Targeting YAP1/TEAD signaling re-sensitizes MAPK/ERK pathway inhibitors in KRAS-driven cancer cells

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Abstract #1931

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Introduction

Several TEAD small-molecule inhibitors (SMIs) have reported initial clinical evaluation on Hippo-mutated cancer types. Studies have also demonstrated that activation of the YAP1/TEAD transcriptional complex can drive resistance to MAPK/ERK pathway inhibitors via a Hippo-independent manner. Here, we elucidated the potential mechanism of TEAD inhibition overcoming MAPK/ERK pathway resistance with a TEAD SMI (hereafter abbreviated as TEADi), which is reported as a direct YAP1/TEAD protein-protein interaction blocker, discovered by Novartis Pharmaceuticals[1].

Objectives

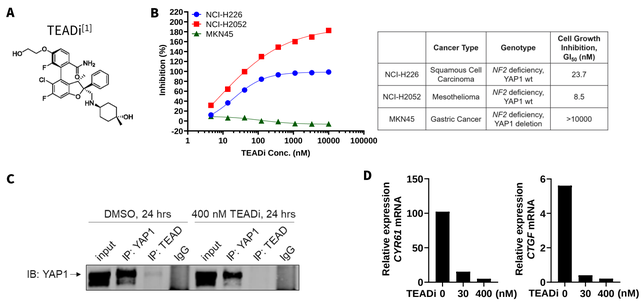
- The mechanism underlying resistance to the targeted therapies.
- How TEAD inhibition restore the sensitivity of resistant cells to targeted therapy

Methods

- **Cell growth assay:** Cell lines were treated with compound(s) for 72 hours and cell viability was measured by CellTiter-Glo (Promega).
- **Co-IP assay:** Compound treated NCI-H226 cells were lysed. The cell lysate was incubated with anti-pan TEAD antibody (CST) and protein complex was pulled down with Protein A/G immobilized sepharose beads (Thermo Scientific). Content of the sample was analyzed by western-blot.
- **qRT-PCR assay:** Cell lines were treated with compound for 24 hours and total mRNA was extracted for downstream gene expression evaluation by qRT-PCR assay.
- **Immunofluoresence assay:** Cells were collected and fixed with 10% neutral buffered formalin (Sigma) for IF assay. Permeabilized cells were incubated with anti-YAP1 antibody (CST) and stained for imaging.
- **Luciferase reporter assay:** The indicated cells were co-transfected with 8xGTIIC-pGL4.25 [luc2CP/minP] and pRL-SV40 (Promega) using Lipofectamine 3000 Transfection Reagent (Invitrogen). After transfection, cells were treated with compound for 24 hours. Luciferase activity was measured with the dual luciferase reporter assay system (Promega).
- **Signaling pathway:** Cell lines were treated with compound for 24 hours and protein expression was measured by western-blotting assay.

Results

Validation of MoA of TEADi in Hippo-mutated cancer types



	Cancer Type	Genotype	Cell Growth Inhibition, GI_{50} (nM)
NCI-H226	Squamous Cell Carcinoma	*NF2* deficiency, YAP1 wt	23.7
NCI-H2052	Mesothelioma	*NF2* deficiency, YAP1 wt	8.5
MKN45	Gastric Cancer	*NF2* deficiency, YAP1 deletion	>10000

Figure 1: TEADi showed potency and selectivity in Hippo-mutated cells *in vitro*.
(A) Chemical structure of TEADi[1]. (B) TEADi inhibited cell growth of NCI-H226 and NCI-H2052 cells but not that of MKN45 cells. (C) TEADi disrupted the YAP1/TEAD interaction in NCI-H226 cells. (D) Two downstream genes of TEAD signaling *CYR61* and *CTGF* were suppressed by TEADi in NCI-H226 cells.

Resistant cells showed more sensitivity to TEADi treatment

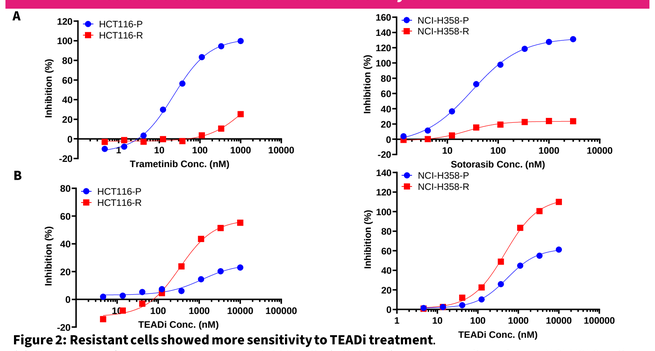


Figure 2: Resistant cells showed more sensitivity to TEADi treatment.
(A) Generation of Trametinib-resistant HCT116 cells (HCT116-R) and Sotorasib resistant NCI-H358 cells (NCI-H358-R). (B) TEADi treatment exerted more inhibitory effects on resistant cells compared to their parental counterparts.

YAP1 nucleus translocation was enhanced in resistant cells

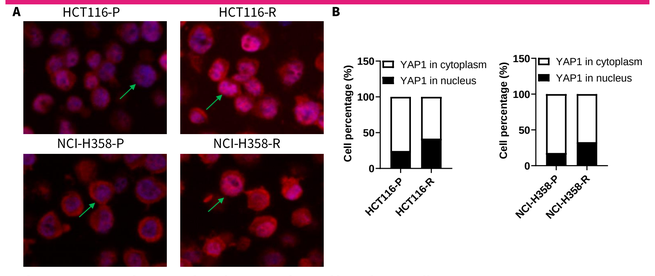


Figure 3: YAP1 nucleus translocation was enhanced in resistant cells.
(A) & (B) IF assay showed that more YAP1 was translocated into nucleus in two resistant cell lines.

YAP1 nucleus translocation promoted TEAD transcriptional activity

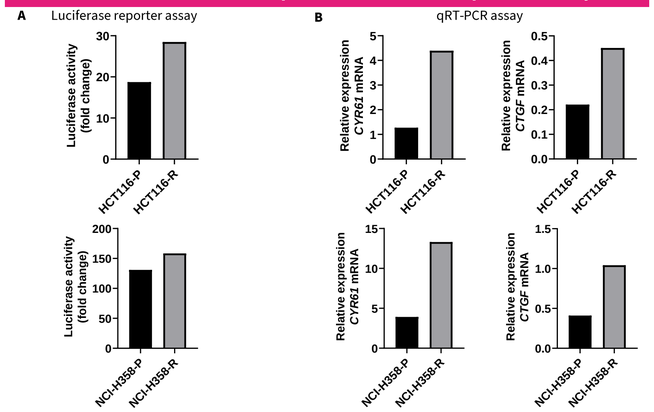


Figure 4: TEAD signaling was activated by YAP1 nucleus translocation.
(A) Increased transcription activities of TEAD and (B) elevated downstream gene expression levels were detected in two resistant cell lines.

TEADi suppressed targeted therapy-induced YAP1/TEAD activation

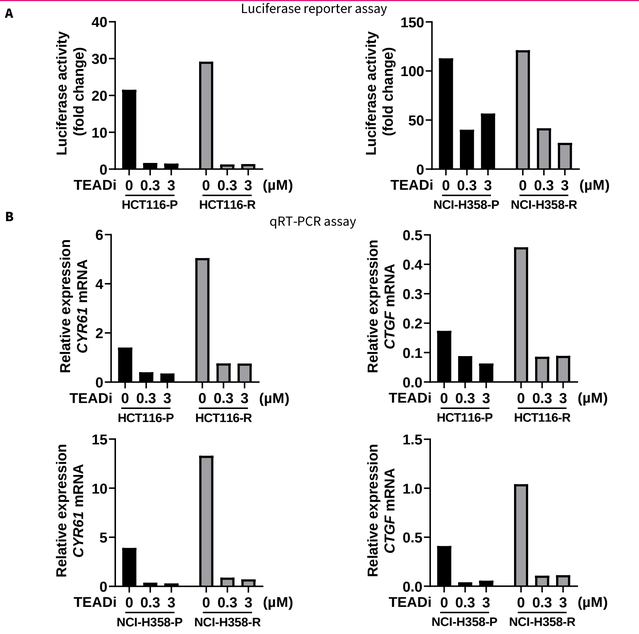


Figure 5: Targeted therapy-induced YAP1/TEAD activation was trans-suppressed with TEADi treatment.
(A) The transcriptional activities of TEAD and (B) two TEAD downstream genes were suppressed by TEADi.

TEADi restored the response of resistant cells to targeted therapy

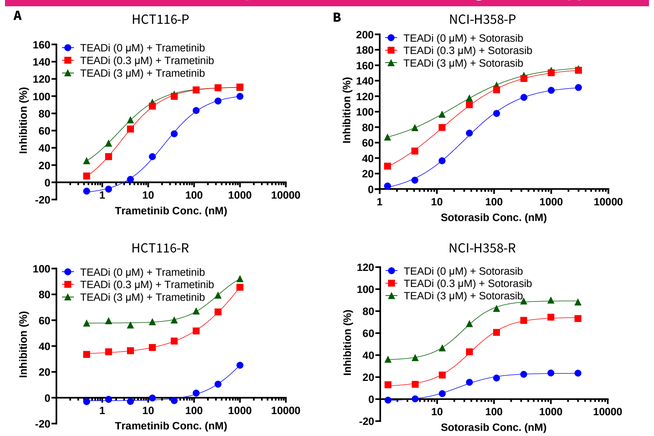


Figure 6: TEADi re-sensitized the two resistant cell lines to targeted therapy.

TEADi had almost no impact on ERK phosphorylation in resistant cells

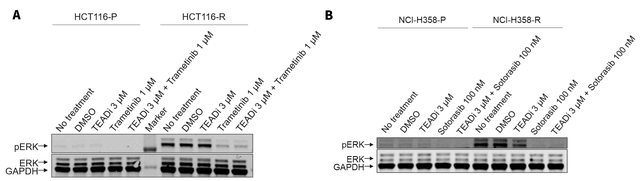


Figure 7: TEADi treatment showed almost no impact on ERK phosphorylation in two resistant cell lines.

Summary

- **TEADi showed potency and selectivity in Hippo-mutated cancer cell lines *in vitro*.**
- **TEADi disrupted the interaction between YAP1 and TEAD, and thus markedly repressed the expression of *CTGF* and *CYR61*, two downstream targets of YAP1/TEAD.**
- **Targeted therapy resistant cells showed more sensitivity to TEADi treatment, indicating the dependency on TEAD/YAP1 signaling.**
- **YAP1 nucleus translocation was enhanced in resistant cells, which is associated with increased transcriptional activity of TEAD.**
- **TEADi treatment restored the response of resistant cells to targeted therapy by trans-suppression of YAP1/TEAD signaling.**

Reference
[1]. WO2021186324A1

Presented at the AACR Annual Meeting, April 5-10, 2024, San Diego, California, USA