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Value of Ferritin Heavy Chain (FTH1) Expression in Diagnosis and Prognosis of Renal Cell Carcinoma

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCEF 1 Huimei Huang
BD 1 Yuyun Qiu
BD 1 Guilian Huang
CF 1 Xiaohui Zhou
AEG 2 Xiaoying Zhou
AEF 1 Wenqi Luo

1 Department of Pathology, Affiliated Tumor Hospital of Guangxi Medical University, Nanning, Guangxi, P.R. China
2 Life Science Institute, Guangxi Medical University, Nanning, Guangxi, P.R. China

Corresponding Author: Wenqi Luo, e-mail: wenqi_luo0429@163.com
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Background: Serum ferritin is a useful tumor marker for renal cell carcinoma (RCC). However, the expression of ferritin heavy chain (FTH1), the main subunit of ferritin, is unclear in primary RCC tissues. In this study, we investigated FTH1 mRNA expression and its diagnostic and prognostic value in RCC.

Material/Methods: The mRNA expression of FTH1 was analyzed using including Oncomine, Gene Expression Omnibus, and Cancer Genome Atlas datasets, while the protein level of FTH1 was analyzed using the Human Protein Atlas database. The associations between FTH1 and clinicopathologic characteristics and survival time and Cox multivariate survival analysis were analyzed using SPSS 22.0 software. A meta-analysis was performed to assess consistency of FTH1 expression. GO, KEGG, and PPI analyses were used to predict biological functions.

Results: According to TCGA data, overexpression of FTH1 was detected in 890 RCC tissues (15.2904±0.63157) compared to 129 normal kidney tissues (14.4502±0.51523, $p<0.001$). Among the clinicopathological characteristics evaluated, patients with increased pathologic T staging, lymph node metastasis, and distant metastasis were significantly associated with higher expression of FTH1. Elevated FTH1 mRNA levels were correlated with worse prognosis of RCC patients. Cox multivariate survival analysis indicated that age, stage, and M stage were predictors of poor prognosis in patients with RCC.

Conclusions: Our data suggest that FTH1 expression is an effective prognostic and diagnosis biomarker for RCC.

MeSH Keywords: **Apoferritins • Carcinoma, Renal Cell • Computational Biology • Diagnosis • Prognosis**

Abbreviations: **RCC** – renal cell carcinoma; **KIRC** – kidney clear cell carcinoma; **KICH** – kidney papillary cell carcinoma; **KIRP** – kidney chromophobe; **FTH1** – ferritin heavy chain; **VHL** – von Hippel-Lindau disease, **HIF-α** – hypoxia-inducible factor-alpha; **SMD** – standardized mean difference

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Background

In 2018, the new incidence of renal cell carcinoma (RCC) ranked sixth among all kinds of tumors, and the death rate ranked eighth [1]. It is estimated that 14 000 people died of RCC in 2012. The incidence of RCC varies geographically [2]. For example, the Czech Republic had the highest incidence in the world. The incidence in Nordic and Eastern Europe, North America, and Australia increased, but was relatively lower in Africa and Southeast Asia [3]. The reasons for the higher incidence in developed countries are not yet clear. Genomics, occupation, environmental exposures, and smoking are implicated [2].

RCC is divided into many different histological types. The clear cell type accounts for 70.90% of all RCC, followed by papilla (10–15%) and chromophobe RCCs (3–5%). Clear cell RCC is worse than papillary or chromophobe RCC, and is more likely to occur in late stage or metastasis [4,5]. In 90% of clear cell RCCs, tumors exhibit alteration the von Hippel-Lindau tumor suppressor (VHL) gene through genetic or epigenetic mechanisms [6,7]. The inactivation of VHL leads to a lower ubiquitination of hypoxia induced factor (HIF-α) and subsequently induces the expression of vascular endothelial growth factor (VEGF), both strictly linked to tumor angiogenesis [8]. VHL and VEGF has been validated as predictive and prognostic markers in RCC [9]. Further insights into the molecular biology of RCC could help find novel molecular biomarkers and potential targets for early diagnosis and precise treatment.

Elevated serum ferritin has been proved to play an important role in iron transport, angiogenesis, inflammation, immunity, signal transduction, and cancer in many human diseases [10]. Ferritin consists of 24 polypeptide subunits of heavy chain (FTH1) and light chain (FTL) [11,12]. In patients with RCC, serum ferritin concentration is significantly higher than in normal controls [13], and even associates with the presence of distant metastasis [14]. It is suggested that serum ferritin may be a useful tumor marker for renal cell carcinoma.

Ferritin consists of the heavy and light chains, encoded by FTH1 and FTL1 genes, respectively. FTH1 is differentially and abnormally expressed in tissues from multiple malignancies, including astrocytic brain tumors [15], prostate cancer [16], and breast cancer [17]. FTH1 has recently been considered a good prognostic protein for triple-negative breast cancer (TNBC) patients [17]. However, the expression of FTH1 is unclear in RCC.

The purpose of this study was to analyze the difference between FTH1 gene expression in RCC and normal renal tissues, and to explore the relationship between FTH1 gene expression and clinical characteristics and prognosis of RCC.

Material and Methods

The Cancer Genome Atlas (TCGA) database analysis

TCGA is a huge repository of high-throughput data of DNA, RNA, and protein in a variety of human cancers, which is helpful in comprehensive analysis of the expression of these components in various cancer types [18]. The data of FTH1 mRNA expression in primary RCC and normal control samples, as well as clinicopathological characteristics of patients, were obtained from TCGA database (*https://xena.ucsc.edu/*). SPSS 22.0 software was used to analyze the differential expression of FTH1 in RCC and the relationship between FTH1 level and clinicopathological parameters and Cox multivariate survival. The survival curve was analyzed using GraphPad software.

Oncomine database analysis

Oncomine databases are online collections of microarrays from various sources, often associated with cancer, and contain many "multiple arrays" (collections of microarrays analyzed in a single study) [19]. The relative expression level of "FTH1" gene was searched in the "kidney cancer" dataset in the analysis type of "cancer *vs.* normal analysis".

Selection of studies and microarrays in GEO datasets

The mRNA expression of FTH1 in RCC was investigated in GEO database, with search terms as follow: 1) "renal cancer", 2) "kidney OR renal AND cancer OR carcinoma OR tumor OR neoplasm* OR malignant*". Microarray was used to examine the expression of FTH1 in RCC tissues and normal tissues, including meta-analysis. The criteria of inclusion were: 1) have more than 6 samples, and 2) sampled FTH1 from human tissues.

Real-time reverse transcription polymerase chain reaction

The transcriptional level of FTH1 was confirmed in normal renal epithelial cell 293 and renal cancer cell 786-0, which were stored in our lab. cDNA of primary renal cell carcinoma tissues and matched adjacent tissues were obtained from Shanghai Outdo Biotech Co. (Shanghai, China; Cat no: MecDNA-HKidE030CS01). The relative expression levels of FTH1 were detected using the Power SYBR Green PCR Master Mix (Foster City, CA, USA, Applied Biosystem) in a QuantStudio 5 Real-Time PCR System (Foster City, CA, USA, Applied Biosystem). After the reactions were completed, the comparative threshold cycle (Ct) method was used to calculate the relative gene expression. The sequences of primers used were as follows:
FTH11-Forward, 5'-AAGCTGCAGAACCAACGAGG-3',
FTH1-Reverse, 5'-AGTCACACAAATGGGGGTCATT-3';
GAPDH1-Forward, 5'-AAGCTCACTGGCATGGCCTT-3',
GAPDH-Reverse, 5'-CTCTCTTCCTCTTGTGCTCTTG-3'.

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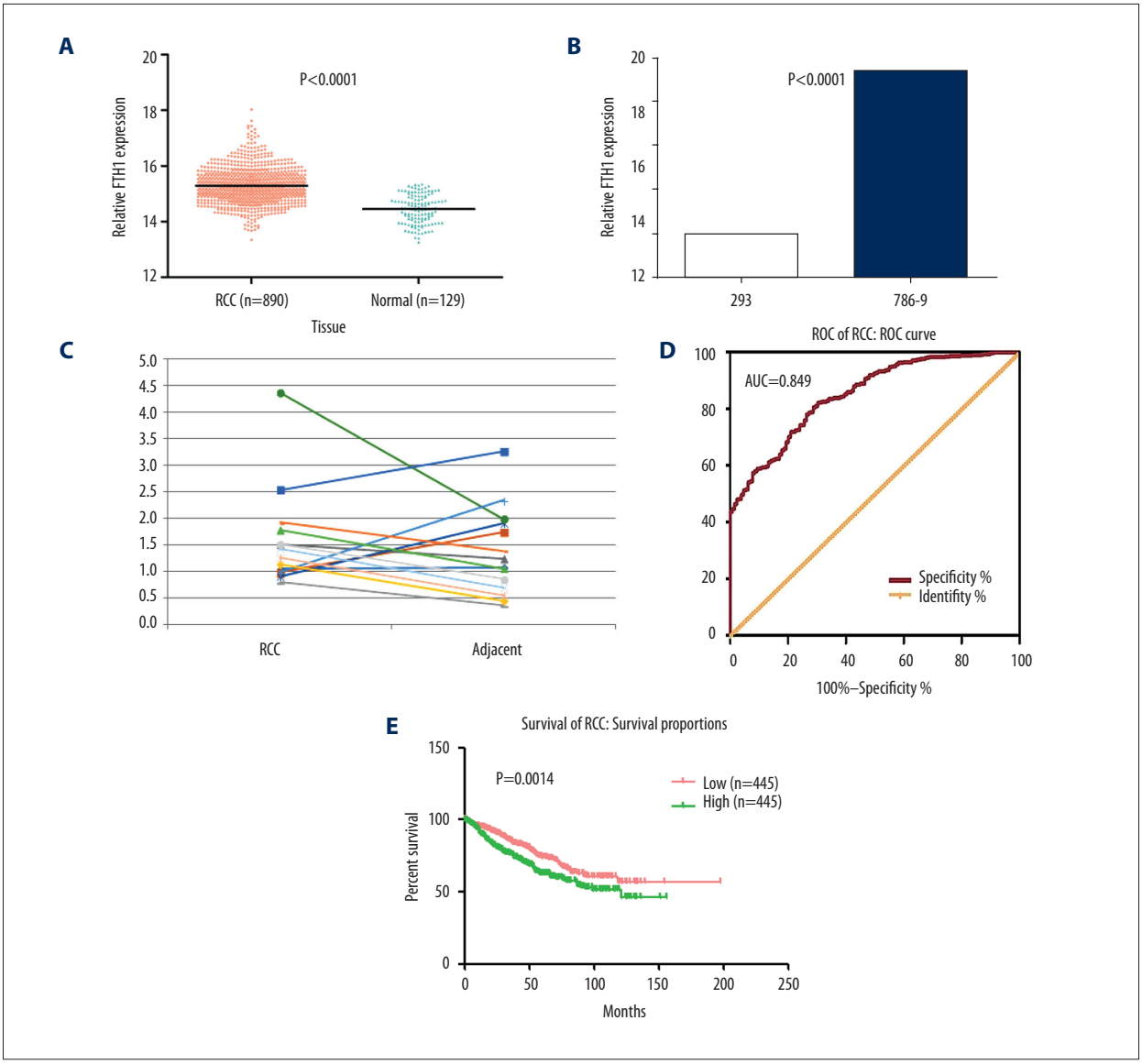

Figure 1. The transcriptional level of FTH1 gene is higher in renal cell carcinoma (RCC) than in normal kidney tissues. (**A**) The mRNA expression of FTH1 in 890 cases of RCC and 129 cases of normal kidney tissues based on TCGA database. (**B**) FTH1 mRNA expression was detected by RT-qPCR in renal cancer cell 786-0 and renal epithelial cell 293, normalized to GAPDH. (**C**) The mRNA expression of FTH1 in 14 primary renal cell carcinoma tissues and matched adjacent tissues. (**D**) The ROC curve for evaluating the diagnostic performance of FTH1 in 890 cases of RCC and 129 cases of normal kidney tissues. The AUC was 0.849. (**E**) The overall survival (OS) of RCC patients with high and low mRNA level of FTH1, which was divided by the median of FTH1 mRNA expression in 890 cases of RCC.

The Human Protein Atlas (HPA)

HPA is a pathology tool that provides a large number of protein expression profiles of human proteins. Clinical tumor tissue samples come from a clinical biobank, including a large number of retrospectively collected patient cohorts and long-term follow-up for research. Here, we used this tool to compare the expression of RCC tissues and normal tissues at the protein level.

cBioPortal for ClueGo

The co-expression genes of FTH1 in KIRC (|Pearson's r|≥0.4 and |Spearman's r|≥0.4) were identified by cBioPortal network tools. Then, genes were loaded into ClueGo in CytoCop3.3.1 to analyze GO and KEGG pathways. Only a path with a p value of 0.05 was included. In addition, co-expressed genes (|Pearson's r|≥0.5 and |Spearman's r|≥0.5) were selected and STING was used for PPI network analysis.

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Table 1. Relationship between the expression of FTH1 and clinicopathological parameters in RCC.

Clinicopathological parameters		n	Relevant expression of FTH1 (log_2X)		
			Mean ±SD	t	p Value
Age (years)	<60	416	15.2286±0.61648	–2.708[a]	0.007*
	≥60	473	15.3431±0.64018		
Gender	Male	598	15.2610±0.62896	–1.933[a]	0.054
	Female	291	15.3481±0.63356		
Lymph node metastasis	Yes	224	15.4004±0.63564	3.083[a]	0.002*
	No	654	15.2505±0.62575		
Stage	I–II	563	15.2102±0.61155	–5.534[a]	0.000*
	III–IV	294	15.4558±0.62696		
T	T1–T2	614	15.2076±0.61946	–5.883[a]	0.000*
	T3–T4	275	15.4724±0.62058		
Pathologic stage	I	460	15.2132±0.61193	F=11.492[b]	0.000*
	II	103	15.1969±0.61267		
	III	189	15.4041±0.62942		
	IV	105	15.5490±0.61454		
Pathologic T	T1	487	15.2095±0.61613	F=12.300[b]	0.000*
	T2	127	15.2004±0.63447		
	T3	258	15.4581±0.60455		
	T4	17	15.6888±0.81950		
M	No	224	15.4004±0.63564	3.083[a]	0.002*
	Yes	654	15.2505±0.62575		

SD – standard deviation; RCC – renal cell carcinoma. [a] A Student's paired or unpaired t test was used for comparison between two group; [b] One-way analysis of variance (ANOVA) was performed. * $p<0.05$ was considered statistically significant.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 software. The correlation between FTH1 gene expression and clinical pathological parameters of RCC patients was evaluated by independent-samples *t* test. The differences in TNM stages were tested using analysis of variance (ANOVA). Cox multivariate survival analysis was performed to predict unfavorable prognosis. The diagnostic value of FTH1 in RCC was evaluated by receiver operating characteristic (ROC) curve. Kaplan-Meier curves and logarithmic rank test were used to analyze the survival of RCC patients. STATA 12 software was used for meta-analysis. $p\leq0.05$ was considered statistically significant.

Results

Association between FTH1 expression and clinicopathological parameters, diagnosis and prognosis of RCC patients

According to TCGA data, over-expression of FTH1 was detected in 890 RCC tissues (15.2904±0.63157) compared to 129 normal kidney tissues (14.4502±0.51523, $p<0.001$; Figure 1A). This was further confirmed in cell lines and tissues by real-time RT-PCR. In contrast with normal renal epithelial cell line 293, the mRNA level of FTH1 was elevated in renal cell carcinoma cell line 786-0 (Figure 1B). We also observed a relatively higher expression of FTH1 in 10 out of 14 primary RCCs than in matched adjacent samples (Figure 1C). There was a significant difference between the expression of FTH1 and age, T stage, M stage, and lymph node metastasis (Table 1). Patients age <60 years showed a lower FTH1 expression compared with those age ≥60 years. The expression of FTH1 was also remarkably

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Table 2. Kaplan-Meier univariate survival analysis of FTH1 and other clinicopathological parameters in RCC patients.

Clinicopathological parameters	Mean survival time (months)	95% CI	P value
FTH1 expression			
Low	135.153	123.535–146.772	0.001*
High	97.873	89.98–105.767	
Age (years)			
<60	147.369	137.543–157.195	0.000*
≥60	93.557	85.903–101.211	
Gender			
Female	103.728	94.55–112.906	0.469
Male	125.38	114.575–136.185	
Lymph			
No	107.774	101.082–114.465	0.000*
Yes	114.567	101.158–127.976	
Stage			
I–II	123.444	116.697–130.191	0.000*
III–IV	85.857	73.647–98.066	
T			
T1–T2	120.245	113.691–126.799	0.000*
T3–T4	87.209	74.525–99.893	
M			
No	112.629	106.22–119.039	0.000*
Yes	103.131	87.961–118.302	

* p<0.05 was considered statistically significant.

Table 3. Cox multivariate analysis of FTH1 and other clinicopathological parameters in RCC patients.

Covariates	HR	95% CI for HR	P value
FTH1 expression level (low *vs.* high)	1.129	0.858–1.486	0.386
Age (<60 *vs.* ≥60 years)	1.655	1.249–2.192	0.000*
Lymph (no *vs.* yes)	1.044	0.78–1.396	0.772
Stage (I–II *vs.* III–IV)	6.032	3.445–10.564	0.000*
T (T1–2 *vs.* T3–4)	0.661	0.394–1.109	0.117
M (no *vs.* yes)	1.615	1.219–2.138	0.001*

* p<0.05 was considered statistically significant.

different in different T and M stages. Patients with lymph node metastasis also had higher FTH1 expression and metastasis.

Kaplan-Meier survival analysis showed that FTH1 expression level, age, lymphatic metastasis, stage, T stage, and M stage were important parameters affecting survival time of RCC patients (Table 2). In addition, Cox multivariate survival analysis was performed, including 6 significant statistical parameters, and demonstrated that age, stage, and M stage were predictors of adverse prognosis in patients with RCC (Table 3).

The P value of ROC curve was <0.001, revealing that the expression of FTH1 is associated with diagnosis of RCC (AUC=0.849, 95% CI: 0.818–0.880, p<0.001; Figure 1D). The Kaplan-Meier curve showed that of RCC patients with high FTH1 expression had worse outcomes (p=0.0014; Figure 1E).

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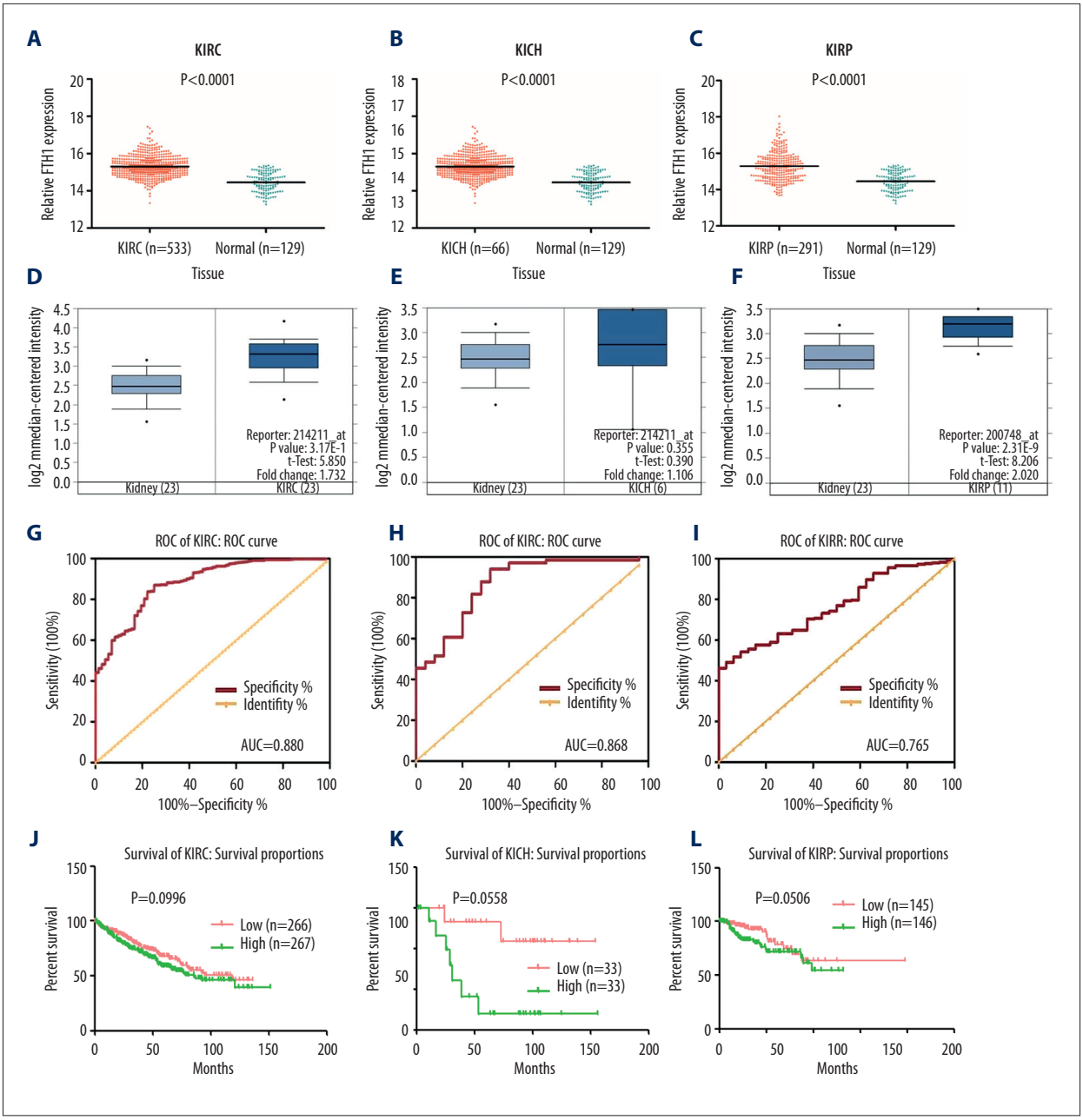

Figure 2. The transcriptional level of FTH1 gene is higher in KIRC, KICH, and KIRP in contrast with normal kidney tissues. Higher expression of FTH1 was associated with poorer prognosis of KIRC patients. (**A–C**) Scatter plot of FTH1 gene expression in normal tissues in contrast with 3 subtypes of RCC. (**D–F**) Validation of FTH1 expression in Jone's study using ONOCMINE database. (**G–I**) ROC curve of FTH1 for patients with KIRC, KICH, and KIRP. The AUC was 0.88 (95% CI: 0.841–0.919, p<0.001), 0.868 (95% CI: 0.786–0.950, p<0.001), and 0.765 (95% CI: 0.697–0.834, p<0.001). (**J–L**) The overall survival (OS) of patients with KIRC, KICH, and KIRP.

Association between FTH1 expression and clinicopathological parameters, diagnosis, and prognosis of KIRC, KICH, and KIRP patients

We extracted 533 cases of KIRC, 66 cases of KICH, and 291 cases of KIRP to analyze the FTH1 expression in subtypes of RCC. In these 3 types of RCC, FTH1 expression was significantly higher than in the 129 normal controls (Figure 2A–2C). To further confirm this finding, we used Oncomine database to analyze the FTH1 expression in 3 types of RCC. Figure 2D–2F shows that FTH1 is overexpressed in KIRC, KICH, and KIRP, but the difference is significant only in KIRC and KIRP.

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Table 4. Relationship between the expression of FTH1 and clinicopathological parameters in KIRC.

Clinicopathological parameters		n	Relevant expression of FTH1 (log_2X)		
			Mean ±SD	t	p Value
Age (years)	<60	245	15.2660±0.51809	−1.534[a]	0.126
	≥60	288	15.3392±0.57304		
Gender	Male	345	15.2739±0.56296	−1.804[a]	0.072
	Female	188	15.3635±0.51938		
Lymph node metastasis	Yes	134	15.4146±0.61153	2.443[a]	0.015*
	No	392	15.2159±0.78573		
Stage	I–II	324	15.2195±0.53435	−4.580[a]	0.000*
	III–IV	207	15.4396±0.54855		
T	T1–T2	342	15.2319±0.53832	−4.209[a]	0.000*
	T3–T4	191	15.4375±0.54507		
Pathologic stage	I	267	15.2250±0.52511	F=8.503[b]	0.000*
	II	57	15.1938±0.57990		
	III	123	15.3756±0.53859		
	IV	84	15.5332±0.55273		
Pathologic T	T1	273	15.2271±0.52558	F=6.478[b]	0.000*
	T2	69	15.2506±0.58975		
	T3	180	15.4251±0.53334		
	T4	11	15.6394±1.71110		
M	No	422	15.2636±0.53297	−3.429[a]	0.001*
	Yes	109	15.4641±0.58600		

SD – standard deviation; RCC – renal cell carcinoma. [a] A Student's paired or unpaired t test was used for comparison between two group; [b] One-way analysis of variance (ANOVA) was performed. * $p<0.05$ was considered statistically significant.

Table 5. Relationship between the expression of FTH1 and clinicopathological parameters in KICH.

Clinicopathological parameters		n	Mean ±SD	t	p value
Age (years)	<60	47	15.2060±0.50464	−0.049[a]	0.961
	≥60	19	15.2130±0.50670		
Gender	Male	39	15.2154±0.52903	0.014[a]	0.888
	Female	27	15.1973±0.48618		
Lymph node metastasis	Yes	35	15.1640±0.54639	0.744[a]	0.459
	No	31	15.2577±0.54639		
Stage	I–II	46	15.1614±0.49987	−1.292[a]	0.205
	III–IV	19	15.3432±0.52252		
T	T1–T2	46	15.1428±0.49759	−1.581[a]	0.123
	T3–T4	20	15.3581±0.51290		

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Table 5 continued. Relationship between the expression of FTH1 and clinicopathological parameters in KICH.

Clinicopathological parameters		n	Mean ±SD	t	p value
Pathologic stage	I	21	15.2598±0.11110	F=2.312[b]	0.085
	II	25	15.0787±0.97330		
	III	13	15.1914±0.51749		
	IV	6	15.6593±0.40624		
Pathologic T	T1	21	15.2598±0.50917	F=1.577[b]	0.209
	T2	25	15.0444±0.47552		
	T3	18	15.3562±0.54161		
	T4	2	15.3752±0.10394		
M	No	34	15.1269±0.53388	–0.555[a]	0.582
	Yes	11	15.2290±0.51903		

SD – standard deviation. [a] A Student's paired or unpaired t test was used for comparison between two group; [b] One-way analysis of variance (ANOVA) was performed.

Table 6. Relationship between the expression of FTH1 and clinicopathological parameters in KIRP.

Clinicopathological parameters		n	Relevant expression of FTH1 (log_2X)		
			Mean ±SD	t	p Value
Age (years)	<60	121	15.1689±0.80649	–2.036[a]	0.043*
	≥60	169	15.3572±0.75462		
Gender	Male	214	15.2485±0.73904	–1.104[a]	0.270
	Female	76	15.3636±0.88799		
Lymph node metastasis	Yes	55	15.5165±0.71347	–2.595[a]	0.010*
	No	231	15.2159±0.78573		
Stage	I–II	193	15.2106±0.74507	–2.963[a]	0.003*
	III–IV	67	15.5353±0.84850		
T	T1–T2	226	15.1841±0.74460	–3.763[a]	0.000*
	T3–T4	64	15.6124±0.81967		
Pathologic stage	I	172	15.1891±0.73811	F=3.326[b]	0.019*
	II	21	15.3870±0.79651		
	III	52	15.5188±0.82226		
	IV	15	15.5928±0.96255		
Pathologic T	T1	193	15.1791±0.73574	F=5.591[b]	0.010*
	T2	33	15.2134±0.80731		
	T3	60	15.5878±0.78798		
	T4	4	15.9815±1.30530		
M	No	95	15.1953±0.73192	–1.429[a]	0.154
	Yes	180	15.3378±0.82060		

SD – standard deviation. [a] A Student's paired or unpaired t test was used for comparison between two group; [b] One-way analysis of variance (ANOVA) was performed. * $p<0.05$ was considered statistically significant.

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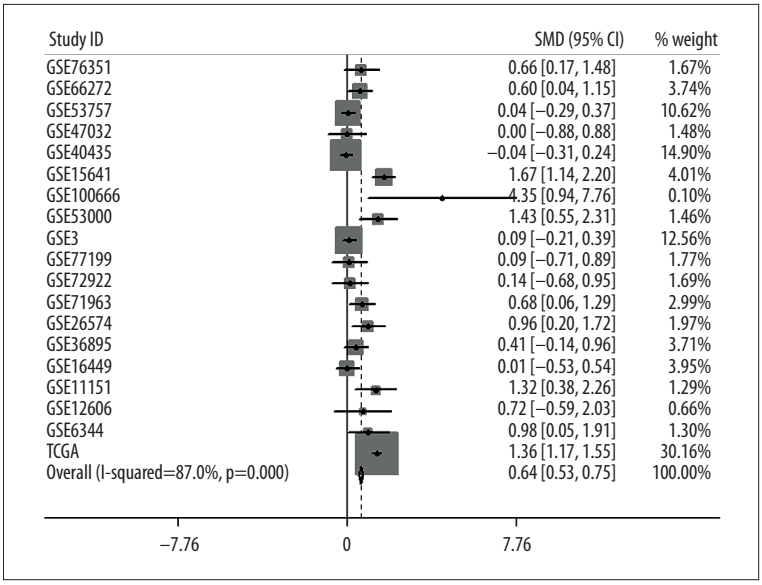

Figure 3. Meta-analysis of FTH1 expression in renal cell carcinoma based on tumor types. A total of SMDs with 95% CI accounted for 0.64 (0.53, 0.75). RCC tissue subgroup was highly heterogeneous (I^2=87.0%, p<0.001).

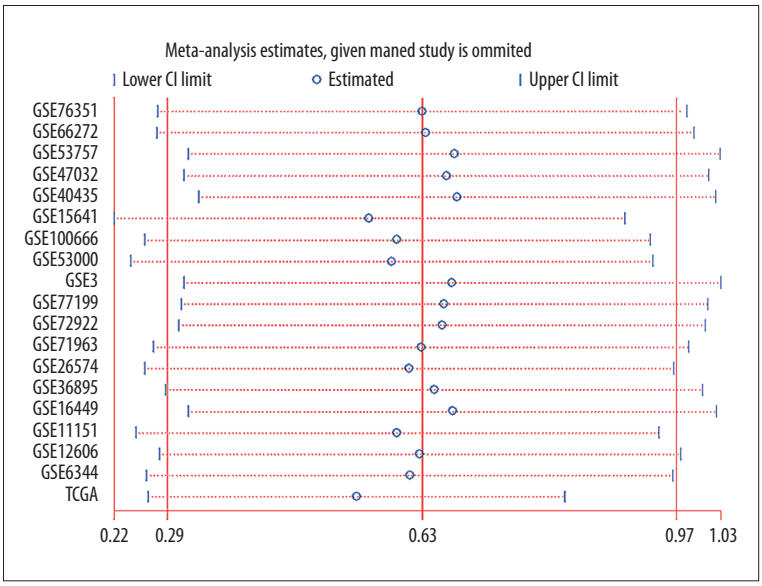

Figure 4. Meta-analysis of FTH1 expression in renal cell carcinoma showed no significant difference in sensitivity analysis.

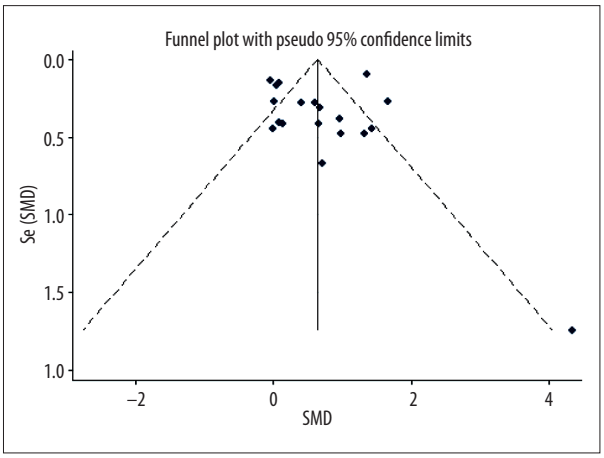

Figure 5. Meta-analysis of FTH1 expression in renal cell carcinoma using Begg funnel map. Symmetric Begg funnel map indicated publication bias (p=0.054).

The expression of FTH1 was also remarkably different in different T stages in KIRC patients. These patients with lymph node or distant metastasis had higher FTH1 expression (Table 4), but no significant difference was found between the expression of FTH1 and any clinical characteristics in KICH patients (Table 5). KIRP patients age <60 years showed lower FTH1 expression compared with those age ≥60 years. The expression of FTH1 was also remarkably different in different T stages. Patients with lymph node or distant metastasis also had higher FTH1 expression (Table 6).

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Table 7. Basic information of all included GEO datasets, array express microarray.

ID	Author	Publish year	Country	Sample type	Cancer N	Cancer M	Cancer SD	Normal N	Normal M	Normal SD
GSE76351	Solodskikh	2015	Russia	Human tissues	12	9.1138	0.2013	12	8.9940	0.1621
GSE66272	Wotschofsky Z	2016	Germany	Human tissues	26	0.0846	0.2991	27	–0.1014	0.3243
GSE53757	von Roemeling CA	2014	USA	Human tissues	72	15.5405	0.5152	72	15.5225	0.3383
GSE47032	Valletti A	2013	Italy	Human tissues	10	4.7872	0.1749	10	4.7872	0.1749
GSE40435	Wozniak MB	2013	France	Human tissues	101	10.3477	0.4258	101	10.3630	0.3850
GSE15641	Jones J	2009	USA	Human tissues	69	11.3989	0.5139	23	10.6087	0.3120
GSE100666	Peng Z	2017	China	Human tissues	3	11.2542	0.0787	3	10.7352	0.1493
GSE53000	Gerlinger M	2014	France	Human tissues	56	10.4677	0.2034	6	10.1719	0.2360
GSE3	Boer JM	2001	Germany	Human tissues	90	5.9354	5.9755	81	5.3819	6.1432
GSE77199	Wragg JW	2016	United Kingdom	Human tissues	12	15.9394	0.5158	12	15.8935	0.4924
GSE72922	De Palma G	2016	Italy	Human tissues	12	10.0338	1.3682	11	9.8243	1.7273
GSE71963	Takahashi M	2016	Japan	Human tissues	32	1.5948	0.7584	16	1.1496	0.3651
GSE26574	Ooi A	2011	USA	Human tissues	57	11.4650	0.6121	8	10.8944	0.4178
GSE36895	Peña-Llopis S	2012	USA	Human tissues	29	13.9418	0.3180	23	13.8316	0.1914
GSE16449	Brannon AR	2010	USA	Human tissues	52	0.0551	0.3741	18	0.0528	0.2364
GSE11151	Yusenko MV	2008	Netherlands	Human tissues	62	15.4108	0.4036	5	14.8845	0.3245
GSE12606	Stickel JS	2008	Germany	Human tissues	6	10.7604	0.0924	4	10.4827	0.6190
GSE6344	Gumz ML	2006	USA	Human tissues	10	13.6205	0.3276	10	13.3191	0.2851
TCGA				Human tissues	890	15.2904	0.6316	129	14.4502	0.5152

N – number; M – mean; SD – standard deviation

The ROC curve was used to assess the diagnostic performance of FTH1 expression in KIRC, KICH, and KIRP (Figure 2G–2I); the AUC was 0.880 (95% CI: 0.841–0.919, p<0.001), 0.868 (95% CI: 0.786–0.950, p<0.001), and 0.868 (95% CI: 0.786–0.950, p<0.001), respectively. This indicates that the transcription of FTH1 could be used as a diagnostic biomarker for all 3 subtypes of RCC.

The Kaplan-Meier curves shown in Figure 2J–2L revealed no predictive value in KIRC, KIRP, or KICH patients.

Meta-analysis of FTH1 expression in RCC

To evaluate the consistency of FTH1 abnormal expression in RCC, 18 microarray studies involving 738 RCC tissues and

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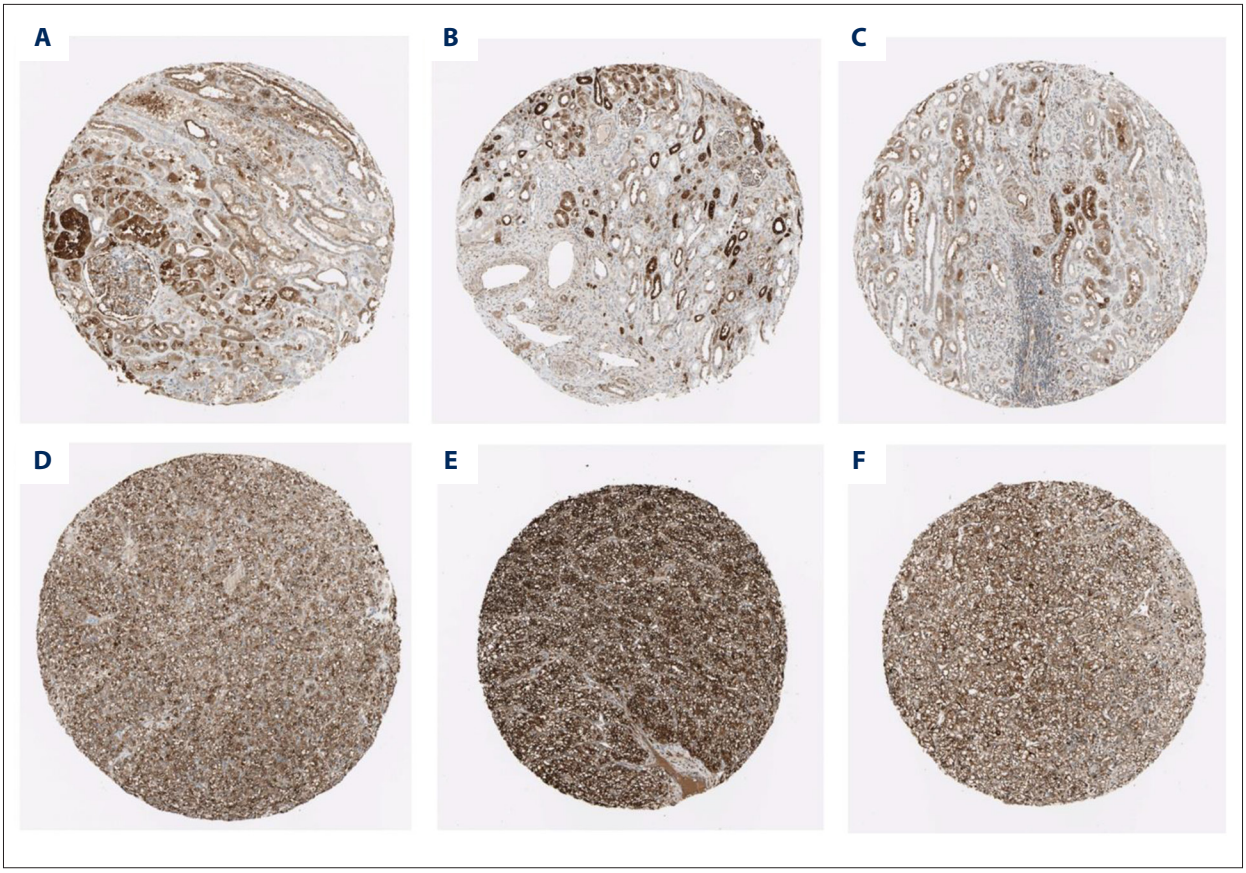

Figure 6. Validation of the protein expression of FTH1 in normal kidney control samples (**A–C**) and RCC samples using the HPA database (**D–F**).

469 normal tissues in GEO database were included for meta-analysis, in which we combined the effective data (GEO and TCGA) and used the random-effects model to obtain the pooled Standard Mean Difference (SMD) as 0.64 (95% CI: 0.53–0.75, p<0.001; Figure 3), and the p value of the heterogeneity test was less than 0.001 (I^2=87.0%). Sensitivity analysis showed that no single study led to significant bias in overall merger results (Figure 4). In addition, no significant publication bias was found in the study (Begg's test: p=0.054; Figure 5). Relevant information was extracted from each study, such as ID number, first author, public year, country, sample type, platform, number of cancer cases, mean (M) and standard deviation (SD) of FTH1 expression in the cancer group, and normal tissue N, M, and SD of FTH1 expression in the normal group (Table 7).

FTH1 protein expression in RCC tissues from HPA

Using the HPA database, we compared 3 normal samples and 3 RCC samples, which showed an elevation of FTH1 protein in RCC (Figure 6).

The GO, KEGG network, and PPI network with co-expressed genes of FTH1

Among these co-expressed genes, 278 genes were selected for GO and pathway analyses (Figures 7–9). These genes are abundantly expressed in positive regulation of the Wnt signal transduction pathway, response to oxygen level, binding of ribosome subunits, and RNA polymerase. In addition, KEGG pathway analysis showed that the expression of FTH1 co-expression gene in hepatocellular carcinoma, proteasome, and ribosome was significantly higher than in the control group (Figure 10). The most important GO items (BP, CC, and MF) are listed in Table 8 and the PPI network is shown in Figure 11.

Discussion

To date, no diagnostic modality for early detection of RCC has been established, other than incidental radiologic discovery. Some promising studies have identified several potential biomarkers in sera and urine. For example, tumor necrosis factor receptor-related factor-1, heat shock protein 27, carbonic anhydrase IX, and ferritin in RCC patients were significantly higher

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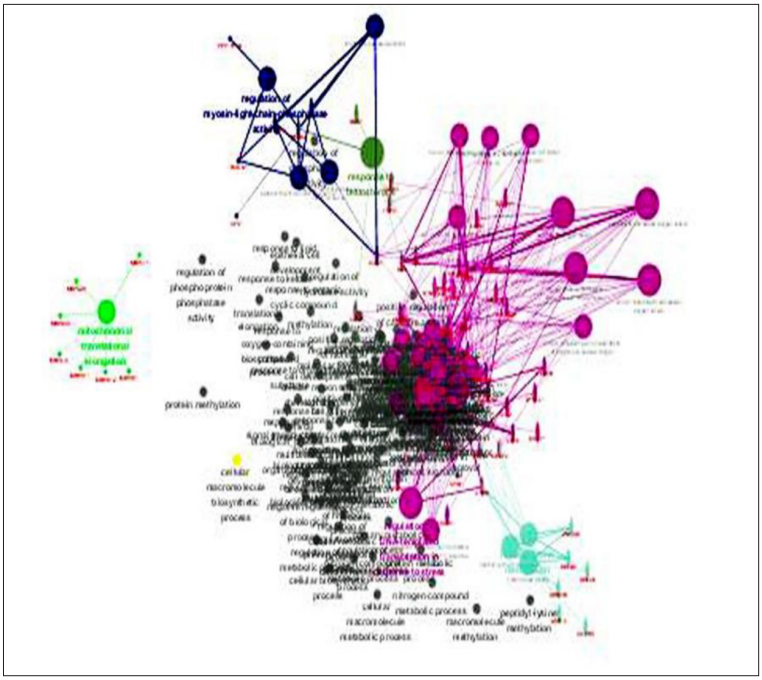

Figure 7. The GO map of BP corresponding to the target gene of FTH1.

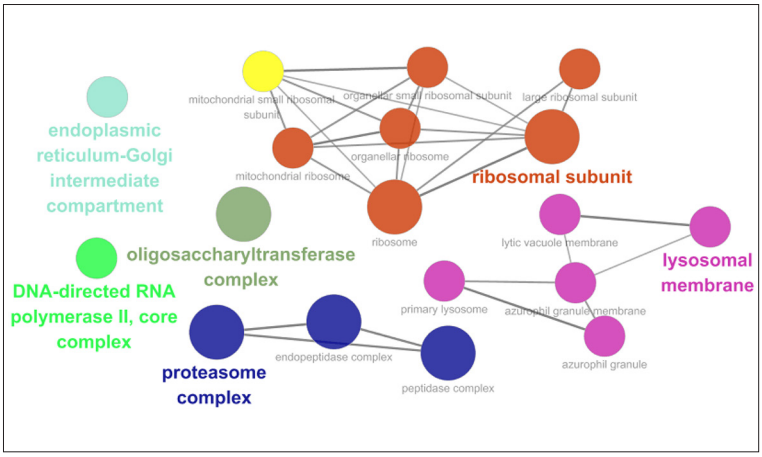

Figure 8. The GO map corresponds to the target gene CC of FTH1.

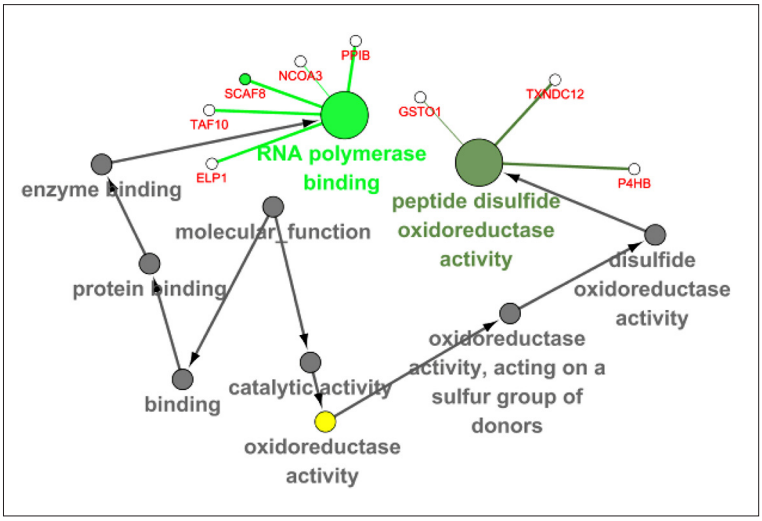

Figure 9. The GO map of MF corresponding to the target gene of FTH1.

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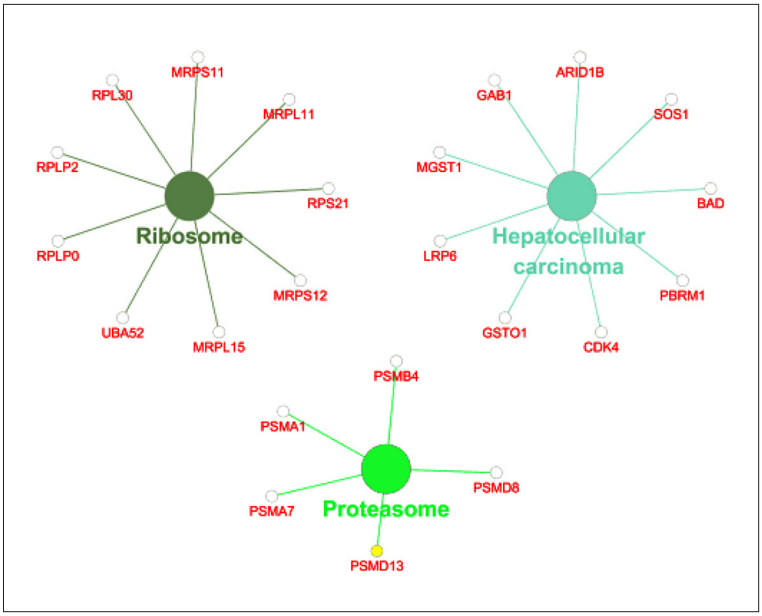

Figure 10. KEGG pathway analysis of co-expression genes of FTH1 target genes.

Table 8. Top 5 enrichment GO terms (BP, CC and MF) of the co-expression genes of FTH1.

GO ID	GO Term	Ontology	Count	P Value
GO: 0030177	Positive regulation of Wnt signaling pathway	BP	11	3.49E-05
GO: 0070482	Response to oxygen levels	BP	19	6.90E-06
GO: 0071456	Cellular response to hypoxia	BP	12	5.29E-05
GO: 0071453	Cellular response to oxygen levels	BP	13	3.58E-05
GO: 0090175	Regulation of establishment of planar polarity	BP	9	6.83E-05
GO: 0044391	Ribosomal subunit	CC	12	2.06E-05
GO: 0008250	Oligosaccharyltransferase complex	CC	4	2.49E-05
GO: 0000502	Proteasome complex	CC	7	5.20E-05
GO: 1905368	Peptidase complex	CC	8	5.45E-05
GO: 1905369	Endopeptidase complex	CC	7	5.71E-05
GO: 0070063	RNA polymerase binding	MF	5	0.001002
GO: 0015037	Peptide disulfide oxidoreductase activity	MF	3	9.22E-04

GO – gene ontology; BP – biological process; CC – cellular component; MF – molecular function.

than those in control serum [20–24], while nuclear matrix protein-22, kidney injury molecule-1, matrix metalloproteinases, aquaporin-1, and perilipin 2 are elevated in urine [25–28]. However, none of these have been used in clinic practice for RCC diagnosis. In this study, we used bioinformatic approaches to reveal the relationship between FTH1 and the clinical characteristics of RCC patients. The RNA-seq data from TCGA showed that FTH1 is overexpressed in RCC tissues. FTH1 transcription level was significantly correlated with pathological T stage, lymph node, and distant metastasis of KIRC, and was significantly correlated with pathological T stage and lymph node metastasis of KIRP, suggesting that FTH1 may be a potential biomarker for clinical stages of these 2 RCC subtypes. Meta-analysis results showed that FTH1 was overexpressed in RCC according to 18 microarray datasets from GEO. However, heterogeneity was moderately high and publication bias was obvious, probably due to small sample size and datasets of varying quality.

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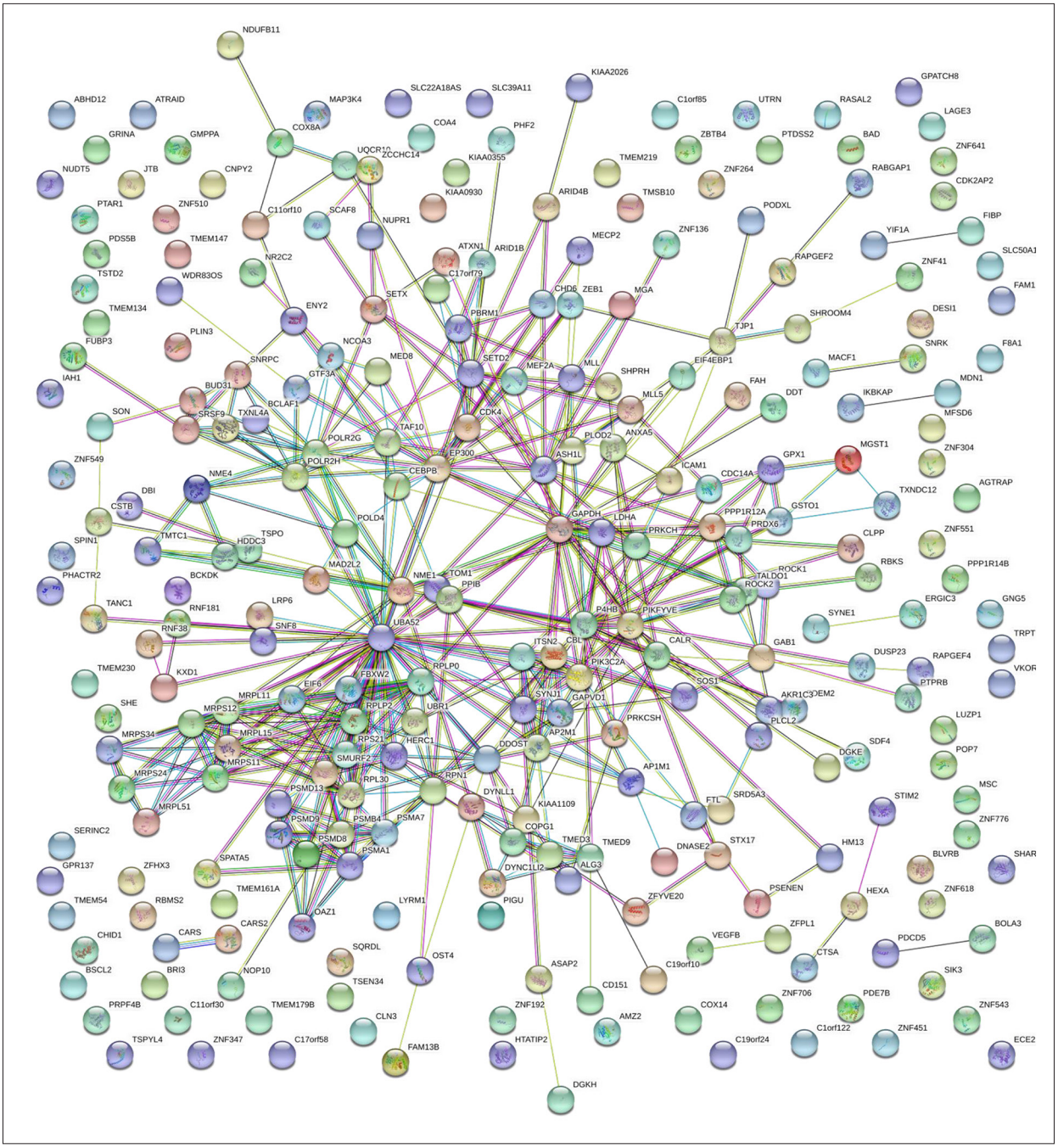

Figure 11. The PPI network of FTH1 target genes.

Currently available biomarkers seem to be most useful as diagnostic tools, prognostic indicators, and follow-up in patients with renal cancer [29]. Steven et al. reported that the positive expression of receptor activator of NF-κB had both worse cancer-specific survival and recurrence-free survival in RCC patients [30]. Increased expression of long noncoding RNA GIHCG is positively correlated with advanced TNM stages, Fuhrman grades, and poor prognosis [31]. In a meta-analysis of 2013 patients, including 22 studies, positive expression of P53 was associated with poor prognosis and advanced clinicopathological features in RCC patients [32]. The nuclear translocation of CXCR4 plays an important role in RCC metastasis and is associated with poor prognosis [33]. Here, we found that RCC patients with higher FTH1 expression in primary RCC were associated with a shorter survival time. Besides, RCC patients with lymph node and distant metastasis had higher FTH1 expression metastasis, which indirectly suggests a poorer prognosis. These finding suggest that the high expression of FTH1

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could be used as a predictor to indicate the poor prognosis of RCC patients.

Dysregulation of iron homeostasis has been linked to numerous diseases, such as cancer and neurodegenerative diseases [34,35]. Cellular iron regulation includes iron uptake, storage, and export. Iron-regulated proteins, such as transferrin receptors in glioblastoma and ferritin in serum, were upregulated, thereby increasing iron uptake [36,37]. Ferritin plays an important role in the storage and release of iron in cells. Ferritin complexes capture intracellular ferrous ions (Fe^2) and convert them into iron ions (Fe^3) by the activity of ferrous oxidase [38]. It consists of 24 subunits of heavy and light ferritin chains (FTH1 and FTL1). In this study, we found that there was no significant correlation between the expression of FTL1 and RCC (data not shown), suggesting that FTH1 might play an important role in the tumorigenesis of RCC. In addition, approaches targeting cellular iron and iron signaling to inhibit tumor growth have been developed and applied in cancer therapy. The application of iron chelators can suppress tumor growth and induce apoptosis, which suggests iron chelators as potential anti-cancer drugs [39,40]. FTH1 controls HIF-induced hypoxia by activating asparagine hydroxylase and affects the expression of HIF-1 target gene [38]. Based on our results of GO analyses, the top enriched functional term of FTH1 genes were regulation of Wnt signaling pathway and response to cellular hypoxia. Overexpressing FTH1 in acute myeloid leukemia (AML) stem cells significantly induced the expression of genes involved in immune and inflammatory response, including NF-κB pathway, oxidative stress, and iron pathways [41]. These findings suggest that FTH1 could be a novel therapeutic target.

The limitations of this study should be considered. The expression of FTH1 in RCC and its correlation with clinical features were analyzed and validated only in TCGA and GEO datasets. Further research is needed to improve our understanding of the functional role of FTH1 in RCC.

Conclusions

In this study, we found that expression of FTH1 is elevated in RCC, which could serve as a potential diagnosis and prognosis biomarker. Our data suggest that higher mRNA levels of FTH1 might contribute to the progression of RCC, and thus could be used as a target for RCC therapy.

Conflict of interest

None.

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