A prognostic index model for assessing the prognosis of ccRCC patients by using the mRNA expression profiles of AIF1L, SERPINC1 and CES1

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Submitted: 13 October 2021 Accepted: 29 November 2021 Published: 19 January 2022

Abstract

Background: Kidney carcinoma is a major cause of carcinoma-related death, with the prognosis for advanced or metastatic renal cell carcinoma still very poor. The aim of this study was to investigate feasible prognostic biomarkers that can be used to construct a prognostic index model for clear cell renal cell carcinoma (ccRCC) patients. Methods: The mRNA expression profiles of ccRCC samples were downloaded from the The Cancer Genome Atlas (TCGA) dataset and the correlation of AIF1L with malignancy, tumor stage and prognosis were evaluated. Differentially expressed genes (DEGs) between AIF1L-low and AIF1L-high expression groups were selected. Those with prognostic value as determined by univariate and multivariate Cox regression analysis were then used to construct a prognostic index model capable of predicting the outcome of ccRCC patients. Results: The expression level of AIF1L was lower in ccRCC samples than in normal kidney samples. AIF1L expression showed an inverse correlation with tumor stage and a positive association with better prognosis. ccRCC samples were divided into high- and low-expression groups according to the median value of AIF1L expression. In the AIF1L-high expression group, 165 up-regulated DEGs and 601 down-regulated DEGs were identified. Three genes (AIF1L, SERPINC1 and CES1) were selected following univariate and multivariate Cox regression analysis. The hazard ratio (HR) and 95% confidence intervals (CI) for these genes were: AIF1L (HR = 0.83, 95% CI: 0.76–0.91), SERPINC1 (HR = 1.33, 95% CI: 1.12–1.58), and CES1 (HR = 0.87, 95% CI: 0.78–0.97). A prognostic index model based on the expression level of the three genes showed good performance in predicting ccRCC patient outcome, with an area under the ROC curve (AUC) of 0.671. Conclusion: This research provides a better understanding of the correlation between AIF1L expression and ccRCC. We propose a novel prognostic index model comprising AIF1L, SERPINC1 and CES1 expression that may assist physicians in determining the prognosis of ccRCC patients.

Keywords: ccRCC; Prognostic index model; AIF1L; SERPINC1; CES1

1. Introduction

Kidney carcinoma is one of the three malignant tumors of the urinary system. It had a global incidence of approximately 431,000 new cases and was responsible for 179,000 related deaths in 2020 [1]. The incidence of kidney carcinoma is much higher in developed regions such as North America and Europe than in Asia and Africa [1,2]. Renal cell carcinoma (RCC) is the most normal histological subtype of kidney carcinoma and represents approximately 90% of all cases. ccRCC is the most common RCC subtype and accounts for 75% of cases [3]. Although the large majority of early, localized RCC can be cured by surgical treatment, the 5-year overall survival rate for advanced and metastatic RCC (mRCC) is only 5–10% [4]. Molecular-targeted therapeutic drugs such as Vascular endothelial growth factor (VEGF)/Vascular Endothelial Growth Factor Receptor (VEGFR) inhibitors and immunotherapy agents such as PD-1 antibodies have markedly improved the clinical prognosis of mRCC patients [5–8]. However, their long-term benefit for patient survival remains unsatisfactory [9,10]. The complexity of tumor heterogeneity and the clonal evolution of tumors ultimately leads to clinical drug resistance [11]. Advanced or metastatic RCC therefore remains as one of the most treatment-resistant cancer...
types. In order to improve patient outcomes, there is an urgent need for well-defined diagnostic biomarkers that can be used for early detection, risk stratification, and to overcome drug resistance.

EF-hand (EFh) domain-containing proteins have been implicated in malignant progression [12]. Allograft inflammatory factor 1 (AIF1, also referred to as IBA1) contains EFh and plays a critical role in the initiation and progression of cancers [13–18]. AIF1L (allograft inflammatory factor 1-like, also referred to as IBA2) is a homolog of AIF1 [19,20] and has a similar overall structure and molecular function [20]. Nevertheless, the two proteins may have diverse functions, as suggested by the different expression patterns seen in different tissues [21]. AIF1 is preferentially expressed in the spleen, tonsil, lymph node, thymus, and lung [22,23], whereas AIF1L is notably expressed in the kidney. A potential role for AIF1L in tumorigenesis of the kidney and the associated molecular mechanisms have yet to be described.

In the present study, AIF1L was found to be significantly downregulated in ccRCC. A total of 539 ccRCC tumors were clustered according to the median value of AIF1L expression value and separated into AIF1L-high and AIF1L-low expression group. Univariate and multivariate Cox regression analysis were then used to identify differentially expressed genes (DEGs) with prognostic value. A prognostic index model based on the expression levels of AIF1L, SERPINC1, and CES1 was then constructed to predict clinical outcome and to guide treatment.

2. Methods

2.1 Data acquisition and pre-processing

Level three sequencing data and clinical follow-up data for 539 clear cell renal cell carcinoma (ccRCC) samples and 72 corresponding healthy kidney samples was extracted from the TCGA dataset. The Fragments Per Kilobase of exon model per Million mapped fragments (FPKM) expression profile was then converted to Transcripts Per Kilobase Million (TPM) based on the sum of expression of all genes in a sample being 100,000. The microarray gene expression profile and related clinical data for GSE40435 [24], containing 101 pairs of ccRCC and adjacent non-tumor renal tissue, was downloaded and used to validate the results of this study.

2.2 Correlation of AIF1L expression with malignancy, pathological stage, and prognosis

The students t-test was used to evaluate statistical differences in mRNA expression between ccRCC and normal tissues. Similarly, paired t-tests were applied between paired ccRCC and adjacent normal tissues. Differences in AIF1L expression between subgroups of various clinicopathological parameters were analyzed by the Kruskal-Wallis test. Survival curves for AIF1L-low and -high expression groups were plotted by Kaplan-Meier analysis and compared using log-rank tests.

2.3 Functional enrichment analysis based on differentially expressed genes (DEGs)

To identify genes associated with AIF1L expression, DEGs between the AIF1L-high and AIF1L-low groups were selected by the “edgeR” package in R language [25]. The median value for AIF1L expression was used to generate the AIF1L-high and AIF1L-low groups. The fold-change and p values were calculated for each gene. Genes with a log2 fold-change >1 and a p-value < 0.05 were selected as DEGs [26,27]. Functional enrichment analysis including Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) 29 was conducted for these DEGs. Pathways with a p-value < 0.05 were regarded as statistically significant.

2.4 Construction of a prognostic index model

DEGs were further selected according to their prognostic value as determined by univariate and multivariate prognostic index Cox proportional hazard regression models [28]. The expression profiles of selected DEGs were then used to construct a prognostic index model. ccRCC patient samples were classified into high and low groups according to the prognostic index’s median cut-off. Overall survival was studied using Kaplan-Meir analysis. The AUC was calculated to assess discrimination of the prognostic index model in the TCGA samples.

2.5 Statistical analysis

All statistical analyses were conducted using R language. Kaplan-Meier survival and univariate and multivariate analyses were performed using the R package “survival”. ROC curves were plotted using the R package “survival ROC”. In all statistical analyses, significance was accepted at a p-value < 0.05.

3. Results

3.1 AIF1L downregulation in ccRCC correlated with malignancy, advanced tumor stage and poor survival

To investigate the relationship between AIF1L and the malignant phenotype in ccRCC, transcriptome data for AIF1L in the TCGA dataset was analyzed for 539 ccRCC tumors and 72 normal kidney samples. AIF1L mRNA expression was markedly lower in ccRCC tissues compared to normal kidney samples (p-value < 0.001, Fig. 1A). The paired students t-test also showed that AIF1L expression was lower in ccRCC samples compared to matched normal samples (p-value < 0.001, Fig. 1B). Patients with advanced stages of ccRCC had significantly lower levels of AIF1L expression than patients with earlier stages (p-value < 0.001, Fig. 1C). Kaplan-Meier survival curves showed that patients with low AIF1L expression had significantly worse overall survival compared to those with high expression (p-value = 0.042, Fig. 1D). A significant difference was also observed for recurrence-free survival (p-value = 0.0017, Fig. 1E). An independent dataset (GSE40435) was
Fig. 1. Correlation of AIF1L expression with malignancy, tumor stage, and prognosis in ccRCC. (A) Student t-test result for the comparison of AIF1L expression between ccRCC and normal kidney samples from the TCGA dataset. (B) Paired t-test result for AIF1L expression between paired ccRCC and normal kidney samples from the TCGA dataset. (C) Kruskal-Wallis test result for AIF1L expression between Stage I, Stage II, Stage III and Stage IV tumor samples. (D) Overall survival analysis for ccRCC samples from the TCGA dataset with high or low AIF1L expression. (E) Recurrence-free survival analysis for ccRCC samples from the TCGA dataset with high or low AIF1L expression.
Fig. 2. Validation in an independent dataset and DEG analysis. (A) Students t-test result for AIF1L expression between ccRCC and normal kidney samples. (B) Paired t-test result for AIF1L expression between paired ccRCC and normal kidney samples. (C) Students t-test result for AIF1L expression in Stage I–II and Stage III–IV samples. (D) Volcano plot visualizing the DEGs. The data for 539 ccRCC samples and 72 corresponding healthy kidney samples was extracted from the TCGA dataset. The vertical lines demarcate the log2 fold-change values, while the horizontal line marks a $-\log_{10} p$-value of 0.05. Red represents the upregulated genes, while blue represents the downregulated genes. (E) Heatmap for the DEGs. The data for 539 ccRCC samples and 72 corresponding healthy kidney samples was extracted from the TCGA dataset. The samples were divided into two groups based on the median value for AIF1L expression. Abbreviations: DEG, differently expressed genes.
Fig. 3. Construction of a prognostic index model based on the expression level of three genes (*AIF1L*, *SERPINC1*, and *CES1*). (A–B) Kaplan-Meier survival plots for *SERPINC1* and *CES1*. High expression of *SERPINC1* and low expression of *CES1* indicated a poorer prognosis. (C) Detailed information on the low and high prognostic index groups in the TCGA dataset (upper); survival status and survival time for the TCGA ccRCC cohort (lower). (D) Heatmap for *AIF1L*, *SERPINC1*, and *CES1* expression in the TCGA dataset. (E) ROC curve estimating the performance of the prognostic index model for predicting first-year survival in the TCGA dataset. (F) Kaplan-Meier survival plots for high- and low-risk groups in the TCGA dataset.
chosen to validate these results. Students t-test and paired t-tests confirmed the high expression level of \textit{AIF1L} in normal kidney tissue compared to ccRCC samples in the independent GSE40435 dataset ($p$-value < 0.001, Fig. 2A–B). Based on the stages of ccRCC samples from GSE40435 dataset, \textit{AIF1L} expression was also significantly higher in stage I–II tumors compared to stage III–IV tumors ($p$-value = 0.018, Fig. 2C). Taken together, these results indicate that downregulation of \textit{AIF1L} expression correlates with malignancy, advanced tumor stage, and worse patient survival.

3.2 DEGs analysis between \textit{AIF1L}-high and \textit{AIF1L}-low groups in TCGA dataset

The data for 539 ccRCC samples and 72 corresponding healthy kidney samples was extracted from the TCGA dataset. The median value for \textit{AIF1L} expression was used to obtain high- and low-expression \textit{AIF1L} groups. In total, 766 DEGs were identified using “edgeR”, comprising 165 increased and 601 decreased DEGs in the \textit{AIF1L}-high group (Fig. 2D). A heatmap was then plotted to reveal the top 50 increased expression and top 50 decreased expression genes (Fig. 2E).

3.3 Enrichment analysis

GO and KEGG enrichment analyses were conducted to identify involved pathways for the DEGs. Cellular Component (CC) enrichment analysis revealed the DEGs were mainly enriched in signaling pathways such as “collagen-containing-extracellular-matrix”, “blood-microparticle”, “endoplasmic-reticulum-lumen”, and “high-density-lipoprotein-particle” (Table 1). In Biological Process (BP), the DEGs were mainly involved in response pathways such as the “humoral-immune-response”, “antimicrobial-humoral-response”, “negative-regulation-of-peptidase”, “hormone-metabolic-process”, and “negative-regulation-of-endopeptidase-activity”. In Molecular Function (MF), the DEGs were mainly involved in inhibitor and binding activities such as “peptidase-inhibitor-activity”, “endopeptidase-inhibitor-activity”, “serine-type endopeptidase-inhibitor activity”, and “endopeptidase-regulator-activity”. KEGG pathway analysis of DEGs further revealed immune-related pathways and metabolism-related pathways such as “complement-and-coagulation-cascades”, “retinol-metabolism” and “metabolism-of-xenobiotics-by-cytochrome-P450” signaling pathways (Table 2).

3.4 Construction of a prognostic model incorporating novel biomarkers

Univariate and multivariate Cox regression analysis was used to evaluate the prognostic significance of DEGs. Three genes (\textit{AIF1L}, \textit{SERPINC1}, and \textit{CES1}) were selected by this analysis. The hazard ratio (HR) and 95% confidence interval (CI) for these were: \textit{AIF1L} (HR = 0.83, 95% CI: 0.76–0.91), \textit{SERPINC1} (HR = 1.33, 95% CI: 1.12–1.58), and \textit{CES1} (HR = 0.87, 95% CI: 0.78–0.97). Kaplan-Meier survival analysis revealed that high \textit{SERPINC1} expression and low \textit{CES1} expression were associated with worse prognosis (Fig. 3A–B). Given their significant association with prognosis, \textit{AIF1L}, \textit{SERPINC1} and \textit{CES1} were regarded as prognosis-related mRNA signatures in order to develop a prognostic index model. The prognostic index of each patient sample was calculated as follows: prognostic index $= (-0.17) \times \text{AIF1L} + (0.28) \times \text{SERPINC1} + (-0.13) \times \text{CES1}$. The detailed prognostic index, survival status, and mRNA expression values for the three genes are shown in Fig. 3C–D. The performance of the prognostic index model for predicting the first-year survival rate of patients from the TCGA-ccRCC dataset was revealed by AUC analysis to be 0.671 (Fig. 3E). Kaplan-Meier survival analysis showed that patients with a high prognostic index had worse overall survival (Fig. 3F).

4. Discussion

Metastasis is found in 25–30% of ccRCC patients at the initial diagnosis [29,30]. Tumor metastasis results in death in >90% of cases and is thus associated with worse patient prognosis [31]. Cancer cells show an inherent ability to migrate, invade adjacent tissues and enter the vasculature, and thus to eventually metastasize. They crawl along extracellular matrix (ECM) fibers toward blood vessels in the primary tumor. By expanding their pseudopodia, the cancer cells generate a force that pulls the cell body forward and drives cell migration along the fibers at the migration front [32].

EF-hand (EFh) domain-containing proteins are associated with numerous disease states, including chronic inflammation and tumor progression [12]. The \textit{AIF1L} protein structure encompasses two central EFh motifs that lack bound Ca$^{2+}$ [18]. \textit{AIF1L} is expressed at high levels in kidney tissues. Previous research has suggested potential associations between \textit{AIF1L} and podocytes. Other studies have revealed extensive accumulation of \textit{AIF1L} within discrete filopodial protrusions [33]. It is well known that filopodia are associated with migration from the primary tumor, degradation of the basal layer, and intravascular infiltration [34]. \textit{AIF1L} has been reported to inhibit the migration and invasion of breast cancer cells by regulating actin remodeling, with low expression of \textit{AIF1L} being associated with poor prognosis [35]. We reached a similar conclusion in the present study of ccRCC samples. \textit{AIF1L} expression was markedly decreased in ccRCC tissues compared to normal kidney samples. Moreover, the \textit{AIF1L} expression level decreased as the tumor stage increased.

This study also identified two DEGs, \textit{SERPINC1} and \textit{CES1}, that are related to \textit{AIF1L}. Results from the TCGA/GEO dataset and validation of gene expression and survival differences confirmed the prognostic significance of \textit{SERPINC1} and \textit{CES1} in ccRCC. \textit{SERPINC1} (serpin peptidase inhibitor clade C member 1), also referred to as an-
Table 1. Gene Ontology (GO) enrichment analysis of DEGs.

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<th>ID</th>
<th>Description</th>
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<td>GO:0062023</td>
<td>collagen-containing extracellular matrix</td>
<td>&lt;0.001</td>
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<td>GO:0072562</td>
<td>blood microparticle</td>
<td>&lt;0.001</td>
<td>33</td>
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<td>GO:0005788</td>
<td>endoplasmic reticulum lumen</td>
<td>&lt;0.001</td>
<td>40</td>
<td>CC</td>
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<td>GO:0034364</td>
<td>high-density lipoprotein particle</td>
<td>&lt;0.001</td>
<td>10</td>
<td>CC</td>
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<td>GO:0042627</td>
<td>chylomicron</td>
<td>&lt;0.001</td>
<td>7</td>
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<tr>
<td>GO:006959</td>
<td>humoral immune response</td>
<td>&lt;0.001</td>
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<td>GO:0019730</td>
<td>antimicrobial humoral response</td>
<td>&lt;0.001</td>
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<td>GO:0010466</td>
<td>negative regulation of peptidase activity</td>
<td>&lt;0.001</td>
<td>34</td>
<td>BP</td>
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<tr>
<td>GO:0042445</td>
<td>hormone metabolic process</td>
<td>&lt;0.001</td>
<td>31</td>
<td>BP</td>
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<tr>
<td>GO:0010951</td>
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<td>GO:0030414</td>
<td>peptidase inhibitor activity</td>
<td>&lt;0.001</td>
<td>31</td>
<td>MF</td>
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<td>GO:0004866</td>
<td>endopeptidase inhibitor activity</td>
<td>&lt;0.001</td>
<td>30</td>
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<td>GO:0004867</td>
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<td>GO:0061135</td>
<td>endopeptidase regulator activity</td>
<td>&lt;0.001</td>
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<td>GO:0005539</td>
<td>glycosaminoglycan binding</td>
<td>&lt;0.001</td>
<td>32</td>
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Table 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DEGs.

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<td>hsa04610</td>
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<td>hsa00830</td>
<td>Retinol metabolism</td>
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<td>hsa00140</td>
<td>Steroid hormone biosynthesis</td>
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<td>Bile secretion</td>
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<td>hsa00980</td>
<td>Metabolism of xenobiotics by cytochrome P450</td>
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<td>Drug metabolism-cytochrome P450</td>
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<td>hsa04979</td>
<td>Cholesterol metabolism</td>
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<td>hsa05204</td>
<td>Chemical carcinogenesis-DNA adducts</td>
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<tr>
<td>hsa04974</td>
<td>Protein digestion and absorption</td>
<td>&lt;0.001</td>
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</table>

To our knowledge, this is the first report of potential prognostic value for AIF1L, SERPINC1, and CES1 expression in ccRCC patients. The prognostic index model based on the three genes revealed a better performance than each gene alone. The performance of this model for the prediction of first-year survival in the TCGA dataset reached 0.671 using AUC analysis, indicating that it can predict the prognosis of ccRCC patients. One limitation of this study is that the model’s risk score was not compared with other clinical parameters (age, histological grade, and pathological stage) for the prediction of overall survival. Furthermore, this research was conducted using retrospective data available from public databases. Further verification will require prospective clinical trials.

5. Conclusions

AIF1L expression is markedly decreased in ccRCC tissues compared to normal kidney tissues. The expression level of AIF1L decreases with increasing tumor stage. A novel prognostic index model based on AIF1L, SERPINC1 and CES1 expression can predict the prognosis of ccRCC.
patients. This study provides additional insight into the potential role of AIF1L in the development and progression of ccRCC. Our proposed prognostic index model may help physicians in assessing the prognosis of ccRCC patients.

**Abbreviations**

RCC, renal cell carcinoma; ccRCC, cell renal cell carcinoma; PRCC, papillary RCC; ChRCC, chromophobe RCC; VEGF, vascular endothelial growth factor; VEGFRs, vascular endothelial growth factor receptors; PD-1, programmed death 1; EFh, EF-hand; AIF1L, allograft inflammatory factor 1 like; FPKM, Fragments Per Kilobase Million; TPM, Transcripts Per Kilobase Million; GEO, Gene Expression Omnibus; DEGs, differentially expressed genes; GO, Gene Ontology; K-M, Kaplan-Meier; AUC, area under the ROC curve; HR, hazard ratio; CI, confidence intervals; ECM, extracellular matrix; SERPINC1, serpin peptidase inhibitor clade C member 1; ATIII, antithrombin III.

**Author contributions**

Dataset downloading and analyses—SZ, ZC, JC, MS, YC. Conception and Design—AY, RL, SZ, JL, FL. Manuscript writing—YW, YX, JZ, WC, HW. Manuscript revision—HW, JL, FF, ZW, CW, BX. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Acknowledgment**

Not applicable.

**Funding**

This work was supported by grants from the Natural Science Foundation of Zhejiang Province (LQ20H160007), the Natural Science Foundation of Fujian Province (2019J01153), the fourth batch of key discipline construction Project fund of Fujian Medical University Union Hospital, and Startup Fund for scientific research, Fujian Medical University (2019QH1053).

**Conflict of interest**

The authors declare no conflict of interest.

**Availability of data and materials**

The datasets supporting the conclusions of this article are included in this article.

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