Down-regulation of BCL2L13 renders poor prognosis in clear cell and papillary renal cell carcinoma

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Abstract

Background: BCL2L13 belongs to the BCL2 super family, with its protein product exhibits capacity of apoptosis-mediating in diversified cell lines. Previous studies have shown that BCL2L13 has functional consequence in several tumor types, including ALL and GBM, however, its function in kidney cancer remains as yet unclearly.

Methods: Multiple web-based portals were employed to analyze the effect of BCL2L13 in kidney cancer using the data from TCGA database. Functional enrichment analysis and hubs of BCL2L13 co-expressed genes in clear cell renal cell carcinoma (ccRCC) and papillary renal cell carcinoma (pRCC) were carried out on Cytoscape. Evaluation of BCL2L13 protein level was accomplished through immunohistochemistry on paraffin embedded renal cancer tissue sections. Western blotting and flow cytometry were implemented to further analyze the pro-apoptotic function of BCL2L13 in ccRCC cell line 786-0.

Results: BCL2L13 expression is significantly decreased in ccRCC and pRCC patients, however, mutations and copy number alterations are rarely observed. The poor prognosis of ccRCC that derived from down-regulated BCL2L13 is independent of patients’ gender or tumor grade. Furthermore, BCL2L13 only weakly correlates with the genes that mutated in kidney cancer or the genes that associated with inherited kidney cancer predisposing syndrome, while actively correlates with SLC25A4. As a downstream effector of BCL2L13 in its pro-apoptotic pathway, SLC25A4 is found as one of the hub genes that involved in the physiological function of BCL2L13 in kidney cancer tissues.

Conclusions: Down-regulation of BCL2L13 renders poor prognosis in ccRCC and pRCC. This disadvantageous factor is independent of any well-known kidney cancer related genes, so BCL2L13 can be used as an effective indicator for prognostic evaluation of renal cell carcinoma.

Keywords: BCL-rambo, Cell death, Renal cancer, Prognosis, ANT

Introduction

Kidney cancer remains one of the malignant tumors with the incidence increased notably in recent years [1]. It is composed of heterogeneous subtypes with histological and molecular abnormalities. Clear cell renal cell carcinoma (ccRCC) accounts for about 75% of all the cases, which is mainly characterized as constitutional chromosome 3p deletion, dysfunctional von Hippel-Lindau (VHL) gene and uncontrolled stabilization of hypoxia
inducible factors (HIFs) by molecular features. Papillary renal cell carcinoma (pRCC), which occupies about 15% of the incidence, usually displays loss of chromosome Y, gain of chromosome 7 and/or chromosome 17. Moreover, different molecular characteristics are assigned to each pathological subtype, including both Type I and Type II pRCC [2, 3]. Chromophobe renal cell carcinoma (chRCC) and some other rare types make up the rest.

Mutations of VHL have been proved to be one of the typical genetic causes of kidney cancer, which often lead to stabilization of HIF1α and HIF2α, creating an illusion of pseudohypoxia in stricken renal tissues [4]. The accumulated HIF1α-HIF1β and HIF2α-HIF1β dimers will cause an elevation in growth factors, including platelet-derived growth factors (PDGFs) and vascular endothelial growth factors (VEGFs), which will prompt tumor angiogenesis [5, 6]. The VHL-HIF-VEGF axis represents one of the canonical pathways for renal carcinogenesis, while some other gene mutations or epigenetic changes have also been reported [7, 8], including mutations of polybromo-1 (PBRM1) [9], SET domain-containing 2 (SETD2) [10] and tuberous sclerosis complex 1/2 (TSC1/2) [11, 12]. Nevertheless, nearly 40% of the resected kidney cancer patients are confronted with risk of recurrence, the effective predictive markers are absent so far [13].

BCL-2-like protein 13 (BCL2L13), also termed BCL-rambo, is encoded by the BCL2L13 gene [14]. It has manifested that BCL2L13 participates in drug resistance in several tumors. For example, BCL2L13 was found elevated in tumors like glioblastoma (GBM) and childhood acute lymphoblastic leukemia (ALL) [15, 16]. In GBM, BCL2L13 interacts with ceramide synthases 2 and 6 (CerS2/6), inhibiting the leakage of cytochrome c into cytoplasm [15]. Augmented BCL2L13, which takes part in L-asparaginase resistance, executes as an independent adverse prognostic factor in ALL [16]. On the other hand, BCL2L13 has been demonstrated to interact with adenine nucleotide translocator (ANT, encoded by SLC25A4), a component of mitochondrial permeability transition pore (MPTP), therefore promoting cytochrome c release from mitochondrial intermembrane space to cytoplasm, resulting in activation of the apoptotic caspase cascade [17].

The prognostic value of BCL2L13 in kidney cancer is still unclearly. In this study, we profiled BCL2L13 across 33 cancer types in the Cancer Genome Atlas (TCGA), and found that its mRNA expression is significantly reduced in ccRCC and pRCC. Moreover, low BCL2L13 expression correlates with lessened survival probability of kidney cancer. It poses an attractive hypothesis that BCL2L13 may be a promising prognosis marker for kidney cancer.

Materials and methods

University of California Santa Cruz (UCSC) genome browser and UCSC Xena

Protein–protein interaction (PPI) network analysis of BCL2L13 was performed using UCSC genome browser (https://genome.ucsc.edu), which offers visualized interconnection of the concerned genes [18, 19]. BCL2L13 mRNA and exon expression were completed on UCSC Xena platform (http://xena.ucsc.edu), complied with data from TCGA [20].

Catalogue of Somatic Mutations in Cancer (COSMIC) and Open Targets Platform

The most frequent somatic mutations in ccRCC and pRCC tissues were queried by COSMIC cancer browser (http://cancer.sanger.ac.uk), showing the top 20 candidate genes curated from published research for each cancer type [21]. Heritable kidney cancer-predisposing syndrome related genes are searched from Open Targets Platform (https://www.targetvalidation.org). The overall target-disease association score is the harmonic sum aggregated from genetics, genomics, drugs, animal models and text mining data [22].

University of Alabama Cancer Database (UALCAN) and cBioPortal for cancer genomics

The BCL2L13 mRNA expression and corresponding effect on tumor prognosis were analyzed on UALCAN (http://ualcan.path.uab.edu/) [23–25]. Lymph node metastasis of renal cancer: N0, No regional lymph node metastasis; N1, Metastases in 1 to 3 axillary lymph nodes; N2, Metastases in 4 to 9 axillary lymph nodes. Mutations and copy number alterations (CNAs) of BCL2L13, the impact of these alterations on patients’ overall survival (OS), and BCL2L13 co-expressed genes in kidney cancer were achieved by cBioPortal for cancer genomics studies (http://www.cbioportal.org/) [26]. Spearman’s r>|0.4 was set to sort out the BCL2L13 co-expressed genes in ccRCC and pRCC.

TargetScan and the encyclopedia of RNA interactomes (ENCORI)

TargetScan (http://www.targetscan.org) was engaged to analyze the potential target microRNA (miRNA) in homo sapiens, conformed to the canonical transcript of BCL2L13, which supported by 736 3P-seq tags [27]. Correlation between BCL2L13 and the target miRNA in kidney cancer were analyzed on ENCORI pan-cancer analysis platform (http://starbase.sysu.edu.cn/panCancer.php), upon the expression data from TCGA [28].
Cytoscape and Search Tool for the Retrieval of Interacting Genes (STRING)
Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and hubs of BCL2L13 co-expressed genes were accomplished within Cytoscape, with the plug-in ClueGO, CluePedia, MCODE and CytoHubba, which realized by the grid topology algorithm density of maximum neighborhood component (DMNC) [29–31]. STRING database (https://string-db.org/) was combined for sifting the hub genes [32].

Transient transfection and apoptosis induction
Full-length of human BCL2L13 was cloned into eukaryotic S-tag fusion expression vector pcDNA3-S-tag via EcoRI/XhoI (TaKaRa) restriction sites. The recombinant plasmids were then harvested from Escherichia coli DH-5α. Transient transfection was performed with Lipofectamine 2000 (Invitrogen, 11668019) according to the manufacturer’s instructions.

HEK293T cells, CAKi-1 cells and 786-0 cells were maintained and grown under a humidified atmosphere (37 °C, 5% CO₂), in Dulbecco’s modified Eagle’s medium (HyClone, SH30243.1), supplemented with 10% fetal bovine serum (HyClone, SH30084.03) and 1% penicillin–streptomycin (Gibco, 15070063). Apoptosis was induced in the transfected 786–0 cells by co-treatment of ABT-263 (navitoclax) (YEASEN, 50804ES08) for 24 h right after transfection.

Western blotting
Whole cell extract was prepared, followed by western blotting analysis with the following primary antibodies: anti-PARP (Cell Signaling Technology, 9542), anti-S-Tag (Cell Signaling Technology, 12774), anti-BCL2L13 (Santa Cruz Biotechnology, sc-390598), anti-GAPDH (Affinity, AF7021) and anti-β-tubulin (Affinity, AF7011), all with a 1:1000 dilution. Gel image system (Tanon, version 4.2) was used for optical densitometric analysis.

Immunohistochemistry (IHC)
Paraffin-embedded pathology specimens from 7 patients with ccRCC were used, including renal cell carcinoma tissues and corresponding paracancerous tissues. IHC was carried out following the standard procedure with diaminobenzidine (DAB) detection kit (M XB Biotechnologies, DAB-0031). Anti-BCL2L13 (Protein tech, 16612-1-AP) was diluted 1:100 in 0.1% goat serum PBS solution, incubated at 4 °C overnight.

Apoptotic assay
The optical microscope (Olympus, CKX53) and micro-camera (TUCSEN, DigiRetina 16, TCapture version 5.1) were used to capture the morphological changes of 786-0 cells after treatments. For quantitative assessment, 786-0 cells were harvested after treatments and stained with both FITC Annexin V and PI (BD Pharmingen, 556547), then subjected to flow cytometry (Beckman Coulter, CytoFLEX). Annexin V+/PI−, Annexin V+/PI+ and Annexin V−/PI+ cells were used to represent the types of early apoptotic, late apoptotic and dead cells, respectively.

Statistical analysis
IBM SPSS statistical software (version 24.0) was used for statistical analysis of the experimental data. All other statistical methods come with the web tools by default. Welch’s test was conducted for differential expression of functional mRNA in UCSC Xena. Poisson test was adopted to measure the correlation of a given gene in Open Targets Platform. Transcripts per million (TPM) values and Student’s t tests were employed to calculate the significance of gene expression divergence between categories in UALCAN. The linear dependence of targeted gene pair was evaluated through Spearman’s correlation coefficient and Pearson’s correlation coefficient in cBioPortal. Log-rank test was implemented in both UALCAN and cBioPortal for comparison of survival curves, which displayed as Kaplan–Meier plot. P < 0.05 is considered significant in this entry (*, P < 0.05; **, P < 0.01; ***, P < 0.001).

Results
BCL2L13 mRNA expression is significantly reduced in ccRCC and pRCC
BCL2L13 expresses anomalously in a variety of tumors, participating in tumor progression with its apoptosis-regulating activity [33–35]. The mRNA expression of BCL2L13 was analyzed in a variety of tumors using the data from TCGA. Significant differences were found between quite a few cancer tissues and their normal counterparts, including BLCA, BRCA, CHOL, COAD, HNSC, chRCC, ccRCC, pRCC, LIHC, LUAD, LUSC, READ, THCA, STAD, UCEC (Additional file 1, Additional file 11). BCL2L13 mRNA is highly expressed in chRCC, but has no effect on prognosis. In contrast, significant reduction of BCL2L13 mRNA and exon expression were both found in ccRCC and pRCC primary tumors, when compared to corresponding normal tissues (Fig. 1). Further analysis in ccRCC and pRCC indicated that the reduced BCL2L13 mRNA is independent of patients’ race, gender, age, lymph node metastasis status, clinical stages and tumor subtypes (Fig. 2, Additional file 1). Moreover, down-regulation of BCL2L13 actively impact on the patients’ survival probability (see below). In other cancers not mentioned herein,
the expression differences between tumor tissues and normal tissues were not significant, which might succumb to the corresponding normal tissues are scarcity in TCGA database.

The clinical proteomic tumor analysis consortium (CPTAC) was then engaged to analyze the protein expression level of \textit{BCL2L13} in renal cell carcinoma. The \textit{BCL2L13} protein expression is consistently lower in ccRCC patients compared to healthy crowd, which is independent of the tumor stage (Additional file 2). However, the relevant data is unavailable for pRCC.

Abnormal methylation of genes often plays an important role in cancer progression. DNA methylation of \textit{BCL2L13} coding regions was not altered in renal cell carcinoma samples (Fig. 1A). Moreover, \textit{BCL2L13} promoter methylation was elevated significantly in ccRCC, but not in pRCC (Additional file 3). Specifically, hypermethylation of \textit{BCL2L13} promoter in ccRCC is independent of patients’ age, gender, race, clinical stages, lymph node metastasis status and tumor grade (Additional file 3).

\textbf{Low expression of \textit{BCL2L13} has significant impact on survival probability in ccRCC and pRCC}

To evaluate how \textit{BCL2L13} expression impact on patient survival, ccRCC and pRCC cases were grouped by their \textit{BCL2L13} mRNA levels. \textit{BCL2L13} low expression significantly correlated with poor prognosis in ccRCC ($P=0.0021$, Fig. 3A), which was independent of patients’ gender or tumor grade (Fig. 3C). In pRCC, similar results were observed, albeit to a lesser extent ($P=0.049$, Fig. 3B). \textit{BCL2L13} expression levels had no obvious impact on patient survival of other cancer types aforementioned by UALCAN (Additional file 4).

Somatic mutations and gene CNAs could also influence the prognosis of cancer patients [36]. Through analysis using data from TCGA, \textit{BCL2L13} copy number altered only 0.2% in ccRCC with deep deletion, and 1.1% in pRCC with deep deletion and missense mutations (Additional file 5). Amino acid mutations of \textit{BCL2L13} were only found in pRCC cases, namely, Ser38 was mutated to Leu and Gly182 was mutated to Ser, in which S38L occurred mainly in the patients with shallow deletion of \textit{BCL2L13} copy number (Additional file 5). \textit{BCL2L13} maintained diploid status in ccRCC and pRCC, while the
other genetic changes (gain, shallow/deep deletion) were found (Additional file 5). Because only a few patients carry BCL2L13 genetic changes, the impact of these changes on prognosis of ccRCC and pRCC is undeterminable due to limited patient numbers (Additional file 6).

**BCL2L13 doesn’t correlate with the kidney cancer related genes or putative target miRNA in ccRCC and pRCC**

Previous studies have found that several genes, including VHL, PBRM1 and TSC1, played an important role in tumorigenesis of kidney cancer [37]. To uncover the mechanisms underlying the aggravated poor prognosis driven by BCL2L13 downregulation in ccRCC and pRCC, the correlation between BCL2L13 and these genes were analyzed. BCL2L13 correlated weakly with the most frequently mutated VHL, PBRM1, SETD2 in ccRCC and VHL, KDM5C, SPEN in pRCC respectively (Fig. 4, Additional file 7). Hereditary kidney cancer patients occupy 5–8% in all the diagnosed ones, and commonly harbor some cancer predisposition genes, such as TSC1/2, CDKN1C and DIS3L2 [38, 39]. BCL2L13 showed frail correlation with the top 5 genes TSC1/2, CDKN1C, VHL and DIS3L2, that associated with inherited kidney cancer-predisposing syndrome (Additional file 12, Fig. 4C) [40].

BCL2L13 can be regulated by miRNA, such as miRNA-874-3p, miRNA-124 and -137 [41–43]. Therefore, the correlation between miRNA and BCL2L13 in kidney cancer were analyzed. Those reported candidate miRNA, however, only have weak correlation with BCL2L13 (Additional file 13).

While only weak correlation was uncovered between BCL2L13 and the known kidney cancer related genes, we found that voltage-dependent L-type calcium channel subunit beta-1 (CACNB1) and numb-like protein (NUMBL) are the two BCL2L13 negatively correlated genes. Compared to the downregulation of BCL2L13, CACNB1 and NUMBL were both significantly upregulated in ccRCC and pRCC, and also affected the prognosis of these patients (Additional file 8).

**BCL2L13 regulates metabolism pathway in ccRCC and pRCC**

BCL2L13 has previously reported to participate in several physiological processes. For example, down-regulated BCL2L13 inclined to relieve brain injury induced by ischemia/reperfusion (I/R) [42]. Mitochondrial dynamics and biogenesis, which is also regulated by BCL2L13, could facilitate the browning process of preadipocytes.
Moreover, low expression of BCL2L13, which was related to weakened oxidative phosphorylation and enhanced glycolysis, was often found in cancer cells [45, 46]. Thus, the cellular functions of BCL2L13 might have latent impact on the poor prognosis of ccRCC and pRCC.

Co-expressed genes of BCL2L13 were investigated for comprehensive grasp [47]. Filtered by |Spearman’s r| > 0.4, 519 and 1318 BCL2L13 co-expressed genes were found in ccRCC and pRCC respectively (Fig. 5A, Additional file 14). KEGG pathway enrichment analysis manifested the physiological characteristics of these genes, including Huntington disease, citrate cycle and substance metabolism for ccRCC (Fig. 5B), and oxidative phosphorylation, citrate cycle, substance metabolism and Huntington disease for pRCC (Fig. 5C). No substantial differences were found between ccRCC and pRCC. While many of these pathways of BCL2L13 co-expressed genes are related to citrate cycle and substance metabolism, these data suggested that BCL2L13 regulated energy and metabolism might be involved in its prognostic role in ccRCC and pRCC patients. ccRCC was selected for further mechanism exploration, because of the more significant prognostic role of BCL2L13 low expression in this kind of cancer.

BCL2L13 has positive correlation with SLC25A4 (ANT) in ccRCC

The PPI network and hub genes were analyzed for BCL2L13 regulated metabolism, and a small-scale of PPI network was found based on this analysis (Fig. 6A). First, a PPI of BCL2L13 and HSP60 (heat shock protein 60, encoded by HSPD1) was found, because BCL2L13 is anchored on the outer mitochondria membrane, while HSP60 is distributed in the mitochondrial matrix, resulting in a fluorescence co-localization [17]. Second, BCL2L13 had PPI connections with UBC, GABARAPL2 and APP respectively, implying that it may engaged in mitophagy [48–51]. Third, BCL2L13 was reported to interact with ANT, the protein localized in mitochondrial inner membrane. DMNC, which can be employed for some covert hubs, was further used for the essential genes calculating in Cytoscape, for its higher hit rate to
key nodes compared to general degree method [52]. Through this analysis, SLC25A4 was found to be one of the senior hub genes that mediate physiological activity of BCL2L13 (Fig. 6B).

ANT is in charge of exchanging ADP for ATP through MPTP, executing a fundamental role in mitochondrial respiration [53]. ANT could induce mitophagy independent of its ADP/ATP exchange activity, though inhibition or ablation of ANT culminate in disparate phenotypic mitophagy [54]. Studies have evinced that ANT substantially interacts with BCL2L13, mediating its pro-apoptotic activity, while further analysis also supported their correlation (Fig. 6C) [17, 55, 56].

**BCL2L13 overexpression promote apoptosis in ccRCC cells**

786-0 cells were used to study the pro-apoptotic activity of BCL2L13. Compared to HEK (human embryonic kidney) 293 T cells, the expression of BCL2L13 is much lower in clear cell renal cell carcinoma cell line 786-0 (Fig. 7A), consistent with the silico-based analyses. Moreover, the IHC results also indicated a low expression of BCL2L13 in tumor tissues (Fig. 7B). After transient transfection for 48 h, BCL2L13 induces apoptosis in 786-0 cells, characterized by increased cleavage of poly-(ADP-ribose) polymerase (PARP) and also the percentage of Annexin V/PI positive cells (Fig. 7C–D, Additional files 9, 10). BCL2/BCLxL/BCLw inhibitor ABT-263 was used to induce mitochondrial mediated apoptosis [57]. Under the treatment of ABT-263 on BCL2L13 overexpressed 786-0 cells, cleaved PARP and the proportion of Annexin-V/PI positive cells were more apparent (Fig. 7C–D, Additional files 9, 10). These data hint that BCL2L13 performs pro-apoptotic function in ccRCC cells.

**Discussions and conclusions**

BCL2L13 belongs to BCL2 protein family, and possesses complete BCL2 homology (BH) 1–4 domains. The BHNo domain embedded between BH regions and the C-terminal transmembrane motif endows BCL2L13 with some
Fig. 5  Enrichment analysis of BCL2L13 co-expressed genes in ccRCC and pRCC. A Co-expressed genes of BCL2L13 were sorted out by |Spearman’s r| > 0.4, 591 target genes in ccRCC and 1318 target genes in pRCC were found respectively. B–C KEGG pathway enrichment analysis of BCL2L13 co-expressed genes in ccRCC (B) and pRCC (C), filtered by P ≤ 0.05.

Fig. 6  BCL2L13 exhibits strong correlation with SLC25A4 (ANT). A Protein–protein interaction network analysis of BCL2L13. B Hubs of BCL2L13 co-expressed genes in ccRCC. C BCL2L13 has high correlation with SLC25A4 in ccRCC. The linear dependence was evaluated by Spearman’s correlation and Pearson’s correlation analysis.
non-canonical characteristics [58–60]. BCL2L13 was reported to induce mitochondria fragmentation that in favor of the caspase activation cascade [55]. In addition, BCL2L13 has been shown to accelerate mitophagy by binding to microtubule-associated protein 1 light chain 3 (MAP1LC3), suggesting a role as a mitophagy receptor for the quality control of mitochondria [60, 61]. BCL2L13 is reported to promote apoptosis, but this function doesn’t relate to any of its four BH domains [14, 17]. The pathological action of BCL2L13 will be the next focal point in this specialism.

Declining BCL2L13-directed poor prognosis in ccRCC is independent of patients’ gender or tumor grade. On the other hand, in ccRCC and pRCC, BCL2L13 has weak correlation with the genes mutated in kidney cancer or the genes associated with inherited kidney cancer predisposing syndrome [62, 63], as well as the BCL2L13 related miRNA. That miRNA may not the proximate cause of reduced BCL2L13 in cancerous renal tissues. Although BCL2L13 does not show strong correlation with these genes or miRNA, SLC25A4 (ANT) exhibits high correlation with BCL2L13, supposed to play as one of the hubs involved in BCL2L13-mediated prognostic consequence of kidney cancer [17]. And the attenuated BCL2L13-ANT pathway may be a possible reason for poor prognosis of ccRCC, that is different from its performance in GBM cells [15].

In addition, less genetic alterations of BCL2L13 in ccRCC and pRCC are observed, complied with the data from TCGA, suggesting that BCL2L13 is relatively genetic stable in kidney cancer patients. Shallow deletion of the copy number in a fraction of patients may result in the low expression of BCL2L13 in pRCC, while promoter hypermethylation of BCL2L13 probably accounts for the low expression in ccRCC. Similar functions of BCL2L13 are presented in ccRCC and pRCC by KEGG pathway enrichment analysis. However, it remains an attractive hypothesis that BCL2L13 maintains a linear dose–effect relationship with its physiological activity.

Taken together, BCL2L13 may act as an independent and desirable prognostic marker for ccRCC and pRCC. In addition to that, the functions of CACNB1 and NUMBL seem antagonistic toward BCL2L13 activity, while further
experimental trial on them may provide new remedies for renal cancer patients.

**Supplementary Information**

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**Additional file 1:** BCL2L13 mRNA expression was evaluated in pan-cancer and corresponding normal tissues, including chRCC, ccrCC and pRCC (A). BCL2L13 mRNA expression is significantly reduced, independent of tumor grade in ccrCC (B) or body mass in pRCC (C). Student’s t tests were employed to calculate the P value.

**Additional file 2:** Protein expression of BCL2L13 was evaluated in several tumor types (A). It is significantly reduced in ccrCC (B), independent of tumor stage (C), with the data from CPTAC. Student’s t tests were employed to calculate the P value.

**Additional file 3:** BCL2L13 promoter methylation is significantly elevated in ccrCC, but not in pRCC (A). Hypermethylation of BCL2L13 promoter in ccrCC is independent of patients’ age (B), gender (C), race (D), clinical stages (E), lymph node metastasis status (F) and tumor grade (G). Student’s t tests were employed to calculate the P value.

**Additional file 4:** BCL2L13 has no effect on survival of BLCA, BRCA, CHOL, COAD, HNSC, KIRP, LAML, LIHC, LUAD, LUOG, MESO, READ, SKCM, THCA, UCEC, STAD and UVM, evaluated by UALCAN. Log-rank test was implemented for the significance.

**Additional file 5:** Genetic alterations of BCL2L13 occurred in ccrCC (A, upper) and pRCC (A, bottom). 2 missense (variants of uncertain significance, VUS) somatic mutations of BCL2L13 occurred in a few pRCC patients (B). Copy-number alterations of BCL2L13 in ccrCC (upper) or pRCC (bottom) were supported by GISTIC (genomic identification of significant targets in cancer), mutations were indicated as indicated (C).

**Additional file 6:** Kaplan–Meier survival plot of ccrCC (left) and pRCC (right) that grouped by BCL2L13 mutations & copy number alterations.

**Additional file 7:** Top 20 most frequently mutated genes in ccrCC (left) and pRCC (right).

**Additional file 8:** CACNB1 and NUMBL were both significantly upregulated in ccrCC (A) and pRCC (B), and affect the prognosis of patients (C, D), analyzed by UALCAN with the data from TCGA. Student’s t tests were employed to compute the P value.

**Additional file 9:** Status of 786–0 cells were captured under microscope (×10 objective): transient transfection of vehicle for 48 h (A), transient transfection of BCL2L13 for 48 h (B), vehicle transient transfection followed by ABT-263 (5.5 μM) treatment for 24 h (C) and BCL2L13 transient transfection followed by ABT-263 (5.5 μM) treatment for 24 h (D).

**Additional file 10:** Uncropped gel images from Fig. 7 A and 7C.

**Additional file 11:** Expression of BCL2L13 in pan-cancer.

**Additional file 12:** Top 25 genes associated with inherited kidney cancer predisposing syndrome.

**Additional file 13:** Correlation of the target miRNA and BCL2L13 in ccrCC, pRCC.

**Additional file 14:** BCL2L13 co-expressed genes in ccrCC, pRCC.

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**Authors’ contributions**

Study design: HD and FM. Data collection: FM and LZ. Contribution of new reagents or analytical tools: KY, WG and YL. Data analysis: HD, JX, FM, HW, ZZ, M2 and WY. Manuscript preparation: HD, FM and JX. All authors read and approved the final manuscript.

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**Availability of data and materials**

The data that support the findings of this study were obtained from open access database indicated in “Materials and Methods”, all the data are available from the corresponding author upon reasonable request.

**Declarations**

**Ethics approval and consent to participate**

No animal experiments or clinical trials were conducted, so we state that there is no ethical problem in this study.

**Consent for publication**

Not applicable.

**Competing interests**

No conflict of interests related to this research.

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