Characterization of Small Molecules that Degrade YAP1 Protein in Cancer

Sudili Fernando[1], Praveen Barrodia[1], Kunal Rai[1]

1. Department of Genomic Medicine, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

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Background

The Hippo Pathway is a signaling pathway that regulates various essential biological processes including cellular proliferation, cell survival, differentiation, organ size, and tissue homeostasis. YAP1 is a transcriptional co-activator and downstream effector in the Hippo pathway[1].

If overexpressed, YAP1 can become a highly potent oncogene that plays a major role tumorigenesis and metastasis in various types of cancer. This gene is overexpressed in 10% of all cancers and seen in many solid tumors either by genetic copy number amplification or by unknown pathways.

YAP1 activation also leads to resistance to common cancer therapies such as cytotoxic chemotherapy, molecular targeted therapy and radiotherapy[2]. Thus, targeting YAP1 requires nontraditional drug and cancer technology.

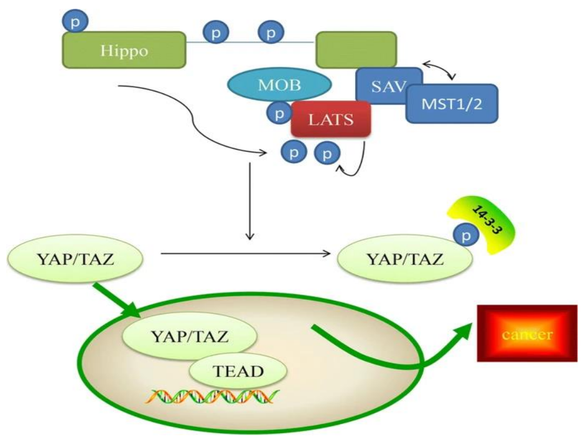


Fig 1. Hippo Signaling Pathway Mechanism
Han Y. Analysis of the role of the Hippo pathway in cancer. J Transl Med. 2019 Apr 8;17(1):116

PROTACs are a novel drug technology that regulate protein function by completely degrading the target protein rather than inhibiting it. PROTACs are sensitive to drug resistant targets, effective at low concentrations, can target undruggable targets, and can influence nonenzymatic functions, making them unique from other drug technologies[4].

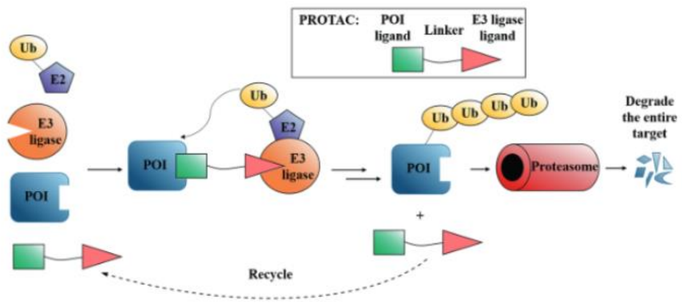


Fig 2. PROTAC structure and mode of Action
Sun et al Nature. (2019) 4:64

Introduction

Since YAP1 plays an oncogenic role in many different cancers, it is important to target this protein for cancer treatment, however, it does not have any targetable enzymatic activity. This makes novel blocking strategies, such as PROTAC's, important for possible treatment. Since YAP1 has been shown to be regulated by proteasomal mediated degradation, we reasoned that it may be possible to identify chemicals/PROTACS that promote YAP1 degradation. To achieve this, we performed a high-throughput chemical screen to identify compounds/PROTACs that could degrade YAP1 proteins. Followed by western blot verification.

Methods

Below is a flow chart outlining the general procedures used in this study to discover and verify the PROTACs most effective in degrading YAP1 proteins.

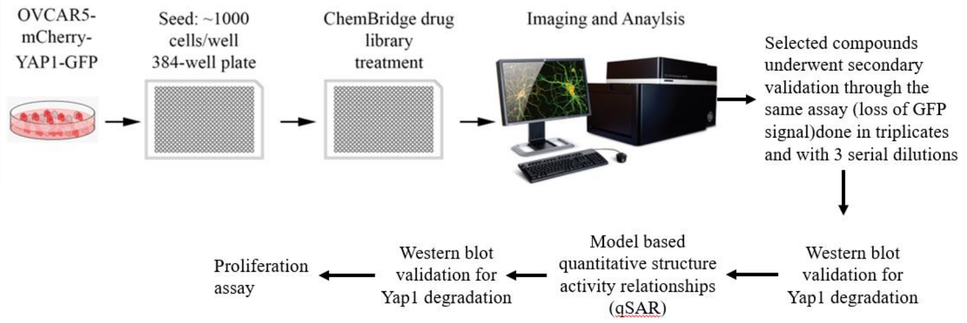


Fig 3. Study methodology

Results

Primary screening followed by secondary validation identified about 32 molecules with potential reduction of GFP levels tagged to the YAP1 protein. Some hits were further validated by YAP1 western blot which suggested that some of the identified compounds degraded YAP1.

CIDD-015087, was identified as a lead compound in degrading YAP1. Through qSAR of this compound three PROTACs, CIDD-0162266, CIDD-0162288, and CIDD-0162289, were synthesized. The efficacy of these four compounds were measured in YAP1 independent and YAP1 dependent cell lines after 4 hours of treatment.

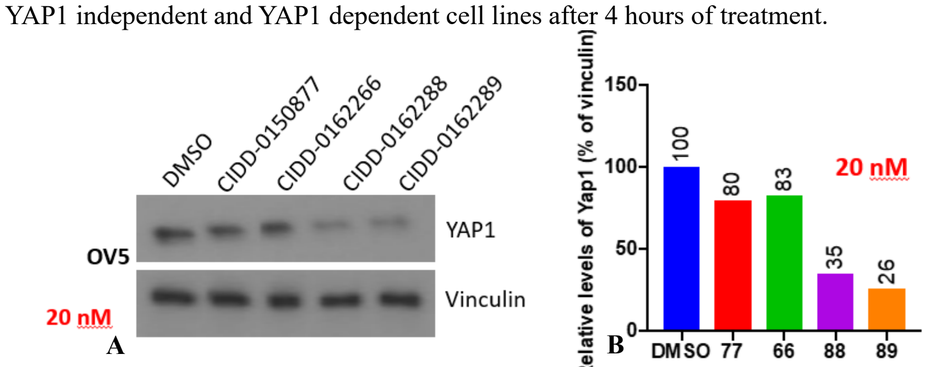


Fig 4 A-B. Western Blot and ImageJ analysis for OV5.

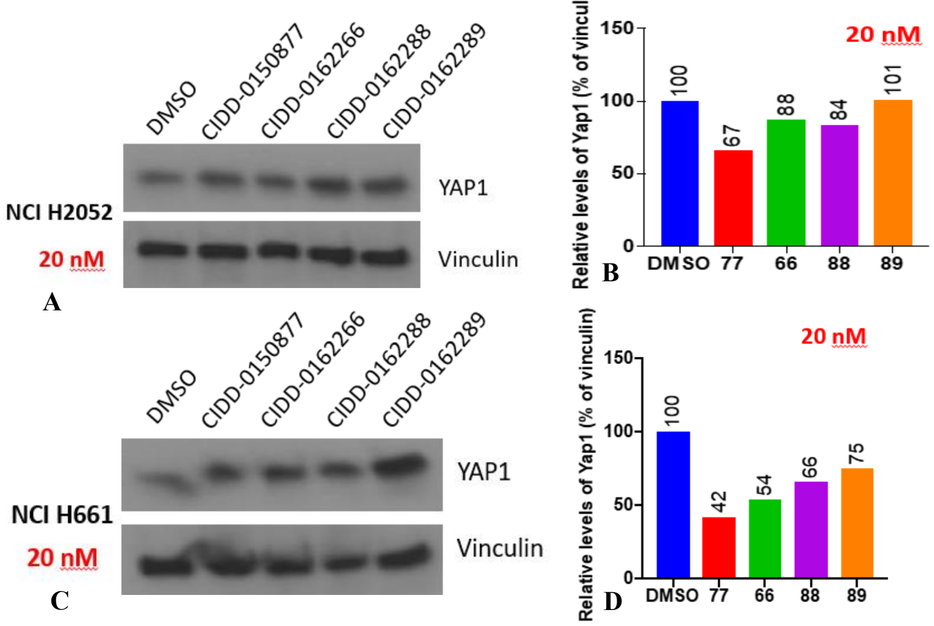
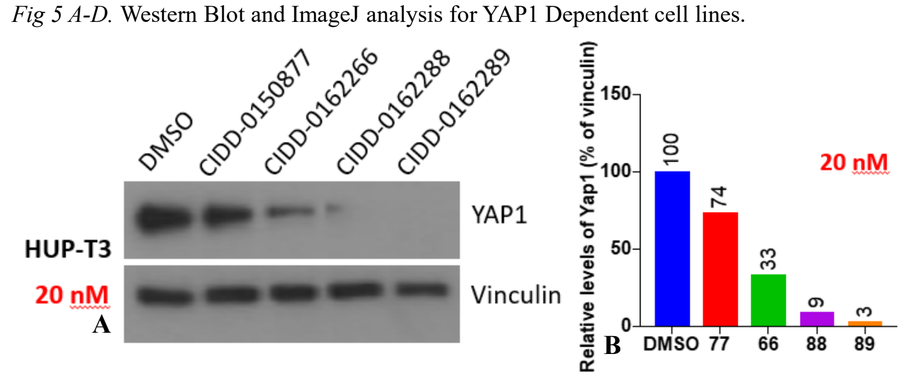


Fig 5 A-D. Western Blot and ImageJ analysis for YAP1 Dependent cell lines.



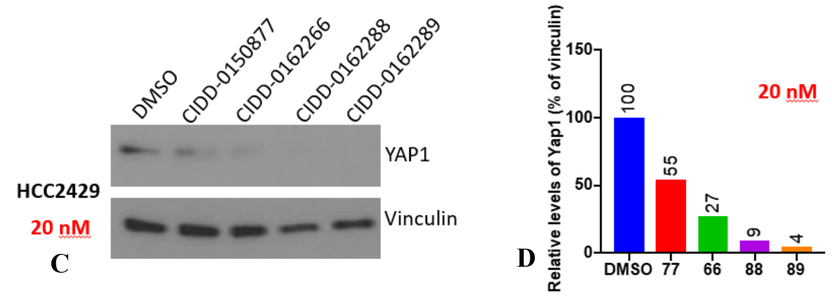


Fig 6 A-D. Western Blot and ImageJ analysis for YAP1 independent cell lines.

With all drugs at 20nM, we saw a reduction in YAP1 protein in both YAP1 dependent and independent cell lines within 4 hours of treatment.

The proliferation assay with the OV5 cell line showed a decrease in proliferation at high concentrations in the CIDD-0150877, CIDD-0162266, and CIDD-0162288 compounds. There was no decrease in proliferation at any concentration for compound CIDD-0162289.

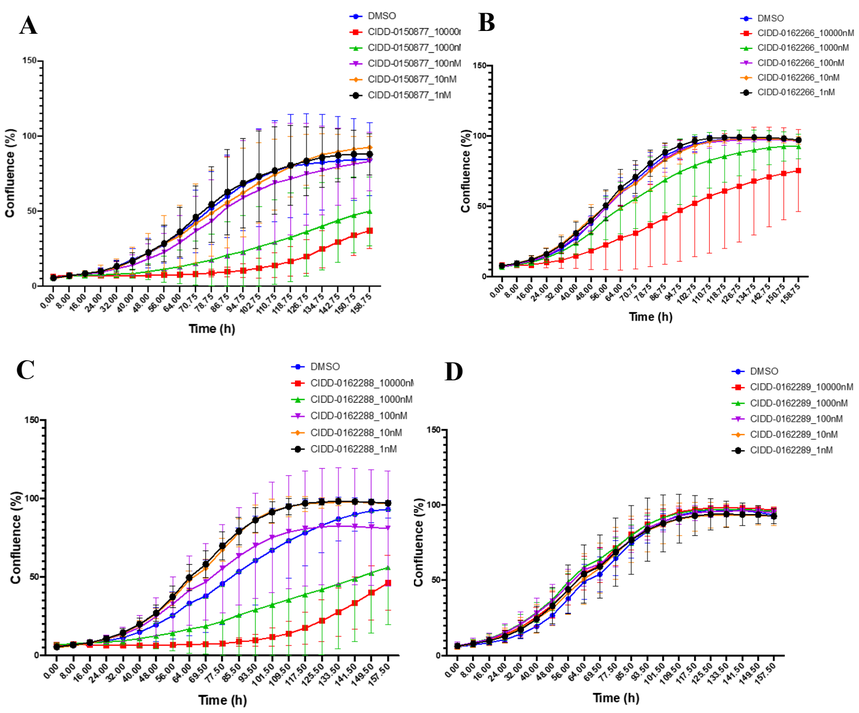


Fig 7. **A:** Proliferation assay results for CIDD-0150877. **B:** Proliferation results for PROTAC CIDD-0162266. **C:** Proliferation results for PRTOAC CIDD-0162288. **D:** Proliferation results for PROTAC CIDD-0162289.

In compounds CIDD-0150877, CIDD-0162266, and CIDD-0162288, the two highest concentrations decreased the viability of the OV5 cells. OV5 cell line proliferation is not dependent on the presence of YAP1, these high concentrations may suggest toxicity to this cell line. The lack of any change at any concentration in the CIDD-0162289 assay suggest that the compound might not be effective.

Conclusion

Our data show that these four novel molecules that can significantly reduce YAP1 protein levels in different cell lines. This data suggests that these compounds could be useful agents to target YAP1-dependent cancers and could offer significant improvements in a short amount of time.

Future Direction

Our future steps include conducting proliferation assays using all the drugs on YAP1 independent and dependent cell lines. After this we hope to validate these results through *in vivo* studies.

References

1. Calses et al Trends in Cancer. (2019) 5: 297-307
2. Kim et al Cell. Mol. Life Sci. (2017) 74:1457–1474
3. Han Y. Analysis of the role of the Hippo pathway in cancer. J Transl Med. 2019 Apr 8;17(1):116
4. Sun et al Nature. (2019) 4:64