Discovery of a Small-Molecule Inhibitor of ADAR1 for Cancer Immunotherapy

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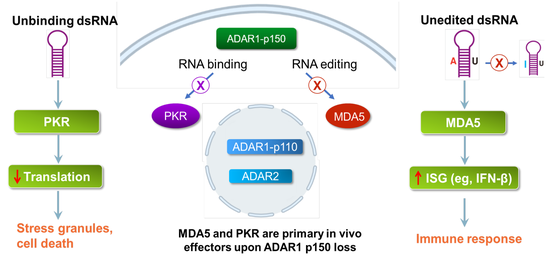
Introduction

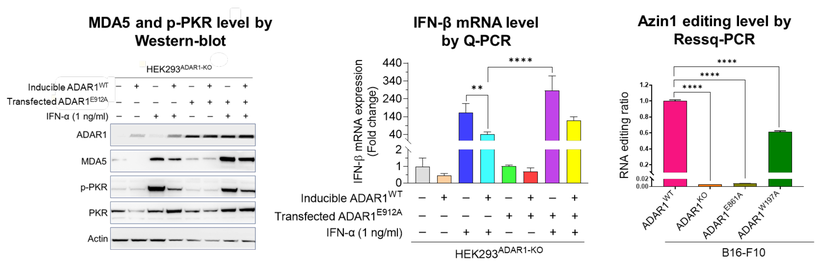
- Adenosine deaminase acting on RNA 1 (ADAR1) is a key enzyme that catalyzes the conversion of adenosine to inosine in double-stranded RNA (dsRNA)
- ADAR1 plays a critical role in preventing the erroneous recognition of endogenous dsRNA by cytoplasmic RNA sensors, such as MDA5, PKR, and ZBP1
- Inhibiting deaminase activity of ADAR1 can activate MDA5-mediated innate immunity and stimulate interferon responses, while inhibition of its RNA-binding activity can induce PKR-mediated translational shutdown and cell death
- A subset of cancer cell lines are dependent on ADAR1 and mounting evidence suggests that silencing ADAR1 render tumors more responsive to immune checkpoint inhibitors
- ADAR1 is thus a key and attractive immuno-oncology target. Leveraging on our state-of-the-art HTS platform and structural biology platform, Risen is developing novel small-molecule inhibitors of ADAR1 with advancing progress for cancer immunotherapy



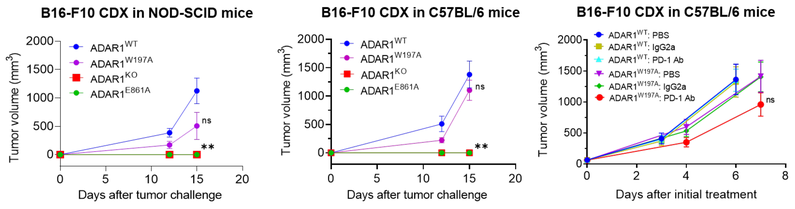
Results

1. Target validation

- ***In vitro*: Loss of editing function of ADAR1 upregulates MDA5 and interferon expression in HEK293 cell**



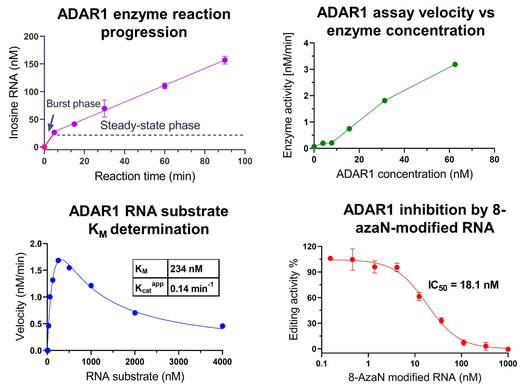
- ***In vivo*: Abolishment of editing function of ADAR1 inhibits B16-F10 melanoma cell growth in mice**



- Abolishment of editing function of ADAR1 (E861A) or knockout of ADAR1 in B16-F10 cells could suppress tumor growth in mice
- While disruption of Z-RNA binding capacity of ADAR1 (W197A) only shows moderate effect in the inhibition of tumor growth, even in combination with PD-1 blockade

2. Risen has developed proprietary FRET based biochemical editing assay for HTS primary screening

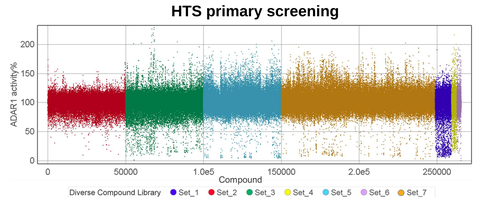
- ADAR1 enzymatic reaction over time was monitored to choose the stead-state phase
- ADAR1 enzymatic reaction velocity increases linearly with enzyme concentration
- Substrate concentration was selected at around K_M in the HTS campaign, and inhibition of velocity was observed at higher concentrations
- The assay was validated by 8-azanebularine-modified dsRNA duplexes control



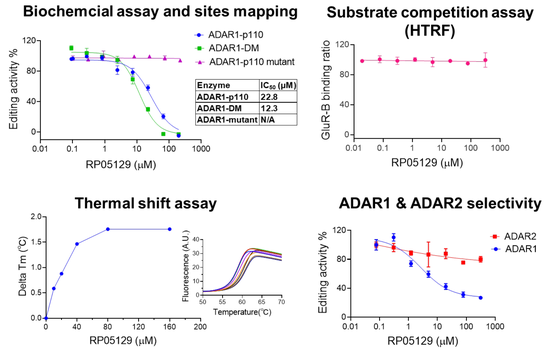
3. Discovery and characterization of the ADAR1 inhibitor

• HTS screening

- 265K compounds were screened against ADAR1-deaminase domain
- 505 primary hits were identified and further subjected to triage validation



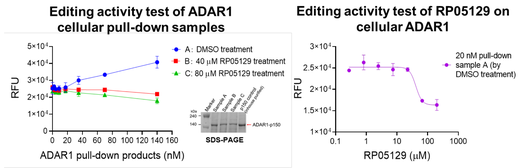
• Biochemical characterization of ADAR1 inhibitor



- RP05129 blocks ADAR1 editing activity and is not substrate-competitive
- Binding sites were mapped out by a series of point mutation experiments
- RP05129 could stabilize ADAR1 protein in a dose-dependent manner
- RP05129 has good selectivity to ADAR1 over ADAR2

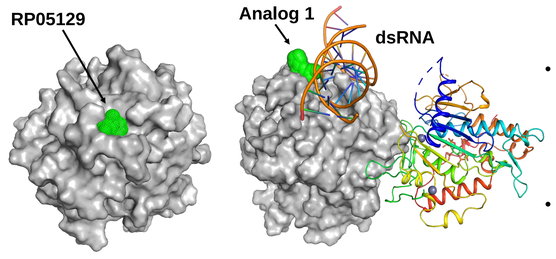
• Cellular target engagement

- RP05129 blocks the editing activity of ADAR1 in cellular complex
- Cellular target engagement and compound bound status were confirmed by mass spectrum analysis (data not shown)



- Flag-tagged ADAR1-p150 plasmid was transfected into HEK293ADAR1-KO
- Transfected cells were treated with DMSO, 40 μM RP05129 and 80 μM RP05129, respectively
- ADAR1 complexes in cell lysates were immune precipitated with anti-flag beads, and editing activities of the 3 pull-down samples were tested followed with the analysis of compound binding by mass spectrum

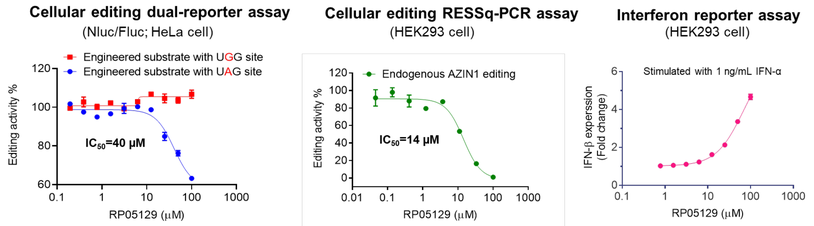
4. High-resolution co-crystal structures of ADAR1 with RP05129 or its analogs have been solved

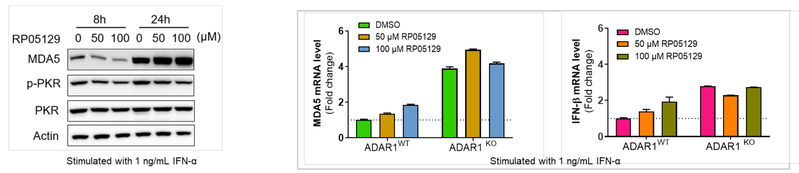


Resolution: 1.52 Å **Resolution: 1.87 Å**

- High-resolution ADAR1 deaminase domain (+/- dsRNA) structures were solved in-house (not shown)
- Several sets of high-resolution ADAR1-compound co-crystal structures in ADAR1's apo state or dsRNA binding states were solved (showcases)
- RP05129 and its analogs bind to a unique pocket that may involve in ADAR1 dimerization and RNA binding

5. RP05129 blocks RNA editing and elicits an IFN response in cells in an ADAR1 dependent manner





- RP05129 blocks ADAR1 cellular editing activity on both engineered substrate and endogenous AZIN1 gene
- RP05129 can upregulate MDA5 but does not activate PKR in cells
- RP05129 triggers downstream interferon-β activation in an ADAR1-dependent manner

Conclusions

- Risen has screened a 265K compound library against ADAR1 deaminase domain and compound RP05129 was identified as an ADAR1 inhibitor
- RP05129 demonstrates the ability to downregulate ADAR1 editing activity and trigger downstream interferon-β activation in an ADAR1 dependent manner
- High-resolution co-crystal structures of ADAR1 with inhibitor RP05129 or its analogs were solved in-house to guide SAR study
- RP05129 and its analogs hold promise for enhancing the efficacy of immune-based therapies in cancers that are dependent on ADAR1