Interferon-inducible protein 16 may be a biomarker and prognostic factor in renal cell carcinoma by bioinformatics analysis

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Abstract

Background: Renal cell carcinoma (RCC) is the 9th most common malignancy in Western countries. Due to the development of surgical procedures and the use of novel drugs, survival has been significantly prolonged. However, current challenges include how to diagnose RCC earlier and how to overcome drug resistance. Methods: We explored the relationship between the transcription level of IFI16 and clinical data in RCC through various online databases, including ONCOMINE, GEPIA, HPA, Timer and COEXPEDIA. Results: In comparison with normal tissues, IFI16 mRNA expression levels were higher in kidney renal clear cell carcinoma (KIRC) and kidney renal papillary cell carcinoma (KIRP) tissues. In KIRC, the higher expression of IFI16 was associated with lower overall survival (P = .037). In KIRP, the higher expression IFI16 was associated with lower disease-free survival and overall survival (P = .037 and P = .011). In contrast, the IFI16 expression was negatively correlated with tumor purity in kidney chromophobe, KIRC and KIRP (all P < .05). In KIRC and KIRP, the expression of IFI16 was positively correlated with tumor-infiltrating immune cells (TIICs) (all P < .05), except macrophages in KIRP. In KIRC, the main TIICs were B cells, CD4+ T cells, neutrophils, and dendritic cells, while the main TIICs in the high amplification state were macrophage (all P < .0001). Functional enrichment analysis by gene ontology and Kyoto Encyclopedia of Genes and Genomes highlighted enrichment of neutrophil degranulation, phagocytosis and vesicle-mediated transport regulation, and pathways including tuberculosis, toxoplasmosis, phagosome, leishmaniasis, and Fc gamma R-mediated. Conclusions: IFI16 is overexpressed in RCC and may be an important oncogene in the progression of kidney. In addition, IFI16 may a marker for RCC diagnosis and prognosis, which may be related to immune infiltration. Abbreviations: DFS = disease-free survival, GO = gene ontology, IFI16 = interferon-inducible protein 16, KEGG = Kyoto Encyclopedia of Genes and Genomes, KICH = kidney chromophobe, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, OS = overall survival, RCC = renal cell carcinoma, TIICs = tumor-infiltrating immune cells. Keywords: biomarkers, interferon-inducible protein 16, immune infiltrates, mRNA, prognosis, renal cell carcinoma

1. Introduction

Renal cell carcinoma (RCC) accounts for 2% to 3% of all human malignancies, and is the 9th most common malignancy 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 comprises a group of heterogeneous cancers with varying genetic and molecular alterations that underlie many histological subtypes. Kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), and Kidney Chromophobe (KICH) are the most common pathological types of kidney cancer, accounting for 85% to 90% of all renal malignancies. In total, there are more than 140,000 RCC-related deaths per year.

Due to the development of surgical procedures and the use of novel drugs, survival has been significantly prolonged. However, current challenges include how to diagnose RCC earlier and how to overcome drug resistance. As such, there is an urgent need for more effective biomarkers and therapeutic targets in RCC. Interferon-inducible protein 16 (IFI16) is an AIM2-like receptor that is able to identify the double stranded (ds)DNA of some viruses to activate the STING-TBK1 pathway to produce IFN-β6 for antiviral defense. In addition, IFI16 has been shown...
to bind with DNA to form an inflammasome-cyttoplasmic protein complex. It responds to various physiological and pathogenic stimuli, including pathogen-associated molecular patterns and damage-associated molecular patterns, and promotes caspase-1 activation and the synthesis of pro-interleukin-1β/interleukin-18. IFI16 is essential for the clean-up of pathogens and 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 cancer cell apoptosis.\[9,9\] Mazibrada et al.\[10\] showed that overexpression of IFI16 significantly reduced tumor volume in head and neck cancer, accompanied by a decrease in tumor neovascularization and an increase in tumor necrosis. Wei Lin et al.\[11\] demonstrated that expression levels of IFI16 are lower in liver cancer tissues compared with healthy tissues. In addition, expression of IFI16 in hepatocellular carcinoma cells promoted apoptosis and decreased cell viability. Studies have shown that overexpression of IFI16 can significantly inhibit the growth of liver cancer cells and reduce tumor volume. However, the expression of IFI16 in oral cancer led to the promotion of cell growth and prevented apoptosis.\[12,13\] Several studies suggest that IFI16 not only functions as a tumor suppressor gene but also functions as an oncogene.\[14,15\] Therefore, we sought to better understand the role of IFI16 in kidney cancer. So far, bioinformatics analysis has not been used to analyze the role of IFI16 in RCC. We aimed to investigate IFI16 expression in relation to prognosis and immune cell infiltration in patients with RCC. Our results have important implications for the identification of biomarkers and adoption of targeted therapy in RCC.

2. Methods

We used a multidimensional analysis to study the expression, mutation, regulation, functional network, and immune infiltration of IFI16 in patients with RCC based on public databases. The ethics committee or institutional review board was not formed for this study because we only acquired and analyzed the data from the public online database, and we did not perform the experiments.

2.1. ONCOMINE analysis

ONCOMINE (www.oncomine.org) is the largest oncogene chip database and integrates a global data mining platform.\[16\] It contains 715 gene expression data sets and 86,733 samples. In this study, we analyzed the mRNA expression of IFI16 in RCC using the ONCOMINE 4.5 database. Using the expression of IFI16 in healthy kidney tissues as a reference, the changes in IFI16 expression in RCC tissues were analyzed through ONCOMINE. P < .05 was used as the cut-off for statistical significance.

2.2. GEPIA (gene expression profiling interactive analysis) dataset

GEPIA uses standard processing pipelines to analyze RNA sequencing expression data, and includes data from 9736 tumors and 8587 normal samples from The Cancer Genome Atlas and The Genotype-Tissue Expression 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.\[17\] We analyzed the correlation between the mRNA expression of IFI16 and prognosis and tumor stage in RCC through the GEPIA database.

2.3. The human protein atlas (HPA)

The HPA\[18\] is a freely available database on antibodies that it produces, tests and characterizes with pathology reports.\[41\] HPA was started 10 years ago at the Swedish Royal Institute of Technology (KTH) and Uppsala University with generous funding from the Knut & Alice Wallenberg Foundation. HPA makes it possible to systematically explore the human proteome using antibody-based proteomics, see. The current release (version 10) contains 14,079 genes with protein expression profiles based on 17,298 antibodies.

2.4. TIMER (tumor immune estimation resource) analysis

TIMER (https://cistrome.shinyapps.io/timer/)[19] is a comprehensive database to examine the immune invasion of various malignancies systematically. This data set evaluates the abundance of 6 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 IFI16 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 < .05 as the cut-off criterion statistically significant.

2.5. COEXPEDIA analysis

IFI16 co-expression genes were analyzed using the COEXPEDIA database[20] (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.

2.6. Functional enrichment analysis

Functional enrichment analysis has become a common method for high-throughput omics data analysis. It is useful in the discovery of biological pathways that play a crucial role in biological processes, thereby revealing the basic molecular mechanisms. IFI16 and the co-expression genes in RCC were analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway and Gene Ontology (GO) enrichment analysis through the R language “GGplot2” package and the “clusterProfiler” package.\[21,22\] A P-value < .05 was considered statistically significant.

3. Results

3.1. Transcriptional levels of IFI16 in patients with RCC

In order to determine the difference between IFI16 expression in RCC and normal renal tissues, we analyzed the transcriptional level of IFI16 via the ONCOMINE database. Figure 1 shows the mRNA expression of IFI16 in 20 different cancer tissues.
Compared with corresponding normal tissues, the mRNA expression levels of IFI16 were higher in 82 data sets, and lower in 15 data sets. In a study of RCC, the mRNA expression level of IFI16 was higher in 7 data sets and lower in 3 data sets. As Figure 2 and Table 1 show, the expression of IFI16 in RCC tissues was significantly higher compared with 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 with corresponding normal tissue, the mRNA expression level of IFI16 in KIRC and KIRP tissues was higher, while in KICH tissues was lower (\(P < 0.05\)) (Fig. 3A and B). We analyzed the relationship between mRNA expression of with clinicopathological parameters in RCC by GEPIA. IFI16 mRNA expression was positively correlated with tumor node metastasis (TNM) stage in KIRC (\(P = 8.56\times10^{-7}\)) and KIRP (\(P = 0.00078\)) (Fig. 3D and E). However, there was no correlation between IFI16 expression and TNM stage in KICH, with the highest IFI16 expression in stage 1 tumors, the lowest expression in stage 2 tumors (Fig. 3C).

### 3.3. The expression level of IFI16 in RCC tissue

In order to further verify the results of the GEPIA database, we also obtained matching RCC tissue immunohistochemistry results (Fig. 4) through the HPA database. The results show that the expression level of IFI16 in ccRCC tissue is higher than that of normal kidney tissue, which is consistent with the results of the GEPIA database.

### 3.4. Prognostic value of IFI16 mRNA expression in RCC

The database divides the expression of IFI16 into high and low expression groups based on median expression. Figure 5 high-
lights the correlation between IFI16 expression and prognosis in RCC. The higher the level of IFI16 expression, the lower the disease-free survival (DFS) in KICH. (Fig. 5A) The OS in KICH is positively correlated with the expression level of IFI16. But the result was not statistically significant. (Fig. 5B). In KIRC, the higher the IFI16 expression, the lower the DFS ($P > .05$, Fig. 5C), and OS (hazard ratio [HR] = 1.4; $P = .037$; Log-rank $P = .037$, Fig. 5D). In KIRP, higher IFI16 expression was associated with lower DFS and OS (Fig. 5E and F) (HR = 1.9, $P = .037$, Log-rank $P = .034$ and HR = 2.3, $P = .011$, Log-rank $P = .0091$, respectively).

Figure 2. The mRNA expression of IFI16 in RCC and adjacent normal tissues (ONCOMINE). IFI16 = interferon-inducible protein 16, RCC = renal cell carcinoma.
3.5. 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. For KICH, the levels of IFI16 expression correlated negatively with tumor purity ($P=8.11\times10^{-10}$), while there was no significant association between IFI16 expression and TIICs (Fig. 6A KICH). For KIRC and KIRP, the levels of IFI16 expression correlated negatively with tumor purity (respectively, $P=8.11\times10^{-10}$, $P=3.41\times10^{-10}$), but positively with TIICs (all, $P<.05$), except macrophages in KIRP (Fig. 6A KIRC/KIRP). We also analyzed the distribution of TIICs in relation to mutated IFI16 (Fig. 6B). In KIRC, arm-level deletion of IFI16 was mainly associated with B cells, CD4+T cells, neutrophil, and dendritic cells, while the main TIICs in the high amplification state were macrophage (all, $P<.0001$).

3.6. GO and the KEGG analysis

To further analyze the specific molecular network of IFI16 in RCC, the IFI16 co-expression network of was predicted using the Coexpedia database. We identified 108 genes co-expressed with IFI16 (Fig. 7A and Table 2). Subsequent functional enrichment analysis showed that neutrophil degranulation, phagocytosis, vesicle-mediated transport regulation, secretory granule membrane, cytoplasmic vesicle lumen, vesicle lumen, actin binding, and peptide binding and amide binding were enriched (Fig. 7B). We also performed KEGG enrichment analysis and showed that tuberculosis, toxoplasmosis, phagosome, leishmaniasis, and Fc gamma R-mediated were enriched (Fig. 7C).

4. Discussion

The IFI16 gene encodes a member of the HIN-200\cite{23} (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 via the Ras/Raf signaling pathway. IFI16 is an amplifier of the DNA-damage response that is involved in cellular senescence and aging-associated inflammatory diseases. [24]

RCC patients are usually asymptomatic, and approximately 30% of patients present with local infiltration or distant metastasis at the time of diagnosis. Therefore, patients can be diagnosed and treated early to improve the prognosis of patients by screening the entire risk population for specific tumor markers.

Although the pathogenesis of RCC is still not fully understood, recent data have confirmed that RCC is basically a metabolic disease.[25] Many researchers have studied genes, proteins,[26] or metabolites,[27,28] of biological samples as potential biomarkers. Lucarelli G et al showed that KTR could serve as a marker of ccRCC aggressiveness and as a prognostic factor for CSS and PFS. The inactivation of this pathway may serve as a novel therapeutic target.[29] Lucarelli G et al also found NDUFA4L2 can promote angiogenesis in clear cell RCC. This protein can be used as a marker of ccRCC aggressiveness and a potential new therapeutic target.[30] Massimo Papale et al. indicated that urinary evaluation of RKIP and p-RKIP may permit ccRCC to be distinguished from other urological malignancies and CKD.[31] Gigante M et a. showed that serum αKlotho can predict cancerspecific mortality and the prognosis of patients in progress.[32]

Few people have studied the relationship between IFI16 and RCC and whether it is a potential biomarker in RCC.

Using bioinformatics analysis, we explored the mRNA expression of IFI16 and showed that it is significantly higher in KIRC and KIRP tissue compared with normal tissue, whilst lower expression was observed in KICH tissues. These findings are similar to previous findings, which showed overexpression of IFI16 in cervical squamous cell carcinoma[12] and oral squamous cell carcinoma.[13] Therefore, we investigated the relationship between the transcriptional level of IFI16 and tumor stage in RCC. IFI16 expression increased in KIRC and KIRP with increasing TNM stage. In summary, IFI16 may play an oncogene role in the progression of kidney cancer and may be a biomarker for early screening in RCC.

In this study, we also explored the relationship between IFI16 expression and survival in RCC. The results indicated a positive correlation between IFI16 expression and DFS and OS in RCC.[13,33] Furthermore, we found that IFI16 expression positively correlated with TIICs in both KIRC and KIRP, but negatively correlated with tumor purity in RCC. It is well known that higher the purity of the tumor, the better the response to treatment and survival. 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.[34] An experimental study has demonstrated that interaction between lung cancer cells and macrophages promotes the invasiveness and matrix-degrading activity of cancer cells.[35] To sum up the 2 results above, IFI16 may be used as a prognostic biomarker for RCC.

Moreover, due to the association between TIICs and tumor 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 are positively correlated with TIICs in RCC and negatively correlated with macrophages in KICH. Existing studies indicate that more TIICs are associated with poorer response to the treatment and decreased survival. Recently, tumor-associated macrophage infiltration has been correlated with angiogenesis and unfavorable prognosis in several types of cancer, including gastric, endometrial, and breast.[36–38] Combined with the above results

Figure 4. Immunohistochemical analysis of IFI16 expression levels in normal renal tissues and RCC tissues (The human protein atlas). (A = normal tissue, B = tumor tissue). IFI16 = interferon-inducible protein 16, RCC = renal cell carcinoma.
and existing research data, we speculate that IFI16 may be used as a biomarker for the diagnosis and prognosis of kidney cancer, and may also be a potential therapeutic target.

Tumor development and progression are associated with multiple genomic aberrations, which in turn may influence TIICs. Tumor mutational burden is known to be associated with response to immune checkpoint inhibition in cancer, while mutation status has been shown to be associated with decreased immune infiltrates across many tumor types. Markers expressed by CD8+ T cells and natural killer cells showed the strongest reduction in tumors with low levels of genomic alterations. CD4+ T cell and regulatory T cells (Treg) specific gene expression is also

Figure 5. Prognostic value of IFI16 mRNA expression 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. DFS = disease-free survival, IFI16 = interferon-inducible protein 16, KICH = kidney chromophobe, KIRC = kidney renal papillary cell carcinoma, OS = overall survival, RCC = renal cell carcinoma.
decreased. The expression of dendritic cells and macrophages is also greatly reduced. In this study, we compared the abundance of TIICs in tumor cells expressing different mutated forms of the IFI16 gene. The result suggested that the central TIICs related to arm-level deletion of IFI16 in KIRC were B cells, CD4+T cells, neutrophils, and dendritic cells, while the primary TIICs related to high amplification of IFI16 were macrophages. High expression of IFI16 is positively correlated with macrophages.

Figure 6. Relationship between the transcriptional level of IFI16 and immune infiltrates in RCC. (A): Relationship between IFI16 mRNA expression and immune infiltration. (B): Relationship between somatic cell copy number change (SCAN) and TIICs. P-value significant codes: 0 ≤ \(*\) < .001 \(*\) < .01 \(*\) < .05.
infiltration, and the role of macrophages in KIRC requires further study.

Elucidating the underlying mechanism of RCC development is of great significance for the effective treatment of patients with RCC.\textsuperscript{[39]} We analyzed the co-expression network of IFI16 using GO and KEGG functional enrichment analysis. The results showed enrichment of neutrophil degranulation, phagocytosis, and regulation of vesicle-mediated transport, as well as certain signal pathways including tuberculosis, toxoplasmosis, phagosome, leishmaniasis, and Fc gamma R-mediated. These results suggest that IFI16 is involved in the immune response and mediates the development of inflammatory diseases. Therefore, it is essential to better understand the interaction between the tumor microenvironment and TIIICs. The characterization of

Figure 7. The co-expression network of IFI16 in RCC was constructed by Coexpedia and functional enrichment analysis was performed using GO and KEGG. (A): Co-expression network of IFI16 in RCC (Coexpedia). (B): GO functional enrichment analysis of the IFI16 co-expression network. (C): KEGG functional enrichment analysis of the IFI16 co-expression network. IFI16 = interferon-inducible protein 16, KEGG = Kyoto Encyclopedia of Genes and Genomes, RCC = renal cell carcinoma.
adaptive immune responses seems to become an indispensable prognostic tool in a wide range of cancers and might be more pertinent than current cancer staging systems. However, this will require further research and exploration.

This study provides evidence for the significance of IFI16 in RCC and the potential role of novel biomarkers. Our results indicate the association between IFI16 expression and clinicopathological features, prognosis, and immune cell infiltration in RCC. At the same time, through functional enrichment analysis, we identified co-expressed genes, which play an important role in immune-related functions and signaling pathways. This suggests that IFI16 may not only be a prognostic biomarker for RCC, but may also be associated with the immune response to RCC. We also found that tumors have a unique IFI16 signature compared with other RCC subtypes, by association with TIIC. As such, we consider KICH a benign tumor with good prognosis.

Our research has some limitations. We have not been able to conform our results using clinical samples. In addition, we did not delve into the mechanisms of IFI16 in RCC progression. Our results indicate an association between IFI16 expression and clinicopathological features, prognosis, and immune cell infiltration in RCC.

5. Conclusion

Overexpression of IFI16 in RCC may lead to progression and worse prognosis. In addition, IFI16 may be a marker for RCC diagnosis and prognosis, which may be related to immune infiltration. This study provides new insights for subsequent molecular pathogenesis studies of RCC and new therapeutic targets. Further clinical research is needed to determine the potential prognostic and diagnostic value of IFI16.

Author contributions

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