# Targeting KEAP1/NRF2 signaling sensitizes KRAS-driven NSCLC to KRAS inhibitors

Abstract #4450

Xianwen Yang, Peiling Ren, Guiping Li, Ying Yu, Guangxiu Dai
HUTCHMED. Building 4, 720 Cai Lun Road, Z.J. Hi-Tech Park, Shanghai, China, 201203

HUTCHMED

## Introduction

The emergence of KRAS G12C inhibitors has paved the way for new therapeutic approaches in NSCLC patients with KRAS mutations. However, the efficacies of these inhibitors have been limited in NSCLC based on clinical trial results. Numerous studies have uncovered that the co-occurrence of additional mutations, particularly in KEAP1, is associated with a poorer prognosis for KRAS G12C-mutated NSCLC patients[1-3]. KEAP1 is a negative regulator of the NRF2 signaling pathway. KEAP1 mutations lead to constitutive activation of NRF2, thus promoting tumor growth and drug resistance. In this study, we found that targeting NRF2 could be a promising therapeutic approach to improve the efficacies of KRAS inhibitors in NSCLC.

## Objectives

- To clarify NRF2 is a critical therapeutic target in KEAP1-mutated NSCLC
- To explore the combinational efficacy of co-targeting NRF2 and KRAS in KRAS-driven NSCLC

## Methods

- **siRNA transfection assay:** Cell lines were treated with NRF2 siRNA (Thermo Fisher) using Lipofectamine™ RNAiMAX Transfection Reagent (Invitrogen) and incubated for 24 hours or 48 hours (Invitrogen) for following analysis.
- **qRT-PCR assay:** Total mRNA was extracted with RNeasy kit (Qiagen) for NRF2 or downstream gene expression evaluation using qRT-PCR Kit (Beyotime).
- **Western blot assay:** Cells were lysed with lysis buffer (CST) and lysate was incubated with anti-NRF2 antibody (Abcam) for NRF2 protein expression detection.
- **Cell growth assay:** Cell lines were seeded into spheroid microplates and incubated for 96 hours after siRNA transfection. For combinational assay, cell lines were treated with KRAS inhibitor for 120 hours after multicellular spheroid formation. Cell viability was measured by CellTiter-Glo 3.0 (Promega).
- **Luciferase reporter assay:** The indicated cells were co-transfected with 8xARE-pGL4.25[luc2CP_minP] and pRL-SV40 (Promega) using Lipofectamine™ 3000 Transfection Reagent (Invitrogen). Luciferase activity was measured with Dual-Luciferase® Reporter Assay System (Promega) after transfection.

## Reference

1. doi:10.1056/NEJMoa2204619
2. doi:10.1056/NEJMoa2103695
3. doi:10.1056/NEJMoa2303810

## Results

### NRF2 protein was accumulated in NSCLC harboring KEAP1 mutations

A

| Cell line | Cancer type | KRAS status | KEAP1 status |
|---|---|---|---|
| A549 | NSCLC | G12S | G333C |
| NCI-H2122 | NSCLC | G12C | A203V/ A203fs |
| NCI-H292 | NSCLC | wt | wt |
| LoVo | CRC | G13D | wt |
| HEK293 | Human Embryonic Kidney Cell | wt | wt |

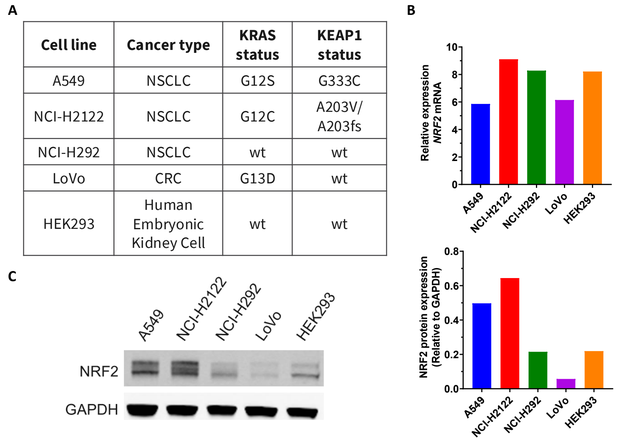


**Figure 1. NRF2 protein was accumulated in NSCLC cells harboring KEAP1 mutations.**
(A) The characterization of 4 cancer cell lines and one normal cell.
(B)-(C) Comparison of the basal mRNA (B) and protein (C) levels of NRF2 among 5 cell lines.

### KEAP1-mutated NSCLC cells showed enhanced trans-activity of NRF2

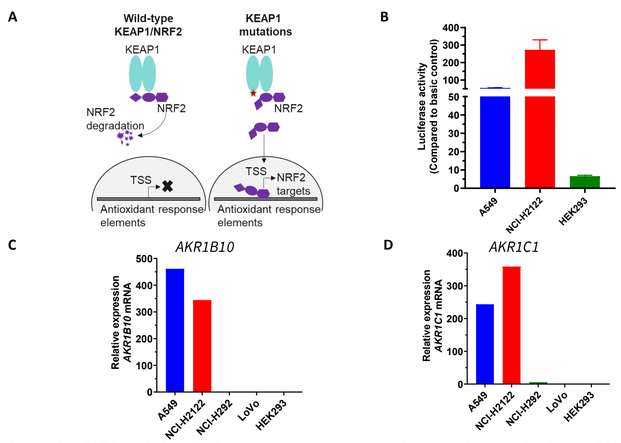


**Figure 2. KEAP1-mutated NSCLC cells showed enhanced transcriptional activity of NRF2.**
(A) Illustration of wild-type or mutated KEAP1/NRF2 signaling pathway.
(B) Increased NRF2 trans-activity was observed in KEAP1-mutated NSCLC cells.
(C)-(D) The expression of *NRF2* downstream genes, *AKR1B10* (C) and *AKR1C1* (D), was higher in KEAP1-mutated cells than in KEAP1-intact cells.

### *NRF2* knockdown inhibited cell growth of NSCLC cells with KEAP1 mutations

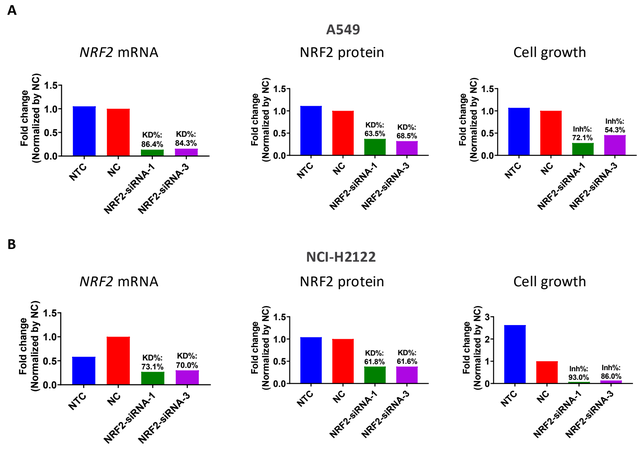


**Figure 3. *NRF2* knockdown inhibited cell growth of NSCLC cells with KEAP1 mutations.**
The Cell growth of A549 (A) and NCI-H2122 (B) cells was inhibited after *NRF2* knockdown. Validation of *NRF2* knockdown efficiency by qRT-PCR (left panel) and western blot (middle panel). NTC, non-treatment control; NC, negative control.

### Analysis of *NRF2* gene dependency in NSCLC and lung cancer

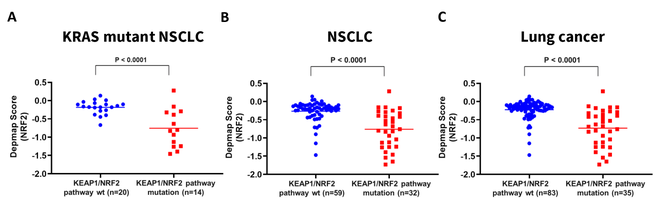


**Figure 4. Analysis of *NRF2* gene dependency in NSCLC and lung cancer using Depmap database.** KRAS mutant NSCLC (A), NSCLC (B) and lung cancer (C) cells with KEAP1/NRF2 pathway mutations demonstrated stronger *NRF2* dependency.

### *NRF2* knockdown down-regulated the expression of its target genes in KEAP1-mutant NSCLC cells

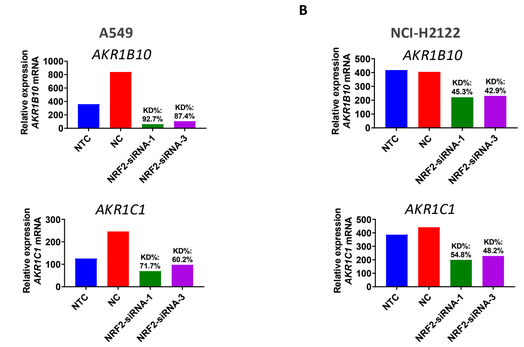


**Figure 5. Down-regulation of *NRF2* target genes were observed in KEAP1-mutant NSCLC cells.** *AKR1B10* and *AKR1C1* were significantly decreased in A549 (A) and NCI-H2122 (B) after NRF2 knockdown. NTC, non-treatment control; NC, negative control.

### Silencing NRF2 enhanced the responsiveness of KRAS inhibitors in KEAP1-mutant NSCLC cells

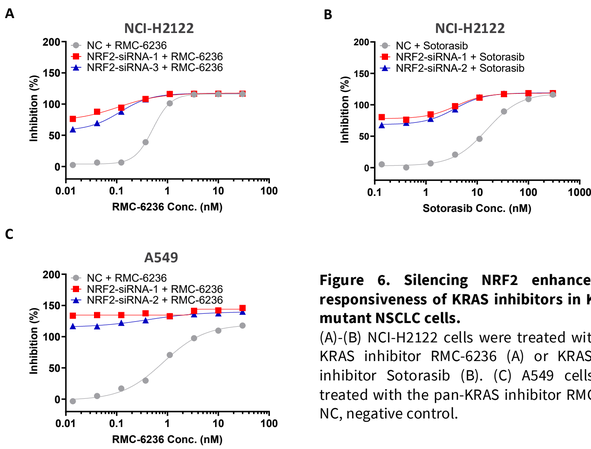


**Figure 6. Silencing NRF2 enhanced the responsiveness of KRAS inhibitors in KEAP1-mutant NSCLC cells.**
(A)-(B) NCI-H2122 cells were treated with pan-KRAS inhibitor RMC-6236 (A) or KRAS G12C inhibitor Sotorasib (B). (C) A549 cells were treated with the pan-KRAS inhibitor RMC-6236. NC, negative control.

## Summary

- **KEAP1 mutations result in NRF2 protein accumulation, which leads to the enhanced transcriptional activity of NRF2 in KRAS-driven and KEAP1-mutated NSCLC.**
- **Knockdown of NRF2 inhibits cell growth of NSCLC cells with KEAP1 mutations, suggesting *NRF2* gene dependency in KEAP1-mutated NSCLC.**
- **Analysis of gene dependency indicates that mutations in KEAP1/NRF2 signaling pathway predict *NRF2* dependency in NSCLC, and even in lung cancer.**
- **Silencing NRF2 sensitizes KRAS-driven NSCLC to KRAS inhibitors, implying that combinational therapy strategy targeting both NRF2 and KRAS may improve patient outcome.**

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