Characterization of Potent, Fully Modified siRNAs That Preferentially Silence KRAS G13D Transcripts in Cancer Cells

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Background

KRAS Oncogene

- KRAS is a GTPase in the MAPK pathway that directs cell survival and replication.
- Remarkably, 31% of lung, 45% of colon and 98% of pancreatic cancers are driven by abnormal KRAS activity.
- KRAS G13D is locked in a constitutively active state, overstimulating the MAPK pathway and leading to enhanced cell division.
  - "Undruggable" for structural and functional hindrances

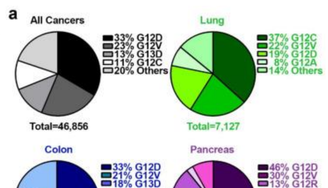


Figure 1. Frequency of KRAS mutations in major cancers

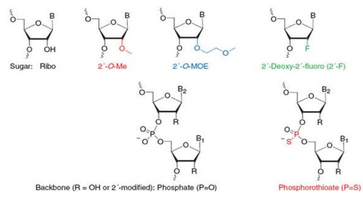


Figure 2. Structures of chemical modifications for siRNAs.

RNAi Interference

- siRNA works with RISC complex to cleave mRNA before translation
- Pecot lab: designed 15 siRNAs with different chemical and mismatch modifications that are increasingly specific to the G13D mutation while sparing wildtype KRAS

Objective: Identify the most potent combination of modifications in siRNA that selectively knockdown G13D KRAS mutant activity and slow down tumorigenesis

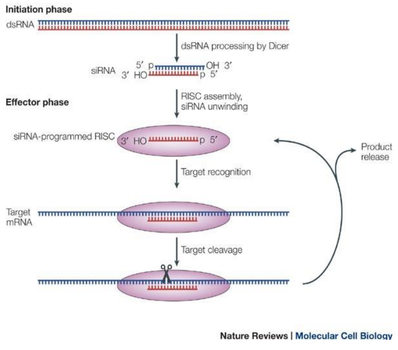
Methodology

Mechanism of siRNA Transfection

- Transfected mutant G13D cancer cells and wildtype cells of A431 line with a lipid nanoparticle: 20 nM for 48hr

Western Blot for Protein Quantification

- Isolated KRAS protein from wildtype and G13D mutants
- Antibody probing for KRAS and a vinculin standard



Reverse qPCR for mRNA Quantification

- Isolated KRAS mRNA from wildtype and G13D mutants
- Conducted reverse qPCR and normalized KRAS levels to 18S gene
- Narrowed from 15 to 4 most selective and most potent siRNAs by comparing to controls
  - **KRAS G13D 2'OMe:** negative control
  - **KRAS Seq2 DV22:** positive control; no wildtype sparing but most potent
  - **KRAS G13D DV22:** positive control; wildtype sparing

Results and Figures

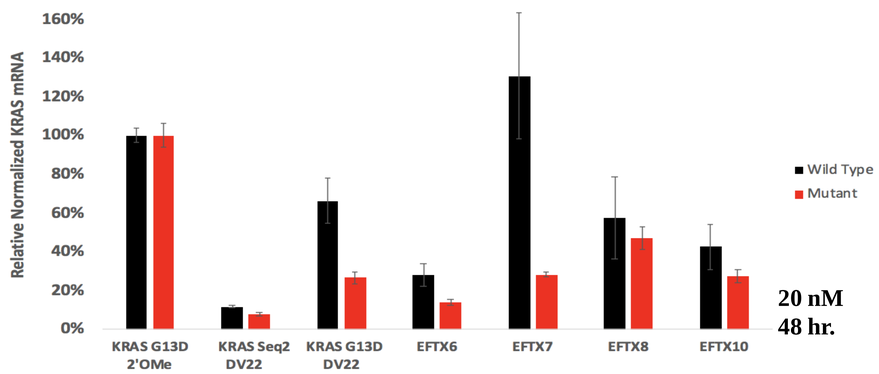


Figure 1. Normalized KRAS mRNA values in mutant vs wild type A431 G13D cell lines transfected by different EFTX siRNAs

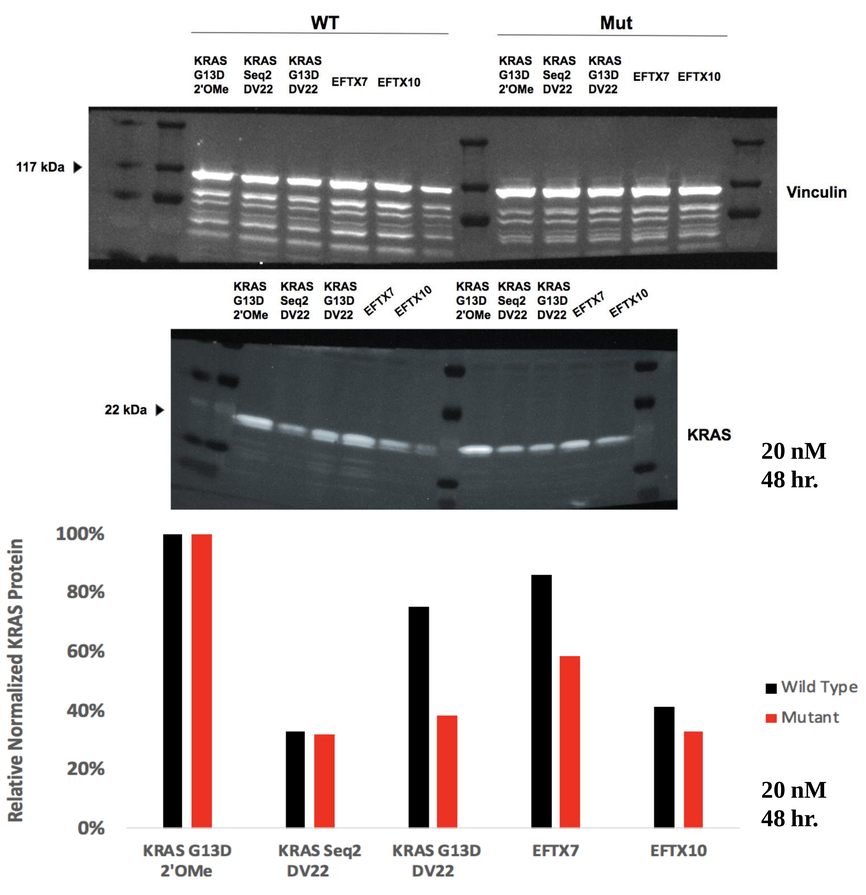


Figure 2. Relative normalized KRAS protein after transfection with EFTX 7 and EFTX 10 in wildtype and mutant G13D cancer cells

Conclusions

Analysis:

- Transfection of different siRNAs in cancer cells shows EFTX7 and EFTX10 have the greatest knockout efficiency of mutant KRAS mRNA, while sparing the wild type.
- Relative normalized KRAS protein after transfection with EFTX7 and EFTX10 confirms wild type sparing of functional KRAS and partial knockdown of the mutant.

Conclusions:

- EFTX7 and EFTX10 have unique designs in preferentially silencing the KRAS oncogene and warrant further investigation as a cancer therapy.
- Protein and mRNA analysis of KRAS can be applied to *in vivo* studies testing if knockdown with the siRNAs produces any off-target effects or cell toxicity.

Future Directions

Next Steps:

- Western blots for downstream effectors RAS, ERK and MEK1/2
- 2D assay to evaluate the proliferative nature of the cancer line compared to wild type before and after siRNA transfection
- 3D spheroid assay to track the tumorigenicity of the G13D mutant in an "in vivo" like environment

Acknowledgements

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Citations:

1.Silencing of Oncogenic KRAS by Mutant-Selective Small Interfering RNA Bjoern Papke, Salma H. Azam, Anne Y. Feng, Christina Gutierrez-Ford, Hayden Huggins, Pradeep S. Pallan, Amanda E. D. Van Swearingen, Martin Egli, Adrienne D. Cox, Channing J. Der, and Chad V. Pecot ACS Pharmacology & Translational Science 2021 *4* (2), 703-712 DOI: 10.1021/acsptsci.0c00165

2.Bumcrot D, Manoharan M, Koteliansky V, Sah DW. RNAi therapeutics: a potential new class of pharmaceutical drugs. Nat Chem Biol. 2006 Dec;2(12):711-9. doi: 10.1038/nchembio839. PMID: 17108989; PMCID: PMC7097247.