APEX2 is a synthetic lethal target for RNASEH2B-deleted or BRCA-mutant tumors

Abstract # B039

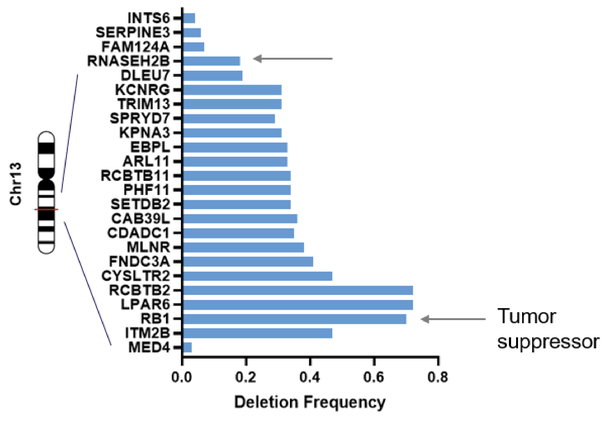
Antoine Simoneau, Steve Lombardo, Ruthie Swain, Serge Gueroussov, Binzhang Shen, Shangtao Liu, Samuel R Meier, Ashley H Choi, Tenzing Khendu, Hannah Stowe, Hsin-Jung Wu, Hongxiang Zhang, Douglas Whittington, Yi Yu, Jannik N Andersen, Teng Teng

Tango Therapeutics, Boston, MA, USA

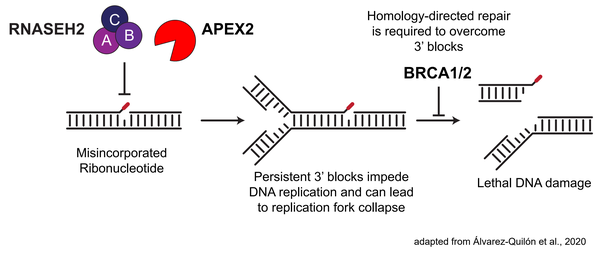
INTRODUCTION

The genomic regions surrounding tumor suppressor genes are often lost as collateral damage during tumorigenesis, providing novel therapeutic vulnerabilities. To systematically identify synthetic lethal pairs of genes within DNA damage repair (DDR) pathways, we conducted a combinatorial genome-wide CRISPR screen. We found that RNASEH2B, a gene frequently co-deleted with RB1 in prostate cancers, emerged as a top vulnerability with knockout of APEX2, which is a nuclease involved in DNA repair. This interaction was validated in RNASEH2B isogenic cell line pairs using whole-genome CRISPR screens and further confirmed across multiple cancer lineages using a focused mini-pool sgRNA library. Single gene knockouts demonstrated that loss of APEX2, specifically its catalytic activity, is sufficient to eliminate RNASEH2B mutant cells both in vitro and in vivo. Importantly, loss of RB1 did not impact the synthetic lethal interaction between RNASEH2B and APEX2. Consistent with prior reports, APEX2 knockout also impairs growth of BRCA1 mutant cells and enhances sensitivity to PARP inhibitors. Overall, these findings position APEX2 as a compelling synthetic lethal target in tumors with RNASEH2B deletion or BRCA1 mutations and support the development of APEX2 inhibitors as a potential monotherapy to improve patient outcome.

RNASEH2B is lost as collateral damage with RB loss, including in ~10-20% of prostate cancer

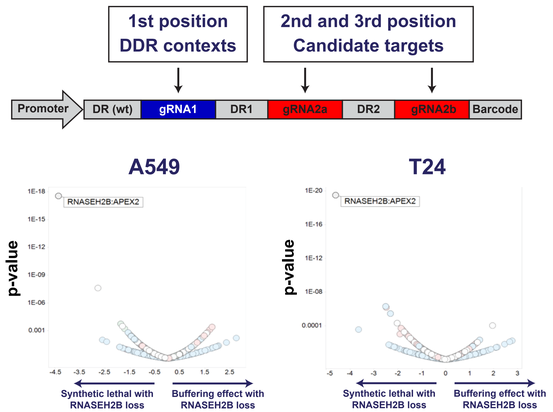


Misincorporated ribonucleotides in absence of RNase H2 can lead to lethal DNA damage if not successfully removed or repaird by APEX2 and BRCA1/2



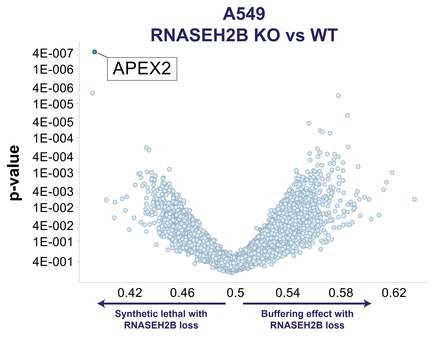
adapted from Álvarez-Quilón et al., 2020

1. FOCUSED COMBO CRISPR SCREENS IDENTIFY APEX2 AS A SYNTHETIC LETHAL TARGET FOR RNASEH2B LOSS



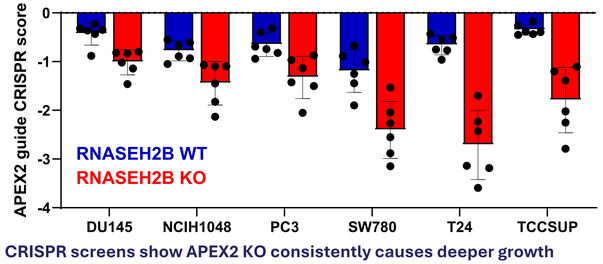
DNA damage response (DDR)-focused CRISPR screen identify genetic interactions between RNASEH2B (context) and APEX2 (target)

2. GENOME-WIDE CRISPR SCREENS CONFIRM APEX2 AS THE TOP SYNTHETIC LETHAL HIT IN RNASEH2B ISOGENIC CELLS



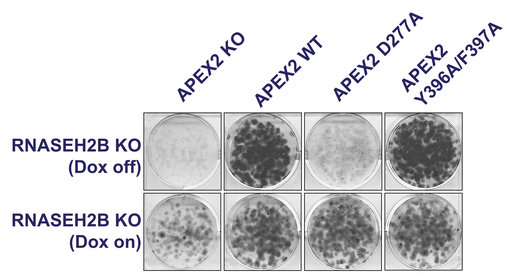
Whole genome CRISPR screen shows that APEX2 KO causes the strongest differential growth defect in RNASEH2B KO cells

3. APEX2 KO CAUSES ROBUST DIFFERENTIAL EFFECTS ACROSS A PANEL OF RNASEH2B ISOGENIC CELL LINES



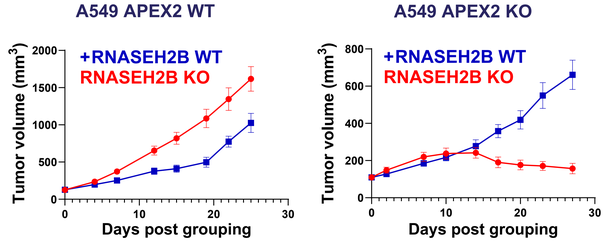
CRISPR screens show APEX2 KO consistently causes deeper growth defects in RNASEH2B KO cells

4. APEX2 CATALYTIC ACTIVITY, BUT NOT PCNA INTERACTION, IS ESSENTIAL FOR VIABILITY OF RNASEH2B-DEFICIENT CELLS



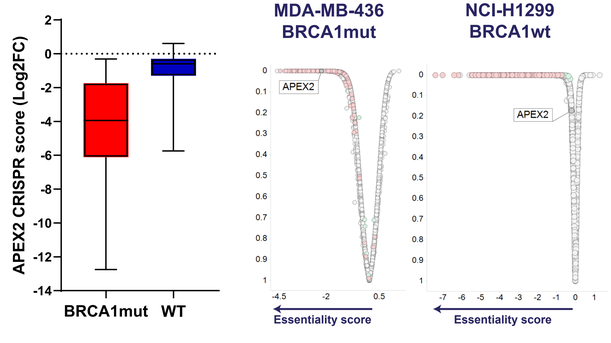
Expression of a catalytic-dead APEX2 mutant (D277A) fail to rescue the viability of endogenous RNASEH2B/APEX2 KO A549 cells, whereas mutations in the PCNA interacting domain restore survival

5. APEX2 KO CAUSES TUMOR GROWTH INHIBITION IN RNASEH2B KO CELLS



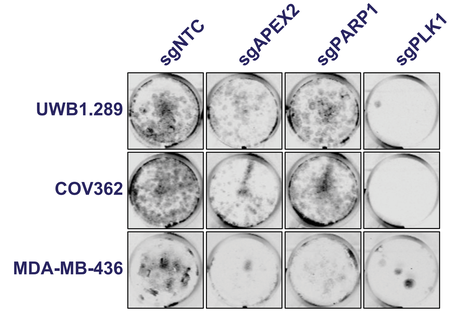
APEX2 KO and RNASEH2B KO tumors showed significant growth defects in vivo and could be rescued by dox-inducible RNASEH2B cDNA

6. BRCA1 MUTANT CELL LINES ARE DEPENDENT ON APEX2



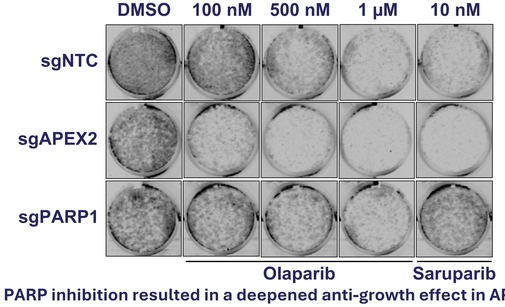
CRISPR screens show that BRCA1 mutant cell lines also have increased dependency on APEX2 for growth

7. BRCA1MUT CELLS ARE ALSO SENSITIVE TO APEX2 KO



APEX2 KO leads to BRCA1 mutant cell growth inhibition to a similar or greater extent than PARP1 KO

8. APEX2 KO IMROVES THE RESPONSE TO PARP INHIBITORS IN BRCA1 MUTANT CELL LINES



PARP inhibition resulted in a deepened anti-growth effect in APEX2 KO cells

SUMMARY

- Collateral loss of RNASEH2B with RB1 deletion is frequent and creates a novel therapeutic vulnerability in prostate and other cancers
- DDR-focused and genome-wide CRISPR screens identify APEX2 as a top synthetic lethal partner of RNASEH2B
- APEX2 loss selectively eliminates RNASEH2B-deficient cells in vitro and in vivo
- Catalytic activity of APEX2 is essential for survival of RNASEH2B-mutant cells
- APEX2 knockout also impairs growth of BRCA1-mutant tumors and enhances PARP inhibitor response
- Findings establish APEX2 as a compelling drug target, supporting development of first-in-class APEX2 inhibitors
- We show strong support for the development of APEX2 inhibitors for the treatment of RNASEH2B and BRCA1mut tumors

ACKNOWLEDGEMENTS

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