Research Square

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Interferon Regulatory Factor 4 (IRF4) is a Prognostic Marker and Related to Immune Infiltration in Breast and Colorectal Cancers

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Keywords: IRF4, breast cancer, colon cancer, immune infiltration

Posted Date: July 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-39448/v1

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Abstract

Background: Interferon regulatory factor 4 (IRF4) is a transcription factor that involves in immune cells differentiation. However, it is not clear the relationship between IRF4 and tumor prognosis and immune infiltration.

Methods: IRF4 expression levels in different cancers and corresponding normal tissues were analyzed by Oncomine database and Tumor Immune Estimation Resource (TIMER). The prognosis value of IRF4 was assessed by PrognoScan and Kaplan-Meier plotter. The correlation between IRF4 and tumor-infiltrating immune cells and immune cells markers was performed by TIMER and Gene Expression Profiling Interactive Analysis (GEPIA). In addition, we explored the genes regulated by IRF4 in Gene Transcription Regulation Database (GTRD) and then put the above genes in Enrich online tool for Gene Ontology (GO) and pathway enrichment analysis.

Results: Decreased expression levels of IRF4 were observed in breast and colorectal cancers. Survival analysis shown that high level of IRF4 was associated with better prognostic outcome in breast and colorectal cancer patients. IRF4 expression was positively correlated with infiltrating levels of B cells, CD8+ T cells, T cells (general), dendritic cells (DCs), Th1, T cell exhaustion and monocytes, and immune cells markers. Beside, functional enrichment analysis of the potential genes regulated by IRF4 indicated that IRF4 may be involved in many important biological processes including immune regulation by regulating various genes.

Conclusions: High expression of IRF4 shown better prognostic outcome for breast and colorectal cancers. IRF4 was associated with immune infiltration in breast and colorectal cancers. Therefore, IRF4 maybe serve as a potential prognostic biomarker in breast and colorectal cancers with immune infiltration.

Background

The tumor microenvironment (TME) is contexture of cancer, in which immune and non-immune cells are found, in addition with factors that have different functions[1]. Evidence has shown that TME, especially immune microenvironment is believed to involve in cancer progression and therapy[2]. With the application of immuno-checkpoint inhibitors such as PD-1/PDL-1, immunotherapy has become a hot direction of tumor intervention. Immune cells infiltration is a characteristic of tumor which relates to tumor progress and outcome as well as chemotherapy and immunotherapy[3]. For example, previous trial study demonstrated that high levels of tumour-infiltrating lymphocytes (TILs) shown benefit from anthracycline chemotherapy in patients with HER2 + breast cancer patients[4]. So, it's necessary to reveal the immunophenotypes of tumor-immune interactions and identify novel therapeutic targets related to cancer immune therapy.

IRF4, a transcription factor member of IRF family, is lymphocyte specific and associated with regulation immune response. It exerts a role for regulating immune cells differentiation and function, such as, helper T (Th) cells differentiation in germinal center[5], B cells class switch and plasma cell maturation[6, 7]. Dysregulation of IRF4 is related to many lymphoid malignancies and autoimmune diseases[8–10]. Recently, study found that IRF4 specifically expressed in CD4 + effector Tregs of non-small cell lung cancer with immune suppression activity[11], suggesting that IRF4 may play a role in regulating tumor immune microenvironment. In addition, IRF4 shown a prognostic role in node-negative breast cancer and IRF4 methylation was identified in colorectal cancer[12, 13]. Therefore, it's needed to evaluate whether IRF4 regulate tumor-immune microenvironment and effect tumor prognosis.

In this study, we comprehensively analyzed the relationship between IRF4 expression and cancer prognosis and the potential role in tumor immune infiltration. Our study help to elucidate the role of IRF4 in cancer prognosis and tumor immune infiltration in breast and colorectal cancers.

Methods

The expression levels of IRF4 in cancers vs normal tissues

Oncomine database (http://www.oncomine.org) was selected to analyze the expression levels of IRF4 in most cancer types. The threshold of Oncomine database was set as follows: P = 0.001, fold change = 2, and gene ranking = Top 10%. Beside, TIMER database (https://cistrome.shinyapps.io/timer/) providing 32 cancer types data from The Cancer Genome Atlas (TCGA)[14], was used to further analyze the expression levels of IRF4 between tumor and adjacent normal tissue.

Survival analysis of IFR4 in different cancers

The PrognoScan (http://www.prognoscan.org/), a database for meta-analysis of the prognostic value of genes, can be used to search the association between target gene and patient prognosis such as overall survival (OS), distant metastasis free survival (DMFS) and disease free survival (DFS)[15]. The Kaplan-Meier plotter database (http://kmplot.com/analysis/) provide survival assessment of 21 cancer types, including source from Gene Expression Omnibus (GEO), European Genome-phenome Archive (EGA) and TCGA, with the purpose to discovery and validate survival biomarkers[16].

These two database were used to evaluate the relationship of the expression of IRF4 and cancer survival. Cox P/logrank P□0.05 was set as the thresholds.

Relationship between IRF4 and immune infiltration

TIMER database is a comprehensive resource for analysis immune infiltration across 32 cancer types. The correlation between IRF4 expression and six immune cells infiltration was estimated, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells (DCs). Moreover, the correlation between IRF4 and gene markers of 16 immune cells was investigated, including markers of B cells, CD8+ T cells, T cells (general), M1 and M2 macrophages, neutrophils, DCs, Th1/2/17 cells, Tfh cells, Tregs, T cell exhaustion, NKs, monocytes and TAM. Another database, GEPIA (http://gepia.cance-pku.cn/index.html) which contains RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects[17], was used to further verified the significantly correlated gene markers in TIMER. The method for correlation analysis was according to Spearman correction. $P < 0.05$ was considered statistically significant. The strength of correlation analysis was set as follow: 0.00-0.19 as 'very weak', 0.20-0.39 as 'weak', 0.40-0.59 as 'moderate', 0.60-0.79 as 'strong', 0.80-1.0 as 'very strong'.

Prediction the potential target genes regulated by IRF4

GTRD (http://gtrd.biouml.org) collected ChIP-seq and DNase-seq data from ENCODE and SRA with the purpose to identify transcription factor binding sites[18]. We used GTRD to predict the potential target genes regulated by IRF4 and then screened according to the following criteria: peak count ≥ 20, exp count ≥ 6, peak-caller count ≥ 4 and removing the duplicate and NA value of gene description.

Functional enrichment analysis for genes regulated by IRF4

Enrichr (http://amp.pharm.mssm.edu/Enrichr) is an online web tool for functional enrichment analysis[19]. The predicted genes from GTRD database were performed Gene Ontology (GO) and pathway enrichment analysis. Biological process

(BP), cellular component (CC) and molecular function (MF), were included in GO functional annotation. $P < 0.05$ was considered statistically significant.

Results

Analysis the IRF4 expression level in cancers

The expression levels of IRF4 in tumors and normal tissues of different cancers were analyzed from Ocomine database. As shown in Fig. 1a, the IRF4 expression was higher in brain and central nervous system (CNS) cancer, kindey cancer, lymphoma, melanoma and myeloma cancers, while lower expression of IRF4 was presented in breast, colorectal, gastric, leukemia and sarcoma cancers, compared to the corresponding normal tissues. To further verify the expression levels of IRF4 in tumors, the RNA-seq data of TCGA was used from TIMER database. The expression levels of IRF4 between tumor and adjacent normal tissues were shown in Fig. 1b. IRF4 expression was significantly higher in HNSC (head and neck cancer), KIRC (kidney renal clear cell carcinoma), LUAD (lung adenocarcinoma), LUSC (lung adenocarcinoma). However, IRF4 expression was significantly lower in BLCA (bladder urothelial carcinoma), BRCA (breast invasive carcinoma), CODA (colon adenocarcinoma), KICH (kidney chromophobe), LIHC (liver hepatocellular carcinoma) and READ (rectum adenocarcinoma).

Prognostic value of IRF4 in Cancers

The prognostic value of IRF4 in cancer patients was assessed by PrognoScan database. IRF4 expression influences the prognosis of 6 type cancers patients (Table S1), especially, breast, colorectal and lung cancers (Fig. 2). GSE2034, GSE4922-GPL96 and GSE2990 breast cancer cohorts shown that high expression of IRF4 was related with better DMFS, DFS and relapse free survival (RFS). Similarly, high expression of IRF4 associated with a better prognosis were also detected in colorectal (GSE17536 and GSE17537) and lung cancer patients (jacob-00182-HLM, GSE31210 and GSE8894).

Kaplan-Meier plotter database was used to evaluate the IRF4 prognostic value based on Affymetrix microarrays. It's shown that higher expression of IRF4 was related with better prognosis in breast cancer (OS, HR = 0.69, 95%CI = 0.55–0.86, logrank P = 0.00072; RFS, HR = 0.68, 95%CI = 0.61–0.76, logrank P = 2.9e-12). Base the above results, it's suggested that increased expression of IRF4 was associated with better prognosis in breast and colorectal cancer.

Correlation analysis between IRF4 and clinical characteristic of breast cancer patient

The relationship of IRF4 and clinical characteristic of breast cancer patient including intrinsic subtype, grade and TP53 status was investigated by Kaplan Meier plotter database. Expression of IRF4 shown a better OS and RFS in breast cancer patient (Table 1). High expression of IRF4 was correlated with better OS in basal subtype (n = 241, HR = 0.47, 95%CI = 0.28–0.78, P = 0.0027) and grade 3 (n = 503, HR = 0.55, 95%CI = 0.39–1.27, P = 0.00042). Interestingly, IRF4 significantly influences RFS of all subtypes and grade 2 and 3 of breast cancer. Therefore, IRF4 can impact the prognosis of breast cancer patient with subtypes and grades.

Table 1
Correlation of IRF4 and clinical prognosis in breast cancer with clinical characteristic by Kaplan-Meier plotter.

Clinical characteristic	OS(n = 1402)			RFS(n = 3955)		
	n	HR	P value	n	HR	P value
subtype						
basal	241	0.47(0.28–0.78)	**0.0027**	618	0.47(0.36–0.62)	**1e-08**
Luminal A	611	0.73(0.51–1.04)	0.081	1933	0.73(0.62–0.87)	**0.00033**
Luminal B	433	0.84(0.58–1.22)	0.35	1149	0.58(0.48–0.7)	**2.5e-08**
HER2+	117	0.64(0.33–1.23)	0.18	251	0.49(0.33–0.74)	**0.00041**
Grade						
1	161	0.76(0.3–1.88)	0.55	345	1.18(0.7–1.98)	0.53
2	387	0.84(0.55–1.3)	0.43	901	0.76(0.6–0.97)	**0.027**
3	503	0.55(0.39–0.77)	**0.00042**	903	0.75(0.6–0.93)	**0.01**

HR Hazard ratio; OS overall survive; RFS relapse free survival; The bold values mean statistically significant.

Immune infiltration correlation analysis between IRF4 and BRCA/COAD

We assessed the correlations of IRF4 expression with immune infiltration levels in BRCA and colon adenocarcinoma (COAD) by TIMER database (Fig. 3). IRF4 is relevant with higher tumor purity in BRCA and its subtypes as well as COAD. IRF4 expression has significant correlations with infiltrating levels of B cell (r = 0.542, P = 1.28e − 75), CD8 + T cell (r = 0.517, P = 6.39e − 68), CD4 + T cell (r = 0.582, P = 1.97e − 88), neutrophils (r = 0.56, P = 1.08e − 79), DCs (r = 0.624, P = 7.86e − 104) in BRCA. In addition, there were positive correlation with infiltration levels of B cell (r = 0.435, P = 4.66e − 20), CD8 + T cell (r = 0.264, P = 6.36e − 08), CD4 + T cell (r = 0.502, P = 4.78e − 27), macrophages (r = 0.327, P = 1.13e − 11), neutrophils (r = 0.517, P = 9.27e − 29), DCs (r = 0.552, P = 1.97e − 33) in COAD. Therefore, these results suggest that IRF4 may play an important role in immune infiltration in BRCA and COAD.

Correlation analysis between IRF4 and immune cells markers in BRCA and COAD

Given the positive correlation of immune infiltration with BRCA and COAD, the correlation between IRF4 and immune cells markers was analyzed by TIMER and GEPIA database. Immune gene markers of B cells, CD8 + T cells, T cells (general), M1 and M2 macrophages, neutrophils, DCs, Th1/2/17 cells, Tfh cells, Tregs, T cell exhaustion, NKs, monocytes and TAM in BRCA and COAD were assessed (Table 2). After adjusted by purity, the results revealed the IRF4 was significantly correlated with most immune marker sets of various immune cells. We found that the expression levels of most marker of B cells, CD8 + T cells, T cells (general), DCs, Th1, T cell exhaustion and monocytes have significant correlations with IRF4 expression in BRCA and COAD. The significant correlations between IRF4 and B cells (CD19, CD79A), CD8 + T cells (CD8A), T cell (general) (CD3D, CD3E, CD2), and monocytes (CD86, CD115) are presented in Fig. 4A-E. In addition, we also analyzed the above immune markers of B cells, CD8 + T cells, T cells (general), and monocytes by the GEPIA database (Table 3) and the most results were consistent with the TIMER database, except CD115 in COAD.

Table 2
Correlation analysis between IRF4 and immune cells markers by TIMER.

Description	Gene markers	BRCA				COAD			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
B cell	CD19	0.807	4.47E-253	0.741	5.80–178	0.746	1.13E-82	0.71	1.79E-63
	CD79A	0.981	0	0.85	3.03E-278	0.87	7.27E-143	0.84	2.10E-109
CD8 + T cell	CD8A	0.797	1.17E-242	0.733	6.70E-168	0.63	5.61E-52	0.568	5.46E-36
	CD8B	0.726	8.64E-181	0.635	8.80E-114	0.35	5.57E-15	0.279	1.02E-09
T cell(general)	CD3D	0.816	4.30E-263	0.743	7.19E-175	0.716	4.78E-73	0.665	4.18E-53
	CD3E	0.828	1.80E-278	0.76	1.64E-187	0.765	2.38E-89	0.72	3.54E-60
	CD2	0.835	1.74E-286	0.774	6.03E-199	0.765	3.73E-89	0.72	3.43E-66
M1 Macrophage	INOS	0.039	0.192	-0.02	0.954	0.288	3.47E-10	0.263	7.16E-08
	IRF5	0.35	5.44E-33	0.285	4.86E-20	0.288	3.17E-10	0.278	1.22E-08
	COX2	0.369	9.28E-37	0.223	1.12E-12	0.295	1.16E-10	0.24	9.80E-07
M2 Macrophage	CD163	0.502	2.18E-71	0.427	2.98E-45	0.583	4.57E-43	0.514	8.71E-29
	VSIG4	0.34	3.51E-31	0.219	3.00E-12	0.522	2.53E-33	0.44	1.25E-20
	MS4A4A	0.574	2.40E-97	0.468	2.46E-55	0.583	4.31E-43	0.51	3.31E-28
Neutrophils	CD66b	0.039	0.199	0.064	0.043	0.083	0.0778	0.14	0.00465
	CD11b	0.422	9.10E-49	0.31	1.42E-23	0.514	2.59E-32	0.438	1.94E-20
	CCR7	0.774	4.25E-220	0.682	5.01E-137	0.758	1.21E-86	0.714	1.44E-64
Dendritic cell	HLA-DPB1	0.648	5.84E-132	0.509	1.66E-66	0.638	1.14E-59	0.566	1.03E-35
	HLA-DQB1	0.558	3.81E-91	0.45	1.26E-50	0.475	3.84E-27	0.407	1.36E-17

BRCA breast cancer; COAD colon adenocarcinoma; Cor R value of Spearman’s correlation; None correlation without adjustment; Purity correlation adjusted by purity; Th T helper cell; Tfh Follicular helper T cell; Treg regulatory T cell; TAM tumor-associated macrophage.

Description	Gene markers	BRCA				COAD			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
	HLA-DRA	0.696	2.08E-160	0.599	1.04E-97	0.612	1.84E-48	0.545	9.26E-33
	HLA-DPA1	0.655	1.21E-135	0.542	3.90E-77	0.648	5.48E-56	0.586	8.59E-39
	BDCA-1(CD1C)	0.586	1.43E-102	0.421	4.69E-44	0.562	1.87E-39	0.499	5.30−27
	BDCA-4(NRP1)	0.246	1.22E-16	0.087	0.0058	0.457	5.66E-25	0.359	8.25E-14
	CD11c(ITGAX)	0.578	6.31E-99	0.473	1.18E-56	0.582	7.85E-43	0.502	2.98E-27
Th1	T-bet(TBX21)	0.823	1.81E-272	0.759	2.25E-187	0.659	2.27E-58	0.615	1.37E-43
	STAT4	0.777	7.35E-223	0.684	8.08E-138	0.669	1.17E-60	0.615	1.11E-43
	STAT1	0.516	6.15E-76	0.509	9.45E-67	0.474	4.54E-27	0.418	1.32E-18
	INF-γ(IFNG)	0.703	1.90E-164	0.644	2.34E-117	0.458	3.86E-25	0.435	3.48E-20
	TNF-α(TNF)	0.377	2.10E-38	0.335	2.01E-27	0.526	5.68E-34	0.478	1.36E-24
Th2	GATA3	-0.341	2.00E-31	-0.244	6.24E-15	0.592	1.06E-44	0.537	1.17E-31
	STAT6	0.1	0.00087	0.048	1.29E-01	0.217	2.70E-06	0.263	7.64E-08
	STAT5A	0.334	3.77E-30	0.194	6.90E-10	0.381	2.68E-27	0.351	3.37E-12
	IL13	0.319	2.15E-27	0.269	6.34E-18	0.304	2.97E-11	0.25	3.52E-07
Tfh	BCL6	0.106	4.11E-04	0.064	0.0425	0.406	1.43E-19	0.323	2.56E-11
	IL21	0.519	9.43E-77	0.472	2.78E-56	0.358	2.90E-15	0.358	1.00E-13
Th17	STAT3	0.11	0.000246	0.084	0.00841	0.459	3.15E-25	0.419	1.02E-18
	IL17A	0.309	8.67E-26	0.234	8.47E-15	0.33	3.99E-13	0.364	3.59E-14
Treg	FOXP3	0.721	7.68E-177	0.66	1.71E-125	0.68	2.26E-63	0.621	1.32E-44

BRCA breast cancer; COAD colon adenocarcinoma; Cor R value of Spearman's correlation; None correlation without adjustment; Purity correlation adjusted by purity; Th T helper cell; Tfh Follicular helper T cell; Treg regulatory T cell; TAM tumor-associated macrophage.

Description	Gene markers	BRCA				COAD			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
	CCR8	0.639	3.05E-127	0.602	3.98E-99	0.6	4.50E-46	0.554	4.26E-34
	STAT5B	0.145	1.36E-06	0.086	0.00648	0.237	2.92E-07	0.236	1.44E-06
	TGFβ(TGFB1)	0.298	4.78E-24	0.108	0.000624	0.517	1.04E-32	0.413	4.01E-18
T cell exhaustion	PD-1(PDCD1)	0.761	1.63E-208	0.682	9.21E-137	0.627	2.12E-51	0.574	6.10E-37
	CTLA4	0.77	9.32E-217	0.715	1.24E-156	0.634	8.56E + 53	0.584	1.62E-38
	LAG3	0.597	2.32E-107	0.549	1.77E-79	0.616	2.90E-49	0.559	9.04E-35
	TIM-3(HAVCR2)	0.514	3.63E-75	0.417	5.01E-43	0.572	3.28E-41	0.495	1.91E-26
	GZMB	0.735	8.16E-188	0.661	5.98E-126	0.24	2.03E-07	0.216	1.09E-05
Natural killer cell	KIR2DL1	0.442	1.08E-53	0.384	3.44E-36	0.257	2.51E-08	0.19	0.000115
	KIR2DL3	0.471	1.06E-61	0.404	2.95E-40	0.25	6.18E-08	0.175	3.79E-04
	KIR2DL4	0.566	2.85E-94	0.521	3.56E-70	0.383	1.84E-17	0.309	2.02E-10
	KIR3DL1	0.511	2.38E-74	0.441	1.36E-48	0.289	2.77E-10	0.195	7.86E-05
	KIR3DL2	0.568	5.20E-95	0.492	7.94E-62	0.393	2.11E-06	0.324	2.39E-11
	KIR3DL3	0.304	5.05E-25	0.266	1.48E-17	0.219	2.31E-06	0.195	7.63E-05
	KIR2DS4	0.408	2.56E-45	0.331	6.72E-27	0.255	3.14E-08	0.231	2.69E-06
Monocyte	CD86	0.611	1.68E-113	0.526	7.84E-72	0.614	9.05E-49	0.536	1.35E-31
	CD115(CSF1R)	0.505	2.20E-72	0.363	2.08E-32	0.654	3.09E-57	0.602	1.96E-41
TAM	CCL2	0.54	3.63E-84	0.433	1.17E-46	0.48	1.00E-27	0.408	1.07E-17
	CD68	0.511	4.34E-74	0.417	3.74E-43	0.466	4.09E-26	0.401	4.14E-17

BRCA breast cancer; COAD colon adenocarcinoma; Cor R value of Spearman's correlation; None correlation without adjustment; Purity correlation adjusted by purity; Th T helper cell; Tfh Follicular helper T cell; Treg regulatory T cell; TAM tumor-associated macrophage.

Description	Gene markers	BRCA				COAD			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
	IL10	0.566	5.16E-94	0.474	7.26E-57	0.581	1.02E-42	0.545	9.37E-33

BRCA breast cancer; COAD colon adenocarcinoma; Cor R value of Spearman's correlation; None correlation without adjustment; Purity correlation adjusted by purity; Th T helper cell; Tfh Follicular helper T cell; Treg regulatory T cell; TAM tumor-associated macrophage.

Table 3
Correlation analysis between IRF4 and immune cells markers by GEPIA.

Description	Gene markers	BRCA				COAD			
		Tumor		Normal		Tumor		Normal	
		R	P	R	P	R	P	R	P
B cell	CD19	0.78	2.20E-227	0.34	0.00021	0.72	3.00E-45	0.39	0.12
	CD79A	0.82	3.70E-100	0.41	9.30E-06	0.87	3.80E-86	0.76	1.50E-06
CD8 + T cell	CD8A	0.75	5.60E-74	0.27	0.0042	0.68	1.40E-38	0.18	0.26
T cell	CD3D	0.77	6.50E-80	0.25	0.0068	0.76	3.30E-53	0.27	0.089
	CD3E	0.87	1.20E-122	0.31	8.00E-04	0.82	1.50E-69	0.38	0.015
	CD2	0.86	3.90E-121	0.39	2.40E-05	0.83	7.40E-70	0.38	0.012
Monocyte	CD86	0.61	1.60E-109	0.15	0.11	0.71	1.90E-43	0.41	0.0075
	CD115(CFS1R)	0.5	1.00E-70	0.26	0.0053	0.75	8.50E-51	0.62	1.80E-05

BRCA breast cancer; COAD colon adenocarcinoma; R value of Spearman's correlation; Tumor correlation analysis in tumor tissue of TCGA; Normal correlation analysis in normal tissue of TCGA.

GO function and pathway enrichment analysis

199 potential target genes were finally obtained from GTRD according to the selection criteria. GO functional and Reactome pathway enrichment analysis were performed on IRF4 and the above target genes. The top 10 items of GO and Reactome pathway enrichment were presented in bar charts (Fig. 5). The positively enriched GO items contained the top 10 positive BP, such as positive regulation of interleukin-2 biosynthetic process, definitive hematopoiesis, regulation of antigen receptor-mediated signaling pathway in BP category; interleukin-6 receptor binding, oxidoreductase activity, oxidizing metal ions, NAD or NADP as acceptor in MF category. The Reactome pathway was positively enriched in beta-oxidation of very long chain fatty acids, cross-presentation of particulate exogenous antigens (phagosomes), MAPK1 (ERK2) activation and MAPK3 (ERK1) activation. These results suggest that IRF4 may be involved in many important biological processes by regulating various genes.

Discussion

IRF4 belongs to the IRF family which were reported relate to infection, protective immunity, and primary immunodeficiencies[20]. In addition, IRF4 plays a role in regulation immune cell development and differentiation.

However, the relationship between the expression level of IRF4 and cancer prognosis and immune cells infiltrate has not been fully reported. In our study, we firstly evaluated the expression levels in various cancer types and found that IRF4 had different expression levels in cancers. High expression levels was found in HNSC, KIRC, LUAD, LUSC and low expression levels was presented in BLCA, BRCA, COAD, KICH, LICH and READ, when compared with adjacent normal tissues. The potential role of IRF4 expression on cancer survival rate was performed via PrognoScan and Kaplan Meier plotter database. Data from PrognoScan shown that high expression levels of IRF4 shown better prognosis in bladder, breast, colorectal and lung cancer. However, higher levels of IRF4 were associated with poor prognosis in follicular lymphoma and ovarian cancer. Results from Kapan Meier plotter shown that high expression of IRF4 was related with better OS and RFS in breast. Therefore, we suggested that lower expression of IRF4 may be a risk factor of breast and colorectal cancer. Besides, high expression of IRF4 demonstrated better prognosis of breast cancer in basal subtype and grade 3 with lower HR for better OS, and basal, luminal A subtype, and grade 2 to 3 with lower HR for better RFS.

Numerous studies emphasized that the crucial role of tumor infiltrating immune cells in cancer therapy and progress[21, 22]. The communication of infiltrating immune cells and cancer cells contributes to cancer heterogeneity and clinical outcome[23]. Given the role of IRF4 in immune regulation, the correlation of IRF4 between infiltration levels of immune cells in BRCA and COAD were assessed by TIMER database. Our results shown IRF4 had a strong correlation with BRCA (Cor = − 0.517, P = 4.76e − 69), compared with COAD (Cor = − 0.396, P = 9.40e − 17). Moreover, the immune infiltration levels in breast cancer subtypes had the strong relationship with expression of IRF4. There is a moderate positive correlations between IRF4 expression and infiltration levels of B cells, CD8 + T cells, CD4 + T cells, neutrophils, while strong positive correlation between IRF4 expression and in infiltration level of DCs in BRCA. In COAD, IRF4 expression demonstrated a moderate positive correlation between infiltration levels of B cells, CD4 + T cells, neutrophils and DCs. Next, we analyzed the correlations of IRF4 and gene makers of immune cells in BRAC and COAD. IRF4 had significantly positive correlation with gene makers of B cells, T cells, Th1, T cell exhaustion and monocyte both in BRCA and COAD, especially, gene markers of B cells (CD19, CD79A) and T cells (CD3D, CD3E, CD2). Additionally, there was strong correlation between IRF4 and CD8 + T cells markers (CD8A, CD8B) in BRCA, whereas, IRF4 exerted a role in regulating polarization of macrophages in COAD.

T cells infiltration is a favorable prognosis feature for cancer that's why limited T cell homing result in cancer immunotherapy failure[24, 25]. CD8 + T cells belong to general T cells, which were proved to show favorable clinical implication in various cancers, including colorectal and breast cancer when infiltrated in cancer tissues[26, 27]. Our results show a positive correlation between IFR4 and general T cells in BRCA and COAD, and strong recruitment of CD8 + T cells in BRCA. Generally, T cells in TME inevitably differentiate into exhausted T cells, so restoring exhausted T cells is a promising strategy for cancer immunotherapy[28]. In addition, some studies revealed that tumor-infiltrating B cells (TIL-B) were associated with better clinical survival for breast and colorectal cancer[29, 30]. These results indicate that IRF4 is capable of recruiting and regulating immune infiltrating cells in BRCA and COAD, and shows different immune mechanisms against different tumors.

To further investigate the functions of IRF4, we used the GTRD database to predict genes regulated by IRF4 and carried out function enrichment analysis for those 199 target genes and IRF4. Our results suggest that IRF4 may be involved in many important biological processes including immunity regulation.

Conclusions

In conclusion, high expression of IRF4 was positively associated with better prognosis in breast and colorectal cancer patients. Moreover, there is positively association between IRF4 and increased immune infiltration levels in B cells, T cells, Th1, T cell exhaustion and monocyte. So, IRF4 may serve as a prognosis biomarker in patients with breast and colorectal cancers and involve in immune cell infiltration.

Abbreviations

IRF4: Interferon regulatory factor; TIMER: Tumor Immune Estimation Resource; GEPIA: Gene Expression Profiling Interactive Analysis; GTRD: Gene Transcription Regulation Database; GO: Gene Ontology; DCs: Dendritic cells; TME: Tumor microenvironment; TILs: Tumour-infiltrating lymphocytes; TCGA: The Cancer Genome Atlas; OS: Overall survival; DMFS: Distant metastasis free survival; DFS: Disease free survival; GEO: Gene Expression Omnibus; EGA: European Genome-phenome Archive; BP: Biological process; CC: Cellular component; MF: Molecular function; HNSC: Head and neck cancer; KIRC: Kidney renal clear cell carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung adenocarcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; CODA: Colon adenocarcinoma; KICH: Kidney chromophobe; LICH: Liver hepatocellular carcinoma; READ: Rectum adenocarcinoma.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by Key Scientific and Technological Project of Meizhou People's Hospital, (Grant No.: MPHKSTP-20180101 to Dr. Zhixiong Zhong) and the Guangdong Provincial Key Laboratory of Precision Medicine and Clinical Translation Research of Hakka Population (Grant No.: 2018B030322003), the Science and Technology Program of Meizhou (Grant No.: 2019B0202001).

Authors' contributions

Zhixiong Zhong and Qingyan Huang designed the study. Qingyan Huang and Yuhong Gan collected data. Qingyan Huang, Heming Wu and Zhikang Yu analyzed the data. Qingyan Huang prepared the manuscript. Heming Wu and Qingyan Huang reviewed the manuscript. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

Acknowledgements

The author would like to thank other colleagues whom were not listed in the authorship of Center for Precision Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-sen University for their helpful comments on the manuscript.

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Figures

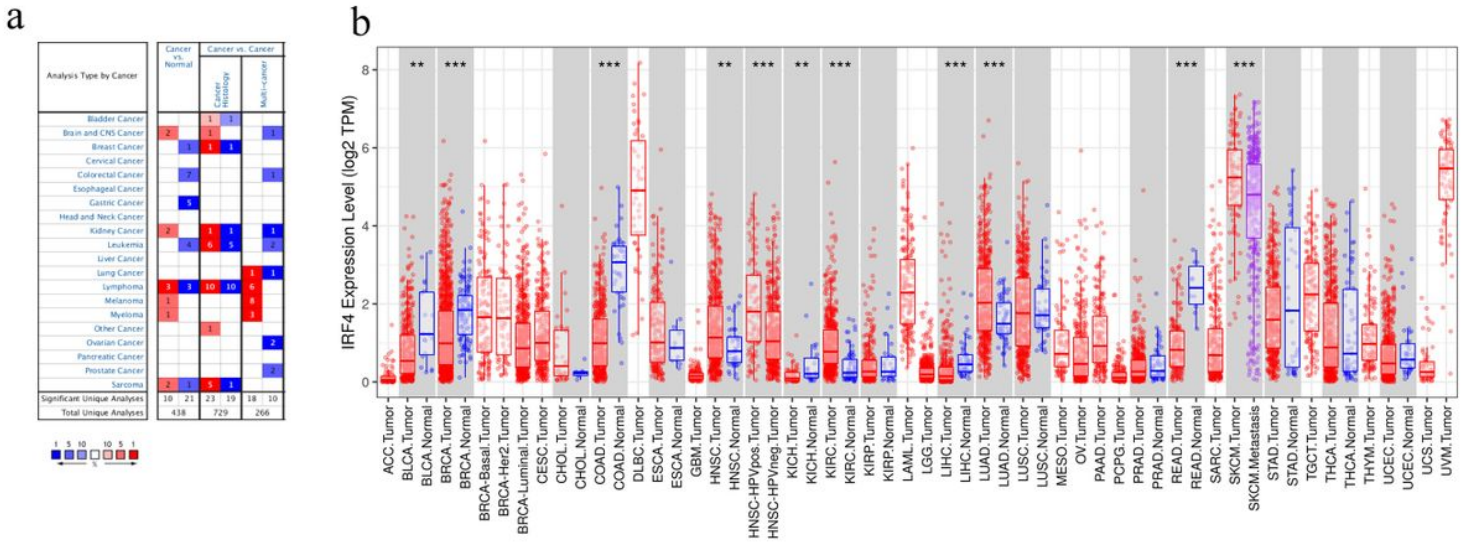

Figure 1

Expression levels of IRF4 in various cancer types. a IRF4 in data sets of different cancers compared with normal tissues in Oncomine database. b The IRF4 expression levels in different tumor types from TCGA database were determined by TIMER (*P < 0.05, **P <0.01, ***P < 0.001).

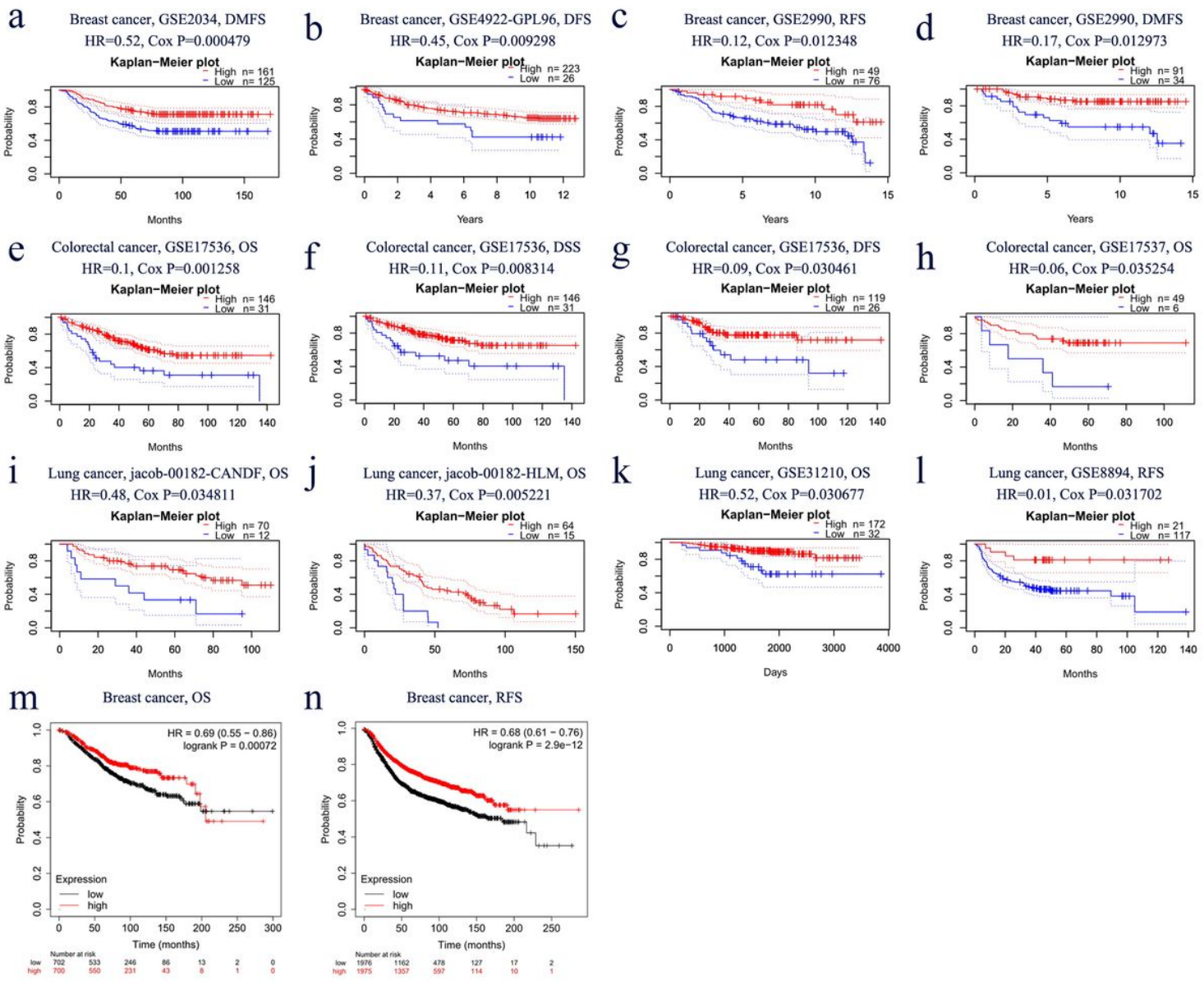

Figure 2

Kaplan-Meier survival curves comparing the high and low expression of IRF4 in different types of cancer in PrognoScan (a-l) and Kaplan-Meier plotter databases (m-n). a-d Survival curves of DMFS, DFS and RFS in three breast cancer cohorts [GSE2034 (n = 286), GSE4922-GPL96 (n=249) and GSE2990 (n=125)]. e-h Survival curves of OS, DSS and DFS in two colorectal cancer cohorts [GSE17536 (n=177) and GSE17537 (n=55)]. i-l Survival curves of OS and RFS in four lung cancer cohorts [jacob-00182-CANDF (n=82), jacob-00182-HLM (n=79), GSE31210(n=204) and GS8894 (n=138)]. m-n OS and RFS survival curves of breast cancer(n=1402, n=3951).

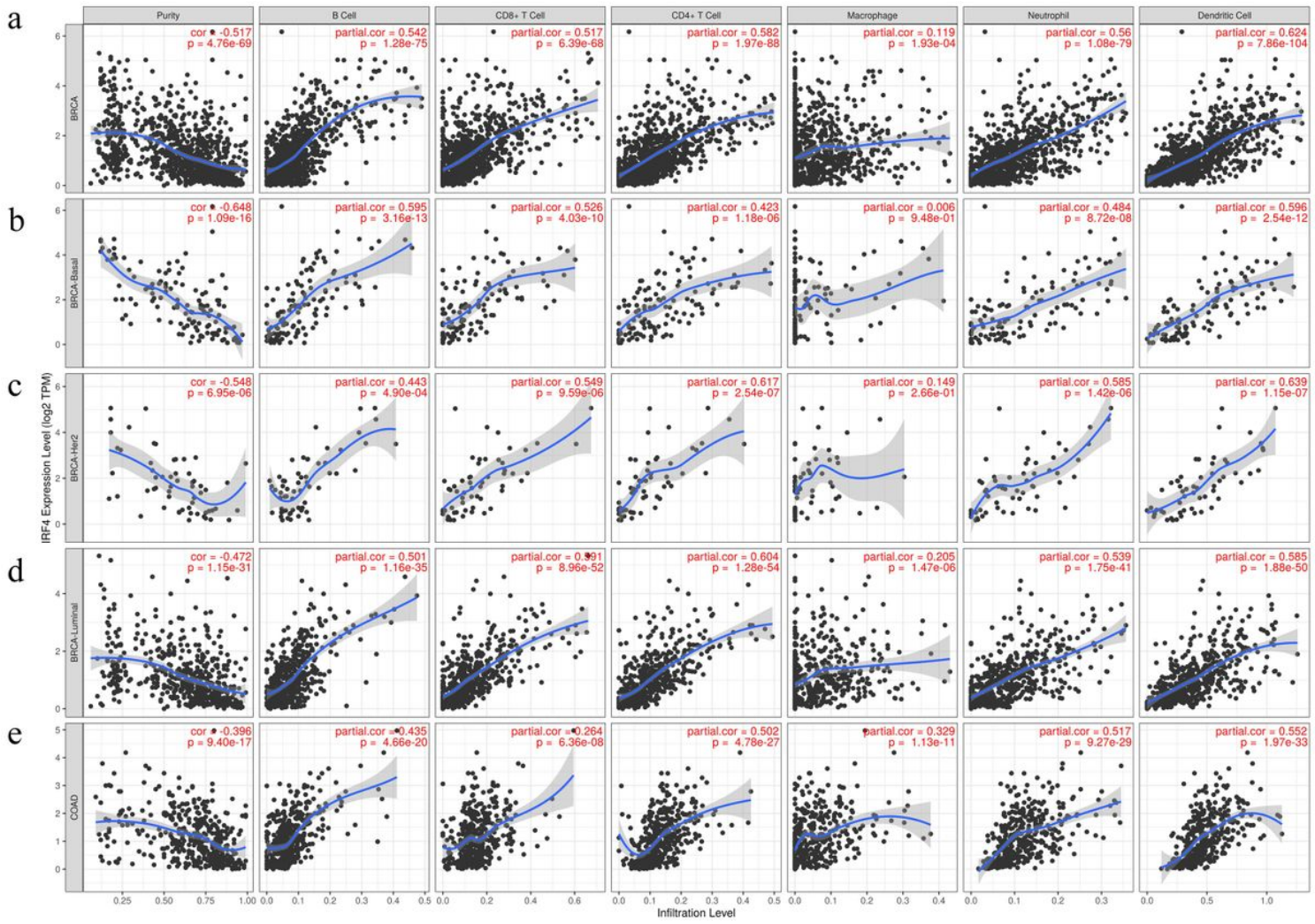

Figure 3

Correlation of IRF4 expression with immune infifiltration level in BRCA (BRCA-Basal, BRCA-Her2 and BRCA-Luminal) and COAD. a-d IRF4 expression was significantly negatively related to tumor purity and had significant positive correlations with the levels of infiltrating B cells, CD8+ T cells, CD4+ T cells, neutrophils, and DCs in BRCA (n = 1093) ,BRCA-Basal(n=139), BRCA-Her2(n=67) and BRCA-Luminal(n=611). e IRF4 expression was significantly negatively related to tumor purity and had significant positive correlations with the levels of infiltrating B cells, CD4+ T cells, macrophages, neutrophils, and DCs in COAD (n = 457).

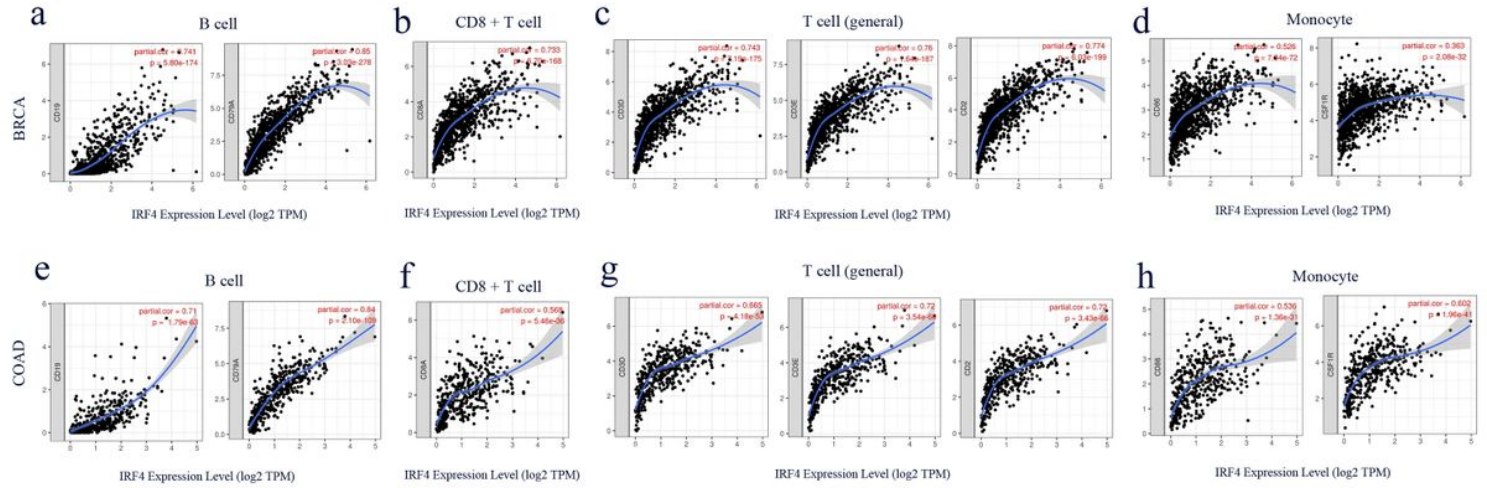

Figure 4

Correlation of IRF4 expression with markers of immune cells (B cells, CD8+ T cells, T cells, monocytes) in BRCA and COAD analyzed by TIMER. a-d Scatterplots of correlation between IRF4 expression and the expression of gene markers of B cells (a), CD8+ T cells (b), T cells (c) and monocytes (d) in BRCA (n=1093). e-h Scatterplots of correlation between IRF4 expression and the expression of gene markers of B cells (e), CD8+ T cells (f), T cells (g) and monocytes (h) in COAD (n=457).

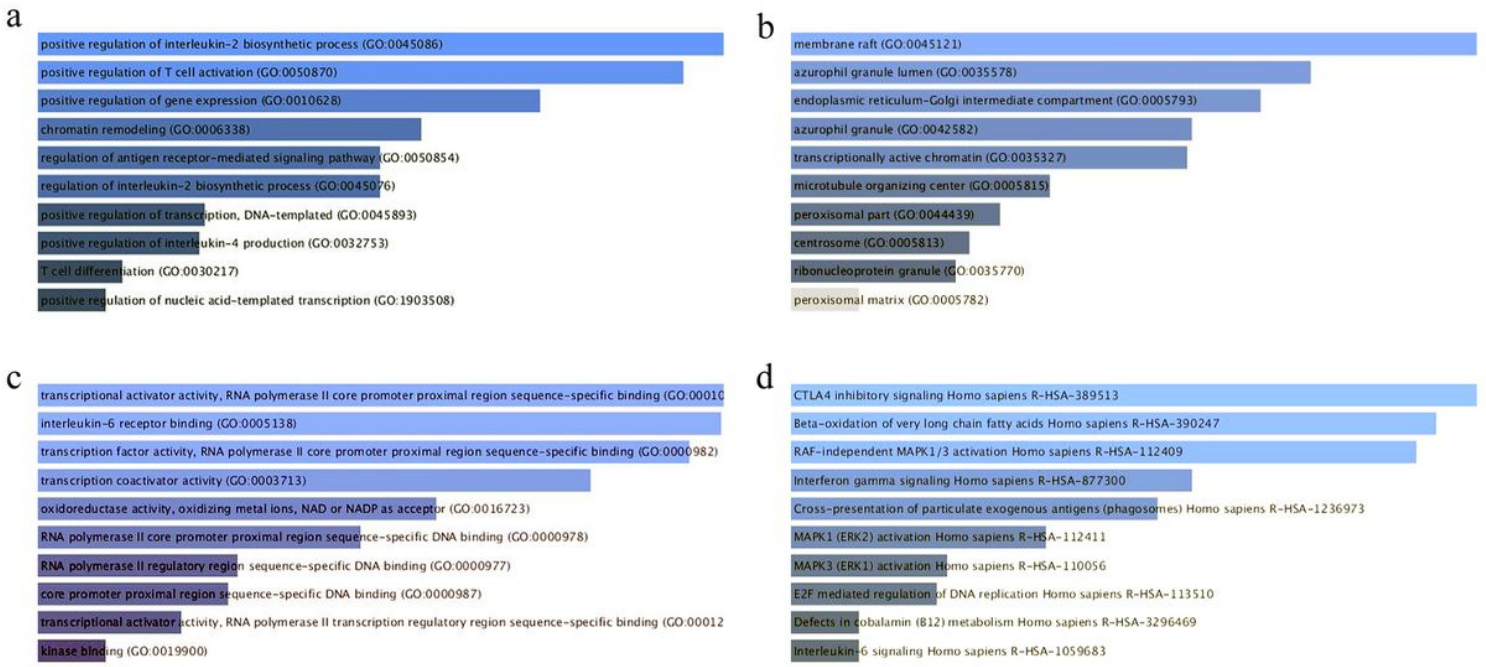

Figure 5

Go and pathway enrichment analysis of potential genes regulated by IRF4. a-d The top 10 enrichment results of biological process, cellular component, molecular function and Reactome pathway category.

Supplementary Files

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- TableS2.xlsx
- TableS1.docx