Interferon-Inducible Protein 16 (IFI16) is a Prognostic Biomarker and Related to Immune Infiltrates in Renal Cell Carcinoma (RCC)

Bao Zhong Yu
Beijing Chaoyang Hospital,Capital Medical University
Jian Dong Zhang
Beijing Chaoyang Hospital, Capital Medical University
Ze Jia Sun
Beijing Chaoyang Hospital
Peng Cao
Beijing Chaoyang Hospital, Capital Medical University
Xiang Zheng
Beijing Chaoyang Hospital, Capital Medical University
Zi Hao Gao
Beijing Chaoyang Hospital, Capital Medical University
Hao Yuan Cao
Beijing Chaoyang Hospital, Capital Medical University
Fei Long Zhang
Beijing Chaoyang Hospital, Capital Medical University
Wei Wang (weiwang0920@163.com)
Beijing chaoyang Hospital,Capital Medical University https://orcid.org/0000-0002-7532-6000

Primary research

Keywords: IFI16, renal cell carcinoma, mRNA, biomarkers, prognosis, immune infiltrates

Posted Date: February 28th, 2020

DOI: https://doi.org/10.21203/rs.2.24819/v1

Abstract

Background: Renal cell carcinoma is one of the most common and lethal types of cancer within the urinary system. the current challenges include how to diagnose RCC in the earlier stage and the drug resistance to chemotherapy or radiotherapy. Therefore, to investigate the survival rate and immune infiltration in RCC base on gene expression of IFI16 in RCC.

Methods: We explored the relationship between the transcription level of IFI16 and clinical data in RCC through various online databases, including ONCOMINE, GEPIA, Timer and COEXPEDIA.

Results: In comparison with the corresponding normal tissues, the mRNA expression levels of IFI16 are higher in KIRC tissues (p 0.05), while lower in KICH tissues (p0.05). The mRNA expression of IFI16 has a positive correlation with TNM stage in KIRC(p=8.56e-07) and KIRP(p=0.00078). In KIRC, the higher expression of IFI16 suggests lower OSHR=1.4; P=0.037; Log-rank p=0.037). In KIRP, the higher expression of IFI16 suggests lower DFS and OS (respectively, HR=1.9, P=0.037, Log-rank p=0.034; HR=2.3, P=0.011, Log-rank p=0.0091). In contrast, the expression level of IFI16 has a negative correlation with the tumour purity in KICH, KIRC and KIRP via the Timer database (all, $p < 0.05$). In KIRC and KIRP, the expression level of IFI16 has a positive correlation with the tumor-infiltrating immune cells (TIICs) (all, $p < 0.05$), except the macrophages in KIRP. In KIRC, the main TIICs in the arm-level deletion state of the IFI16 gene were B cell, CD4+T cell, Neutrophil, and Dendritic cell, while the main TIICs in the high amplification state were Macrophage (all, $p < 0.0001$). 108 genes are co-expressed with the IFI16 gene. Function enrichment analysis by GO and KEGG mainly enriched on some functions, including neutrophil degranulation, phagocytosis, vesicle-mediated transport regulation and enriched on some signal pathways, including tuberculosis, toxoplasmosis, phagosome, leishmaniasis, and Fc gamma R-med8ted.

Conclusions: IFI16 acts as an oncogene in the progression of kidney cancer which is overexpressed in the RCC tissues. Apart from that, IFI16 has been determined as a marker of diagnosis and prognosis in RCC, which might be associated with the immune infiltration.

1. Background

Renal cell carcinoma accounts for 2–3% of all human malignancies [1]and ranks ninth in the most common malignancies in Western countries. It is estimated that more than 338,000 people are diagnosed with RCC each year, with a 22% increase projected by 2020. RCC includes a group of heterogeneous cancers with different genetic and molecular changes that underlie many histological subtypes[2]. Kidney renal clear cell carcinoma(KIRC), kidney renal papillary cell carcinoma(KIRP), and Kidney Chromophobe (KICH) are the most common pathological types in kidney cancer, accounting for 85-90% of all renal malignancies[3]. There are more than 140,000 RCC-related deaths per year[4].

Due to the development of the surgical procedures and the use of new drugs, the RCC patients' survival rate has been significantly prolonged[5]. However, the current challenges include how to diagnose RCC in

the earlier stage and the drug resistance to chemotherapy or radiotherapy. Therefore, there is an urgent need for more effective biomarkers and therapeutic targets.

IFI16 is an AIM2-like receptor that can sense the dsDNA of some viruses to activate the STING-TBK1 pathway to produce IFN-β6 for antiviral defense[6]. Besides, it has been found to bind with the DNA and form an Inflammasome-cytoplasmic protein complex. It responds to various physiological and pathogenic stimuli, including PAMP and DAMP, and promotes caspase-1 activation and the synthesis of pro-IL-1β/IL-18[7]. It is essential to clean pathogens or damaged cells. Further evidence indicates that IFI16 had been shown to enhance the activation of known p53 target genes (including p21, Hdm2, and bax), thereby inducing p53-mediated cell cycle arrest and human cancer cell apoptosis[8, 9].

Mazibrada et al. [10]established that it significantly reduced the tumor volume of head and neck cancer when IFI16 was overexpressed in tumor cells, accompanied by a decrease in tumor neovascularization and an increase in tumor necrosis area. Wei Lin et al. [11] demonstrated that the expression levels of IFI16 are lower in the liver cancer tissues compared to the healthy tissues. When forced expression of IFI16 in hepatocellular carcinoma promoted apoptosis and decreased cell viability. Studies have shown that the overexpression of IFI16 can significantly inhibit the growth of liver cancer and reduce tumor volume. But, it was found that the expression of IFI16 in oral cancer is up-regulated by activating NF-κB signaling, thereby promoting cell growth and preventing apoptosis[12 13].The current research suggests IFI16 not only functions as a tumour suppressor gene but also functions as an oncogene. So how does it function in kidney cancer? So far, the bioinformatics analysis has not been used to analyze the role of IFI16 in RCC. Therefore, in this study, we aimed to investigate the expression, prognosis, and immune infiltration of IFI16 in RCC patients via different databases. Our results have important implications to screen biomarkers and guide target therapy in RCC.

2. Methods

We used a multidimensional analysis strategy to study the expression, mutation, regulation, functional network, and immune infiltration of IFI16 in patients with RCC on the basis of different open databases.

2.1 ONCOMINE analysis

ONCOMINE (www.oncomine.org) is the largest oncogene chip database and integrates a global data mining platform[14]. It contains 715 gene expression data sets and 86,733 samples. In this study, we analyzed the mRNA expression of IFI16 in RCC in ONCOMINE 4.5 database. Taking the expression of IFI 16 in healthy kidney tissues as a reference, the changes of IFI 16 expression in RCC tissues were analyzed through ONCOMINE. $P < 0.05$ was used as the cut off standard, which was statistically significant.

2.2 GEPIA (Gene Expression Profiling Interactive Analysis) dataset

GEPIA uses standard processing pipelines to analyze the RNA sequencing expression data from 9,736 tumors and 8,587 normal samples from the TCGA and GTEx projects. GEPIA provides customizable features such as dimension reduction analysis, correlation analysis, tumor / normal differential expression analysis, analysis based on cancer types or pathological stages, similar gene detection, and patient survival analysis[15]. We analyzed the correlation between the mRNA expression of IFI16 and the prognosis and tumor stage in RCC through the GEPIA database.

2.3 TIMER (Tumor Immune Estimation Resource) analysis

TIMER (https://cistrome.shinyapps.io/timer/)[16] is a comprehensive database to examine the immune invasion of various malignancies systematically. This data set evaluates the abundance of six immune infiltrates cells (CD8 $^{+}$ T cells, B cells, CD4 $^{+}$ T cells, macrophages, neutrophils, and dendritic cells) by using the statistical methods and the pathological estimation methods. We used the database to explore the relationship between the expression of IFI 16 and the abundance of immune infiltrates in RCC. We also explored the relationship between the somatic cell copy number change and the immune infiltration by considering $P < 0.05$ as the cut-off criterion statistically significant.

2.4 COEXPEDIA analysis

Co-expression genes of IFI16 were analyzed using the COEXPEDIA database[17] (http://www.coexpedia.org/), which is a database of context-associated co-expression networks inferred from individual series of microarray samples for human and mouse samples based on Gene Expression Omnibus data (https://www.ncbi.nlm.nih.gov/geo/). The generated network was a filtered network for the medical subject heading term ‘RCC'. The score for each gene is a summation of edge-weights (Log-likelihood score) to all connected genes in the network.

2.5 Function enrichment analysis

Function enrichment analysis has become a common method for high-throughput omics data analysis. It is expected to discover biological pathways that play a crucial role in biological processes, thereby revealing and understanding the basic molecular mechanisms of biological processes. IFI16 and the obtained co-expression genes of IFI16 in RCC were analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG) signalling pathway and Gene Ontology (GO) enrichment analysis through the R language “GGplot2” package and the “cluster Profiler” package[18, 19], the *p*-value < 0.05 was set as the cut-off criteria.

3. Results

3.1 Transcriptional levels of IFI16 in patients with RCC.

In order to determine the difference in IFI16 expression in RCC and normal renal tissues, we analyzed the transcriptional level of IFI16 via the ONCOMIINE database. Figure 1 shows the mRNA expression of IFI16 was observed in 20 different cancer tissues. Compare to the corresponding normal tissues, the mRNA expression levels of IFI16 appear high in 54 study data sets, while appear low in 6 study data sets. In the study of RCC, the mRNA expression level of IFI16 appear high in 7 study data set and only appear low in 1 study data set. As Figure 2 and Table 1 show, the expression of IFI16 in RCC tissues exist significantly higher apart from the healthy renal tissues, and the fold change is greater than 2.

3.2 Relationship between the transcriptional level of IFI16 and clinical pathology and tumor stage in RCC.

Through the GEPIA database, we investigated the expression of IFI16 in different pathological types of RCC. Compared to the corresponding normal tissue, the mRNA expression level of IFI16 in KIRC tissues seem to be higher (p<0.05), while in KICH tissues seem to be lower (p<0.05) (Figure 3A-B). We analyzed the relationship between the mRNA expression of IFI16 with clinicopathological parameters in RCC by GEPIA. The mRNA expression level of IFI16 has a positive correlation with TNM stage in KIRC(p=8.56e-07) and KIRP(p=0.00078) (Figure 3D-E). However, there is no correlation between IFI16 expression and TNM stage in KICH, such as the highest IFI16 expression in stage 1 tumor, the lowest IFI16 expression in stage 2 tumor (Figure 3C).

3.3 Prognostic value of mRNA expression of IFI16 in RCC.

Figure 4 highlights the correlation between the level of IFI16 expression and the prognosis in RCC. Higher the level of IFI16 expression suggests lower DFS and OS with no statistical significance in KICH (Figure 4A-B). In KIRC, higher the IFI16 expression suggests the lower DFS with no statistical significance (Figure 4 C), and the lower OS (Figure 4 D) with statistical significance（HR=1.4; P=0.037; Log-rank p=0.037）. In KIRP, the higher the IFI16 expression suggests the lower DFS, and OS (Figure 4E-F) with statistical significance (respectively, HR=1.9, P=0.037, Log-rank p=0.034; HR=2.3, P=0.011, Log-rank p=0.0091).

3.4 Relationship between the transcriptional level of IFI16 and immune infiltrates in RCC.

Through the Timer database, we investigated the relationship between the transcriptional level of IFI16 and immune infiltration in RCC. In KICH, the levels of IFI16 expression correlate negatively with the tumor purity(p=8.11e-10), while there's no significant association between the IFI16 expression and the TIICs

(Figure 5A- KICH).In KIRC and KIRP, the levels of IFI16 expression correlate negatively with the tumour purity(respectively, p=8.11e-10, p=3.41e-10), but correlate positively with the TIICs (all, $p < 0.05$), except macrophages in KIRP(Figure 5A- KIRC/ KIRP). Furthermore, we analyzed the abundant distribution of TIICs under different mutation states of the IFI16 gene (Figure 5B). In KIRC, the main TIICs in the arm-level deletion state of IFI16 gene were B cell, CD4+T cell, Neutrophil and Dendritic cell, while the main TIICs in the high amplification state were Macrophage (all, $p < 0.0001$).

3.5 Gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

To further analyze the specific molecular network of IFI16 in RCC, the co-expression of genes connected with IFI16 functionally was predicted by using the Coexpedia database. As a result, there are 108 genes co-expressed with the IFI16 gene (Figure 6A and Table 2). The co-expression network of IFI16 has performed the functional enrichment analysis. The results were mainly enriched in certain functions, including neutrophil degranulation, phagocytosis, vesicle-mediated transport regulation, secretory granule membrane, cytoplasmic vesicle lumen, vesicle lumen, actin binding, peptide binding and amide binding (Figure 6B). The co-expression network of IFI16 has also performed the functional KEGG enrichment analysis. The results were mainly enriched in certain signal pathways, including tuberculosis, toxoplasmosis, phagosome, leishmaniasis, and Fc gamma R-med8ted (Figure 6C).

4. Discussion

The gene of IFI16 encodes a member of the HIN-200[20] (hematopoietic interferon-inducible nuclear antigens with 200 amino acid repeats) family of cytokines. The encoded protein contains domains involved in DNA binding, transcriptional regulation, and protein-protein interactions. It modulates p53 function and inhibits cell growth in the Ras/Raf signaling pathway. IFI16 is an amplifier of DNA-damage response and involves in cellular senescence and aging-associated inflammatory diseases[21]. Studies have shown that IFI16 is poorly expressed in normal kidney tissue.

In bioinformatics analysis, we explored the mRNA expression of IFI16 are significantly higher in RCC tissues than the normal renal tissues, and the fold change is greater than 2, which strongly suggests that IFI16 is overexpressed in RCC. By comparing with the corresponding normal kidney tissues, the mRNA expression level of IFI16 expresses higher in KKIRC and KIRP tissues, while lower in KICH tissues. These findings are similar to some previous studies. For example, Overexpression IFI16 was observed in cervical squamous cell carcinoma[12] and oral squamous cell carcinoma[13]. Therefore, we also investigated the relationship between the transcriptional level of IFI16 and the tumor stage in RCC. Due to the increasing TNM stage the level of IFI16 expression are increases in KIRC and KIRP. To sum up, it's reasonable to assume that IFI16 acts as an oncogene in the progression of kidney cancer. In this case, IFI16 can be used as a biomarker for early screening in RCC.

In this article, we also explored the relationship between IFI16 expression and survival in RCC. The results indicate the positive correlation between the IFI16 expression with DFS and OS in RCC[13, 22]. Furthermore, our study manifested the levels of expression IFI16 positively correlated with TIICs in KIRC and KIRP, while negatively correlated with the tumor purity in RCC. It is well known that higher the purity of the tumor, better the response to treatment and survival will be. Mast cell density has been reported to be highly correlated with the extent of both normal and pathologic angiogenesis, such as the angiogenesis observed in chronic inflammatory diseases and tumors, including gastric cancer and endometrial cancer[23]. An experimental study has demonstrated that interaction between lung cancer cells and macrophages promotes the invasiveness and matrix-degrading activity of cancer cells[24].To sum up the two results above, IFI16 can act as prognostic biomarker for RCC.

Moreover, due to the association between the TIICs and the tumour prognosis, we investigated the relationship between the transcriptional level of IFI16 and immune infiltration in RCC. The results revealed that the levels of IFI16 expression have a positively correlation with TIICs in RCC, while the negative correlation with macrophages in KICH. Existing studies indicated more TIICs reflects on less response to the treatment and survival. Recently, tumor-associated macrophage infiltration is correlated with angiogenesis and unfavorable prognosis in several kinds of cancer, including gastric cancer, endometrial cancer, and breast cancer[25-27].Combined with the above research results and existing research data, we assume that IFI16 can be used as a biomarker for the diagnosis and prognosis of renal cancer, apart from that it can be used as a potential therapeutic target.

Tumor development and progression are associated with multiple genomic aberrations, which in turn may influence TIICs. In this study, we compared the abundance of TIICs under different mutation states of the IFI16 gene. The result of analysis in KIRC prompt the central TIICs in the arm-level deletion state of IFI16 were B cell, CD4+T cell, neutrophil, and dendritic cell, while the primary TIICs in the high amplification state of IFI16 was macrophages. The high expression of FI16 is positively correlated with the infiltration of macrophages, and the role of macrophages in KIRC require further studies.

Elucidating the underlying mechanism of RCC development is of great significance for the effective treatment of patients with RCC [28]. The co-expression network of IFI16 is used to perform GO and KEGG function enrichment analysis. The results enriched in certain functions including neutrophil degranulation, phagocytosis, the regulation of vesicle-mediated transport, and enriched in certain signal pathways including tuberculosis, toxoplasmosis, phagosome, leishmaniasis and Fc gamma R-med8ted. These results also suggest that IFI16 involves in the immune response and mediates the development of the inflammatory diseases. Therefore, it is essential for us to better understand the interaction between the tumor microenvironment and the TIICs. The characterization of adaptive immune responses seems to become an indispensable prognostic tool in a wide range of cancers and might be more important than the current cancer staging systems[29, 30]. It requires further research and exploration.

This study provides evidence for the significance of IFI16 in renal cell carcinoma and the potential role of novel biomarkers. Our results indicate the association between the IFI16 expression with the

clinicopathological features, prognosis, and immune cell infiltration in RCC. At the same time, we also identified the co-expressed genes of IFI16 in RCC. These genes play an important role in immune-related functions and signaling pathways through the functional enrichment analysis, which suggests the gene of IFI16 is not only a prognosis biomarker for RCC patients but also has the possibility to associate with the immune response to RCC. At the same time, our research has some limitations. On the one hand, our results have not been confirmed by the clinical samples. On the other hand, we did not delve into the mechanism of IFI16 in RCC progression. Our results indicate the association between the IFI16 expression with the clinicopathological features, prognosis, and immune cell infiltration in RCC.

5. Conclusion

As of now, we demonstrated that IFI16 expression can act as a marker of diagnosis and prognosis for patients with RCC, providing important guidelines for the management of RCC patients, which might be associated with the immune infiltration.

Abbreviations

IFI16, Interferon-Inducible Protein 16.

RCC, Renal Cell Carcinoma.

DFS, suggests shorter disease-free survival.

OS, overall survival.

KIRC, Kidney renal clear cell carcinoma.

KIRP, kidney renal papillary cell carcinoma.

KICH, Kidney Chromophobe.

KEGG, Kyoto Encyclopedia of Genes and Genomes.

GO, Gene Ontology.

TIICs, tumor-infiltrating immune cells.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent of publication All authors have reviewed the manuscript and agree to publish it in its current form.

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: Additional Supporting Information may be found in the online version of this article.

Authors' contributions:

conceived and supervised the study: Zhang JD

Analyzed data: Yu BZ

Wrote the manuscript: Yu BZ

All authors read and approved the final manuscript.

Acknowledgements: This study was supported by grants from the National Natural Science of China Programs (8177060840).

References

1. Siegel, R. L.; Miller, K. D.; Jemal, A., Cancer statistics, 2018. *CA Cancer J Clin* **2018,** *68* (1), 7-30.
2. Shuch, B.; Amin, A.; Armstrong, A. J.; Eble, J. N.; Ficarra, V.; Lopez-Beltran, A.; Martignoni, G.; Rini, B. I.; Kutikov, A., Understanding pathologic variants of renal cell carcinoma: distilling therapeutic opportunities from biologic complexity. *Eur Urol* **2015,** *67* (1), 85-97.
3. Srinivasan, R.; Ricketts, C. J.; Sourbier, C.; Linehan, W. M., New strategies in renal cell carcinoma: targeting the genetic and metabolic basis of disease. *Clin Cancer Res* **2015,** *21* (1), 10-7.
4. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D. M.; Forman, D.; Bray, F., Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* **2015,** *136* (5), E359-86.
5. Capitanio, U.; Montorsi, F., Renal cancer. *Lancet* **2016,** *387* (10021), 894-906.
6. Unterholzner, L.; Keating, S. E.; Baran, M.; Horan, K. A.; Jensen, S. B.; Sharma, S.; Sirois, C. M.; Jin, T.; Latz, E.; Xiao, T. S.; Fitzgerald, K. A.; Paludan, S. R.; Bowie, A. G., IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol* **2010,** *11* (11), 997-1004.
7. Sharma, D.; Kanneganti, T. D., The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. *J Cell Biol* **2016,** *213* (6), 617-29.
8. Fujiuchi, N.; Aglipay, J. A.; Ohtsuka, T.; Maehara, N.; Sahin, F.; Su, G. H.; Lee, S. W.; Ouchi, T., Requirement of IFI16 for the maximal activation of p53 induced by ionizing radiation. *J Biol Chem* **2004,** *279* (19), 20339-44.

9. De Andrea, M.; Gioia, D.; Mondini, M.; Azzimonti, B.; Reno, F.; Pecorari, G.; Landolfo, V.; Tommasino, M.; Accardi, R.; Herold-Mende, C.; Landolfo, S.; Gariglio, M., Effects of IFI16 overexpression on the growth and doxorubicin sensitivity of head and neck squamous cell carcinoma-derived cell lines. *Head Neck* **2007,** *29* (9), 835-44.
10. Mazibrada, J.; De Andrea, M.; Ritta, M.; Borgogna, C.; Dell'eva, R.; Pfeffer, U.; Chiusa, L.; Gariglio, M.; Landolfo, S., In vivo growth inhibition of head and neck squamous cell carcinoma by the Interferon-inducible gene IFI16. *Cancer Lett* **2010,** *287* (1), 33-43.
11. Lin, W.; Zhao, Z.; Ni, Z.; Zhao, Y.; Du, W.; Chen, S., IFI16 restoration in hepatocellular carcinoma induces tumour inhibition via activation of p53 signals and inflammasome. *Cell Prolif* **2017,** *50* (6).
12. Imadome, K.; Iwakawa, M.; Nakawatari, M.; Fujita, H.; Kato, S.; Ohno, T.; Nakamura, E.; Ohkubo, Y.; Tamaki, T.; Kiyohara, H.; Imai, T., Subtypes of cervical adenosquamous carcinomas classified by EpCAM expression related to radiosensitivity. *Cancer Biol Ther* **2010,** *10* (10), 1019-26.
13. Kondo, Y.; Nagai, K.; Nakahata, S.; Saito, Y.; Ichikawa, T.; Suekane, A.; Taki, T.; Iwakawa, R.; Enari, M.; Taniwaki, M.; Yokota, J.; Sakoda, S.; Morishita, K., Overexpression of the DNA sensor proteins, absent in melanoma 2 and interferon-inducible 16, contributes to tumorigenesis of oral squamous cell carcinoma with p53 inactivation. *Cancer Sci* **2012,** *103* (4), 782-90.
14. Rhodes, D. R.; Kalyana-Sundaram, S.; Mahavisno, V.; Varambally, R.; Yu, J.; Briggs, B. B.; Barrette, T. R.; Anstet, M. J.; Kincead-Beal, C.; Kulkarni, P.; Varambally, S.; Ghosh, D.; Chinnaiyan, A. M., Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* **2007,** *9* (2), 166-80.
15. Tang, Z.; Li, C.; Kang, B.; Gao, G.; Li, C.; Zhang, Z., GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* **2017,** *45* (W1), W98-W102.
16. Li, T.; Fan, J.; Wang, B.; Traugh, N.; Chen, Q.; Liu, J. S.; Li, B.; Liu, X. S., TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res* **2017,** *77* (21), e108-e110.
17. Yang, S.; Kim, C. Y.; Hwang, S.; Kim, E.; Kim, H.; Shim, H.; Lee, I., COEXPEDIA: exploring biomedical hypotheses via co-expressions associated with medical subject headings (MeSH). *Nucleic Acids Res* **2017,** *45* (D1), D389-D396.
18. Yu, G.; Wang, L.-G.; Han, Y.; He, Q.-Y. J. O. a. j. o. i. b., clusterProfiler: an R package for comparing biological themes among gene clusters. **2012,** *16* (5), 284-287.
19. Wickham, H., *ggplot2: elegant graphics for data analysis*. Springer: 2016.
20. Ludlow, L. E.; Johnstone, R. W.; Clarke, C. J., The HIN-200 family: more than interferon-inducible genes? *Exp Cell Res* **2005,** *308* (1), 1-17.
21. Choubey, D.; Panchanathan, R., IFI16, an amplifier of DNA-damage response: Role in cellular senescence and aging-associated inflammatory diseases. *Ageing Res Rev* **2016,** *28*, 27-36.
22. Cai, H.; Yan, L.; Liu, N.; Xu, M.; Cai, H., IFI16 promotes cervical cancer progression by upregulating PD-L1 in immunomicroenvironment through STING-TBK1-NF-kB pathway. *Biomed Pharmacother* **2019,** *123*, 109790.

23. Ozdemir, O., Can a mast cell subtype (MC(T)) play a role in the progression of endometrial cancer through angiogenesis? *Am J Obstet Gynecol* **2006,** *195* (6), e24; author reply e25.
24. Chen, J. J.; Lin, Y. C.; Yao, P. L.; Yuan, A.; Chen, H. Y.; Shun, C. T.; Tsai, M. F.; Chen, C. H.; Yang, P. C., Tumor-associated macrophages: the double-edged sword in cancer progression. *J Clin Oncol* **2005,** *23* (5), 953-64.
25. Tsutsui, S.; Yasuda, K.; Suzuki, K.; Tahara, K.; Higashi, H.; Era, S., Macrophage infiltration and its prognostic implications in breast cancer: the relationship with VEGF expression and microvessel density. *Oncol Rep* **2005,** *14* (2), 425-31.
26. Koga, J.; Kakeji, Y.; Sumiyoshi, Y.; Kimura, Y.; Shibahara, K.; Emi, Y.; Maehara, Y.; Sugimachi, K., [Angiogenesis and macrophage infiltration in Borrmann type IV gastric cancer]. *Fukuoka Igaku Zasshi* **2001,** *92* (9), 334-9.
27. Hashimoto, I.; Kodama, J.; Seki, N.; Hongo, A.; Miyagi, Y.; Yoshinouchi, M.; Kudo, T., Macrophage infiltration and angiogenesis in endometrial cancer. *Anticancer Res* **2000,** *20* (6C), 4853-6.
28. Rabjerg, M., Identification and validation of novel prognostic markers in Renal Cell Carcinoma. *Dan Med J* **2017,** *64* (10).
29. Galon, J.; Costes, A.; Sanchez-Cabo, F.; Kirilovsky, A.; Mlecnik, B.; Lagorce-Pages, C.; Tosolini, M.; Camus, M.; Berger, A.; Wind, P.; Zinzindohoue, F.; Bruneval, P.; Cugnenc, P. H.; Trajanoski, Z.; Fridman, W. H.; Pages, F., Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **2006,** *313* (5795), 1960-4.
30. Kawai, O.; Ishii, G.; Kubota, K.; Murata, Y.; Naito, Y.; Mizuno, T.; Aokage, K.; Saijo, N.; Nishiwaki, Y.; Gemma, A.; Kudoh, S.; Ochiai, A., Predominant infiltration of macrophages and CD8(+) T Cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. *Cancer* **2008,** *113* (6), 1387-95.

Tables

Table 1. The significant changes of IFI16 expression in transcription level between different types of Kidney Cancer and Kidney tissues (ONCOMINE database)

GENE	Types of Kidney cancer vs. Normal	Fold change	P value	t-test	Ref
IFI16	Clear Cell Renal Cell Carcinoma vs. Normal	4.454	5.12E-12	17.235	Gumz Renal
IFI16	Clear Cell Renal Cell Carcinoma vs. Normal	5.099	6.37E-9	11.758	Yusenko Renal
	Papillary Renal Cell Carcinoma vs. Normal	2.801	3.84E-6	6.052	Yusenko Renal
IFI16	Non-Hereditary Clear Cell Renal Cell Carcinoma vs. Normal	3.617	2.32E-10	9.599	Beroukhim Renal
	Hereditary Clear Cell Renal Cell Carcinoma vs. Normal	3.907	9.39E-10	12.105	Beroukhim Renal
IFI16	Clear Cell Renal Cell Carcinoma vs. Normal	2.160	6.71E-5	5.904	Lenburg Renal
IFI16	Clear Cell Renal Cell Carcinoma vs. Normal	3.863	1.52E-15	12.157	Jones Renal

Table2 coexpression gene list of DAB2IP in COEXPEDIA http://www.coexpedia.org

GENE						
CASP1	DOCK2	LAMA4	PSMB10	RASSF2	C1QA	GMFG
VIM	CXCR4	LAIR1	PLXNC1	RAC2	BIRC3	GBP2
TYROBP	CTSS	KIAA0930	PLEK	RAB31	BID	FYB
TRIM22	CSTA	ITGB2	OLFML2B	RAB27A	ARPC5	FXYD5
TREM2	CORO1A	ITGA4	NNMT	PYGL	ARPC1B	FKBP11
TOP2A	CLEC2B	ISG20	NCKAP1L	PYCARD	ARL4C	FCGR2A
TNFAIP8	CEP170	IL10RA	MYOF	PTPRC	ARHGDIB	FCGR1B
TMSB10	CD86	IGSF6	MSR1	PSMB8	ALOX5	FCER1G
TLR2	CD84	IFNGR1	MS4A6A	LYN	ADGRE2	EVI2B
TIMP1	CD53	IFNAR2	MNDA	LY96	ADA	ENTPD1
TGFBI	CD163	HLA-DRA	MARCKS	LY86	TAGLN2	EMP3
TGFB1	CD14	HLA-DQB1	CAPZA1	LST1	SLC1A3	ELF4
CAV1	CCR5	HLA-DMA	C3	LOXL2	SLAMF8	HCK
CASP4	CAV2	HCLS1	C1QB	LIMS1	SLA	GZMA
LHFPL2	RNASE6	RHOH	LCP2	LILRB2	SAMSN1	GPR65
LAPTM5	RECQL	RCN1				

Figures

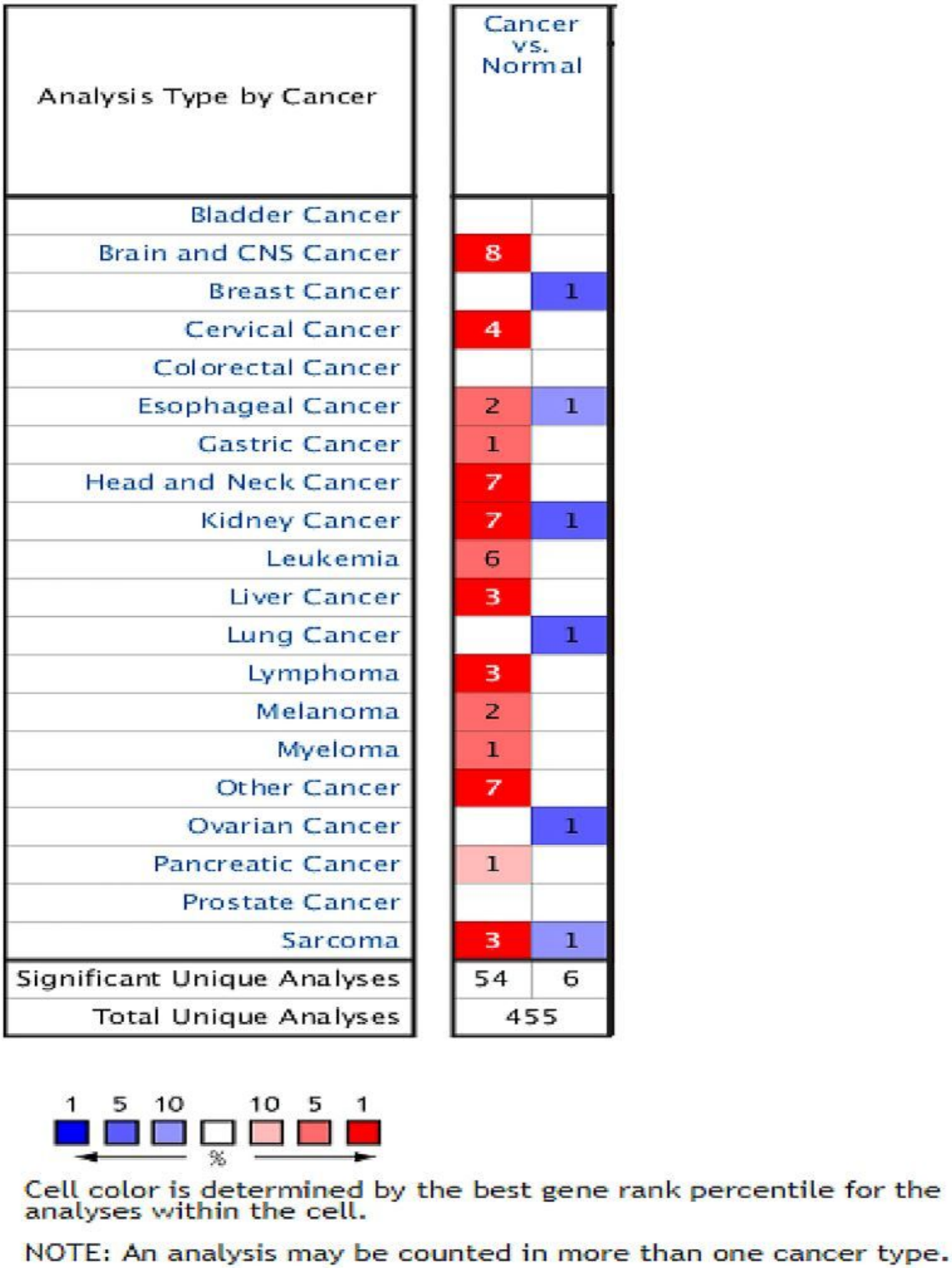

Figure 1

The transcriptional levels of IFI16 in different types of cancers (ONCOMINE).

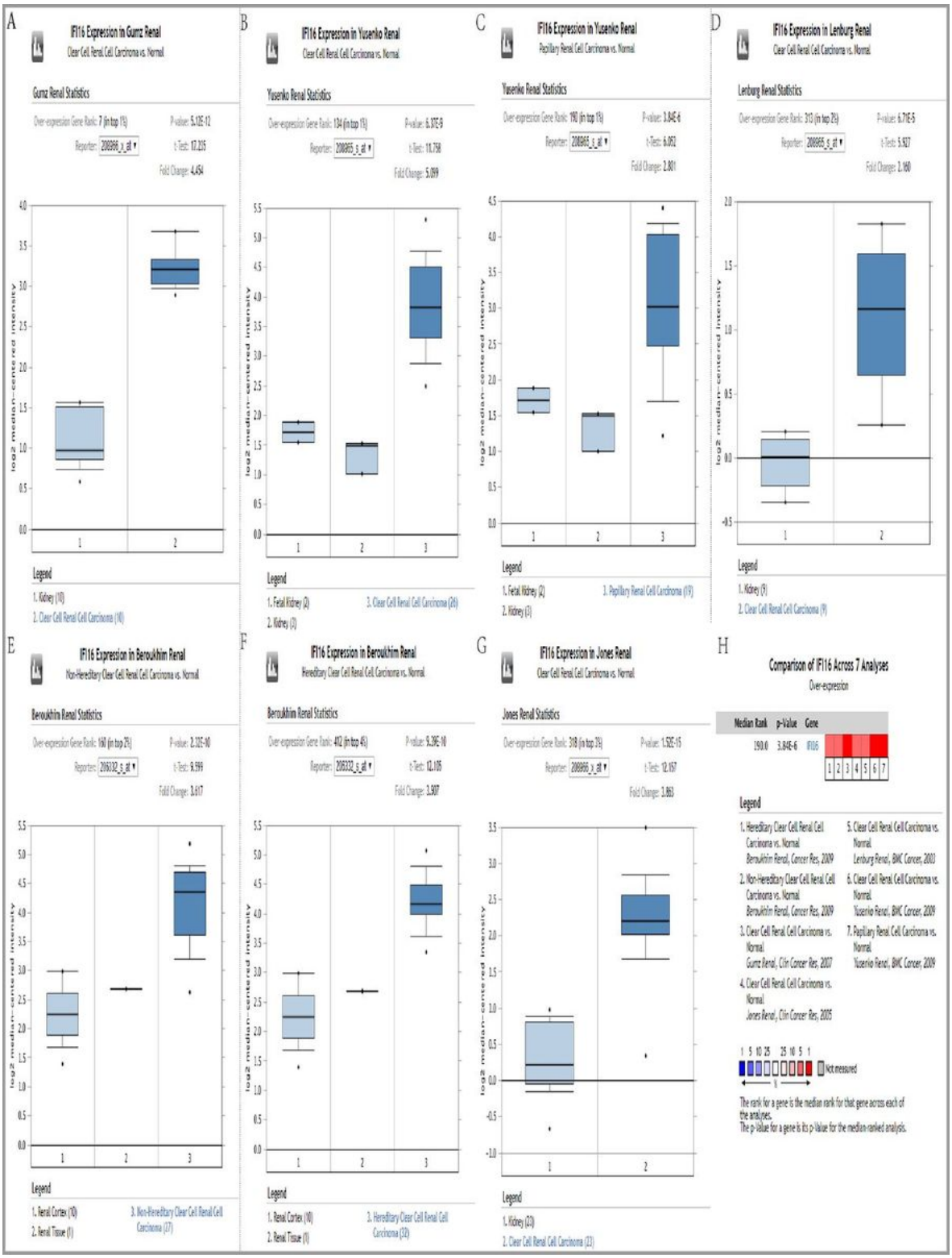

Figure 2

The mRNA expression of IFI16 in RCC tissues and adjacent normal kidney tissues (ONCOMINE).

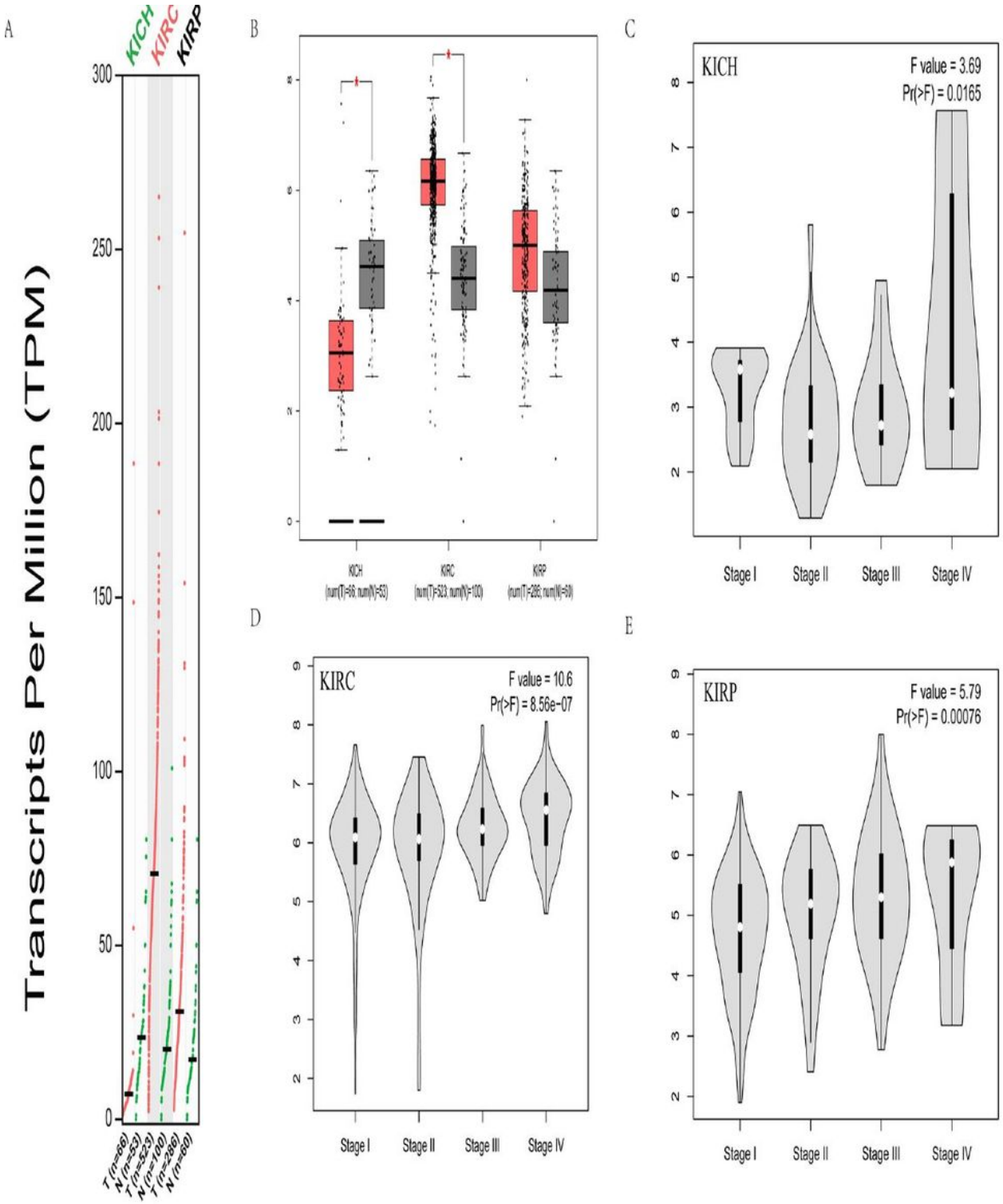

Figure 3

(A-B)The mRNA expression of IFI16 in RCC (GEPIA). * means p is less than 0.05. (C-E): The relationship between IFI16 expression and TNM stage in RCC (GEPIA).

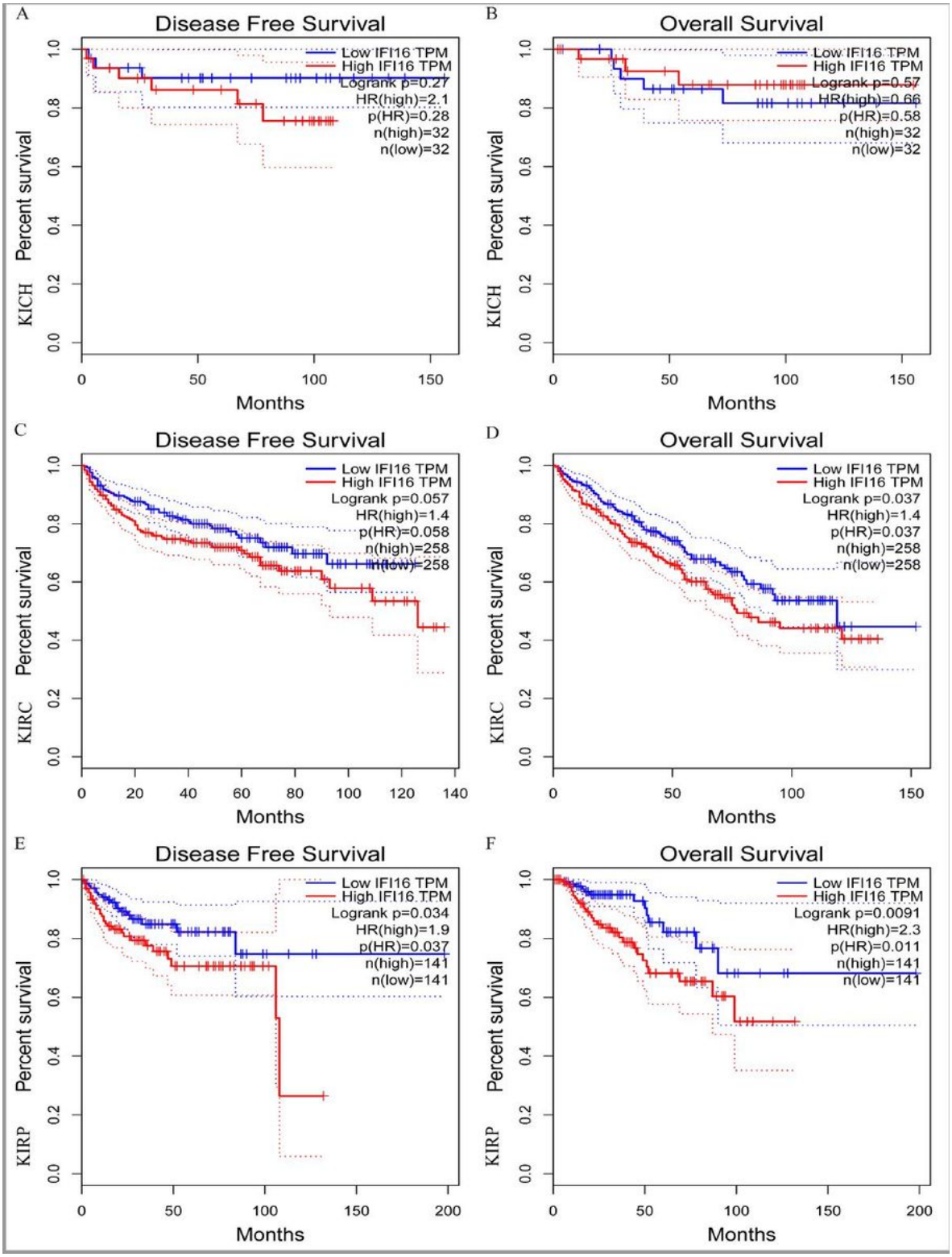

Figure 4

rognostic value of mRNA expression of IFI16 in RCC (GEPIA). (A, B): Correlation of IFI16 expression with DFS and OS in KICH. (C, D): Correlation of IFI16 expression with DFS and OS in KIRC. (E, F): Correlation of IFI16 expression with DFS and OS in KIRP.

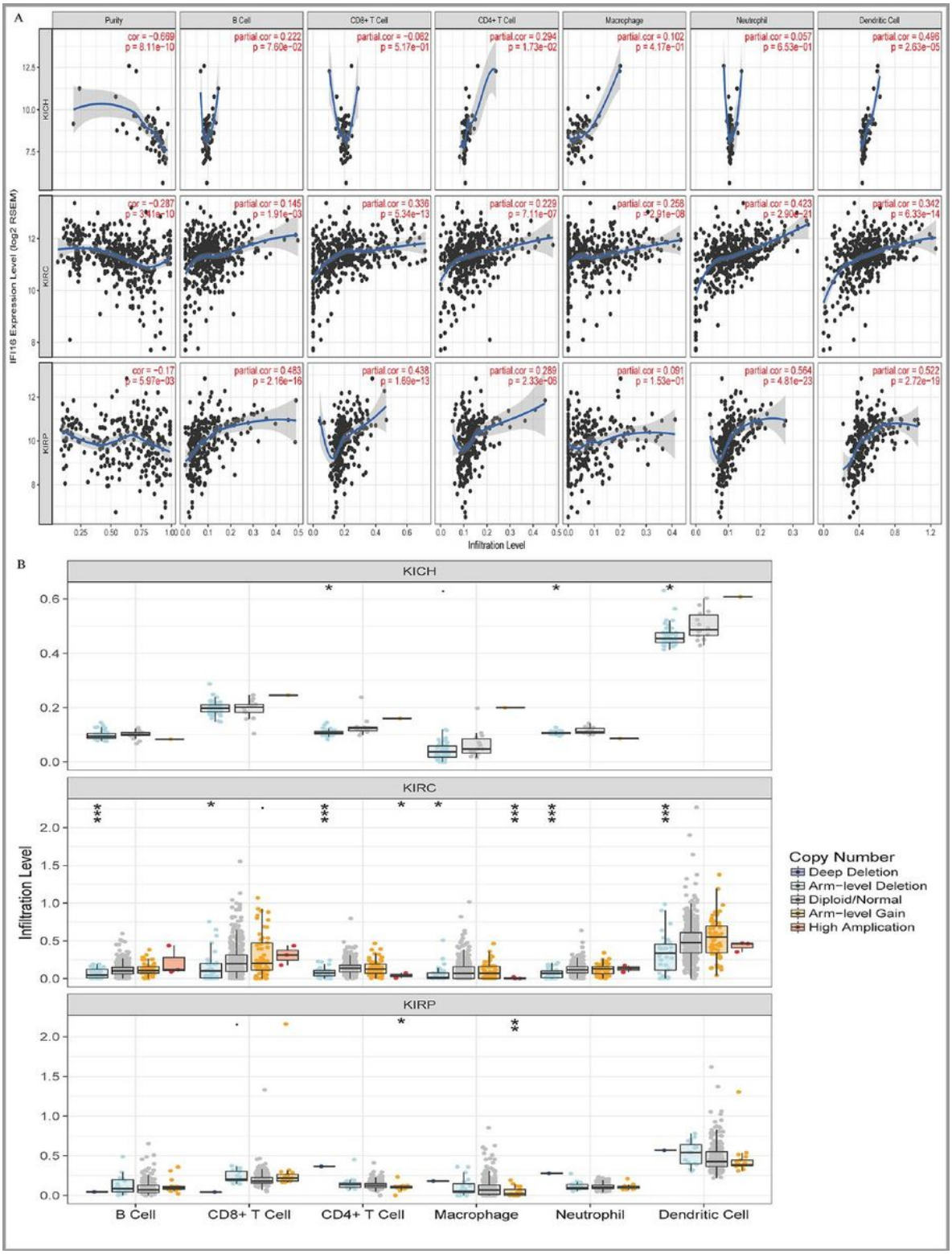

Figure 5

Relationship between the transcriptional level of IFI16 and immune infiltrates in RCC. (A): Relationship between the mRNA expression of IFI16 and immune infiltration. (B): Relationship between somatic cell copy number change (SCAN) and TIICs. P-value Significant Codes: 0 ≤ *** < 0.001 ≤ ** < 0.01 ≤ * < 0.05.

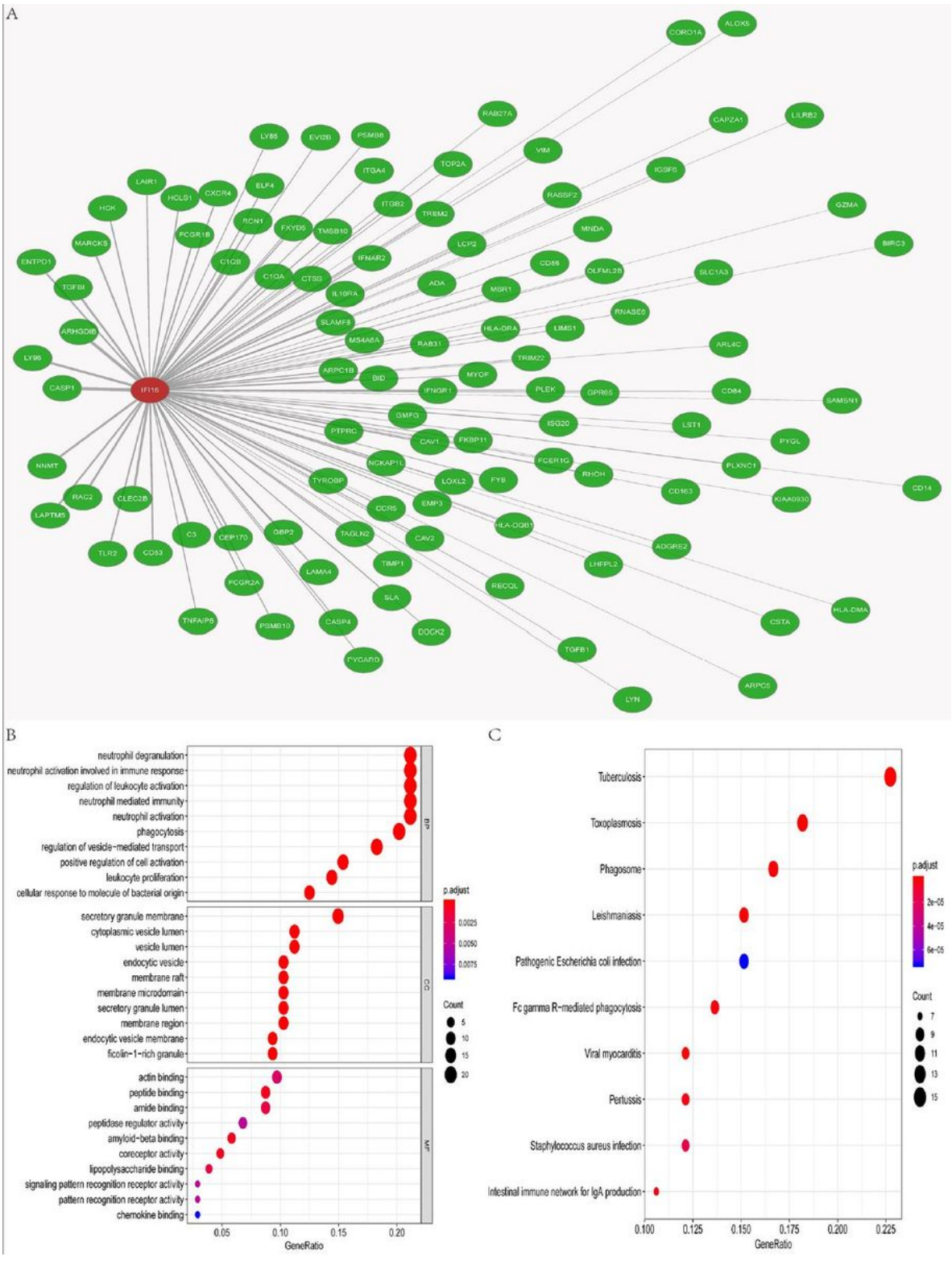

Figure 6

Co-expression network of IFI16 was constructed by Coexpedia and performed function enrichment analysis by GO and KEGG in RCC. (A): Co-expression network of IFI16 in RCC (Coexpedia). Co-expressed genes with IFI6 in RCC are ranked according to Coexpedia's online instructions. (B): Co-expression network of IFI16 was performed function enrichment analysis by GO. (C): Co-expression network of IFI16 was performed function enrichment analysis by KEGG.