Identification of molecular vulnerabilities in aggressive renal cell carcinoma by a small molecule

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ABSTRACT

There is an urgent need to develop new therapeutic options for metastatic renal cell carcinoma (RCC) patients with a dismal prognosis due to a lack of effective treatment options. Our small molecule screening effort has identified a new chemical compound that specifically inhibited the growth of RCC cells over normal kidney cells. The anti-RCC potential of our compound was confirmed by inhibiting RCC colony-forming ability and RCC proliferation, inducing cell cycle arrest and apoptosis, and synergizing iron-related cell death. We are profiling transcriptional changes and binding proteins after our compound treatment on RCC cells using high-throughput sequencing and proteomics techniques, which will assist us to identify molecular vulnerabilities of RCC. Furthermore, we aim to validate the translational potential of our compound and develop/test new chemical analogs of our compound using patient-derived xenografts and cell-line derived xenografts.

BACKGROUND

Among patients with renal cell carcinoma (RCC), one out of three individuals experience metastatic spread. The average survival period for metastatic RCC patients is 6 to 12 months, with a 2-year survival rate ranging from 10% to 20%. Metastatic RCC patients are treated with systemic therapies, including targeted and immunotherapy. Currently, seven FDA-approved drugs are being applied in clinical treatment as monotherapy or combination. However, despite the availability of various treatment approaches, the prognosis remains poor. Therefore, developing effective therapeutic agents is an urgent need for RCC patients.

RESULT

Figure 1. Scheme of a chemical compound screening by using an assay measuring cell viability

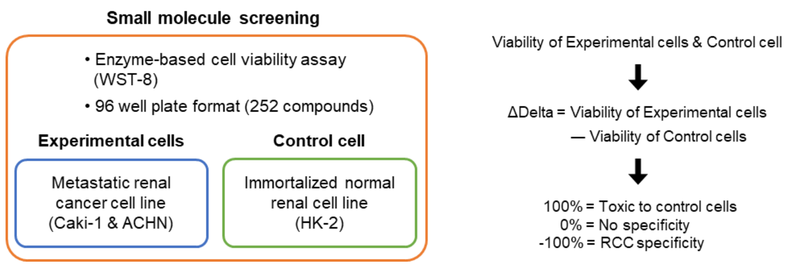
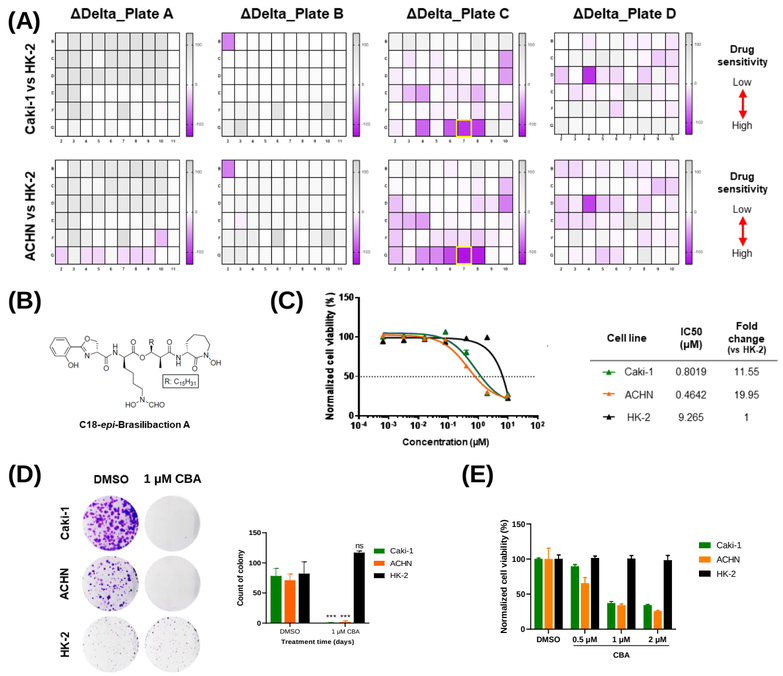
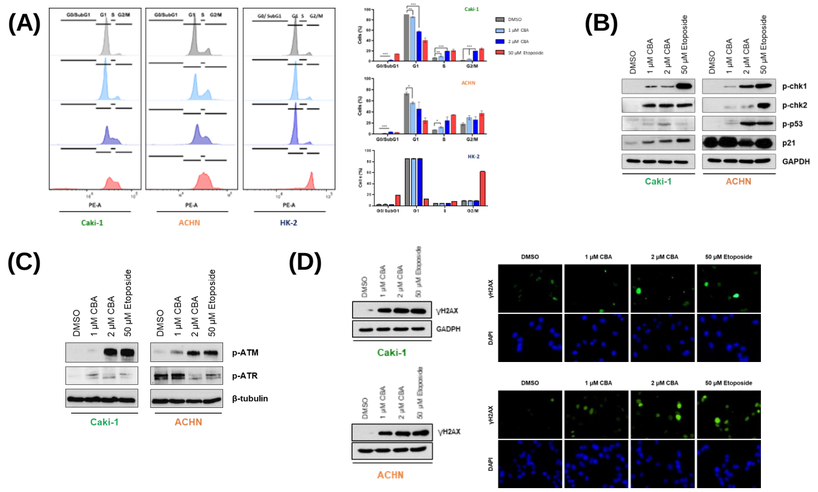


Figure 2. C18-*epi*-Brasilibaction A (CBA) specifically inhibit the cell viability and proliferation on RCC



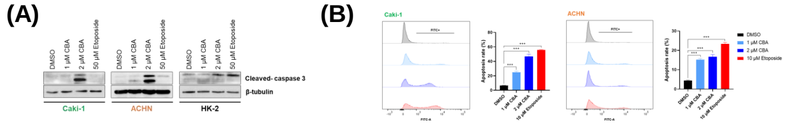
(A) Cell viability of renal cell lines. All cells were treated various compound for 48 hours. (B) Structure of CBA (C) IC50 concentration of renal cell lines by CBA. (D) Colony forming assay of renal cell lines by CBA. (E) Cell viability by single concentration of CBA.

Figure 3. CBA induce cell cycle arrest and DNA damage



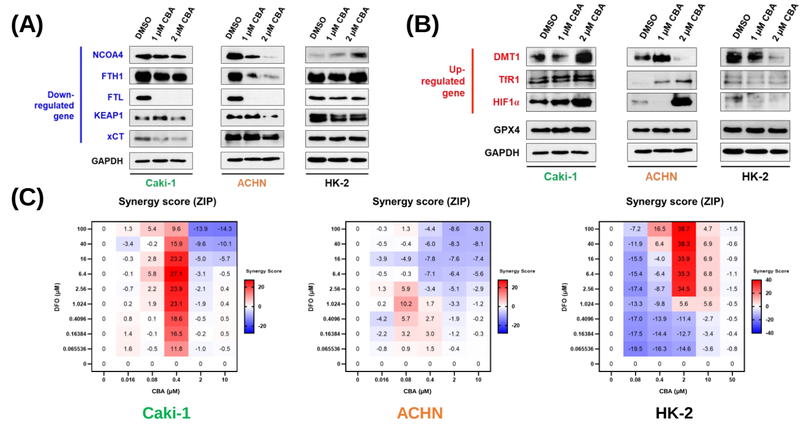
(A) Cell cycle assay of renal cell lines using PI staining. The percentage shows the cell population rate. (B) Representative immunoblots of RCC cell lines with antibodies against cell cycle-related genes. (C)) Representative immunoblots of p-ATM and p-ATR. (D) Representative immunoblots and fluorescence images of γH2AX.

Figure 4. CBA induce apoptosis on RCC



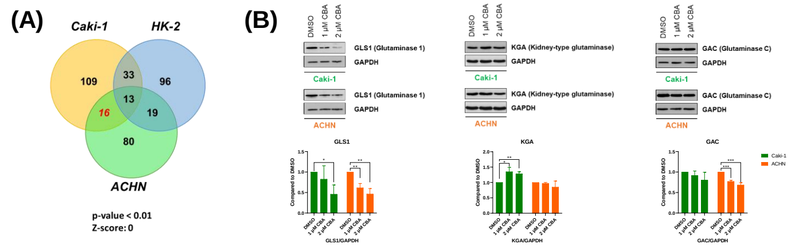
(A) Cleaved caspase-3 expression of RCC by CBA (B) Annexin V staining of RCC

Figure 5. CBA de-regulated the iron-related proteins and CBA have the synergy effect with deferoxamine (DFO)



(A) Up-regulated (B) down –regulated iron-related genes by CBA. (C) Synergy effect of CBA and DFO on renal cell lines. All cells were treated for 48 hours

Figure 6. CBA regulated the glutaminase expression



(A) TPP assay to confirm the de-stabilized protein by CBA. (B) Representative immunoblots image of glutaminase and isoforms.

CONCLUSION

In this study, a new chemical compound, CBA, which showed specific anticancer effects on metastatic RCC cell lines was discovered. The mechanism of action of CBA may tackle multiple cellular and molecular processes based on our findings, which will provide insight into discover new therapeutic vulnerabilities in metastatic RCC cells. Currently, we aim to validate the translational potential of CBA and develop/test new chemical analogs of our compound using patient-derived xenografts and cell-line derived xenografts.