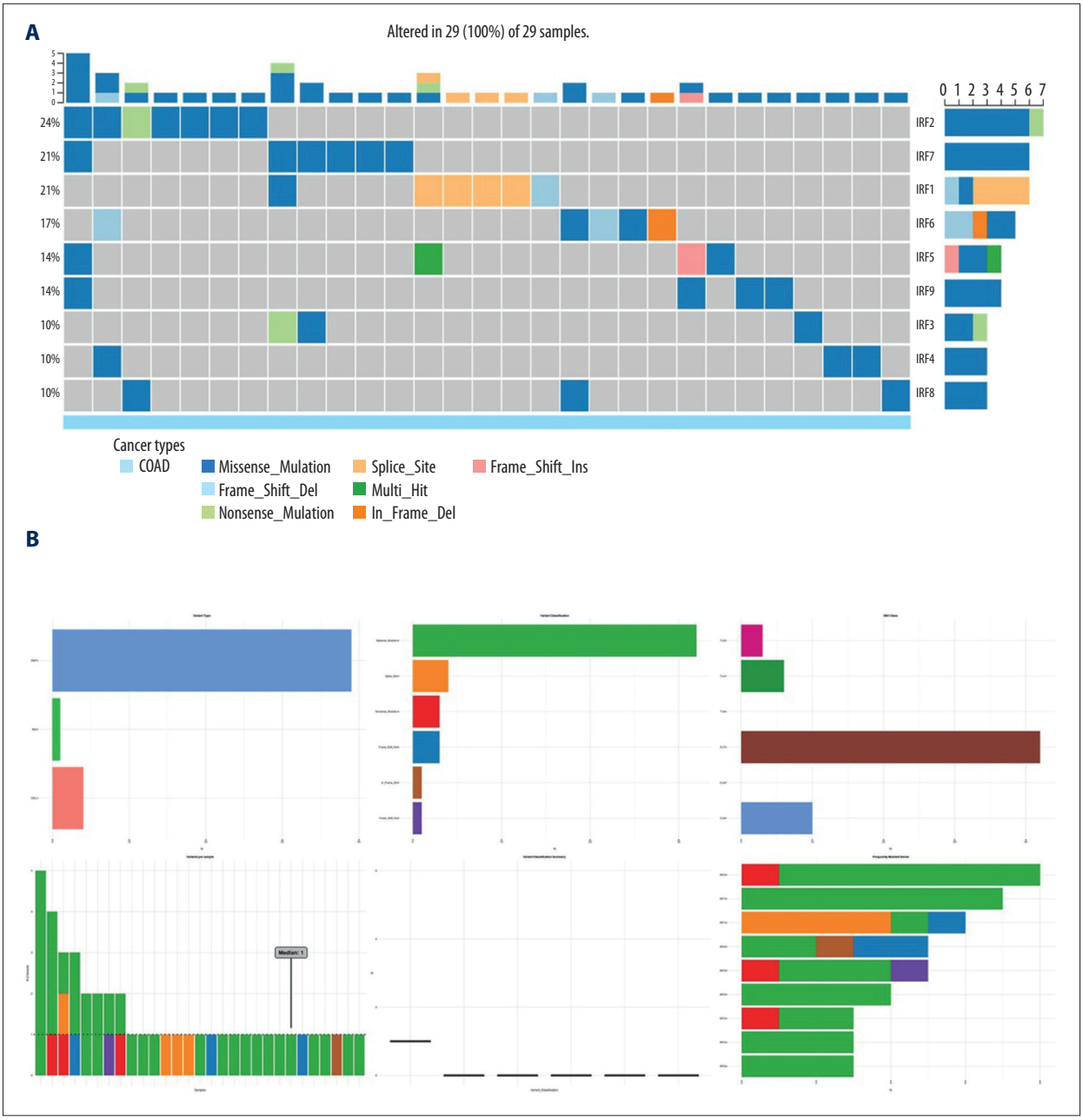

Figure 6. The genetic variation analysis of the IRF family in COAD. (**A**) Summary plot displays genetic variation frequency and variant types of IRF family in COAD. (**B**) Waterfall plot shows the genetic variation distribution of IRF family in COAD and a genetic variation classification.

that the IRF family and adjacent genes participate in the JAK-STAT signaling pathway and tuberculosis (Figure 11E, 11F).

Kinase and miRNA targets of IRF family in COAD

To determine the role of IRFs in COAD, we then explored the kinase target and miRNA target of the IRF family in COAD (Table 5). The results suggested that kinase LCK and LYN are common targets of IRF1/4/7/8/9. The kinase target of IRF5 is SYK and FYN. Kinase ATR as well as STK are kinase targets for IRF6. The kinase targets of IRF3 are IKBKB and PLK3. The miRNA targets of IRFs are shown in Table 6. The (TGTATGA) MIR-17-5P, MIR-20A, MIR-106A, MIR-106B, MIR-20B, MIR519D, and (TGTATGA) MIR-485-3P were suggested to be the miRNA targets of IRF3. (CCAGGGG) MIR-331 and (CAGTCAC) MIR-134 were suggested to be the miRNA targets of IRF7.

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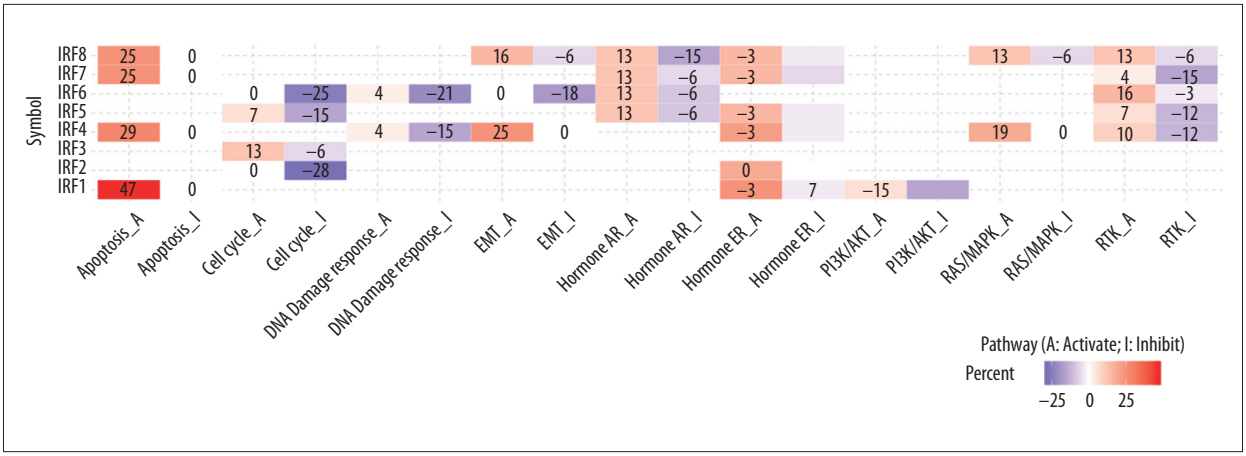

Figure 7. Cancer-related pathways analysis of the IRF family in COAD.

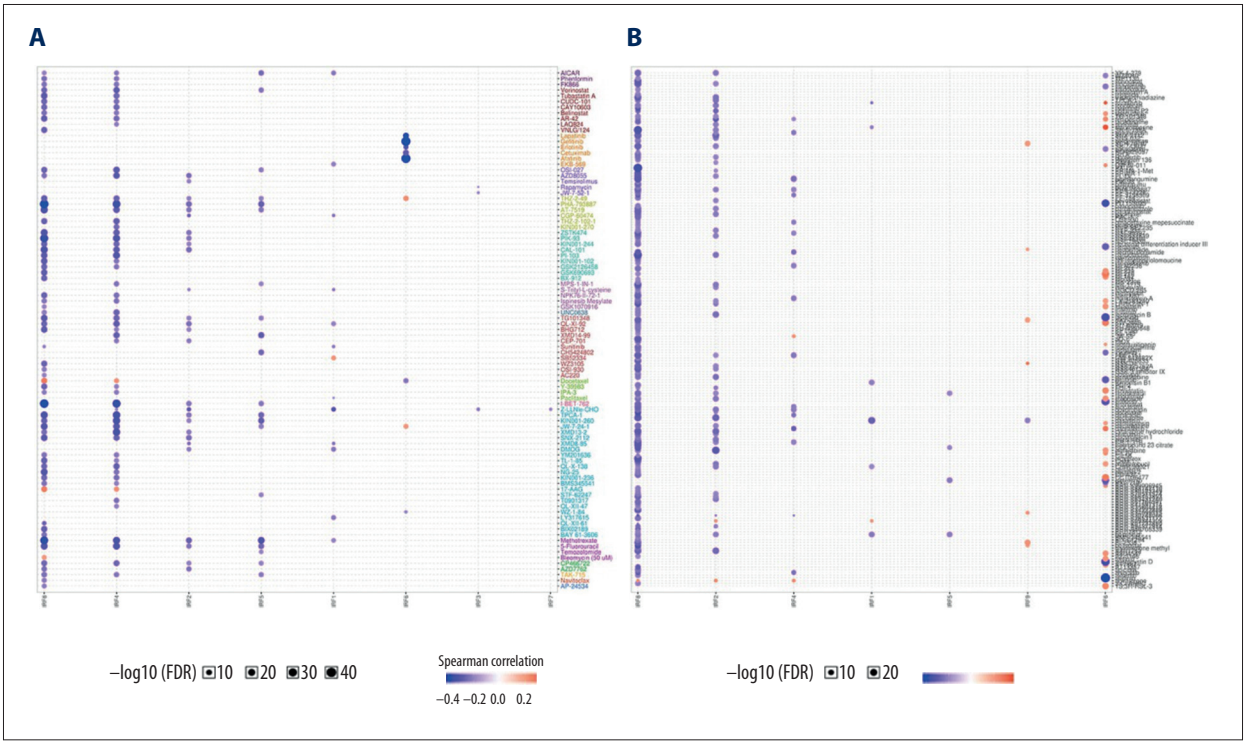

Figure 8. (**A, B**) Role of IRF family members in drug sensitivity. A positive correlation indicates that high gene expression is resistant to drugs and vice versa.

Discussion

The prognosis of COAD mainly depends on the extent of disease, and lack of reliable biomarkers results in late diagnosis and high mortality in COAD [19]. If a genetic diagnosis can be used to detect COAD at an early stage for effective intervention, the prognosis of patients will be greatly improved. The IRF family plays an important function in cancer immunobiology. During tumorigenesis, each member strictly controls the production and function of cells involved in the antitumor immune response [20]. The diverse role of IRFs in cancers has been reported, suggesting that IRFs modulate tumor progression and could be used as biomarkers. However, for COAD, there is no such specific description of the correlation between IRFs and COAD.

Firstly, we explored the transcription level of IRFs in COAD. IRF2/4/6 was downregulated in COAD patients regarding all kind of clinic pathologic features, while only IRF3 was highly expressed in COAD tissues. IRF1/5/8/9 showed the result was not statistically significant; however, a few studies showed that increased IRF1 and IRF2 levels were found in CRC tissues, and

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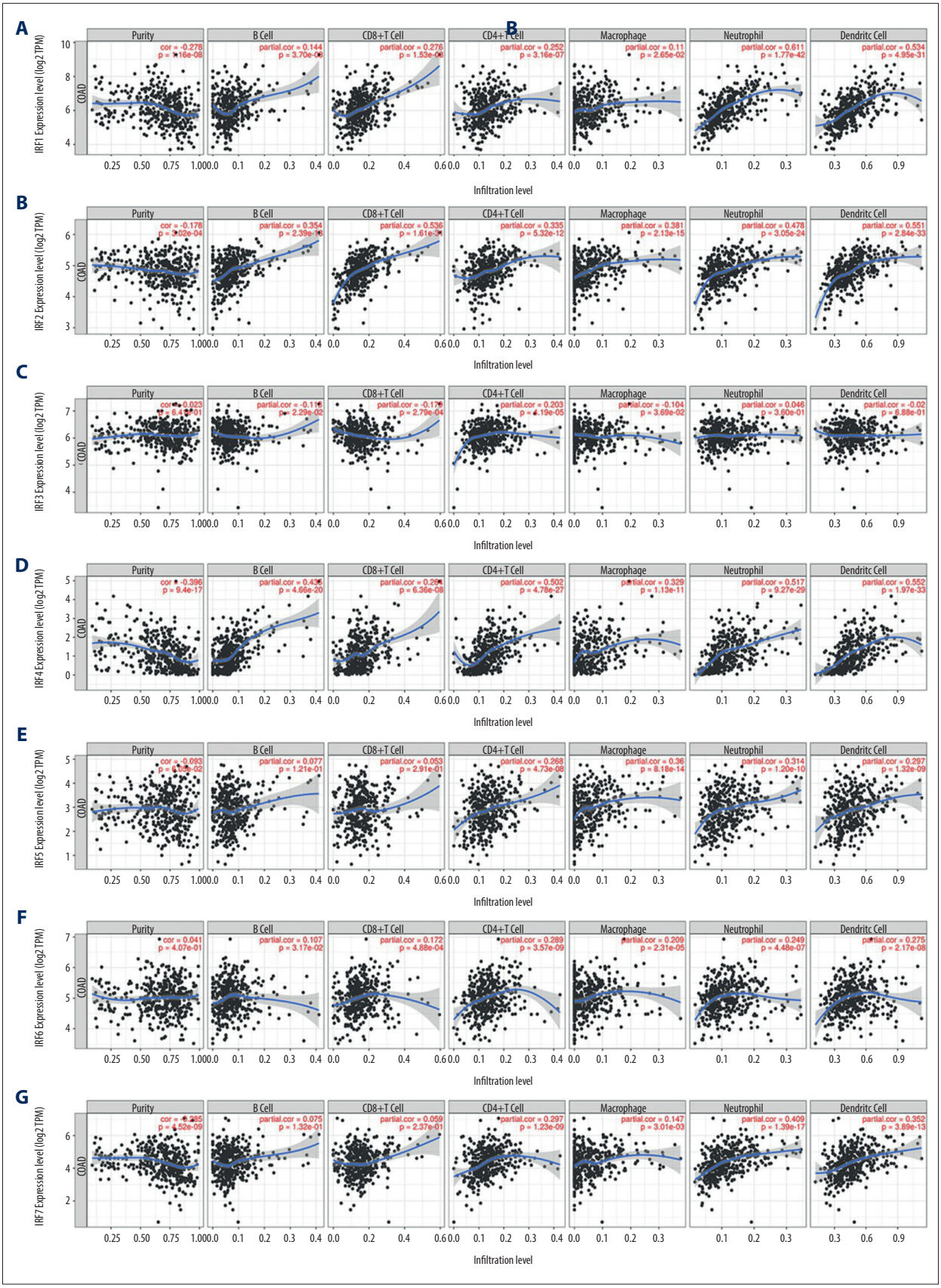
A
B
C
D
E
F
G
Purity
B Cell
CD8+T Cell
CD4+T Cell
Macrophage
Neutrophil
Dendritc Cell
COAD
IRF1 Expression level (log2 TPM)
IRF2 Expression level (log2 TPM)
IRF3 Expression level (log2 TPM)
IRF4 Expression level (log2 TPM)
IRF5 Expression level (log2 TPM)
IRF6 Expression level (log2 TPM)
IRF7 Expression level (log2 TPM)
Infiltration level

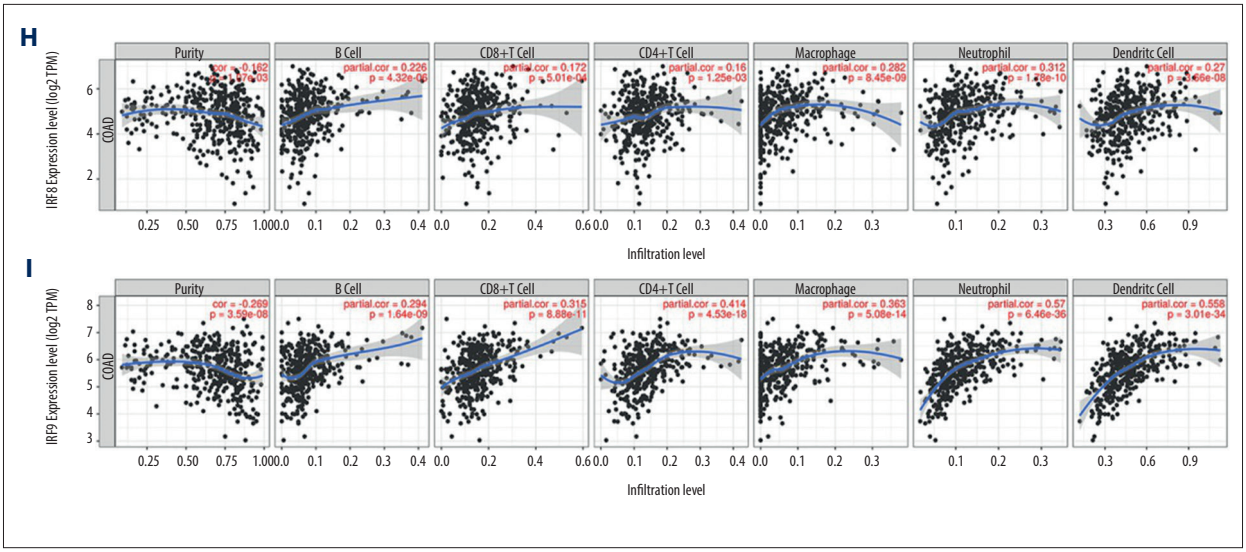

Figure 9. Correlation of IRFs expression with immune infiltration level in COAD tissues (TIMER). The scatter plots (**A–I**) identify the different profiles of immune cells associated with IRFs.

Table 1. Comparison of gene expression and immune cell landscape.

	Tumor purity	B cells	CD8+T cells	CD4+T cells	Macrophages	Neutrophils	Dendritic cells
	r	r	r	r	r	r	r
IRF1	(–)	(+)	(+)	(+)	(+)	(+++)	(+++)
IRF2	(–)	(++)	(+++)	(++)	(++)	(++)	(+++)
IRF3	(–)	(–)	(–)	(+)	(–)	(–)	(–)
IRF4	(– –)	(++)	(+)	(+++)	(++)	(+++)	(+++)
IRF5	(–)	(–)	(–)	(+)	(++)	(++)	(+)
IRF6	(–)	(+)	(+)	(+)	(+)	(+)	(+)
IRF7	(–)	(–)	(–)	(+)	(+)	(++)	(++)
IRF8	(–)	(+)	(+)	(+)	(+)	(++)	(+)
IRF9	(–)	(+)	(++)	(++)	(++)	(+++)	(+++)

Correlation of immune cell landscape of COAD compared with TCGA gene expression of IRFs (TIMER). r – categorized Pearson's correlation coefficient; (– –): –0.5 to –0.3, weak negative association; (–): –0.3 to 0.1, little association; (+): +0.1 to 0.3, little association; (++): +0.3 to +0.5, weak positive association; (+++): +0.5 to +1.0, strong positive association.

the high expression level of IRF2 was related to a more than 2-fold increase in the risk of all-cause mortality in CRC patients [21,22]. Although IRF7 was downregulated in COAD tissues, IRF3 and IRF7 were significantly associated with poorer overall survival. These data indicate that differentially expressed IRFs may play a significant role in COAD. As a recent study suggests, β-catenin is overexpressed in colorectal cancer and its expression level is positively associated with the level of IRF3 in CRC cells [23].

The present study demonstrates the molecular characteristics of IRFs in COAD. In COAD, the frequent genetic alterations in IRFs were differentially expressed. Genomic aberrations could serve as potential biomarkers for drug screening and affect clinical responses to treatment. Drug sensitivity analysis shows the expression levels of IRF2/4/8 were negatively associated with drug resistance, indicating that they are potential novel markers for drug screening.

Next, we focused on immune cell infiltration. Interestingly, in COAD patients, IRF3/5/7 had a weak correlation with B cells and CD8+ T cells infiltration level, while IRF1/2/4/6/8/9 showed a strong correlation with infiltration levels of all 6 types of immune cells (neutrophils, CD8+ T cells, dendritic

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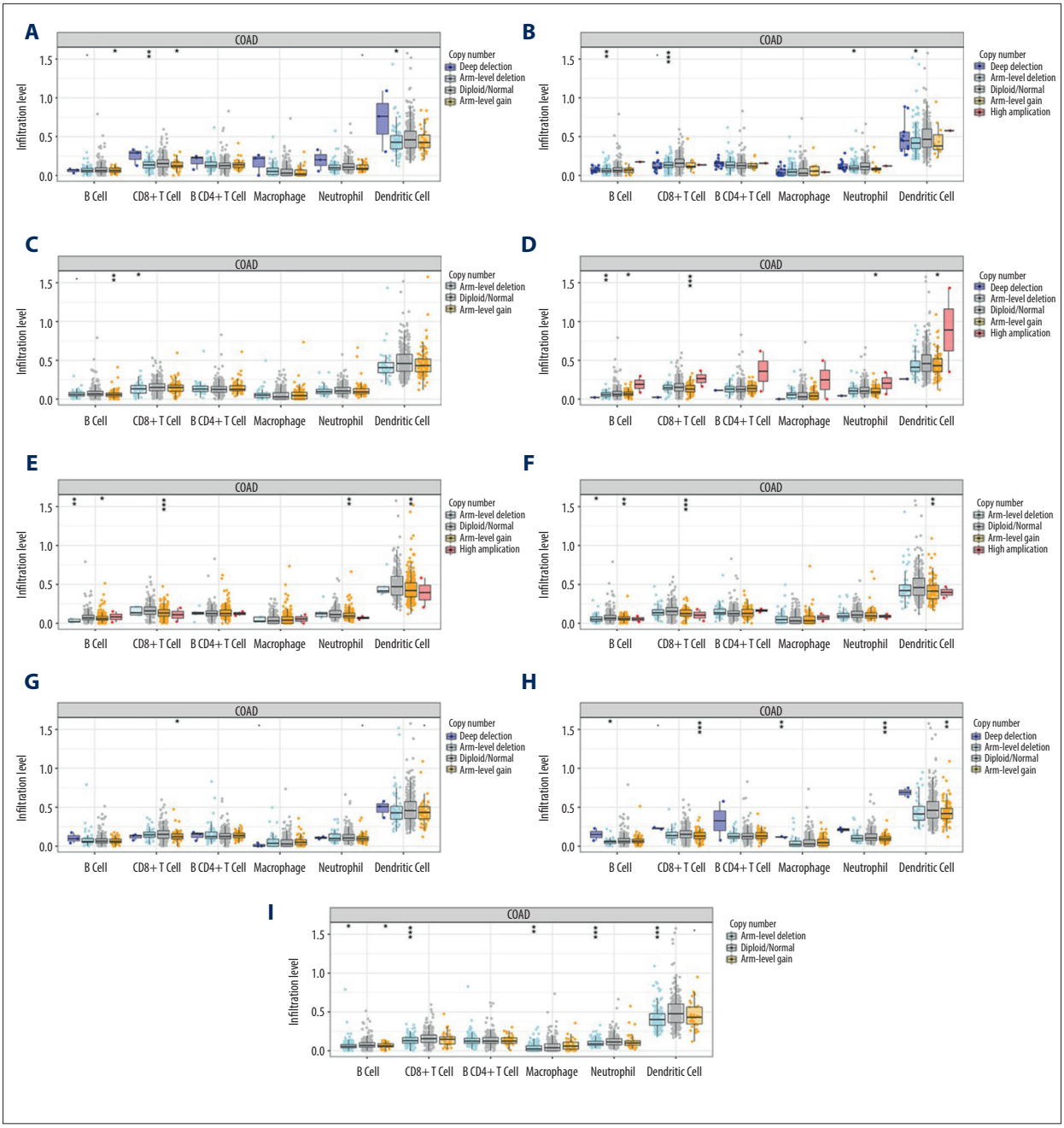

Figure 10. (**A–I**) Effect of copy number variation of the IRF gene family on the level of immune cell infiltration.

cells, macrophage, B cells, and CD4+ T cells), and the change of copy number in the IRF family inhibits the level of infiltrating immune cells. Unlike the other IRF members, IRF3 expression had a very weak correlation with infiltration levels of B cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells, but had a significant correlation with the CD4+T cells infiltration level in COAD patients. Studies revealed that, compared with normal colon tissues, there were more CD4+T cells in colorectal cancer tissues [24], and the correlation between IRF3 and CD+4 T cells suggested its role as a biomarker of IRF3 in COAD.

With GO enrichment analysis and KEGG pathway enrichment analysis, we found a potential role of IRFs in COAD development. The IRF family was mostly enriched in the type I interferon signaling cascade, which was expected because, expect for IRF6, the other members are all the primary regulator of type I IFN activation. The type I IFN signaling pathway is important for innate antiviral immunity, and pathway damage is related to increased risk of tumorigenesis. IRF9 can enhance the p53 pathway when cells are exposed to endogenous induced or exogenous type I interferon, suggesting that IRF9

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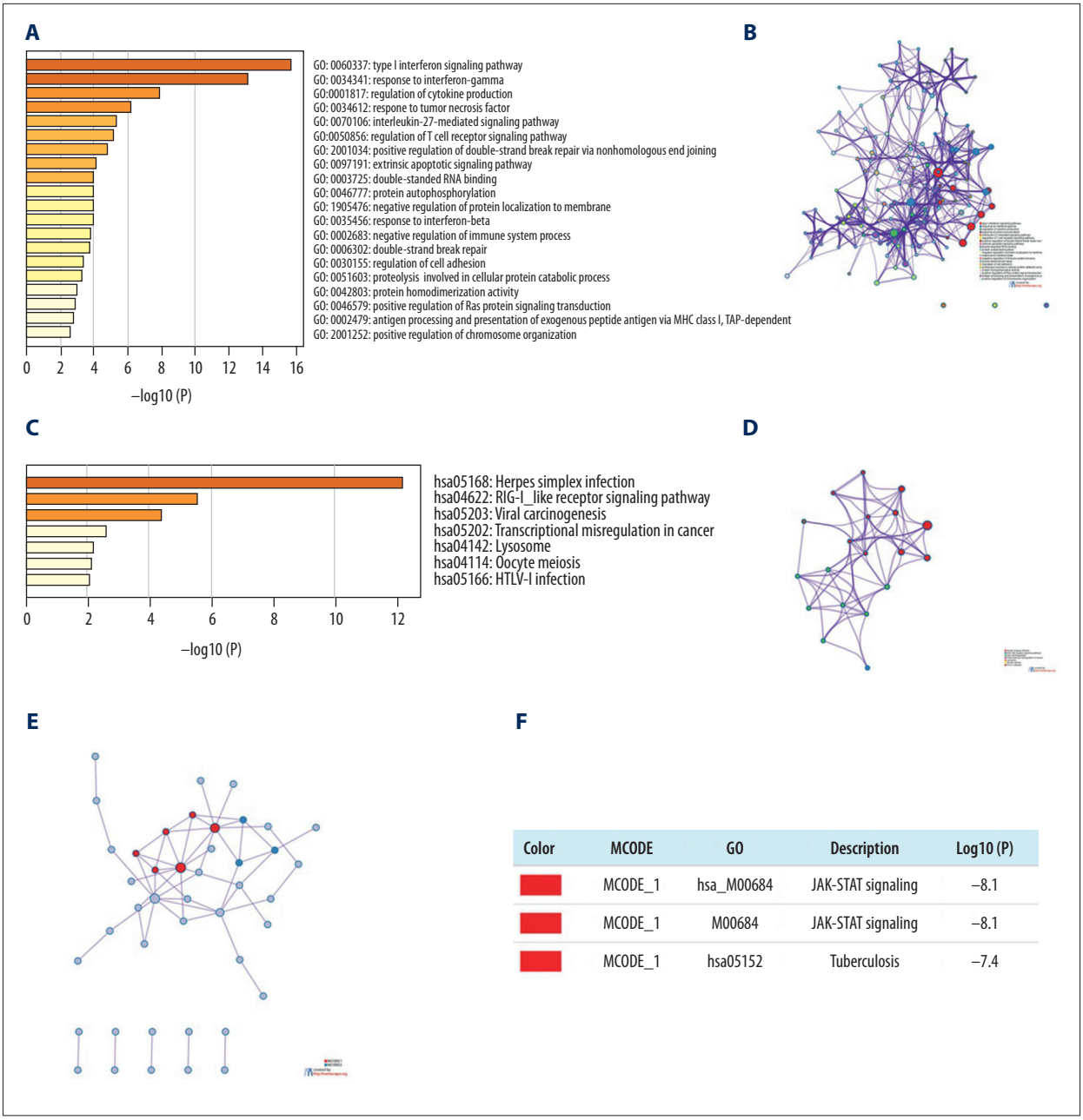

Figure 11. Functional enrichment analysis of IRFs in COAD (Metascape). (**A, B**) The enriched terms in GO analysis, colored by *P* value; (**C, D**) The enriched terms in KEGG pathways analysis, colored by *P* values. (**E**) PPI network and 3 most significant MCODE components. (**F**) Independent functional enrichment analysis of 3 MCODE components.

has an anti-proliferation effect [7,20]. Results also showed that the IRFs were mainly associated with response to tumor necrosis factor, transcription misregulation in cancer, and the JAK-STAT signaling pathway. The JAK-STAT pathway is a common signal transduction pathway, but under pathological conditions, the activation of this pathway is associated with the proliferation of many malignant tumors [25]. An immunohistochemical experiment showed that the expression levels of JAK-1 and STAT-3 proteins were upregulated in colon cancer tissues, and the levels were an independent risk factor for the prognosis of colon cancer [26]. As our study showed, IRFs are closely related to the JAK-STAT pathway, which suggests that the high expression level of IRF3/7 in COAD patients may have a deeper relationship with the JAK-STAT signaling pathway in tumor development.

Finally, due to the different interactions of IRFs and the SRC family tyrosine kinases (LCK, LYN, and FYN), they play different roles

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Table 2. Top 10 correlated genes of each member of IRF family in COAD (GEPIA).

IRF1	UBE2L6, GBP1, TAP1, STAT1, C5orf56, GBP4, PSMB9, PARP14, ETV7, SAMD9L
IRF2	CASP3, CAMK2D, ATP10D, CYLD, TLR3, CTSO, TNFSF10, LITAF, JAK1, JAK2
IRF3	PNKP, SNRNP70, PTOV1-AS2, AC018766.4, LENG1, SMG9, PRKD2, SUV420H2, CLASRP, PPP1R12C
IRF4	LAX1, PRR33, GPR174, KCNA3, CTD-2020K17.1, ZNF80, TRAF3IP3, RP11-686D22.10, NCF1B, UBASH3A
IRF5	AP1M1, GDI1, C17orf62, RP11-1072A3.3, IKBKG, TFE3, SCPEP1, MAP3K3, TBC1D25, SAMHD1
IRF6	LPGAT1, C1orf106, PLEKHA6, F11R, KDM5B, PPP2R5A, ETV3, BROX, GOLPH3L, PIK3C2B
IRF7	XAF1, MX1, ISG15, IFIT1, OAS2, IFI44, IRF9, DHX58, HSH2D, AP001610.5
IRF8	NUB1, RP11-542M13.2, MCM4, NBN, MAX, IMPA1, SSX2IP, TCEA1P2, CDC27, TRAF3
IRF9	PARP9, XAF1, OAS2, DDX60, SP100, PARP14, IFI44, IFIT3, SP110,USP1

Table 3. GO function enrichment analysis of IRF family members and neighbor genes in COAD (Metascape).

GO	Category	Description	Count	%	Log10(P)	Log10(q)
GO: 0060337	GO biological processes	Type I interferon signaling pathway	12	15.58	–15.70	–11.73
GO: 0034341	GO biological processes	Response to interferon-gamma	13	16.88	–13.12	–9.61
GO: 0001817	GO biological processes	Regulation of cytokine production	15	19.48	–7.87	–4.69
GO: 0034612	GO biological processes	Response to tumor necrosis factor	9	11.69	–6.14	–3.09
GO: 0070106	GO biological processes	Interleukin-27-mediated signaling pathway	3	3.90	–5.30	–2.42
GO: 0050856	GO biological processes	Regulation of T cell receptor signaling pathway	4	5.19	–5.14	–2.34
GO: 2001034	GO biological processes	Positive regulation of double-strand break repair via nonhomologous and joining	3	3.90	–4.77	–2.02
GO: 0097191	GO biological processes	Extrinsic apoptotic signaling pathway	6	7.79	–4.09	–1.44
GO: 0003725	GO Molecular Functions	Double-stranded RNA binding	4	5.19	–4.01	–1.37
GO: 0046777	GO biological processes	Protein autophosphorylation	6	7.79	–3.97	–1.36
GO: 1905476	GO biological processes	Negative regulation of protein localization to membrane	3	3.90	–3.93	–1.33
GO: 0035456	GO biological processes	Response to interferon-beta	3	3.90	–3.88	–1.31
GO: 0002683	GO biological processes	Negative regulation of immune system process	8	10.39	–3.82	–1.27
GO: 006302	GO biological processes	Double-strand break repair	6	7.79	–3.74	–1.21
GO: 0030155	GO biological processes	Regulation of cell adhesion	9	11.69	–3.35	–0.90
GO: 0051603	GO biological processes	Proteolysis involved in cellular protein catabolic process	9	11.69	–3.29	–0.87
GO: 0042803	GO Molecular Functions	Protein homodimerzation activity	8	10.39	–2.98	–0.62
GO: 0046579	GO biological processes	Positive regulation of RAS protein signal transduction	3	3.90	–2.87	–0.54
GO: 0002479	GO biological processes	Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	3	3.90	–2.75	–0.46
GO: 2001252	GO biological processes	Positive regulation of chromosome organization	4	5.19	–2.54	–0.34

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Table 4. KEGG function enrichment analysis of IRF family members and neighbor genes in COAD (Metascape).

Items	Category	Description	Count	%	Log10(P)	Log10(q)
hsa05168	KEGG pathway	Herpes simplex inflection	12	15.58	–12.17	–9.48
hsa04622	KEGG pathway	RIG-I-like receptor signaling pathway	5	6.49	–5.53	–3.61
hsa05203	KEGG pathway	Viral cacinogenesis	6	7.79	–4.35	–2.56
hsa05202	KEGG pathway	Transcriptional misregulation in cancer	4	5.19	–2.58	–1.23
hsa04142	KEGG pathway	Lysosome	3	3.90	–2.14	–0.87
hsa04114	KEGG pathway	Oocyte meiosis	3	3.90	–2.14	–0.87
hsa05166	KEGG pathway	HTLV-I infection	4	5.19	–2.04	–0.81

Table 5. Kinase target networks of the IRF family in COAD.

IRFs	Enriched kinase target	Description	Leading edge num	P value
IRF1	Kinase_LCK	LCK proto-oncogene, Src family tyrosine Kinase	25	0
	Kinase_LYN	LYN proto-oncogene, Src family tyrosine Kinase	26	0
IRF2	Kinase_LCK	LCK proto-oncogene, Src family tyrosine Kinase	24	0
	Kinase_SYK	spleen associated tyrosine Kinase	18	0
IRF3	Kinase_IKBKB	Inhibitor of nuclear factor Kappa B Kinase subunit bata	6	0
	Kinase_PLK3	polo like Kinase 3	5	0
IRF4	Kinase_LCK	LCK proto-oncogene, Src family tyrosine Kinase	22	0
	Kinase_LYN	LYN proto-oncogene, Src family tyrosine Kinase	22	0
IRF5	Kinase_SYK	Spleen associated tyrosine Kinase	22	0
	Kinase_FYN	FYN proto-oncogene, Src family tyrosine Kinase	31	0
IRF6	Kinase_ATR	ATR serine/threonine Kinase	30	0
	Kinase_STK4	serine/threonine Kinase	5	0
IRF7	Kinase_LYN	LYN proto-oncogene, Src family tyrosine Kinase	20	0
	Kinase_LCK	LCK proto-oncogene, Src family tyrosine Kinase	28	0
IRF8	Kinase_LYN	LYN proto-oncogene, Src family tyrosine Kinase	27	0
	Kinase_LCK	LCK proto-oncogene, Src family tyrosine Kinase	21	0
IRF9	Kinase_LYN	LYN proto-oncogene, Src family tyrosine Kinase	21	0
	Kinase_LCK	LCK proto-oncogene, Src family tyrosine Kinase	25	0

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Table 6. miRNA target networks of the IRF family in COAD.

IRFs	Enriched miRNA target	Leading edge num	P value
IRF1	GTATTAT, MIR-369-3p	42	0
	CTTGTA, MIR-381	44	0
IRF2	GTGTTGA, MIR-505	25	0
	GCACCTT, MIR-18A, MIR-18B	44	0
IRF3	GCACTTT, MIR-17-5P, MIR-20A, MIR-106A, MIR-106BMIR-20B, MIR519D	229	0
	TGTATGA, MIR-485-3P	63	0
IRF4	CAGTATT, MIR-200B, MIR-200C, MIR-429	166	0.042
	ACTGTGA, MIR-27A, MIR-27B	152	0.060
IRF5	ATGCTGC, MIR-103, MIR-107	67	0.002
	TCCAGAG, MIR-518C	46	0.002
IRF6	GACAATC, MIR-219	53	0
	GCACTTT, MIR-17-5P, MIR-20A, MIR-106A, MIR-106B, MIR-20B, MIR-519D	203	0
IRF7	CCAGGGG, MIR-331	21	0
	CAGTCAC, MIR-134	17	0
IRF8	CACTTTG, MIR-520G, MIR-520H	43	0
	AAGCAAT, MIR-137	57	0
IRF9	CCAGGTT, MIR-490	21	0
	TATCTGG, MIR-488	16	0

in oncogenesis. The common kinase targets of IRF1/4/7/8/9 are LCK and LYN. LCK is important in tumorigenesis because the expression level of LCK is elevated in colorectal cancer cells, suggesting that LCK has a cancer-promoting role in CRC [27,28]. As the common kinase target of IRF5/6, SYK has been found to be a cancer suppressor in colorectal cancer [29]. PLK3, which is the kinase target of IRF3, contributes to regulation of cell proliferation and apoptosis, and studies showed that PLK3 was overexpressed in breast and ovarian cancer, but there is little evidence of the role of PLK3 in COAD [30]. The miRNA targets of IRF3 are upregulated in human colon cancer. For example, MIR-17-5p and MIR-20a are both highly expressed in colon cancer tissues, and the MIR-106 family was also found to be closely involved in the initiation and development of colorectal cancer [31,32]. On the contrary, the miRNA target of IRF7, MIR-331, was reported to be a tumor suppressor in colorectal carcinoma [33].

This study is the first to systematically demonstrate the association between the IRF family and COAD; however, it has some limitations. Because all the information was obtained from public databases, there are many influencing factors, such as the size and location of the tumor, and the medical parameters are incomplete, which could influence the results. Since the IRFs are correlated with cell cycle control and apoptosis, carcinogenesis, and immune responses, further studies are needed to elucidate the molecular mechanism involved.

Conclusions

Overall, these results indicate that IRF3 and IRF7 are prognostic biomarkers in COAD, and IRF family members are associated with immune cell infiltration and gene regulation networks. These results add to the growing evidence of the significant role of IRFs in COAD, and contribute to developing the prognostic value of IRFs in COAD.

Conflict of interests

None.

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