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In silico repurposing the Rac1 inhibitor NSC23766 for treating PTTG1-high expressing clear cell renal carcinoma

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ABSTRACT

The pituitary tumor-transforming gene 1 (PTTG1), also known as Securin, is considered an oncogene. This study aimed to investigate the role of PTTG1 in clear cell renal cell carcinoma (ccRCC) using *in silico* bioinformatics approaches. A pan-cancer analysis using The Cancer Genome Atlas (TCGA) data indicated that among all cancer types copy number amplification of PTTG1 gene was most frequently found in ccRCC. However, amplification of PTTG1 gene copy number did not correlate with the increase of mRNA level in ccRCC, and did not predict the patients' overall survival. Instead, ccRCC was correlated with overexpression of PTTG1 mRNA, and its expression level was stage-dependent increased in cancer patients. An outlier analysis using the Oncomine database suggested that PTTG1 mRNA expression served as a good biomarker for ccRCC. Pathway analysis for upregulated genes enriched in PTTG1-high expressing ccRCC patients found that PTTG1 overexpression was associated with mitotic defects. Mining drug sensitivity data using the Cancer Therapeutics Response Portal (CTRP) discovered that PTTG1-high expressing ccRCC cell lines were susceptible to a Rac1 (Ras-related C3 botulinum toxin substrate 1) inhibitor NSC23766. Therefore, this study provides an *in silico* insight into the role of PTTG1 in ccRCC, and repurposes the Rac1 inhibitor NSC23766 for treating PTTG1-high expressing ccRCC.

1. Introduction

Renal cell carcinoma (RCC) belongs to the most common type and accounts for more than 90% of kidney cancers. RCC can be categorized into more than 10 histological and molecular subtypes, of which clear cell RCC (ccRCC) accounts for about 75˜80% of all RCC cases. Other

Abbreviations: APC, anaphase promoting complex; AR, androgen receptor; AURK, aurora kinase; BAP1, BRCA1-associated protein-1; ccRCC, clear cell renal cell carcinoma; CDC20, cell division cycle 20; CDK1, cyclin-dependent kinase 1; chRCC, chromophobe renal cell carcinoma; CMap, Connectivity Map; COL23A1, collagen type XXIII alpha 1 chain; CTRP, Cancer Therapeutics Response Portal; EGFR, epidermal growth factor receptor; FoxM1, forkhead box M1; GEPIA, Gene Expression Profiling Interactive Analysis; HIF, hypoxia-inducible factor; KIRC, kidney renal clear cell carcinoma; LOX, lysyl oxidase; MoA, mechanisms of action; mTOR, mammalian target of rapamycin; NF-Y, nuclear factor-Y; PD-1, programmed cell death protein 1; PDGFR, platelet-derived growth factor receptor; PLK1, polo-like kinase 1; pRCC, papillary renal cell carcinoma; PTTG1, pituitary tumor-transforming gene 1; Rac1, Ras-related C3 botulinum toxin substrate 1; RCC, Renal cell carcinoma; SETD2, SET domain containing 2; STAT3, signal transducer and activator of transcription 3; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; SQSTM1, sequestosome 1; SREBP1c, sterol regulatory element-binding protein 1c; TCF, β-catenin/T cell factor; TCGA, The Cancer Genome Atlas; TGFBI, transforming growth factor, beta-induced gene; TKI, tyrosine kinase inhibitor; VCAN, versican; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; VHL, Von Hippel-Lindau

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major subtypes are papillary RCC (pRCC) and chromophobe RCC (chRCC) with the incidence of 7˜14% and 6˜11%, respectively [1–3]. About 90% of ccRCC harbor genetic defects of the Von Hippel-Lindau (VHL) tumor suppressor gene, the negative regulator of hypoxia-inducible factor (HIF) proteins [2]. Consistently, upregulation of HIF-responsive genes mediates angiogenesis and tumor growth. Therefore, current therapies for patients with advance disease include anti-angiogenesis agents such as multi-targeted tyrosine kinase inhibitors (TKIs) and vascular endothelial growth factor (VEGF) antagonists, and mammalian target of rapamycin (mTOR) inhibitors. In addition, immune checkpoint inhibitors such as programmed cell death protein 1 (PD-1) inhibitors are also emerged as effective therapeutic options [3]. Because the 5-year survival rate for advance ccRCC is still very low (about 11%) [4], it is still urgent to develop better therapeutic strategy.

The pituitary tumor-transforming gene 1 (PTTG1), also known as Securin, is initially isolated from rat pituitary tumor cells [5]. PTTG1 functions in the regulation of sister chromatid separation [6], DNA damage/repair [7], gene transcription [8], organ development and metabolism [9–11]. PTTG1 is considered an oncogene. It is frequently overexpressed in human cancers and correlates with tumor invasiveness and poor prognosis [12]. Accumulating evidences support an essential role of PTTG1 in the drug sensitivity of cancer cells. For example, PTTG1 loss enhances colon cancer cells' sensitivity to ionized radiation, doxorubicin, and histone deacetylase inhibitors [13,14]. In contrast, high PTTG1 expression is associated with the resistance of cancer cells to an epidermal growth factor receptor (EGFR) inhibitor gefitinib and a SRC inhibitor saracatinib [15,16]. Therefore, inhibition of PTTG1 is a promising strategy to overcome anticancer drug resistance.

In this study, we aimed to investigate the role of PTTG1 in ccRCC and identify anticancer therapy for PTTG1-high expressing ccRCC via *in silico* bioinformatics approaches. We have found that PTTG1 mRNA upregulation, but not copy number amplification, served as a prognostic biomarker and a therapeutic target in ccRCC. In addition, a Rac1 inhibitor NSC23766 was a potential therapeutic agent to selectively target to PTTG1-high expressing ccRCC.

2. Materials and methods

2.1. cBioPortal for cancer genomics analysis

cBioPortal (http://www.cbioportal.org/) is a website to visualize and analyze large-scale The Cancer Genome Atlas (TCGA) or other cancer genomics datasets [17,18]. For pan-cancer analysis of PTTG1 genetic alterations, the "TCGA, PanCancer Atlas" datasets were selected. For the comparison of RCC subtypes, the "TCGA, Provisional" datasets for ccRCC, pRCC, and chRCC were selected. For the PTTG1 mRNA expression in ccRCC, "mRNA z-Scores (RNA Seq V2 RSEM)" was selected and set to "2". Other parameters not mentioned were set as default.

2.2. Gene Expression Profiling Interactive Analysis (GEPIA)

GEPIA (http://gepia.cancer-pku.cn/) is a web server for cancer and normal gene expression profiling and interactive analysis [19]. For the comparison of PTTG1 mRNA expression in normal and cancer tissues, kidney renal clear cell carcinoma (KIRC) and match normal datasets were selected. For the impact of PTTG1 on ccRCC patients' overall survival, KIRC datasets were selected. The parameter "Group Cutoff" was set to "Median". Other parameters not mentioned were set as default.

2.3. Oncomine analysis

The Oncomine (https://www.oncomine.org/) database collects and standardizes cancer gene expression profiles, and provides analytic modules for data assessment [20]. For the comparison of PTTG1 mRNA expression in RCC subtypes, "Beroukhim Renal" and "Jones Renal" datasets were selected [21,22].

2.4. Functional enrichment

FunRich software version 3.1.3 [23] was downloaded from http://funrich.org/. It is used for functional enrichment and interaction network analysis of genes and proteins. Both up- and down-regulated genes in Table S1 were inputted independently for query. The functional module "Enrichment analysis (compare multiple datasets" was used for "Biological pathway" enrichment. The "Number of items to show on chart" was set to "5".

2.5. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) analysis

The STRING (http://string-db.org/) is a database that collects and integrates functional interactions beween the expressed proteins based on text-mining, and known and predicted protein–protein association data for a large number of organisms [24]. The upregulated genes in Table S1 were inputted into the STRING for query. The "Organism" was set to "Homo sapiens". The "meaning of network edges" was set to "evidence". The "active interaction source" was set to "Experiments" and "Databases". The "minimum required interaction score" was set to "high confidence (0.700)". The "max number of interactors to show" was set to "none". The "network display mode" was set to "interactive svg". The "display simplifications" was set to "hide disconnected nodes in the network".

2.6. Cancer Therapeutics Response Portal (CTRP) analysis

The CTRP version 2 (https://portals.broadinstitute.org/ctrp.v2.1/) links genetic, lineage, and other cellular features of cancer cell lines to small-molecule sensitivity, which can be used to identify cancer dependencies with small molecules [25–27]. For the drugs correlated with PTTG1 mRNA expression in ccRCC, the feature "PTTG1" was selected. Sensitivity correlation with gene-expression levels was selected. The filter was set to ccRCC cell lines with adherent growth mode for calculation.

2.7. CellMinerCDB analysis

The CellMinerCDB (https://discover.nci.nih.gov/cellminercdb/) is an interactive web-based portal for querying the relationship between genomic and pharmacological data from large-scale cancer cell lines [28]. For the correlation between PTTG1 mRNA and NSC23766 drug activity, both "x- and y-Axis Cell Line Set" was set to "CTRP". The "x-Axis Data Type" and "y-Axis Data Type" were set to "exp: mRNA Expression **(log2)**" and "act: Drug Activity (AUC)", respectively. The identifiers for x- and y-axis were set to "PTTG1" and "NSC23766", respectively. The "Kidney: Renal Cell Carcinoma (RCC): Renal Clear Cell Carcinoma (CCRCC)" was selected for analysis.

2.8. Statistical analysis

Statistical analysis was performed by built-in programs in each database or software used in this study, and p values of < 0.05 were considered significant.

3. Results

3.1. PTTG1 copy number amplification is not associated with its mRNA expression and overall patients' survival in ccRCC

Due to the advances of cancer genomics provided by The Cancer Genome Atlas (TCGA), a comprehensive gene analysis across various

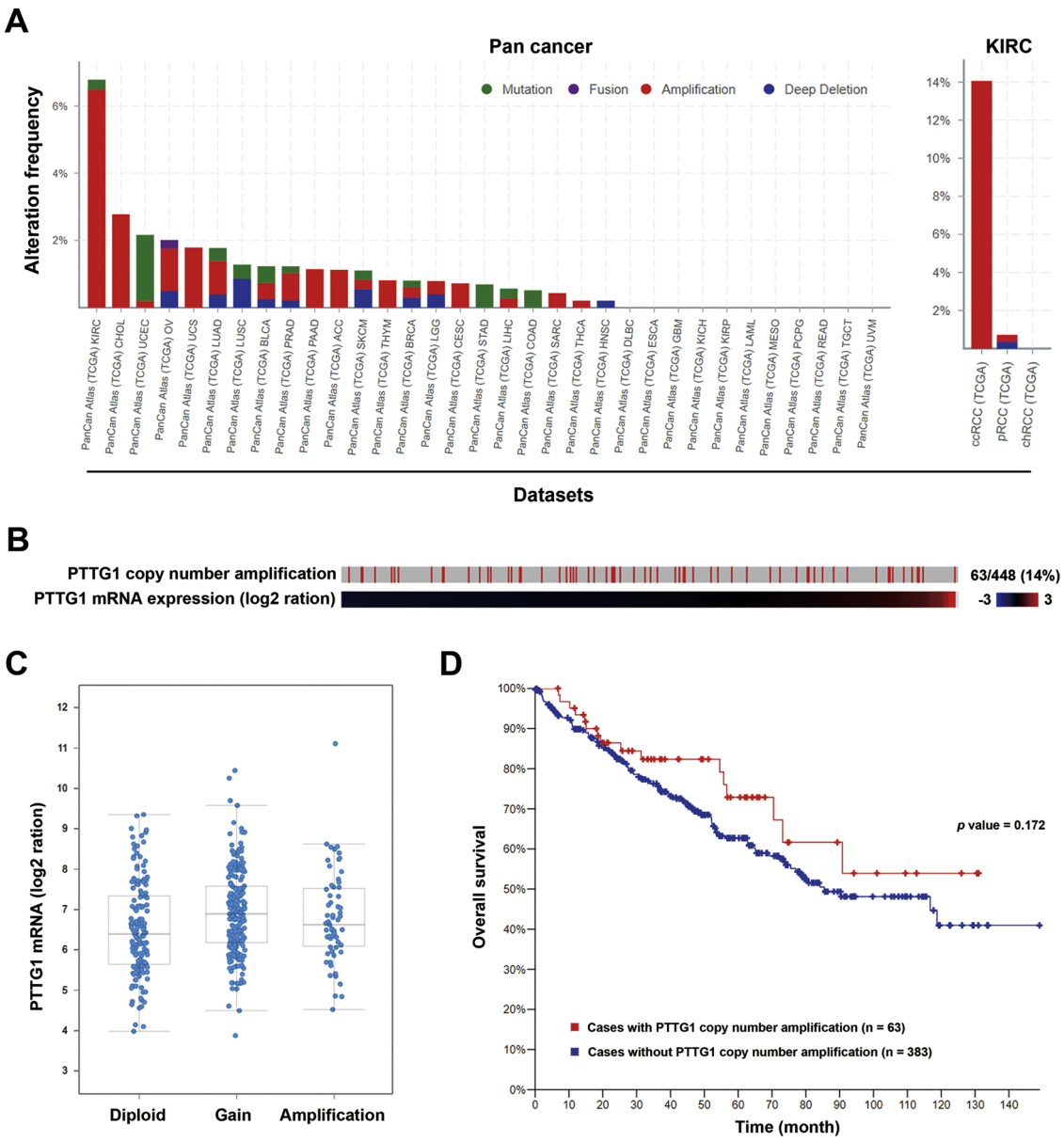

Fig. 1. Copy number amplification of PTTG1 gene in ccRCC. (A) PTTG1 gene was analyzed for copy number alterations and mutation status in various cancer types using "TCGA, PanCancer Atlas" and "TCGA, Provisional (ccRCC, pRCC, and chRCC)" data in the cBioPortal cancer genomics database. (B) A bar code plot for the comparison of PTTG1 copy number amplification and mRNA expression in ccRCC. (C) A scatter plot for the comparison of PTTG1 copy number amplification and mRNA expression in ccRCC. Diploid, two alleles present; Gain, low-level gene amplification event; Amp, high-level gene amplification. (D) The impact of PTTG1 copy number amplification on the overall survival of ccRCC patients.

cancer types becomes feasible nowadays. To gain more insights into the role of PTTG1 in cancers, a pan-cancer analysis was performed using "TCGA, PanCancer Atlas" data in the cBioPortal website (http://www.cbioportal.org/) for the frequency of copy number alterations and mutation status of PTTG1 gene [17,18]. As shown in Fig. 1A (the left part), the copy number of PTTG1 gene was mostly amplified in 31 (6.05%) out of 512 cases in the kidney renal clear cell carcinoma (KIRC) data. When different RCC subtypes were compared using "TCGA, Provisional" data, we found that 14.13% (76 out of 538 cases) of PTTG1 copy number amplification in ccRCC and low frequency in pRCC and chRCC (Fig. 1A, the right part). These results indicate that PTTG1 gene is frequently amplified in ccRCC.

Previous studies on the role of PTTG1 in ccRCC is limited. It is identified as an oncogene amplified on chromosome 5q and overexpressed in ccRCC [29]. Overexpression of PTTG1 is associated with aggressiveness and poor prognosis in ccRCC [29,30]. Our analysis was consistent with Wondergem's study showing the copy number amplification of PTTG1 in ccRCC [29]. However, we found that copy number amplification of PTTG1 genes was not positively correlated with its mRNA expression (Fig. 1B and C). In addition, ccRCC patients with PTTG1 copy number amplification had similar overall survival rate with those without PTTG1 copy number alterations (Fig. 1D). Therefore, PTTG1 copy number amplification in ccRCC does not result in the increased expression of PTTG1 mRNA and cannot predict patients' survival.

3.2. Overexpression of PTTG1 mRNA is associated with a poor overall survival in ccRCC patients

Overexpression of PTTG1 mRNA has been shown in a previous study [29]. Consistently, TCGA analysis also indicated that PTTG1 mRNA was significantly upregulated in KIRC data (Fig. 2A, the left part). In

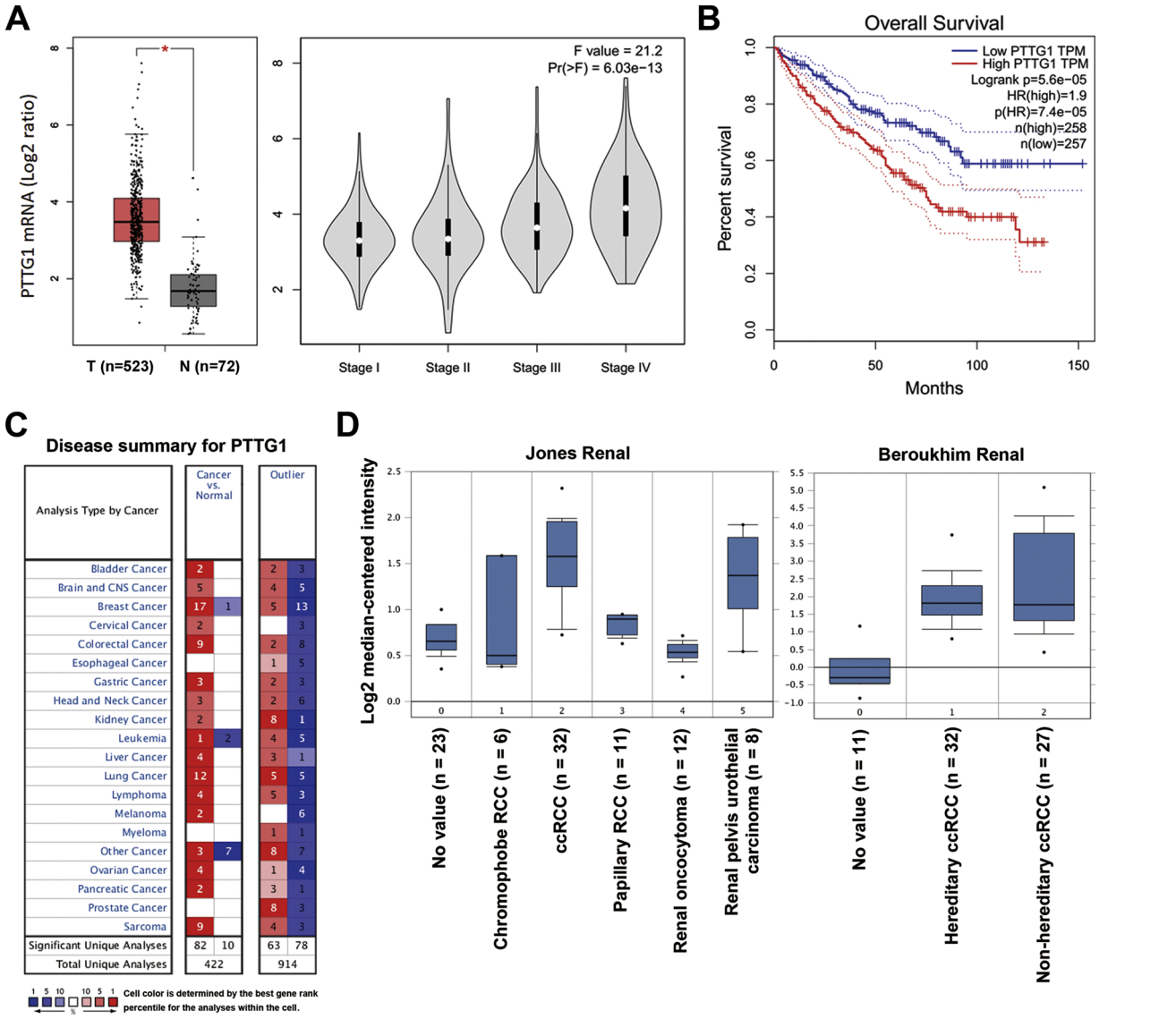

Fig. 2. Overexpression of PTTG1 mRNA in renal cancer. (A) PTTG1 mRNA expression in renal cancer and normal renal tissues and in different tumor stages in renal cancer was analyzed using the cBioPortal cancer genomics database. (B) The impact of PTTG1 mRNA on the overall survival of renal cancer patents. (C) A summary view of PTTG1 gene expression profiles in human tumors using published human cancer microarray database (Oncomine). The number in each cell under "Cancer vs. Normal" corresponded to the amount of cancer types that contained a significantly different level of PTTG1 compared to normal corresponding tissue. Thresholds for significance were: fold expression > 2; p-value < 0.05 and ranking of gene in the analyses > top 10%. Red signified the gene overexpression in the analyses; blue represented the gene underexpression. Intensity of color signified the best rank of gene in those analyses. (D) PTTG1 mRNA expression in tissues of various types of renal cancer patients were obtained from the Oncomine database (http://www.oncomine.org/).

addition, the mRNA level of PTTG1 was increased during the progression stage of KIRC (Fig. 2A, the right part), suggesting that PTTG1 plays a role in the tumorigenesis of RCC. Furthermore, RCC patients with higher PTTG1 mRNA expression had a lower overall survival (Fig. 2B), which was consistent with the previous studies [29,30].

To further support the prognostic value of PTTG1 gene, a pan-cancer analysis was performed using the Oncomine (https://www.oncomine.org) that is a database collecting human cancer microarray data [20]. As shown in Fig. 2C, PTTG1 mRNA was frequently overexpressed in 82 out of 422 cancer microarray data (cancer vs. normal), especially in breast (17 analyses) and lung (12 analyses) cancers. Although only 2 kidney cancer analyses showed overexpression of PTTG1, there was an outlier in 8 renal cancer analyses, suggesting that PTTG1 mRNA serves as a good biomarker for kidney cancer. Again, we compared PTTG1 mRNA expression in various kidney cancer types in two microarray data of the Oncomine database. As shown in Fig. 2D, PTTG1 mRNA was mostly upregulated in ccRCC, including hereditary and nonhereditary subtypes. Therefore, ours and previous studies suggest that PTTG1 mRNA, but not copy number alterations, is a prognosis marker for ccRCC, and may serve as a therapeutic target for treating ccRCC.

3.3. Genetic alterations of mitotic cell cycle progression in ccRCC

To investigate the impact of PTTG1 overexpression, 15 ccRCC patients with the highest PTTG1 mRNA (mRNA z-scores more than 2) was selected, and then the most significant coexpressed genes with PTTG1 (as indicated in the red boxes in Fig. 3**A** and the full gene list was shown in Table S1) in these patients were obtained using the "enrichments" tool of the cBioPortal website. Pathway enrichment of these genes were performed by the Functional Enrichment (FunRich) software [23]. As shown in Fig. 3B, the pathways enriched in downregulated genes were not statistically significant. However, pathways related to cell cycle, especially mitosis, were enriched in upregulated genes. The network of

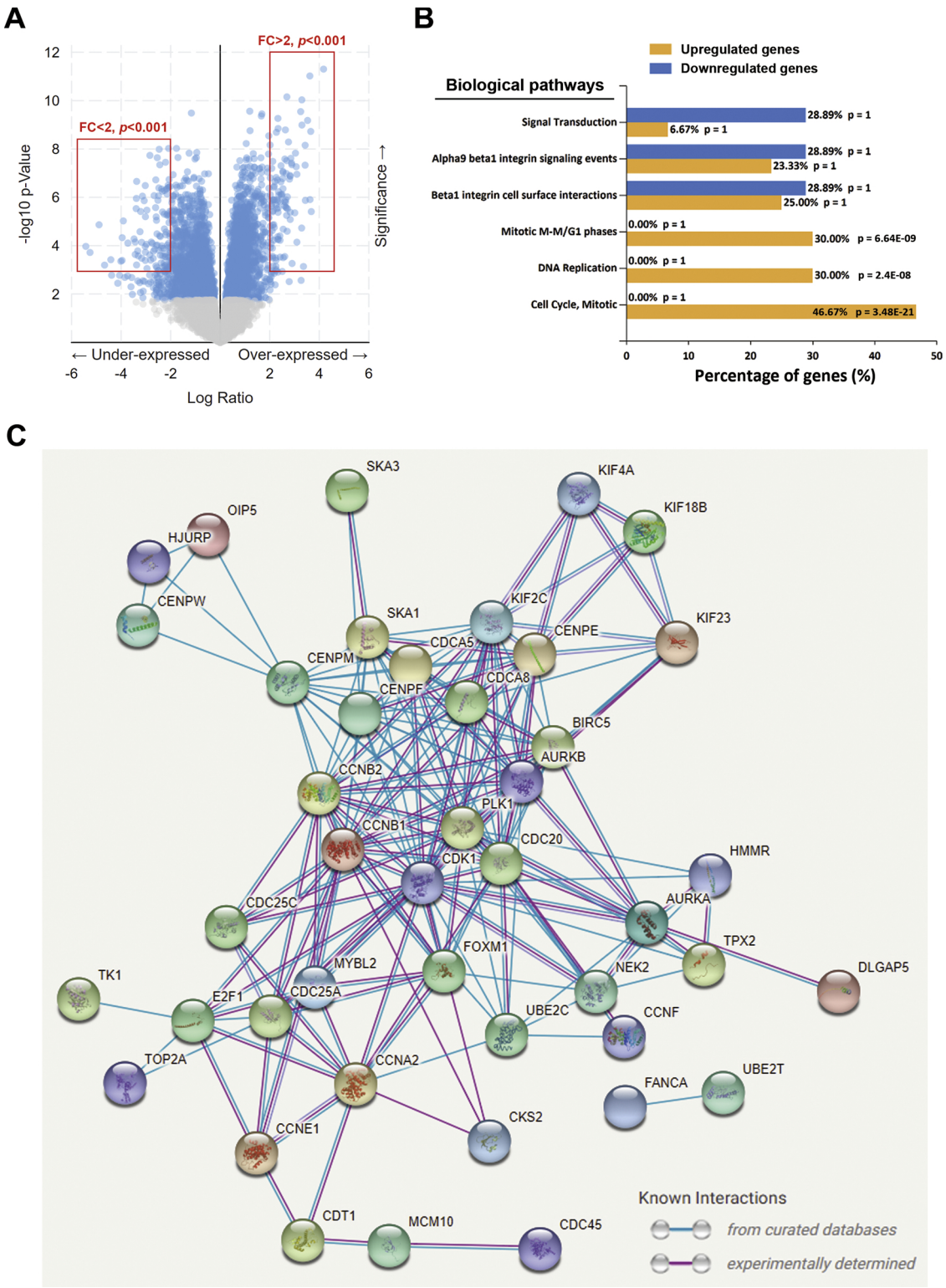

Fig. 3. Coexpression of PTTG1 with mitosis-regulatory genes in ccRCC. A volcano plot for over- and under-expressed genes enriched in PTTG1-high expressing ccRCC patients (A). The red boxes indicated the mostly significant (fold change more or less than 2 or -2, respectively, and *p* value less than 0.001) genes selected for further pathway analysis by the FunRich v3.1.3 (B). The network of over-expressed genes were further constructed by the STRING v10.5 (C).

the upregulated genes, together with PTTG1, was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org) [24]. As shown in Fig. 3C, the major network consisted many genes which play essential roles during mitosis. For example, cell division cycle 20 (CDC20) activates anaphase promoting complex (APC) that initiates chromatid separation through UBE2C-mediated PTTG1/Securin degradation, then promoting the entrance into anaphase [31]. Several kinases for mitotic entry, including cyclin-dependent kinase 1 (CDK1), polo-like kinase 1 (PLK1), and aurora kinase A and B (AURKA/AURKB) were also upregulated. Their upregulation may result in chromosome instability [32]. Thus, PTTG1 overexpression may be associated with the mitotic defects in ccRCC.

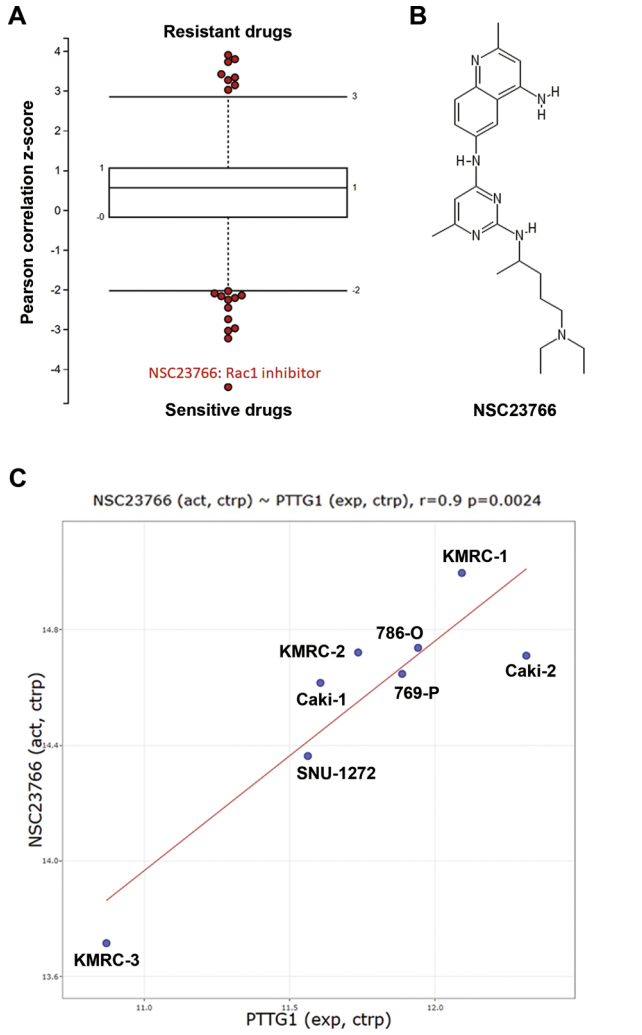

Fig. 4. PTTG1 mRNA expression was highly correlated with the drug sensitivity of NSC23766 in ccRCC cell lines. (A) The drug response profiles correlated with PTTG1 mRNA expression were analyzed by the CTRP v2.1. (B) The chemical structure of NSC23766. (C) A scatter plot generated by the CellMinerCDB showed the correlation between PTTG1 mRNA expression and the drug activity of NSC23766 in ccRCC cell lines.

3.4. Higher PTTG1 mRNA expression is sensitive to a Rac1 inhibitor NSC23766 in ccRCC cell lines

So far, no specific PTTG1 small-molecule inhibitors are developed. However, its expression could be suppressed by several therapeutic agents, including the Chinese herbal medicine berberine [33], the BRAF inhibitor dabrafenib [34], the Hsp90 inhibitor dicoumarol [35], and the CDK inhibitor PHA-848125 [36]. Whether these agents could be used to target PTTG1 and selectively treat PTTG1-high expressing ccRCC is unknown. Here we attempted to search novel therapeutic drugs to selectively kill PTTG1-high expressing ccRCC cells by mining the Cancer Therapeutics Response Portal (CTRP; https://portals.broadinstitute.org/ctrp.v2.1/) [25–27]. The CTRP is developed based on the idea that differential basal gene expression in cancer cell lines could be correlated with the patterns of drug sensitivity. Thus, it is suitable for the prediction of unknown or novel mechanisms of action (MoA) of small molecules [25–27]. As shown in Fig. 4A, higher PTTG1 mRNA expression was positively correlated with the sensitivity of ccRCC cell lines to a Rac1 inhibitor NSC23766 (its chemical structure was shown in Fig. 4B). A scatter plot further visualized the strong positive correlation (Pearson's correlation coefficient $r = 0.9$ and p value $= 0.0025$) between PTTG1 mRNA expression and NSC23766 drug activity. Therefore, our results identify and repurpose the Rac1 inhibitor NSC23766 for treating PTTG1-high expressing ccRCC.

Whether PTTG1 serves as a target of NSC23766 is unclear. Connectivity Map (CMap) database collects gene signatures from human cancer cell lines treated with small molecules and can be used to explore MoA of drugs by comparing the similarity of gene signatures [37]. By querying the L1000-based CMap data via an online website, the L1000FWD (http://amp.pharm.mssm.edu/L1000FWD) [38], we found that inhibition of CDK is a potential MoA of NSC23766 (Table S2). Supportively, a CDK inhibitor PHA-848125 has been shown to downregulate PTTG1 expression that is partially dependent on the p53 function [36]. Whether NSC23766 inhibits CDK activity and suppresses PTTG1 expression warrants further investigation.

4. Discussion

ccRCC is characterized by chromosome 3p deletions and chromosome 5q amplifications. Chromosome 3p harbors several tumor suppressor genes including VHL, BRCA1-associated protein-1 (BAP1), and SET domain containing 2 (SETD2). These three genes have been reported to participate in the regulation of mitosis. It has been found that VHL functions in the control of mitotic fidelity in mouse kidney cells. Loss of VHL results in mitotic checkpoint impairment such as spindle misorientation and aneuploidy. Moreover, VHL-deficient kidney displays ccRCC precursor lesions [39]. SETD2 methylates microtubule during mitosis and its loss causes mitotic spindle and cytokinesis defects, micronuclei, and polyploidy [40]. In ccRCC, loss of a single allele of SETD2 is sufficient to serve as an early driver of chromosome instability and promote oncogenesis [41]. BAP1 is a deubiquitination enzyme of γ-tubulin that is required for microtubule nucleation, which is required to prevent mitotic defects and chromosome instability [42]. In addition, BAP1 is essential for kidney function and cooperates with VHL in renal tumorigenesis [43]. Therefore, the defects of these tumor suppressor genes in ccRCC imply that mitotic defects might be the driver for ccRCC tumorigenesis. PTTG1 is located in chromosome 5q and, as a Securin protein, its overexpression can also interfere mitosis, leading to aneuploidy [44]. However, the relationship between PTTG1 and these tumor suppressor genes is still unknown.

Although chromosome 5q gain is the second most common cytogenetic abnormality in ccRCC, its clinical significance is less clear than chromosome 3p loss that is an early event and is involved in tumor initiation. More and more oncogenes on chromosome 5q are discovered, such as sequestosome 1 (SQSTM1; also known as p62) [45], lysyl oxidase (LOX) [46], PTTG1 [29], transforming growth factor, beta-induced gene (TGFBI) [47], collagen type XXIII alpha 1 chain (COL23 A1) [48], and versican (VCAN) [49]. These findings support the role of chromosome 5q gain in tumor progression. However, it has been reported that gain of chromosome 5q31-qter has a favorable outcome [50]. It is possible that chromosome 5q gain is statistically significant in VHL-mutated ccRCC and VHL mutations in chromosome 3p are associated with good prognosis [47,51,52]. Furthermore, VHL deficiency has been viewed as therapeutic target for ccRCC. Several agents targeting to HIF proteins and their transcriptional targets such as VEGF, VEGF receptor (VEGFR), and platelet-derived growth factor receptors (PDGFR), have been approved or investigated [53–56]. In addition, VHL-deficient ccRCC is more sensitive to chemotherapy such as anthracyclines [57]. The association between the oncogenes on chromosome 5q and VHL deficiency is still unclear. Further investigation of this concept may shed light on precision therapy for ccRCC.

PTTG1 upregulation in other cancer types can be directly regulated by transcription factors such as E2F1, Sp1, nuclear factor-Y (NF-Y), β-catenin/T cell factor (TCF), signal transducer and activator of transcription 3 (STAT3), androgen receptor (AR), forkhead box M1 (FoxM1) [58–65]. However, the mechanism for PTTG1 upregulation in ccRCC is still unresolved. Whether these transcription factors also involve in the

upregulation of PTTG1 in ccRCC warrant further investigation. Recently, PTTG1 has been identified as a novel target gene of sterol regulatory element-binding protein 1c (SREBP1c) that is a key transcription factor in *de novo* lipogenesis, and SREBP1c/PTTG1 signaling may serve as a bridge between aberrant lipid metabolism and cell cycle progression in ccRCC [66,67]. Because ectopic lipid accumulation is a hallmark of ccRCC [68], upregulation of SREBP1c may be responsible for PTTG1 overexpression during ccRCC tumorigenesis.

This study conducts an *in silico* bioinformatics analysis to investigate the role of PTTG1 in ccRCC. Our results suggest that both copy number and mRNA of PTTG1 are frequently increased in ccRCC; however, only PTTG1 mRNA overexpression predicts a poor prognosis in ccRCC patients. The PTTG1 is coexpressed with several mitotic regulators, which may lead to mitotic defects in ccRCC. In addition, a Rac1 inhibitor NSC23766 is repurposed for treating PTTG1-high expressing ccRCC. Taken together, our study provides an *in silico* insight into the role of PTTG1 in ccRCC and repurposes the Rac1 inhibitor NSC23766 for treating PTTG1-high expressing ccRCC.

Conflict of interests

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.prp.2019.03.002.

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