CENPA promotes clear cell renal cell carcinoma progression and metastasis via Wnt/β-catenin signaling pathway

Qi Wang1†, Jiaju Xu1†, Zhiyong Xiong1, Tianbo Xu1, Jingchong Liu1, Yuenan Liu1, Jiaping Chen3, Jian Shi1, Yi Shou1, Changjie Yue1, Di Liu1, Huageng Liang1, Hongmei Yang2, Xiong Yang1* and Xiaoping Zhang1*

Abstract
Clear cell renal cell carcinoma (ccRCC) is the most common malignant tumor of the kidney. New and reliable biomarkers are in urgent need for ccRCC diagnosis and prognosis. The CENP family is overexpressed in many types of cancers, but its functions in ccRCC have not been fully clarified. In this paper, we found that several CENP family members were highly expressed in ccRCC tissues. Also, CENPA expression level was related to clinicopathological grade and prognosis by weighted gene co-expression network analysis (WGCNA). CENPA served as a representative CENP family member as a ccRCC biomarker. Further in vitro experiments verified that overexpression of CENPA promoted ccRCC proliferation and metastasis by accelerating the cell cycle and activating the Wnt/β-catenin signaling pathway. The elevated β-catenin led by CENPA overexpression translocated to nucleus for downstream effect. Functional recovery experiment confirmed that Wnt/β-catenin pathway was essential for ccRCC progression and metastasis. Developing selective drugs targeting CENPA may be a promising direction for cancer treatment.

Keywords: Kidney renal cell carcinoma, Biomarker, Gene set enrichment analysis, Metastasis, CENPA, Targeted therapy

Background
Renal carcinoma is a malignancy in urinary system with high incidence. As reported by the American Cancer Society’s most recent estimates about renal malignancies in the United States for 2020, approximately 76,080 new cases of kidney and renal pelvis cancer would be diagnosed, and approximately 13,780 people would die from this disease [1]. Renal cell carcinoma (RCC) accounts for 90% of all renal malignancies [2]. Clear cell renal cell cancer (ccRCC) is a major and malignant subtype of renal carcinoma, accounting for approximately 3/4 of RCC [3]. Although ccRCC’s diagnostic technique has been greatly improved, approximately one in three patients have advanced tumor when first diagnosed still have distant metastasis at the time of diagnosis [4]. These patients may have a worse prognosis due to missing the timing for surgery. Beyond surgery, radiotherapy and traditional chemotherapy are not as effective for ccRCC, which is why targeted therapy has been developed. However, insensitiveness and resistance could present problems for the use of traditional molecular targeted antitumor drugs, including sunitinib, a widely applied drug for RCC. Researchers are striving for new targets [5, 6], yet few are sufficiently effective for clinical research. As a result, it is imperative to look for new biomarkers for early diagnosis and targeted therapy.

Sustained proliferative signaling is a distinctive feature of tumors [7]. Mitotic defects accumulation of finally lead...
to chromosomal instability (CIN) [8]. Cancers are frequently aneuploid [9], and the alteration of oncogenes or tumor suppressors that regulates changes in chromosome number may contribute to tumorigenesis, progression, metastasis, and prognosis of patients [10–12]. Accurate duplication and segregation of our chromosomes depend on precise assemblies of the kinetochore protein complex on centromeric chromatin [13], but abnormal segregation leads to chromosomal instability and aneuploidy [14]. Centromere protein A, namely CENPA or CenH3, is recognized as a marker of centromeric location, as it exists in all active centromeres [15]. Overexpression of CENPA promotes aneuploidy with karyotypic heterogeneity [16]. In contrast, CENPA deficiency drives apoptosis and induces cell cycle arrest [17–20].

Extensive studies have uncovered elevated CENPA levels in tumors and their effect in tumorigenesis, including colorectal cancer [12, 21], breast cancer [22, 23], gastric cancer [24], prostate adenocarcinoma [25], and lung cancer [26]. However, though researchers claimed that CENPA may play a role in kidney cancer through bioinformatics analyses [27–30], the relationship between CENPA and ccRCC has not been unearthed by in vitro experiment yet.

Here we systematically analyzed the role of CENP family members in ccRCC. We found that CENPA, a representative of CENP family member, was highly expressed and could be a diagnostic and prognostic biomarker of ccRCC. In addition, downregulation or upregulation of CENPA could inhibit or promote the proliferation, migration and invasion of ccRCC in vitro. With further exploration, we found that CENPA accelerated cell cycle and activated the Wnt pathway. Finally, functional rescue experiments indicated that CENPA promoted ccRCC cell proliferation and metastasis by activating the Wnt/β-catenin pathway.

Results

CENPA was identified as a hub gene in ccRCC via WGCNA

To find the hub genes for ccRCC from the analyzed gene set, 6137 genes were identified as differentially expressed genes (DEGs) by the “limma” package according to the cutoff criterion. WGCNA was used to screen hub modules closely related to clinical traits. In our study, a scale-free network was ensured with the soft threshold β = 4 (Fig. 1A). Based on the gene expression pattern, we identified 25 modules shown in Fig. 1B. For correlation coefficient between modules were all less than 0.8 (Fig. 1C, D), no modules needed to be merged. As shown in Fig. 1E, we selected the pink module (T stage: r = 0.3, p = 3e−12; N stage: r = 0.061, p = 0.2; M stage: r = 0.19, p = 2e−5; Stage: r = 0.27, p = 2e−10) as the hub module for further analysis. The relationships between the genes in the pink module for M stage, T stage, N stage, stage, and G grade were presented in Fig. 1F. Notably, the top 10 hub genes included CENPI and CENPA, which are members of the CENP family (Fig. 1G). Then, we focused our interest on the CENP family. Eight members (CENPA, CENPE, CENPF, CENPH, CENPI, CENPK, CENPM, CENPU) of the CENP family were all upregulated in KIRC (kidney renal clear cell carcinoma) cohort from TCGA (The Cancer Genome Atlas) database (Fig. 2A). The plot of genetic alteration suggests that genes in CENP family seldomly mutate (Additional file 2: Figure S2I). From the Kaplan–Meier curve for overall survival (OS) and the disease-free survival (DFS) of the eight genes in TCGA-KIRC, we found that neither CENPI nor CENPU had prognostic significance (Fig. 2B–I). Although the remaining 5 genes (CENPA, CENPAF, CENPH, CENPK, and CENPM) had prognostic value, they were not hub genes by WGCNA analyses. Then subgroup analysis according to age, gender, Stage and G grade were consistent with the previous (Additional file 1: Fig. S1D–G). Above all, CENPA was not only a hub gene, but could also predict the prognosis of ccRCC patients, indicating it to be a representative gene of the CENP family in ccRCC.

The expression level of CENPA was significantly associated with clinicopathological features

Next, we aimed to investigate the aberrant expression of CENPA in ccRCC. Firstly, we investigated the mutations and copy-number alterations (CNAs) of CENPA. As illustrated above, there exist no genetic alterations for CENPA in TCGA-KIRC cohort (Additional file 2: Figure S2I). We then explored the CCLE (Cancer Cell Line Encyclopedia), finding that no mutations or CNAs were detected in kidney cancer cell lines. Transcriptomically, CENPA was overexpressed in tumor tissues in the TCGA-KIRC project (Fig. 3A-B), GEO (gene expression omnibus) database (Fig. 3C, D) and Oncomine database (Fig. 3E) [31–33]. Also, CENPA...
Fig. 1 (See legend on previous page.)
Fig. 2 Expression and survival curve of 8 members of CENP family. 

A The expression heatmap of eight members (CENPA, CENPE, CENPF, CENPH, CENPI, CENPK, CENPM, CENPU) of CENP family in TCGA ccRCC. Left: 535 ccRCC tissues; right: 72 cancer-adjacent tissues. 

B–I The OS and DFS curve of the eight CENP family members. In each analysis, all patients were sorted in ascending order based on corresponding gene expression, then they were divided into two groups with the same sample size. The OS and DFS of patients in two groups were visualized by Kaplan–Meier plot. OS: overall survival; DFS: disease free survival
overexpression is not acquired from treatment as we can see a similar result when we eliminated samples from patients with adjuvant therapy prior to the surgery (Additional file 1: Figure S1L-M). As shown in Fig. 3F–H and Additional file 1: Figure S1A-B, the expression of CENPA was positively correlated with multiple clinical stages (T stage, N stage, M stage and TNM stage and G stage). Similar results were obtained in other datasets (Fig. 3I–K). Higher CENPA expression indicated shorter survival time and higher tumor grade and stage (Table 1). Univariate and multivariate analyses were conducted showing that CENPA was one of the independent prognostic markers of ccRCC (Table 2). In addition, ROC (receiver operator characteristic) curve analysis showed that CENPA could be used as a good diagnostic marker (Fig. 3L and Additional file 1: Figure S1C). Furthermore, the ROC curve analyses were conducted between clinicopathological subgroups such T1+2 vs T3+4, M0 vs M1, Stage I+II vs Stage III+IV, and G1+2 vs G3+4 (Additional file 1: Figure S1H–K), which indicated good diagnostic value of CENPA expression for clinicopathological subgroups. Thus, CENPA can serve as a potential diagnostic and prognostic biomarker in ccRCC.

**CENPA was upregulated in ccRCC tissues and cells**

To verify the expression levels of CENPA in ccRCC tissues, qRT-PCR (reverse transcription-quantitative polymerase chain reaction) and IBT (immunoblotting test) were performed. It was observed that CENPA expression levels were notably elevated in tumor tissues in comparison with their corresponding adjacent normal tissues (Fig. 3M, N). The immunohistochemistry (IHC) results of cancer/para-cancer pairs also suggested that CENPA was upregulated in cancer tissues (Fig. 3O). Furthermore, we confirmed that the mRNA and protein levels of CENPA were higher in RCC cell lines (786-O, A498, ACHN, Caki-1 and OSRC-2) than in the normal renal cell line HK-2 by qRT-PCR and IBT (Fig. 3P, Q). Generally, these results collectively pinpoint the fact that CENPA is overexpressed in ccRCC.

**CENPA promoted the proliferation, invasion and migration of ccRCC cells in vitro**

To investigate the effect of CENPA on the biological behaviors of ccRCC, ccRCC cell lines were transfected with si-CENPA or CENPA plasmid to down- or upregulated the expression of CENPA. The mRNA and protein expression levels decreased or increased significantly in A498 and Caki-1 cells compared with the corresponding negative control (Fig. 4A–C). CCK-8 (cell counting kit-8) assays suggested that tumorous cells downregulated or upregulated in CENPA inhibited or promoted proliferation, respectively (Fig. 4D–G). The colony formation assays confirmed this finding (Fig. 5A, B). In addition, transwell assays (Fig. 4H) and wound healing assays (Fig. 5C–F) collectively indicated that the level of CENPA was positively correlated with the migration and invasive abilities of the cells. These results provided us with solid evidence that CENPA promoted the proliferation, migration and invasion of ccRCC cells, which is significant in the cascade of tumor metastasis.

**CENPA activated the Wnt/β-catenin pathway and accelerated the cell cycle**

To determine how CENPA is involved in ccRCC pathogenesis, functional enrichment analyses were performed using the TCGA-KIRC cohort to identify ccRCC-related pathways and biochemical processes affected by differentially expressed CENPA. GSEA (Gene Set Enrichment Analysis) results indicated that the high expression of the CENPA group was mainly enriched in cell cycle pathways and Wnt pathways (Fig. 6A). The results of GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses also included cell cycle pathways (Additional file 2: Figure S2A, B). We found that the expression of CENPA was positively related to WNT5A using GEPIA in TCGA-KIRC, which encodes a member of the Wnt family that signals through both the canonical and noncanonical Wnt pathways (Fig. 6B). To verify the presumption of bioinformatics analysis, the western blotting assays were performed, and we found that the silencing of CENPA could significantly downregulate the expression of β-catenin (CTNNB1) and its target gene.
Fig. 3 (See legend on previous page.)
cyclin D1 (CCND1) in ccRCC (Fig. 6C), whereas the overexpression of CENPA could upregulate the expression of β-catenin (CTNNB1) and cyclin D1 (CCND1) in ccRCC (Fig. 6D). Then, the β-catenin (CTNNB1) nuclear accumulation was observed with CENPA overexpression whereas CENPA knockdown reduced nuclear proportion of β-catenin (CTNNB1) (Fig. 6E). In addition, cell cycle assays showed that Caki-1 cells were accumulated in G0/G1 phase, suggesting an inhibition of cell cycle from G0/G1 to S phase (Fig. 6F). The cell cycle arrest blocked cell growth. In contrast, overexpression of CENPA promoted entry of more cells into S phase so that the cell proliferation rate increased (Fig. 6G).

The Wnt/β-catenin pathway is involved in CENPA-mediated proliferation and metastasis

To further test whether the Wnt/β-catenin pathway was required for the downstream effect of CENPA on cell proliferation and metastasis, we conducted in vitro rescue experiments. We used Si-CTNNB1 to knock down the expression of β-catenin (CTNNB1) (Additional file 2: Figure S2D, G). And the Wnt/β-catenin inhibitor XAV-939 or activator CHIR-99021 trihydrochloride were utilized to inhibit or activate the Wnt/β-catenin pathway respectively. To explore their effects on β-catenin (CTNNB1), a series of concentration gradients for 24 h and time gradients for 10 μM of XAV-939 and CHIR-99021 trihydrochloride were employed Caki-1 (Additional file 2: Figures S2C, E, F, H). Then, 10 uM and 24 h were considered to be a proper drug treatment condition. Following depletion of β-catenin by transfection of siRNAs, the function of CENPA on cell proliferation was reduced, as shown by CCK-8 assays (Fig. 7A, D). A similar result was also obtained by using XAV-939 (Fig. 7B, E). Moreover, following activation of Wnt/β-catenin signaling with the CHIR-99021 trihydrochloride, the proliferative ability of CENPA-depleted cells was enhanced (Fig. 7C, F). Therefore, we believe that CENPA promotes the progression of ccRCC through activating the Wnt/β-catenin signaling pathway. In addition, to investigate whether CENPA exerted its effects during ccRCC metastasis in the context of the Wnt/β-catenin pathway, we conducted transwell assays. Similar to CCK-8 assays, inhibition of β-catenin reduced CENPA-mediated migration and invasion (Fig. 7G). In contrast, activation of the Wnt/β-catenin pathway enhanced the abilities in CENPA-depleted cells (Fig. 7H). These results indicated that CENPA promoted ccRCC cell proliferation and metastasis by activating the Wnt/β-catenin pathway.

**Discussion**

Here, we uncovered CENPA as a new ccRCC biomarker and demonstrated that CENPA acts crucially in ccRCC. First, through performing WGCNA analyses, we found that eight CENP family members (CENPA, CENPE, CENPF, CENPH, CENPI, CENPK, CENPM, CENPU) were closely related to clinical stage and grade with a similar expression pattern in pink module. Second, we selected the CENPA as a representative of the eight CENP family members. Functional experiments proved that CENPA could accelerate RCC cell proliferation and metastasis. Additionally, our experimental evidence showed that upregulating CENPA could accelerate the cell cycle and trigger the Wnt/β-catenin pathway. Finally, functional rescue experiments indicated that CENPA promoted ccRCC cell multiplication and metastasis through triggering the Wnt/β-catenin pathway.

| Parameter | Number | CENPA mRNA expression | P-value |
|-----------|--------|------------------------|---------|
| Low (n = 261) | High (n = 261) |
| Age (years) | | | |
| < 60 | 235 | 120 | 115 | 0.725 |
| ≥ 60 | 287 | 141 | 146 | |
| Sex | | | |
| Female | 183 | 108 | 75 | 0.0033 |
| Male | 339 | 153 | 186 | |
| T stage | | | |
| T1 or T2 | 336 | 197 | 139 | < 0.0001 |
| T3 or T4 | 186 | 64 | 122 | |
| N Stage | | | |
| N0 or Nx | 507 | 258 | 249 | 0.0326 |
| N1 | 15 | 3 | 12 | |
| M Stage | | | |
| M0 or Mx | 445 | 242 | 203 | < 0.0001 |
| M1 | 77 | 19 | 58 | |
| G grade | | | |
| G1 or G2 or Gx | 245 | 157 | 88 | < 0.0001 |
| G3 or G4 | 277 | 104 | 173 | |
| TNM stage | | | |
| I + II | 318 | 190 | 128 | < 0.0001 |
| III + IV | 204 | 71 | 133 | |

The four-grid tables were made according to clinicopathological characteristics and the CENPA expression level. Statistical analyses were conducted via Pearson’s χ2 test. P < 0.05 was considered statistically significant.

Table 1 Correlation between CENPA mRNA expression and clinicopathological parameters of ccRCC patients
with karyotypic heterogeneity [36]. Therefore, we deduced that chromosomal aneuploidy caused by CENPA overexpression is an important cause of ccRCC.

Due to the limitations of current treatment, researchers have focused on targeted therapy, and research on ccRCC pathogenesis and the search for new therapeutic targets are increasing; nonetheless, few of them achieve clinical usage. Thus, we aimed to uncover a new mechanism of ccRCC progression and discuss its potential application for developing new drugs. According to our results, CENPA acts crucially in ccRCC genesis and progression, so it maybe a potential target. Presently, some antitumor drugs that target key molecules in cell division have been developed. PF-2771 [37] and GSK923235 [38] are both CENPE inhibitors. Ispinesib could specifically inhibit kinesin spindle protein [39]. But they are all investigational. Due to CENPA overexpression in several types of cancers, it is expected to be a broad-spectrum anti-tumor target in clinical use. Research on anti-cancer drugs target Wnt/β-catenin pathway has been advancing. Schultz-Hausmann et al. confirmed that ethacrynic acid, ciclopirox olamine and piroctone olamine had cytotoxic effect on RCC cell lines via Wnt/β-catenin pathway [40], but they are not specific inhibitors of the pathway. The side effects of them are unable to predict. Several researchers have developed more specific drugs target Wnt/β-catenin pathway such as MSAB [41] and CWP232228 [42], yet they are still far from the patients. New drug research and development target CENPA and Wnt/β-catenin pathway will be a follow-up issue worthy of attention.

Histone variants are considered critical in malignant transformation in several cancer types. As one of the histone H3 variants, CENPA acts crucially in mitosis and contributes to tumor occurrence and development [13, 21, 25, 43]. Past research has mainly focused on the changes and functions during mitosis [44]. With regard to diseases or cancer, it is currently thought that CENPA functions downstream of the pathway or axis rather than upstream [45]. Jeffery et al. uncovered that CENPA overexpression impacted epidermal-mesenchymal transition or radiosensitivity depends on p53 status in cervical or colorectal cancer cell lines [46]. Some studies have shown that histone variants, including CENPA,  --

### Table 2 Univariate and multivariate Cox regression analyses of CENPA mRNA level and patient overall survival (OS)

| Variable | Univariate analysis | Multivariate analysis<sup>c</sup> |
|----------|---------------------|-------------------------------|
|          | HR<sup>a</sup>    | 95% CI<sup>b</sup> | P    | HR | 95% CI | P    |
| Overall survival (n = 522) |          |                   |     |     |       |     |
| CENPA Low (n = 261) | 2.384 | 1.722–3.3 | 0.003 | 1.656 | 1.17–2.344 | 0.004 |
| High (n = 261)      |          |                   |     |     |       |     |
| Age <60 (n = 235)   | 1.641 | 1.196–2.252 | 0.002 | 1.391 | 1.003–1.928 | 0.048 |
| ≥ 60 (n = 287)      |          |                   |     |     |       |     |
| Gender Female (n = 183) | 1.071 | 0.783–1.464 | 0.669 | 1.239 | 0.894–1.717 | 0.198 |
| Male (n = 339)      |          |                   |     |     |       |     |
| T stage T1 or T2 (n = 336) | 3.184 | 2.336–4.329 | <0.001 | 1.626 | 1.127–2.347 | 0.009 |
| T3 or T4 (n = 186)  |          |                   |     |     |       |     |
| N stage N0 or NX (n = 507) | 3.96 | 2.143–7.315 | <0.001 | 2.087 | 1.101–3.956 | 0.024 |
| N1 (n = 15)         |          |                   |     |     |       |     |
| M stage M0 or MX (n = 445) | 4.378 | 3.199–5.992 | <0.001 | 2.52 | 1.752–3.625 | <0.001 |
| M1 (n = 77)         |          |                   |     |     |       |     |
| G grade Gx or G1 or G2 (n = 245) | 2.681 | 1.901–3.782 | <0.001 | 1.638 | 1.129–2.377 | 0.009 |
| G3 or G4 (n = 277)  |          |                   |     |     |       |     |

<sup>a</sup> Hazard ratio, estimated from Cox proportional hazard regression model

<sup>b</sup> Confidence interval of the estimated HR

<sup>c</sup> Multivariate models were adjusted for T, N, M stage, G grade, age and gender
can act as transcription factors [25, 47]. In kidney cancer, many scholars have already screened out CENPA as a diagnostic and prognostic biomarker through bioinformatics analysis, including chromophobe [27] and ccRCC [28–30]. However, how CENPA functions in ccRCC has not been completely determined. In this study, one of our major contributions is our discovery that artificially regulating the expression of CENPA can not only affect the proliferation and metastasis in ccRCC but change the activity of the Wnt signaling pathway.

The Wnt pathway is involved in many biological processes, including cell differentiation, proliferation,
migration, and cell adhesion. Dysregulation of Wnt signal transduction is suggested to be related to various human cancers, including RCC [48]. Many Wnt members were identified as biomarkers for RCC, and some of them were verified as participants in RCC development [49–52]. Piotrowska et al. compared the activation of Wnt/β-catenin pathway among ccRCC, papillary RCC and chromophobe RCC via immunohistochemistry, finding that the Wnt pathway pronouncedly activated in ccRCC [53]. A classical mechanism of the Wnt pathway is to decrease the amount of phosphorylated GSK3β and cytoplasmic β-catenin as well as upregulate many transcription factors that could upregulate oncogene MYC and CCND1 [54]. The Wnt pathway abnormally activates during RCC genesis, and inhibition of the pathway can reduce invasion, migration and drug resistance [55, 56]. In this study, we determined that a high level of CENPA promoted the multiplication and metastasis of RCC by activating
CENPA accelerated the cell cycle and activated the Wnt signaling pathway. A The GSEA results of CENPA using TCGA-KIRC expression dataset. S33 tumoral samples was divided into two groups based on CENPA level. Genes expression patterns of two groups was different in cell cycles and Wnt pathway. B The expression of WNT5A is positively correlated to the expression of CENPA in TCGA-KIRC ccRCC tissues. C, D The expression of cyclin D1 (CCND1) and β-catenin (CTNNB1) were down-regulated or upregulated in ccRCC cells with knockdown or overexpression of CENPA. E Western blot revealed the expression of CTNNB1 in the cytoplasm and nucleus. β-actin and Lamin B1 were used as internal references in the cytoplasm and nucleus, respectively. F–G Cell cycle distribution was analyzed by PI staining in Caki-1 cells after transfection by Si-CENPA or CENPA plasmid for 48 h. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Error bars indicate mean ± SD.

Fig. 6 CENPA accelerated the cell cycle and activated the Wnt signaling pathway. A The GSEA results of CENPA using TCGA-KIRC expression dataset. S33 tumoral samples was divided into two groups based on CENPA level. Genes expression patterns of two groups was different in cell cycles and Wnt pathway. B The expression of WNT5A is positively correlated to the expression of CENPA in TCGA-KIRC ccRCC tissues. C, D The expression of cyclin D1 (CCND1) and β-catenin (CTNNB1) were down-regulated or upregulated in ccRCC cells with knockdown or overexpression of CENPA. E Western blot revealed the expression of CTNNB1 in the cytoplasm and nucleus. β-actin and Lamin B1 were used as internal references in the cytoplasm and nucleus, respectively. F–G Cell cycle distribution was analyzed by PI staining in Caki-1 cells after transfection by Si-CENPA or CENPA plasmid for 48 h. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Error bars indicate mean ± SD.
The Wnt/β-catenin pathway is involved in CENPA-mediated proliferation and metastasis. A. A498 and Caki-1 cells with CENPA overexpression were treated with Si-CTNNB1 for 48 h. Then, expression of CTNNB1 was measured by western blotting. B, C. A498 and Caki-1 cells with CENPA overexpression or knockdown were treated with Wnt-pathway inhibitor XAV-939 (10 μmol/L) or agonist CHIR-99012 (10 μmol/L) for 24 h. Then, western blotting determined that the drugs were effective to Wnt pathway. D–F. Cell viability was assessed in A498 and Caki-1 cells with CENPA overexpression and Wnt pathway inhibition/excitation via CCK-8 assays (n=4 per group). G, H. Caki-1 cells with overexpression or knockdown of CENPA were treated with Si-CTNNB1 for 48 h or CHIR-99021 (10 μmol/L) for 24 h as indicated and subjected to migration assay and invasion assay (magnification: 200×, n=3 per group). NC: negative control; n.s: not significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Error bars indicate mean ± SD.
the Wnt pathway. CENPA overexpression up-regulates β-catenin, promoting its accumulation in the nucleus and transactivating Cyclin D1. The possible Wnt subunit that CENPA activates may be WNT5A. Functional recovery experiment confirmed that Wnt/β-catenin pathway was essential for ccRCC progression and metastasis.

Due to the important role CENPA plays in cell division, we can easily associate it with cell cycle regulation. According to our results, we uncovered CENPA could up-regulate CCND1, which is a downstream target gene of Wnt pathway. The CCND1 is always overexpressed in cancer [57, 58] and regulates cell cycle transition from G1 phase to S phase along with CCND2 and CCND3 [59]. Consistent with the results above, we found CENPA could progresses cell cycle from G0/G1 phase to S phase that mediated by CCND1.

Our study has some limitations. We only verified the tumor-promoting effect of CENPA through in silico and in vitro experiments without in vivo data. Regarding the mechanisms, we did not determine how Wnt pathway and cell cycle-related proteins regulated by CENPA. The above issues will be the focus of our further research.

**Conclusion**

In conclusion, our study unearthed that high-level CENP family genes were related to adverse survival and high clinicopathological stage in ccRCC patients as determined by WGCNA analyses. High-level CENPA could increase the multiplication, migration and invasion ability of ccRCC cells via activating Wnt/β-catenin pathway in vitro. Our effort disclosed that CENPA is an important renal cancer biomarker and a possible highly specific therapeutic target.

**Materials and methods**

**Dataset**

The data we analysed were obtained from TCGA project (https://portal.gdc.cancer.gov/), UCSC Xena browser (https://xenabrowser.net/), GEO database (GSE44035, GSE66272; https://www.ncbi.nlm.nih.gov/geo/), Oncomine database (Jones Renal dataset; https://www.oncomine.org), and CCLE, which included mutations and CNA data, gene expression datasets (RNA sequencing, RNA-seq), corresponding clinicopathological information and survival (including DFS and OS) information of KIRC patients [60].

**WGCNA**

DEGs was acquired by “limma” package [61] under the condition of p < 0.05 and |log FC| (|log Fold Change|) > 1.0. The “WGCNA” package was used to construct the co-expression network in R [62]. WGCNA analysis was conducted based on the previously described standard method [6]. All of the above were performed by R 4.0.2. The ten top hub genes were identified by Betweenness method in CytoHubba plugin using Cytoscape software [63].

**Survival and ROC curve analysis**

The ccRCC samples were classified into two groups with the same sample size based on the median CENPA mRNA level. DFS and OS were visualized by Kaplan–Meier plot using GraphPad 8.01. Meanwhile, ROC curves were also drawn among two groups. Then, p < 0.05 was considered statistically significant.

**ccRCC tissue samples**

120 pairs of ccRCC and their tumor-adjacent renal tissues were acquired from patients at the Department of Urology, Union Hospital in Wuhan during 2015 and 2018. All patients did not undergo adjuvant therapy before the surgery. The clinicopathological features of the 120 ccRCC patients were collected in Additional file 3: Table S1. Our study comply with the regulations of the Human Research Ethics Committee of Huazhong University of Science and Technology. All the procedures in our research obeyed the Declaration of Helsinki. The tumor-adjacent normal renal tissues were taken more than 2.5 cm away from the cancer tissue. The RNAs from 42 paired samples were analyzed by qRT-PCR and proteins extracted from 12 pairs were analyzed via immunoblotting test. Three pairs of samples were analyzed via IHC.

**Cell culture**

The human normal cell line HK-2, and five types of ccRCC cell lines (786-O, ACHN, A498, Caki-1 and OSRC-2) got from the institution named American Type Culture Collection were employed in the research. A kind of commonly used culture medium, high glucose Dulbecco’s Modified Eagle’s Medium (Gibco, USA) was used to culture the cells. Before use, we add 10% fetal bovine serum (Gibco in USA) to the medium. Usually, cells were incubated with 5% CO₂ at 37.3 °C.

**Immunoblotting test (IBT)**

Tissues and cells were lysed in RIPA Buffer (Beyotime, China) including protease inhibitor PMSF (Servicebio, China) for 30 min. The nucleoproteins and plasma proteins were extracted by PARIS™ Kit Protein and RNA Isolation System (Invitrogen, Carlsbad, Canada) directed by the manufacturer’s protocols. Then, BCA Protein Assay Kit (Beyotime in China) was applied for protein quantification. In IBT assays, the condition of 12% gel (SDS-PAGE) at 90 V for 30 min and 120 V for 50 min was used for electrophoresis and the condition
of 250 mA for 50 or 90 min was employed for trans-

ferring to membrane. After blocked with 5% BSA
for 1–2 h at 20 °C, the membrane was incubated with
specific CENPA primary antibody (1:2000; Abclonal in
China, A15995), beta-actin (1:5000; Proteintech in
China, 66009-1-lg), CCND1 (1:2000; Abclonal in China,
A15995), LaminB1 (1:1000; Abclonal in China, A11512)
and CTNNB1 (1:2000; Abclonal in China, A11512)
at 4 °C for 12 h. After washed with 0.1% PBST (phosphate
buffered saline tween) for 10–15 min thrice, the mem-

branes were immersed with specie-matched secondary
antibodies (1:2500; Abclonal, China, AS014 and AS003)
for 2 h at 25 °C. Finally, following washed with 0.1%
PBST for 30–40 min, the bands for each protein were
shown with Electrochemiluminescence IBT Substrate
(Ultra sensitivity; Biosharp, China) via ChemiDoc-
XRS+ (Bio-Rad, China). All the original western blot
pictures were included in Additional file 4.

RNA extraction and qRT-PCR
Directed by the manufacturer’s protocols, we extracted
total RNA from tissues or cells using Ultrapure RNA
Kit (CoWin Biosciences, China). Then the concentra-
tion was measured by a multi-wavelength microplate
reader Tecan’s Infinite M200 Pro (Thermo Fisher Scien-
tific, USA). Afterwards, PrimeScript™ RT Master Mix
(Takara, Japan) was applied to transform the RNA solu-
tion into cDNA solution. The qPCR conditions were
seen in the manufacturer’s protocols. GAPDH was con-
sidered as an endogenous control. All qRT-PCR assays
in the paper were conducted in triplicate. TSINGKE
provides us with the forward or reverse primers for
CENPA and GAPDH. The primer sequences used for
qPCR were: CENPA: 5′-GTG TGG ACT TCA ATT
GGC AAG-3′ (forward) and 5′-TGC ACA TCC TTT
GGG AAG AG-3′ (reverse); CTNNB1: 5′-AAA GCG
GCT GTT AGT CAC TGG-3′ (forward) and 5′-CGA
GTC ATT GCA TAC TGT CCA T-3′ (reverse); GAPDH:
5′-CGT GGA AGG ACT CAT GAC CA-3′ (forward)
and 5′-GCC ATC ACG CCA CAG TTT C-3′ (reverse).

IHC assay
Briefly, immunohistochemical was stained with 4 µm
formalin-fixed paraffin-embedded tissue sections. The
slices were then reacted with a rabbit antibody against
CENPA (1:100) for 12 h at 4 °C. Then the section was
washed with PBS, immunodetection was performed with
50 µl DAKO secondary antibody per section and
cultured with secondary antibodies at 25 °C for about
2 h. Three randomly fields were selected to observe

under a light microscope (Olympus in Japan) at
200× and 400× magnification.

Transient transfection for overexpression or knockdown
of CENPA and/or CTNNB1
Plasmids overexpressing CENPA, siRNA targeting
CENPA (si-CENPA) oligonucleotide sequences and
their corresponding negative controls were constructed
in Vigene Biosciences (Shandong, China). The siRNA
targeting CTNNB1 and the corresponding negative
control siRNA were synthesized with Wuhan Qijing Bio-
technological Limited Technology (Wuhan, China). Lipofectamine
3000 (Invitrogen, Carlsbad, CA) reagent was employed
for transfection directed by the manufacturer’s proto-
col while the ccRCC cells were at 30–50% fusion. 5 µg
per well of plasmids (vector or CENPA) or 0.1 nmol per
well of siRNAs (si-CENPA, si-CTNNB1 or si-NC) were
used directed by the manufacturer’s protocols. Finally,
cells were stored for further experiments after 48 h
transient transfection. Si-CENPA sequence was as fol-

ows: sense 5′-GCA GAA GCA UUU CUA GUU
TT-3′; antisense 5′-AAC UAG AAA UGC UUC UGC
UGC TT-3′. The si-CTNNB1 sequence was as follows:
sense 5′-GGA GGU AUC CAC AUC CTC-3′; antisense 5′-UGG GGA GGU AUC CAC AUC CTC-3′.

Cell proliferation assays and cell cycle analysis
After transient transfection for at least 48 h, 1 × 10^3
cells were cultured in 96-well plate with 200 µl of
medium. The cell proliferation assays were measured
by CCK-8 (MedChemExpress, USA) at a 1:10 dilution with
serum-free medium every 24 h for four days directed
by the manufacturer’s protocols. Finally, OD450 of cells
over four days was shown by GraphPad Prism to reflect
the ability of cell proliferation. As for cell cycle analysis,
Caki-1 cells were labeled with PI/RNase Staining (BD
Bioscience) Buffer after transected with Si-CENPA for
48 h. The DNA content was measured using flow (Beck-
man FC500, USA) cytometry and displayed by Modfit
software.

Cell migration and invasion assays
The standard steps are as described in the previous
[64]. Notably serum-starved cells (A498: 2 × 10^4; Caki-
1: 10^5) were used for migration assays. For invasion
assays, the cells were double.

Bioinformatics analyses
The ccRCC samples were classified into two groups
with the same sample based on the median CENPA
expression. The GSEA (http://www.broadinstitute.org/
gsea) analysis was conducted for enrichment analysis
according to the grouping. The p < 0.05 and the false discovery rate (FDR) value < 0.25 were thoughted as the relevant enriched pathways [65]. The KEGG and GO analyses of differential expressed genes form two CENPA expression groups were conducted by R 4.0.2.

The activation or inhibition of Wnt signaling pathway

10 μmol/L Wnt/β-catenin inhibitor XAV-939 or agonist CHIR-99021 trihydrochloride (MedChemExpress, USA, HY-15147 and HY-10182B) were mixed in cells in 6-well plates for 24 h when confluence reached 60%. Then the proteins were collected for further analysis.

Wound healing assay

The same amounts of cells were cared in the 6-well plates. When the fusion reached 100%, cells were wounded with the same size. Pictures of wounds were observed at 24 h.

Colony formation assays

1000 A498 (Si-NC, Si-CENPA, Vector, CENPA) and Caki-1 (Si-NC, Si-CENPA, Vector, CENPA) cells were cultured into a well of 6-well plates. After about 12 days, the cells were fixed for 30 min. After washed by PBS the cells were then dyed with crystal violet for 40 min.

Statistical analyses

The group data were presented with mean and standard deviation (SD). The differences between groups were evaluated using a Student’s t-test or paired Student’s t-test. The relationships between CENPA expression and clinicopathological characteristics of ccRCC samples were analyzed by Pearson’s χ2 test. To conduct univariate and multivariate Cox regression analyses, we assigned clinicopathological features as binary variables, including CENPA mRNA expression levels and set OS as dependent variable. Then the analysis were conducted by SPSS 25.0. Significance was determined at P < 0.05. All of experiments were repeated for three times. Except the qRT-PCR data of tumor and normal tissues were represented as mean, the rest were represented as the mean ± SD. All analyses above were conducted by GraphPad Prism (GraphPad Software, San Diego, California in USA) as seen in previous article [66].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-021-03087-8.

Additional file 1: Figure S1. CENPA was closely related to clinical traits. (A–B) The expression of CENPA elevated with N stage and M stage in TCGA-KIRC cohort. (C) The ROC curve of CENPA in TCGA KIRC N/T pairs. (D–G) The subgroup analysis of survival curve according to age, gender, Stage and G grade. (H–K) The subgroup analysis of ROC curve according to T, M, Stage and G grade. (L–M) CENPA expression of KIRC patients without adjuvant therapy prior to the surgery.

Additional file 2: Figure S2. The GO and KEGG analysis of CENPA. (A–B) The results of the GO and KEGG analysis about CENPA. TCGA-KIRC ccRCC samples were divided into two groups based on CENPA expression levels, then DEGs between two groups were enriched referred to GO and KEGG database. (C, E, F and H) To explore their effects on β-catenin (CTNNB1), a series of concentration gradients for 24 h and time gradients for 10 μM of XAV-939 and CHIR-99021 trihydrochloride were employed Caki-1. Then 10 μM and 24 h were considered to be a proper drug treatment condition (D and G) The mRNA and protein expression of CTNNB1 in A498 and Caki-1 cells after transfected with si-CTNNB1. (I) The genetic alteration information of eight CNEP family members.

Additional file 3: Table S1. The clinicopathological features of the 120 ccRCC patients.

Additional file 4. The original western blot pictures.

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No.

Authors’ contributions

QW, JX and XPZ designed this study. QW, TBJ, JCL, YNL, JPC, JS, YS, and CJY performed the experiments. ZYX, DL, HGL, XY and HMY analyzed the data. QW and JX wrote the paper. All authors read and approved final manuscript.

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Availability of data and materials

The datasets and data used in this study can be obtained from official website or corresponding author.

Declarations

Ethics approval and consent to participate

The Institutional Review Board of Huazhong University of Science and Technology has approved our research procedures.

Consent for publication

Yes.

Competing interests

The authors declare no conflict of interests.

Abbreviations

RCC: Renal cell carcinoma; ccRCC: Clear cell renal cell carcinoma; DEG: Differentially expressed genes; CENP: Centromere protein; WGCNA: Weighted gene co-expression network analysis; KIRC: Kidney renal clear cell carcinoma; TCGA: The Cancer Genome Atlas; CNA: Copy-number alteration; OS: Overall survival; DFS: Disease free survival; CLEC: Cancer Cell Line Encyclopedia; GEO: Gene expression omnibus; ROC: Receiver operator characteristic; qRT-PCR: Reverse transcription-quantitative PCR; IHC: Immunohistochemistry; IBT: Immunoblotting test; CCK-8: Cell Counting Kit-8; GSEA: Gene set enrichment analysis; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; CTNNB1: β-Catenin; CCND1: Cyclin D1; DEG: Differential expressed gene; RNA-seq: RNA sequencing; AJCC: American Joint Committee on Cancer; PBST: Phosphate Buffered Saline Tween; FDR: False discovery rate; SD: Standard Deviation.
Author details
1 Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China. 1 Department of Pathogen Biology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. 1 Department of Thoracic, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China.

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References
1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30.
2. Campbell S, Uzzo RG, Allal ME, Bass EB, Caddeo JA, Chang A, Clark PE, Davis BJ, Derweesh IH, Giambraresi L, et al. Renal mass and localized renal cancer: AUAG guideline. J Urol. 2017;198(3):S20–9.
3. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO classification of tumours of the urinary system and male genital organs – part a: renal, penile, and testicular tumours. Eur Urol. 2016;70(1):93–105.
4. Gong J, Masa MC, Dizman N, Govindarajan A, Pal SK. Metastasis in renal cell carcinoma: biology and implications for therapy. Asian J Urol. 2016;3(4):286–92.
5. Xiong Z, Yuan C, Shi J, Xiong W, Huang Y, Xiao W, Yang H, Chen K, Zhang X. Restoring the epigenetically silenced PCK2 suppresses renal cell carcinoma progression and increases sensitivity to sunitinib by promoting endoplasmic reticulum stress. Theranostics. 2020;10(25):11444–61.
6. Xu T, Ruan H, Gao S, Liu J, Liu Y, Song Z, Cao Q, Wang K, Bao L, Liu D, et al. ISG20 serves as a potential biomarker and drives tumor progression in clear cell renal cell carcinoma. Aging. 2020;12(21):1808–27.
7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
8. Mahile MA, Necheima-Arbel Y. Guarding the genome: CENP-A-chromatin in health and cancer. Genes. 2020;11(7):810.
9. Rajagopalan H, Lengauer C. Aneuploidy and cancer. Nature. 2004;432(7015):338–41.
10. Funk LC, Zasadil LM, Weaver BA. Living in CIN: mitotic infidelity and its consequences for tumor promotion and suppression. Dev Cell. 2016;39(6):638–52.
11. Bakhoum SF, Cantley LC. The multifaceted role of chromosomal instability and centromere protein-A in cancer: AUA guideline. J Urol. 2017;198(3):520–9.
12. Bolhaqueiro ACF, Ponsioen B, Bakker B, Klaasen SJ, Kucukkose E, van der Raaij ES, et al. Identification and analysis of novel biomarkers involved in chromophobe renal cell carcinoma by integrative bioinformatics analyses. Biomed Res Int. 2020;2020:2671281.
13. Parasarmana M, Serie DJ, Asmarn YW, Eckel-Passow JE, Castle EP, Stanford ML, Leibovich BC, Thompson RI, Thompson EA, Parker AS, et al. Validation of gene expression signatures to identify low-risk-clear cell renal cell carcinoma patients at higher risk for disease-related death. Eur Urol Focus. 2016;2(6):608–15.
14. Wang Y, Chen L, Wang G, Cheng S, Qian K, Liu X, Wu CL, Xiao Y, Wang X. Fifteen hub genes associated with progression and prognosis of clear cell renal cell carcinoma identified by coexpression analysis. J Cell Physiol. 2019;234(7):10225–37.
15. Wu Y, Cai J, Yan J, Deng A, Cao Y, Zhu X. Identification of novel glycolysis-related gene signatures associated with prognosis of patients with clear cell renal cell carcinoma based on TCGA. Front Genet. 2020;11:598663.
16. Rhodes DR, Yu J, Shanker K, Deshpande V, Varambally R, Barretina J, Paner SE, Rimm DL, et al. Oncomine: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004;6(1):1–6.
17. Wotchofski Z, Gummlich L, Liep J, Stephan C, Kilic E, Jung K, Billaud JM, Meyer HA. Integrated microRNA and mRNA signature associated with the transition from the locally confined to the metastasized clear cell renal cell carcinoma exemplified by mir-146–5p. PloS ONE. 2016;11(2):e0148746.
18. Wozniak MB, Le Calvez-Kelm F, Abdi-Addeki B, Byrnes G, Durand C, Carreira C, Michelon J, Janout V, Holcatova I, Foretova L, et al. Integrative genome-wide gene expression profiling of clear cell renal cell carcinoma in Czech Republic and in the United States. Plos ONE. 2013;8(3):e57886.
19. Zeitlin SG, Shelby RD, Sullivan KF. CENP-A is phosphorylated by Aurora B kinase and plays an unexpected role in completion of cytokinesis. J Cell Biol. 2001;155(7):1147–57.
20. Husein F, Erfardt S, Blower MD, Weiss S, Skora AD, Karpen GH. Mislocalization of the Drosophila centromere-specific histone CID promotes formation of functional ectopic kinetochores. Dev Cell. 2006;10(3):303–15.
21. Shrestha RL, Rossi A, Wangsa D, Hogan AK, Zaladana KS, Suva E, Chung YJ, Sanders CL, Dillipantoni S, Karpova TS, et al. CENP-A overexpression promotes aneuploidy with karyotypic heterogeneity. J Cell Biol. 2021;212(4):e202007195.
22. Kung PP, Martinez R, Zhu Z, Zager M, Blasina A, Rymer I, Hallin J, Xu M, Carroll C, Chiosis G, et al. Chromogenic evaluation of the mitotic kinesin CENP-E reveals a critical role in triple-negative breast cancer. Mol Cancer Ther. 2014;13(8):2104–15.
23. Wood KW, Lad L, Luo L, Qian X, Knight SD, Nevins N, Breck K, Sutton D, Gilmartin AG, Chua PH, et al. Antitumor activity of an allelostatic inhibitor of centromere-associated protein-E. Proc Natl Acad Sci USA. 2010;107(13):5893–44.
24. Lad L, Luo L, Carson JD, Wood KW, Hartman JJ, Copeland RA, Sakowicz R. Mechanism of inhibition of human KSP by ispinesib Biochemistry. 2008;47(11):3575–85.
40. Von Schulz-Hausmann SA, Schmeel LC, Schmeel FC, Schmidt-Wolf IG. Targeting the Wnt/beta-catenin pathway in renal cell carcinoma. Anticancer Res. 2013;34(8):4101–8.
41. Hwang SY, Deng X, Byun S, Lee C, Lee SJ, Suh H, Zhang J, Kang Q, Zhang T, Westover KD, et al. Direct targeting of beta-catenin by a small molecule stimulates proteasomal degradation and suppresses oncogenic Wnt/beta-catenin signaling. Cell Rep. 2016;16(1):28–36.
42. Jang GB, Hong IS, Kim RJ, Lee SY, Park SJ, Lee ES, Park JH, Yun CH, Chung JI, Lee KJ, et al. Wnt/beta-catenin small-molecule inhibitor CWP232228 preferentially inhibits the growth of breast cancer stem-like cells. Can Res. 2015;75(8):1691–702.
43. McGovern SL, Qi Y, Pusztai L, Symmans WF, Buchholz TA. Centromere protein-A, an essential centromere protein, is a prognostic marker for relapse in estrogen receptor-positive breast cancer. Breast Cancer Res. 2012;14(3):R72.
44. Nechemia-Arbel Y, Miga KH, Shoshani O, Aslalian A, McMahon MA, Lee YJ, Fachinetti D, Yates JR 3rd, Rien B, CLEVELAND DW. DNA replication acts as an error correction mechanism to maintain centromere identity by restricting CENP-A to centromeres. Nat Cell Biol. 2019;21(6):743–54.
45. Shirakawa J, Fernandez M, Takatani T, El Ouaamari A, Jungtrakoon P, Okawa ER, Zhang W, Yi P, Doria A, Kulkarni RN. Insulin signaling regulates the FoxM1/Pleck1/CENP-A pathway to promote adaptive pancreatic beta cell proliferation. Cell Metab. 2017;25(4):868-882.e865.
46. Jeffery D, Gatto A, Punsalpan K, Renaud-Pageot C, Ponce Landete R, Jeffery D, Gatto A, Podsypanina K, Renaud-Pageot C, Ponce Landete R, Thomas S, Myszczyszyn A, Elezkurtaj S, Erguen B, et al. Inhibiting WNT and NOTCH signaling promotes distinct fates in human cells, depending on p53 status. Commun Biol. 2021;4(1):417.
47. Vardabasso C, Gaspar-Maia A, Hasson D, Pünzeler S, Valle-Garcia D, Straub T, Keilhauer EC, Stadlbauer T, Deng X, Byun S, Lee C, Lee SJ, Suh H, Zhang J, Kang Q, Zhang T, Westover KD, et al. Direct targeting of beta-catenin by a small molecule stimulates proteasomal degradation and suppresses oncogenic Wnt/beta-catenin signaling. Cell Rep. 2016;16(1):28–36.
48. Jeffery D, Gatto A, Podsypanina K, Renaud-Pageot C, Ponce Landete R, Thomas S, Myszczyszyn A, Elezkurtaj S, Erguen B, et al. Inhibiting WNT and NOTCH signaling promotes distinct fates in human cells, depending on p53 status. Commun Biol. 2021;4(1):417.
49. Nusse R. Wnt signaling in disease and in development. Cell Res. 2005;15(1):28–32.
50. Fendler A, Bauer D, Busch J, Jung K, Wulf-Goldenberg A, Kunz S, Song K, Myszczyszyn A, Elezkurtaj S, Enguen B, et al. Inhibiting WNT and NOTCH in renal cancer stem cells and the implications for human patients. Nat Commun. 2020;11(1):929.
51. Kruck S, Eyrich C, Scharpf M, Sievert KD, Fend F, Stenzl A, Bedike J. Impact of an altered Wnt1/beta-catenin expression on clinicopathology and prognosis in clear cell renal cell carcinoma. Int J Mol Sci. 2013;14(6):10944–57.
52. Urakami S, Shiina H, Enokida H, Hirata H, Kawamoto K, Kawakami T, Kikuno N, Tanaka Y, Majid S, Nakagawa M, et al. Wnt antagonist family genes as biomarkers for diagnosis, staging, and prognosis of renal cell carcinoma using tumor and serum DNA. Clin Cancer Res. 2006;12(23):6989–97.
53. Saini S, Majid S, Davya R. The complex roles of Wnt antagonists in RCC. Nat Rev Urol. 2011;8(22):690–9.
54. Piotrowska Z, Niezgoda M, Mlynczak G, Acewicz M, Kasacka I. Comparative assessment of the WNT/beta-catenin pathway, CacyBP/SIP, and the immunoproteasome subunit LMP7 in various histological types of renal cell carcinoma. Front Oncol. 2020;10:566337.
55. Jang GB, Hong IS, Kim RJ, Lee SY, Park SJ, Lee ES, Park JH, Yun CH, Chung JI, Lee KJ, et al. Wnt/beta-catenin small-molecule inhibitor CWP232228 preferentially inhibits the growth of breast cancer stem-like cells. Can Res. 2015;75(8):1691–702.
56. Ross-Macdonald P, Walsh AM, Chasalow SD, Ammar R, Papillon-Cavanagh S, Szabo PM, Choueiri TK, Sznl M, Wind-Rotolo M. Molecular correlates of response to nivolumab at baseline and on treatment in patients with RCC. J Immunother Cancer. 2021;9(3):e001506.
57. Findlay JM, Middleton MR, Tomlinson I. A systematic review and meta-analysis of somatic and germline DNA sequence biomarkers of esophageal cancer survival, therapy response and stage. Ann Oncol. 2015;26(4):624–44.
58. Qie S, Diehl JA. Cyclin D1, cancer progression, and opportunities in cancer treatment. J Mol Med. 2016;94(12):1313–26.
59. Kato J, Matsushima H, Hiebert SW, Ewen ME, Sheer CJ. Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. Genes Dev. 1993;7(3):331–42.
60. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17(6):1471–74.
61. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. Lmima powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
62. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinform. 2008;9:559.
63. Shannon R, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikiowski B, Ideker T. CytoScape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504.
64. Liu Y, Cheng G, Huang Z, Bao L, Liu J, Wang C, Xiong Z, Zhou L, Xu T, Liu D, et al. Abdominal lymph node RNA SHG-H12 promotes tumour progression and sunitinib resistance by upregulating CDC43 in renal cell carcinoma. Cell Death Dis. 2020;11(7):1515.
65. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005, 102(43):15545–50.
66. Xu J, Liu Y, Liu J, Xu T, Cheng G, Shou Y, Tong J, Liu L, Zhou L, Xiao W, et al. The identification of critical miRNA methyltransferase regulators as malignant prognosis factors in prostate adenocarcinoma. Front Genet. 2020;11:602485.

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