Integrative Bioinformatics Analysis Reveals New Prognostic Biomarkers of Clear Cell Renal Cell Carcinoma

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BACKGROUND: The outcome of clear cell renal cell carcinoma (ccRCC) is still unpredictable. Even with new targeted therapies, the average progression-free survival is dismal. Markers for early detection and progression could improve disease outcome.

METHODS: To identify efficient and hitherto unrecognized pathogenic factors of the disease, we performed a uniquely comprehensive pathway analysis and built a gene interaction network based on large publicly available data sets assembled from 28 publications, comprising a 3-prong approach with high-throughput mRNA, microRNA, and protein expression profiles of 593 ccRCC and 389 normal kidney samples. We validated our results on 2 different data sets of 882 ccRCC and 152 normal tissues. Functional analyses were done by proliferation, migration, and invasion assays following siRNA (small interfering RNA) knockdown.

RESULTS: After integration of multilevel data, we identified aryl-hydrocarbon receptor (AHR), grainyhead-like-2 (GRHL2), and KIAA0101 as new pathogenic factors. GRHL2 expression was associated with higher chances for disease relapse and retained prognostic utility after controlling for grade and stage [hazard ratio (HR), 3.47, \( P = 0.012 \)]. Patients with KIAA0101-positive expression suffered worse disease-free survival (HR, 3.64, \( P < 0.001 \)), and in multivariate analysis KIAA0101 retained its independent prognostic significance. Survival analysis showed that GRHL2- and KIAA0101-positive patients had significantly lower disease-free survival (\( P = 0.002 \) and \( P < 0.001 \)). We also found that KIAA0101 silencing decreased kidney cancer cell migration and invasion in vitro.

CONCLUSIONS: Using an integrative system biology approach, we identified 3 novel factors as potential biomarkers (AHR, GRHL2 and KIAA0101) involved in ccRCC pathogenesis and not linked to kidney cancer before.

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ubiquitin protein ligase (VHL)<sup>6</sup> inactivation (3). A number of additional molecular changes have been recently reported in ccRCC at the mRNA, protein, and microRNA (miRNA) levels; although these studies represent a step forward, they focus on only one type of molecule, whereas cancer development necessitates an interaction between different levels of molecular changes. More recently, “integrated genomics” holds the promise of a much better understanding of the pathogenesis of ccRCC through analyzing the interaction between the different levels of molecular changes. Furthermore, integrated analyses could help in overcoming the problem of tumor and patient heterogeneity, a recently recognized challenge in cancer research (4). A number of recent studies have shown the great advantages of the integrating approach in RCC (5) (6, 7).

The goal of this study was to obtain greater knowledge of the complex biological background of kidney cancer tumor development by using a systems biology approach to integrate molecular changes of ccRCC at the mRNA, miRNA, and protein expression levels to identify novel pathways and new pathogenetic factors that can be of clinical utility as prognostic biomarkers or potential therapeutic targets.

Materials and Methods

PATIENT COHORTS

The discovery set included high-throughput gene, miRNA, and protein expression data from 593 ccRCC and 389 normal kidney samples. The first validation set comprised the Cancer Genome Atlas data of 470 ccRCC and 69 normal kidneys (mRNA sequencing data) and 499 ccRCC and 66 normal samples (miRNA sequencing data). For the second validation set, we constructed a tissue microarray of 383 primary ccRCC, and 85 matched normal tissues (Fig. 1A).

Details of the applied data sets, RNA isolation, quantitative PCR, and bioinformatics analysis are presented in Fig. 1, B and C, and the Supplementary Materials and Methods file and Supplementary Table 1 in the Data Supplement that accompanies the online version of this report at http://www.clinchem.org/content/vol60/issue10.

CELL PROLIFERATION, MIGRATION, AND INVASION ASSAYS FOLLOWING siRNA TRANSFECTION

The primary 786-O and metastatic CAHN and Caki-2 kidney cancer cell lines were purchased from American Type Culture Collection. Cells were transfected with 1 of 2 different Locked Nucleic Acid<sup>5</sup> small interfering RNAs (siRNAs), an siRNA against KIAA0101 (Sylencer Select s18861, s18863) (30 nmol/L) (Life Technologies), or a negative-control siRNA (30 nmol/L) using Lipofectamine RNAiMAX (Life Technologies). Transfections were optimized by BLOCK-it fluorescent oligo (Life Technologies). Gene knockdown was verified by quantitative PCR using the previously published primers 5’-AGGTTGTCCTCCATAGATCTG-3’ and 5’-CAGTTGCAAGGACATGCTC-3’ (8) after RNA isolation (miRNAs kit, Qiagen) and by Western blot using a published mouse monoclonal antibody against KIAA0101 (H00009768-M01; Abnova) (9). Cell proliferation/viability was controlled by WST-1 cell proliferation/viability reagent (Roche Applied Science) according to the manufacturer’s protocol, and cell number was counted after trypsinization. Cell behavior was investigated by migration and invasion assays (BD-Bio coat), as previously described, on 8.0-μm chambers (10).
Fig. 1. Study design and analysis pipelines.
(A), Outline of study design. (B), Pathway analysis pipeline. (C), Network analysis pipeline. Coloring on B–C: medium gray: process; light gray: database; white: software. See details in the text.
TISSUE MICROARRAY
Tissue microarrays (TMAs) were constructed from 383 primary cSrc and 85 matched normal tissues. Samples were collected from St. Michael’s Hospital (Toronto, Canada) after we obtained Research Ethics Board approval. For TMA construction, two 1.0-mm cores were obtained from each tumor. Immunohistochemistry was performed using primary antibodies against aryl-hydrocarbon receptor (AHR) (Novus Biologicals), grainyhead-like-2 (GRHL2) (Novus Biologicals), and KIAA0101 (Abnova). The detailed description of TMA construction, immunostaining, and scoring is documented in the online Supplementary Materials and Methods.

Results

PATHWAY ANALYSIS
First, we identified mRNAs, proteins, and miRNAs that are commonly differentially expressed between ccRCC and normal kidney tissues (see online Supplementary Tables 2 and 3). After performing pathway analysis, we found that the most commonly altered signaling were metabolic pathways related to glucose metabolism (“glycolysis”), fatty acid-retinoic acid (“LXR/RXR activation”), and amino-acid metabolism (“valine degradation”) (see online Supplementary Table 4). We also identified “renal cell cancer,” “hypoxia signaling,” and “angiopoietin signaling” among the significant pathways, and “cellular movement” and “cell death and survival” on the basis of the gene expression profile, with the functional terms “renal cancer,” “kidney development,” “angiogenesis,” “migration and proliferation of vascular endothelial cells,” and “vascularization” among BioFunction categories.

“AHR signaling” was also identified as significant (Fig. 2; also see online Supplementary Fig. 1 for a version of this image that can be enlarged). This pathway is involved in xenometabolism, cell cycle, differentiation, and apoptosis, but has not yet been linked to ccRCC pathogenesis; thereby we selected it for further validation.

NETWORK ANALYSIS
We built an interaction network using the most significant molecules. This network is based on an unbiased approach that relies only on molecular interactions and thus differs from pathway analysis that performs gene set enrichment on a predefined gene set. The network structure is formed of basic elements (mRNAs or proteins), designated nodes, and the physical interactions or relationships between these nodes (edges) (11). Interactions are directionally based on their type (such as activation and inhibition). The relationships between different members are predicted by computational algorithms and/or literature-documented experimentally validated interactions (e.g., protein–protein, protein–DNA, RNA–miRNA). The highest-degree nodes based on the number of interactions are generally defined as hubs. In our ccRCC network, 5 nodes were determined as hubs (cyclin D1 [CCND1], cyclin dependent kinase inhibitor 2A [CDKN2A]), v-ets avian erythroblastosis virus E26 oncogene homolog 1 [ETS1], ubiquitin-like modifier [ISG15], and KIAA0101 [KIAA0101]). In the functional annotation of the network members, we found “proliferation of kidney cells” and “angiogenesis” categories to be significant (data are not shown).

Based on directional interactions, a network hierarchy contains 3 (or more) layers of nodes (see online Supplementary Fig. 2A). Genes in the “top” layer(s) are considered to be the master regulators of the network because they are not affected by other nodes, and these genes act as regulators of all others, and as such, they should influence the whole network through their downstream targets. The second, “core” layer(s) usually contain the majority of the hubs, which determine the basic structure of the network. This layer plays a central role in the regulation of signal propagation and modulation (the signal can be enhanced, attenuated, or buffered). Genes in the third, “bottom” layer(s) are directly regulated effector molecules (12). The top-layer genes are potential drug targets, being “directors” of the network and having only a few further connections; therefore, targeting of these molecules should be associated with fewer side effects (13). Regarding our network among top members, the only individual genes were F-box protein (FBXO21) and grainyhead like-2 (GRHL2), whereas the other members were molecular groups/families (Fig. 3), and the core layer was the most abundant layer, containing all of the hubs.

miRNA CONTRIBUTION TO ccRCC PATHOGENESIS
Integrating another level of regulation, we subsequently identified tissue-specific miRNAs that are predicted to target network members (see online Supplementary Table 5). The miRNAs miR-124, miR-139-5p, and miR-204-5p had the most important effect on the expression of network members, with each of them targeting 4 different transcripts (see online Supplementary Fig. 2B). Transcription factor 4 (TCF4), zinc finger E-box binding homeobox 1 (ZEB1), and cyclin D2 (CCND2) were the genes found to be most influenced by these miRNAs.

We also identified 11 miRNAs that are predicted to target the AHR signaling pathway, among these, miR-203–3p and miR-124–3p can target the aryl hydrocarbon receptor (AHR) gene itself (Fig. 2).
VALIDATION OF GENE DEREGULATION IN ccRCC

For validation of our results, we selected 3 critical genes, AHR, which is a major component of AHR signaling that was significantly affected in ccRCC in our pathway analysis, GRHL2, which is located in the top level of the network, and KIAA0101, which is among the highest-degree hubs in our network.

In the first step, we used the Cancer Genome Atlas (TCGA) data set to assess gene expression at the mRNA level of the network, and KIAA0101, which is among the highest-degree hubs in our network.

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We assessed the prognostic significance of AHR, KIAA0101, and GRHL2 in ccRCC at both mRNA and protein levels.

**DIFFERENTIAL EXPRESSION OF AHR, GRHL2, AND KIAA0101 IN ccRCC COMPARED TO NORMAL KIDNEY TISSUE**

ROC analysis showed the potential utility of AHR to distinguish cancer from normal kidney tissues on the basis of mRNA expression levels [area under the ROC curve (AUC) 0.825 with 80% sensitivity and 80% specificity at a cutoff of expression of 1413.4 (read number)] (Fig. 4B). Also, the lower mRNA expression of GRHL2 was highly specific for the tumors [AUC, 0.963 (P < 0.0001); cutoff of expression, 130.2 (read number)] (Fig. 4G). KIAA0101 was also found to be a potential diagnostic biomarker (AUC, 0.956, P < 0.001) [at cutoff of expression 30.9 (read number), 94.4% sensitivity and 91.3% specificity] (Fig. 4L).

**THE IDENTIFICATION OF POTENTIAL PROGNOSTIC MARKERS FOR ccRCC**

We assessed the prognostic significance of AHR, KIAA0101, and GRHL2 in ccRCC at both mRNA and protein levels.

**GRHL2 AS A PROGNOSTIC MARKER IN ccRCC**

Lower mRNA expression levels for GRHL2 were associated with significantly better survival (Fig. 4H). GRHL2 protein expression in cancer tissues is positively associated with tumor grade (P < 0.001) (see online Supplementary Table 7) and with tumor size (GRHL2-positive staining in 19% of tumors <4 cm vs 48% in those ≥4 cm, P = 0.001) (Supplementary Table...
Fig. 3. Hierarchical molecular network of ccRCC.
mRNA level: higher expression was associated with higher tumor stages with 26% positivity in stage 1 tumors compared to 51% in higher stages (P = 0.008) (see online Supplementary Table 8).

In univariate analysis, GRHL2 protein expression was associated with higher chances for disease relapse [hazard ratio (HR), 3.81; 95% CI, 1.54–9.42; P = 0.004] (Table 1). The GRHL2 protein expression association with shorter overall survival, although positive, did not reach statistical significance (HR, 2.79; 95% CI, 0.531–14.67; P = 0.225). In multivariate analysis, GRHL2 retained its prognostic utility after controlling for grade and stage (HR, 3.47; 95% CI, 1.32–9.15; P = 0.012). Kaplan–Meier survival analysis showed that GRHL2-positive patients had significantly lower disease-free survival than those who were GRHL2 negative (P = 0.002) (Fig. 4). No association was found between GRHL2 expression and overall survival (data not shown).

KIAA0101 AS A PROGNOSTIC MARKER IN ccRCC

KIAA0101 had significant influence on prognosis at the mRNA level: higher expression was associated with worse overall survival (P < 0.0003) (see online Supplementary Fig. 3A). On the protein level, no statistically significant association was found between KIAA0101 expression and tumor stage (see online Supplementary Table 7). Univariate analysis showed that KIAA0101-positive patients had worse disease-free survival (HR, 3.64; 95% CI, 2.09–6.31, P < 0.001) (Table 1). Although there was an association between KIAA0101 and worse overall survival, this was not statistically significant (P = 0.094) (Table 1). In the multivariate analysis, KIAA0101 retained its independent prognostic significance after controlling for tumor size and grade (HR, 2.76, P < 0.001) or grade and stage (HR, 2.73, P = 0.002). Kaplan–Meier survival analysis indicated that KIAA0101-positive patients had reduced disease-free survival (P < 0.001) (Fig. 4O). Those patients also had lower overall survival, although this was not statistically significant (P = 0.087) (see online Supplementary Fig. 3B).

AHR AS A PROGNOSTIC MARKER IN ccRCC

At the mRNA level, higher expression of AHRR was associated with better overall survival (Fig. 4C). AHRR protein expression in cancer tissues was negatively associated with tumor grade (P = 0.047) (see online Supplementary Table 7). Univariate and multivariate analyses showed no significant association.
Fig. 4. AHR, GRHL2, and KIAA0101 expression.

(A, F, K), AHR, GRHL2, and KIAA0101 expression in ccRCC vs normal tissue. (B, G, L), ROC analysis for AHR, GRHL2, and KIAA0101. (C, H), Survival analysis of AHR and GRHL2. (D, I, N), AHR, GRHL2, and KIAA0101 immunostaining. (E), AHR protein expression was higher in ccRCC (black boxes) compared to normal tissue (open boxes). (J, O) GRHL2 and KIAA0101 staining is associated with worse prognosis. (M), KIAA0101 Western blot of ccRCC vs normal tissue. *, Statistical significance; N, normal; C, cancer; DFS, disease-free survival; OS, overall survival.
with either progression-free or overall survival (data not shown).

**THE EFFECT OF KIAA0101 ON RCC TUMOR CELL BEHAVIOR**

Because KIAA0101 was one of the most significant hubs (node having the most connection with other members) in our network, we explored its potential effect on ccRCC tumor cell behavior using cell-line models. Loss-of-function experiments were done using KIAA0101-specific siRNA (see online Supplementary Fig. 3, C–E). There was no significant effect of KIAA0101 knockdown on cell proliferation and viability in the primary 786-O and the metastatic ACHN and Caki-2 kidney cancer cell lines (Fig. 5A). However, silencing KIAA0101 significantly reduced cell migration and invasion by 39.8% and 78.8% compared to the nontargeting control siRNA (P < 0.05) (Fig. 5, B–C).

**Discussion**

In this present work we performed a uniquely comprehensive integrative analysis and built a new gene interaction network on the basis of large publicly available data sets assembled from 28 publications, comprising a 3-prong approach with high-throughput mRNA, miRNA, and protein expression profiles of 593 ccRCC and 389 normal kidney samples. For this aim we used Ingenuity Pathway Analysis (IPA) software that employs a curated database [the IPAKB (IPA Knowledge Base)], which contains scientific data from numerous journal articles, textbooks, and other data sources and canonical pathways, similarly to GeneGo or Pathway Studio (14). These databases/software programs can help to translate omics data (e.g., mRNA, miRNA microarray, metabolic, proteomics) by pathway and network analysis using specific directional interactions between protein–protein, protein–DNA, and protein–RNA, drug targeting, and bioactive molecules and their effects.

The main power of this analysis is the large number of samples, the combination of different patient sets and platforms, and the use of 2 different validation sets, in addition to the discovery cohort. Literature data suggest that combination of results from similar biological experiments analyzed on different microarray platforms gives a more complete transcriptomic profile and is more informative than considering them individually (15). It has also been suggested that this kind of combination of studies, by increasing the number of replicates, adds robustness to the experimental design and limits undesired effects and bias in expression profiles, especially when comparing 2 or more platforms” (16). Additionally, the present work relies more on mRNA than protein data, because the numbers of identified proteins of the included studies are relatively smaller than those for mRNA data because of the limitations of the high-throughput protein expression analysis methods used (see online Supplementary Table 1). With this approach we tried to identify those pathways which were significant in the most independent studies as common features, rather than to analyze tumor heterogeneity among individual samples.

**Table 1. AHR, KIAA0101, and GRHL2 expression and patient survival.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Disease-free survival</th>
<th>Overall survival</th>
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<tbody>
<tr>
<td></td>
<td>HR* 95% CI*</td>
<td>HR* 95% CI*</td>
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<tr>
<td>AHR expression</td>
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<tr>
<td>Negative</td>
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<td>1.00</td>
</tr>
<tr>
<td>Positive</td>
<td>1.30 0.596–2.85</td>
<td>1.56 0.550–4.41</td>
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<td>KIAA0101 expression</td>
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<tr>
<td>Negative</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Positive</td>
<td>3.64 2.09–6.31</td>
<td>2.09 0.881–4.99</td>
</tr>
<tr>
<td>GRHL2 expression</td>
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<td></td>
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<tr>
<td>Negative</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Positive</td>
<td>3.81 1.54–9.42</td>
<td>2.79 0.531–14.67</td>
</tr>
<tr>
<td>Tumor size positive</td>
<td>7.02 3.38–14.58</td>
<td>1.93 0.963–3.87</td>
</tr>
<tr>
<td>Grade (ordinal)</td>
<td>4.02 2.49–6.50</td>
<td>3.78 1.92–7.44</td>
</tr>
<tr>
<td>Pathological stage (ordinal)</td>
<td>5.15 3.107–8.54</td>
<td>3.11 1.64–5.92</td>
</tr>
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*a Univariate analysis (n = 385).

*b HR, estimated from Cox proportional hazard regression model.

*c CI of the estimated HR.
Our findings are in keeping with previous data indicating that metabolic pathways are critical in ccRCC pathogenesis (17), and we showed that AHR signaling is altered in ccRCC. We identified and validated AHR as an overexpressed gene that is regulated by the underexpressed miR-124 in ccRCC, an interaction that already has been experimentally validated in other tumors (18). AHR can mediate either tumor-promoting or -inhibiting effects. In mammary tumors, high levels of AHR were detected (19). In addition, overexpression of AHR resulted in increased hepatic and stomach tumor development, and its knockdown reduced tumor growth and metastasis. In contrast, AHR null mice have hyperplastic phenomena (hyperkeratosis of the skin, hyperproliferation of hair follicles, hyperplasia in the gastric pylorus, hyperproliferation of portal blood vessels) and develop liver and lung adenocarcinoma (20). Active AHR expression led to growth inhibition and apoptosis, and caused cell-cycle arrest (21, 22). It was also reported that AHR activation has a promigratory effect in epithelial cells and enhances cancer invasion in urothelial cancer (23). AHR can also represent a therapeutic target. Callero et al. reported that activating AHR with an agonist inhibited cell growth in a dose-dependent manner in RCC cell lines (24). It was also found that sunitinib, a tyrosine kinase inhibitor used for metastatic ccRCC, induced CYP1A1 expression through AHR in breast cancer (25). Aminoflavone, which has an antiproliferative effect on human breast cancer cells mediated by AHR, is also applied in clinical trials as a new anticancer drug. It was found to inhibit cell growth and to induce apoptosis in several but not all renal cancer cell lines as well (24).

By characterizing the ccRCC disease network, we identified the most significant molecules in the pathogenesis as hubs (KIAA0101, CCND1, ETS1, CDKN2A).

Fig. 5. In vitro functional validation of the role of KIAA0101 in RCC cells. (A), KIAA0101 knockdown has no effect on proliferation and viability. (B, C), Inhibition of KIAA0101 function by siRNA significantly decreased the migration and invasion ability of RCC cells (P < 0.05). siKIAA0101, siRNA against KIAA0101.
Among these, CCND1 and ETS1 were already linked to kidney cancer. ETS1 was found to correlate with microvascular density in ccRCC (26). We found that GRHL2 was underexpressed in tumors compared to normal kidney both for mRNA and protein levels and can be a potential prognostic marker based on immuno-staining. GRHL2 is a transcription factor playing roles in development, regulation of epithelial mesenchymal transition (EMT), and restoring the sensitivity to anoikis (27). GRHL2 overexpression was detected in several cancers; however, in certain subclasses of breast cancer, GRHL2 was downregulated (27, 28). This dual, context-dependent effect of GRHL2 might be explained by operation through transforming growth factor β (TGF-β) signaling, which also has both tumor-promoting and -suppressing effects in a context-dependent manner. It was shown that GRHL2 interferes with TGF-β signaling by repressing the ZEB1 promoter and by interfering with SMAD family member 2 (SMAD2)- and SMAD family member 3 (SMAD3)-mediated transcriptional activation (27). Its overexpression suppressed primary tumor growth in xenograft assay and tumor cells sensitized to chemotherapy-induced cytotoxicity (27). In breast cancer, GRHL2 was found to be downregulated in the context of TGF-β/EMT-driven tumor types and its loss was associated with a mesenchymal phenotype (27); thereby, GRHL2 was considered as an “oncogenic restriction point” (27). The role of GRHL2 downregulation in ccRCC has not been investigated, but its target, ZEB1, which is a direct suppressor of GRHL2, is overexpressed in kidney cancer. The GRHL2 targets miR-200a and-b are underexpressed in ccRCC samples (29), which supports the role of GRHL2 in ccRCC. These, and GRHL2 being a top-layer gene in the network, suggest that restoring of the function of GRHL2 (including its downstream targets) may be a new, potentially therapeutic direction in addition to being a potential diagnostic marker. KIAA0101 encodes a PCNA (proliferating cell nuclear antigen)-associated factor, also referred to as PAF15 (proliferating cell nuclear antigen-associated factor). The KIAA0101 protein plays a role in controlling cell-cycle progression through affecting DNA replication. Its role in the cell cycle and DNA damage response was also described (30). The APC/C (cell cycle anaphase-promoting complex/cyclosome) controls degradation of substrate proteins, such as KIAA0101, at mitotic exit and throughout the G1 phase (30). This may explain the relationship between high KIAA0101 expression and cancer. KIAA0101 was found to be overexpressed in several malignancies, such as breast, gastric, hepatocellular, adrenal, lung, and pancreatic carcinoma. We found that KIAA0101 was significantly overexpressed at both the mRNA and protein levels in ccRCC, similarly to other cancers. In line with previous reports, we found that knockdown of KIAA0101 led to reduced migration and invasion ability of kidney cells but did not affect the cell proliferation similarly to adrenal cancer (31). In several cell types, proliferation and migration/invasion are distinct, supporting the “go or grow” hypothesis (32). Our results suggest that KIAA0101 is involved in malignant behavior and development of metastasis of ccRCC cells rather than tumorigenesis. We also showed that the increased expression of KIAA0101 protein was positively associated with poor prognosis, in agreement with recent reports in other tumors (33). Interestingly, circulating KIAA0101 mRNA has also been shown to be a predictive marker for hepatic cancer (33).

MiR-124, miR-139, and miR-204 were downregulated and we considered them to be the most critical miRNAs influencing our ccRCC network having 4 targets each. MiR-124 was characterized as tumor suppressor in several tumor types. It has an essential role in differentiation of neural stem cells and neural progenitor cells (34), and its downregulation promotes growth, invasiveness, and metastasis (35) by targeting laminin, gamma 1 (LAMC1), integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MD2F, MSK12) (ITGB1), cyclin–dependent kinase 6 (CDK6), and SRY (sex determining region Y)-box 9 (SOX9) (36). Similarly, miR-204 loss enhanced migration and stem cell phenotype in vivo (37). MiR-139 expression was shown to differ between different kidney cancer subtypes (38) and was also linked to metastasis and prognosis in ccRCC (39). In our network, miR-139 targets ETS1, TCF4, ZEB1, and CCND2. This miRNA was found to be downregulated in other cancers and target chemokine (C-X-C motif) receptor 4 (CXCR4) and FB murine osteosarcoma viral oncogene homolog (FOS) (40, 41). All 3 miRNAs (miR-124, miR-204, and miR-139) target TCF4 and CCND2. The overexpressed ETS1 and ZEB1 are both targeted by 2 of these three miRNAs. On the basis of their role in the RCC disease network, we propose that these 3 miRNAs have the most important role in ccRCC pathogenesis.

In conclusion, using an integrative systems biology approach, we determined that AHR signaling, and a number of critical molecules, including GRHL2 and KIAA0101, are involved in ccRCC pathogenesis. To the best of our knowledge, these pathogenic factors have not previously been linked to kidney cancer.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.
References