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REVIEW

# KRAS Cold Turkey: Using microRNAs to target KRAS-addicted cancer

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**Human cancers are driven by genetic mutations which cause aberrant activation of pro-growth pathways. Although cancers are uniquely dependent on the pro-growth signaling from oncogenic pathways, efforts to directly target these have been largely unsuccessful. One of the most common and drug resistant oncogenic drivers in colon cancer is the GTPase KRAS. It has been shown that colon cancers with KRAS driver mutations are also 'addicted' to proteins outside of the KRAS pathway due to aberrant re-wiring of cell signaling. A number of genes with a synthetic lethal relationship to mutant KRAS have been previously identified by RNAi screens. MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression, and their expression is frequently dysregulated in cancers. Recently, we have used an innovative functional miRNA screening approach to identify miRNAs that inhibit the survival of KRAS-mutant cells but not KRAS-wild-type cells. MiR-126 was one of the miRNAs that displayed this selective effect. We found that miR-126 induced synthetic lethality in KRAS-Mutant cells via the down-regulation of the polo-like kinase signaling network and a number of genes specifically necessary for the growth of KRAS-Mutant tumors. This study offers a new way forward for exploiting the regulatory power of miRNAs to specifically target aberrant cell signaling in cancer.**

***Keywords:*** miRNA; miR-216; KRAS; RAS; synthetic lethality; colorectal cancer

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In studies of model organism genetics, mutations which are individually harmless may result in a lethal phenotype when combined. This combinatorial manifestation of loss-of-function is termed 'synthetic lethality,' with synthetic being used in the Greek sense, meaning 'placed together' [1]. This phenomenon is a result of phenotypic robustness in biological systems conferred by redundant or parallel pathways meant to buffer genetic variation and thereby reduce the chances that a mutation will have adverse effects on fitness [2]. In cancer, this phenotypic robustness is often lost, and instead cancers depend on a single oncogenic pathway for sustaining malignant growth [3]. For example, the monomeric GTPase KRAS is one of the most commonly activated oncogenes in human cancer, and many tumors have been shown to be selectively sensitive to its inhibition. In other words, tumors with activated KRAS become 'addicted' to its oncogenic signaling. In contrast to the redundant pathways that safeguard embryonic development, tumor growth relies so heavily on the mutated KRAS pathway that its inhibition results in death and involution of the tumor [4].

This so-called oncogene addiction has been an area of intense research for the development of targeted gene therapies, with the idea that tumors are uniquely sensitive to disruption of the signaling pathway to which they are

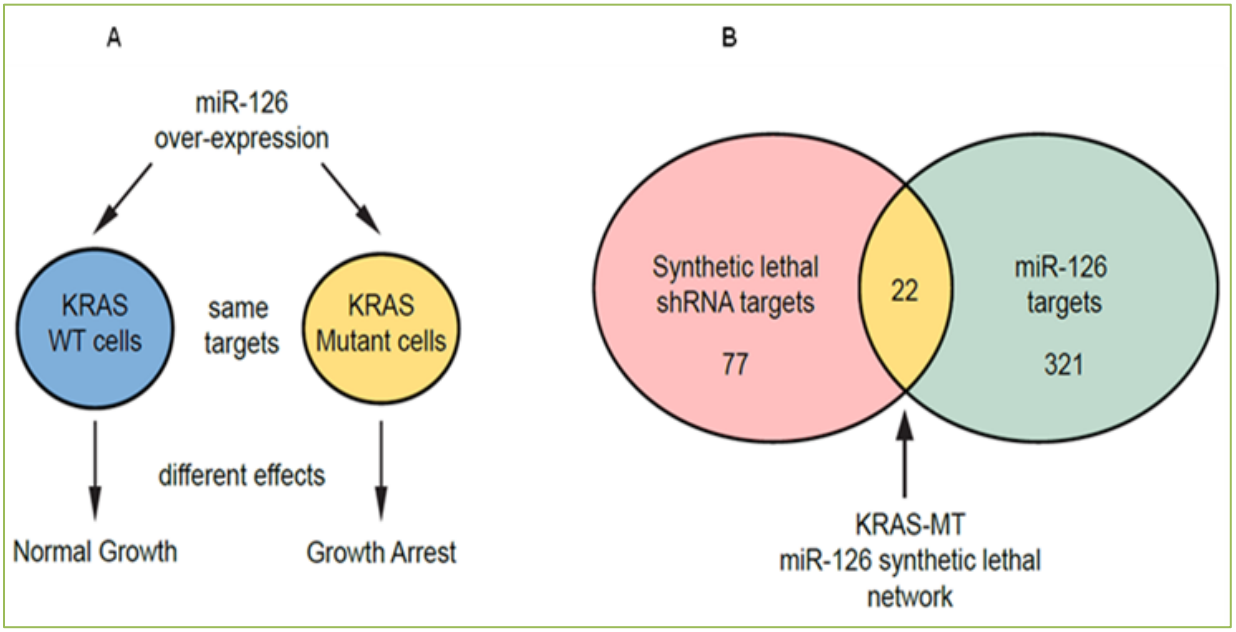


**Figure 1. A**) We performed microarray gene expression profiling in an isogenic pair of HCT116-derived cell lines with either wild type (KRAS-WT) or mutant KRAS (KRAS-Mutant) following miR-126 mimic transfection. Although a highly similar set of mRNAs was significantly repressed in both cell types, the effects of miR-126 expression were strikingly different, with the wild-type cells growing normally and the KRAS mutant cells suffering a significant reduction in growth and tumorigenicity. **B**) We compared the genes significantly down-regulated by miR-126 to a list of KRAS synthetic lethal genes previously discovered by an RNAi screen. Nearly one-third of the RNAi candidates were significantly repressed by miR-126, suggesting that miR-126 over-expression has a synthetic lethal phenotype in KRAS-Mutant cells due to its silencing of genes uniquely required for the viability KRAS-Mutant cells.

addicted [5]. However, in the case of KRAS, disrupting the signaling pathway has proven technically difficult for a variety of reasons [6]. In fact, KRAS itself is often held to be an 'undruggable target' for several reasons: unusually tight association between KRAS and GTP which prevents direct inhibition; compensatory protein modifications foil drugs targeting the lipid tags necessary for KRAS localization to the inner leaflet of the plasma membrane; and evolution of activating mutations in downstream KRAS effectors such as *BRAF* circumvent efforts to cut KRAS off at the legs [7]. Recently, several studies have attempted to determine whether KRAS-dependent tumors, resistant to frontal assault, can be outflanked using the principle of synthetic lethality to exploit a tumor's addiction to non-oncogenic proteins. High throughput siRNA screens have been used to determine whether KRAS mutation rewires cellular pathways resulting in increased dependence of a tumor on other genetic pathways, such that inhibition of that pathway in combination with KRAS mutation would specifically confer lethality. A number of studies have shown that KRAS tumors depend on, for example, the cell cycle kinase PLK1, the transcription factor GATA2, and the NF-κB activator TBK1 [8-11].

Previous high-throughput screens have focused on using siRNAs or shRNAs to inhibit the expression of individual genes in KRAS mutant cancer cells. Our recent study has investigated the potential of microRNAs (miRNAs) to mediate synthetic lethality [12]. This approach presents an apparent advantage over the siRNA strategy: namely that a miRNA may post-transcriptionally inhibit the expression of dozens of target genes [13], especially when over-expressed, and can therefore influence the expression of gene networks rather than just individual genes [14]. We began by screening the effects of 879 human miRNAs on the viability of KRAS-Mutant HCT116 cells (colorectal cancer) compared to the isogenic KRAS-wild-type (WT) line. MiR-126 was observed to specifically reduce cell growth in KRAS-Mutant HCT116. This context-dependent effect was validated in 6 additional colorectal cancer cell lines with differential KRAS mutation status. Moreover, the expression of miR-126 was significantly reduced in The Cancer Genome Atlas (TCGA) colorectal tumor samples with mutant KRAS (n=28) compared to wild type (n=57), indicating that down-regulation of miR-126 confers a selective advantage to KRAS mutant tumors. MiRNAs primarily act to reduce the stability and translation of their target mRNAs by binding to partially complementary sites in the mRNA 3'untranslated region (3'UTR) [15,16]. The KRAS-3'UTR does not contain any predicted miR-126 target sites, and indeed miR-126 over-expression did not alter mutant KRAS mRNA or protein expression. This strongly suggested that the effect of

miR-126 on the growth of KRAS mutant cancer cells was not due to direct inhibition of KRAS expression. In other words, the effect was more likely a reflection of a synthetic lethal interaction with KRAS than a withdrawal symptom of oncogene addiction.

We then identified the target genes of miR-126 by microarray expression profiling combined with bioinformatic analysis of 3'UTR sequences for miR-126 seed matches. The direct targets of miR-126 were verified by 3'UTR reporter assays. Interestingly, we did not detect significant differences between the miR-126 regulated transcriptome in KRAS-WT and KRAS-Mutant cells despite the starkly different functional effects of miR-126 over-expression in these cell types (Fig. 1A). This observation raised the hypothesis that miR-126 targets genes that are synthetically lethal with mutant KRAS. To test this, we cross-referenced miR-126 targets detected by microarray and 3'UTR assay with hits from previous KRAS synthetic lethality RNAi screens. We discovered that miR-126 mediates the repression of 22 genes that had previously been reported as synthetic lethal interactors with KRAS mutation in colon cancer, and we reasoned that these 22 genes were likely to be the effectors of the context dependent miR-126 phenotype (Fig. 1B). Many of these had roles in cell-cycle regulation, with the intersection of the KRAS synthetic lethal and miR-126 target gene sets being enriched for mitotic polo-like kinases. As expected from the results of the KRAS siRNA screen, we found that knockdown of miR-126 targets with siRNAs resulted in a significant repression of clonogenicity of KRAS-Mutant cells, but a greater, synergistic effect was observed with miR-126 transfection. We explain this as a consequence of the context-dependent regulation of a coherent gene network by miR-126 that is required for the viability of KRAS-Mutant cells. The effect of miR-126 on KRAS mutant cancers is likely to be due to down-regulation of polo-like kinase signaling: silencing of polo-like kinase 1 (PLK1) has previously been shown to be synthetically lethal with KRAS mutations [9]. In addition to PLK1, we also showed that several genes such as *UBQLN2* and *SLC39A6* not involved in canonical RAS signaling played a role in the selective growth suppression mediated by miR-126 in KRAS-Mutant cells.

The therapeutic promise of synthetic lethal approaches to cancer treatment is easy to see: targeting a specific genetic vulnerability of cancer would likely constitute a significant improvement in therapeutic index (dose required for therapy divided by dose required for toxicity) relative to conventional chemotherapy [1]. As miRNAs slowly but surely make their way from the bench to the clinic [17], it will be important to find miRNAs that exhibit context-dependent specificity for targeting cancer cell growth. Our work on miR-126 and mutant KRAS has uncovered at least one such miRNA. Several other miRNAs in our screen also seemed to have a selective inhibitory effect on KRAS mutant cells. For example, miR-122 was a very potent inhibitor of HCT116 KRAS-Mutant cell growth, but was not endogenously expressed in HCT116 KRAS-WT or KRAS-Mutant cells. We omitted miR-122 from further analysis, reasoning that it was unlikely to play a significant role in the biology of HCT116 cells, but it may yet be an effective biomolecule for therapeutic inhibition of KRAS-mutant tumors. It is likely that a similar approach will uncover more KRAS synthetic lethal miRNAs as well as miRNAs that selectively target other tumor types or oncogenic networks.

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### References

1. Kaelin WG. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer 2005; 5:689-98.
2. Hartman JL, Garvik B, Hartwell L. Principles for the buffering of genetic variation. Science 2001; 291:1001-4.
3. Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heal of cancer. Science 2002; 297:63-4.
4. Luo J, Solimini NL, Elledge SJ. Principles of Cancer Therapy: Oncogene and Non-oncogene Addiction. Cell 2009; 136:823-37.
5. Sharma SV, Settleman J. Oncogene addiction: setting the stage for molecularly targeted cancer therapy. Genes Dev 2007; 21:3214-31.
6. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. Nat Rev Mol Cell Biol 2008; 9:517-31.
7. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS:Mission Possible? Nat Rev Drug Discov 2014; 13:828-51.
8. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, *et al*. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature 2009; 462:108-12.
9. Luo J, Emanuele MJ, Li D, Creighton CJ, Schlabach MR, Westbrook TF, *et al*. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell 2009; 137:835-48.
10. Kumar MS, Hancock DC, Molina-Arcas M, Steckel M, East P, Diefenbacher M, *et al*. The GATA2 transcriptional network is requisite for RAS oncogene-driven non-small cell lung cancer. Cell 2012; 149:642-55.
11. Weng M-T, Lee J-H, Wei S-C, Li Q, Shahamatdar S, Hsu D, *et al*. Evolutionarily conserved protein ERH controls CENP-E mRNA splicing and is required for the survival of KRAS mutant cancer cells. Proc Natl Acad Sci USA 2012;109:E3659-67.
12. Hara T, Jones MF, Subramanian M, Li XL, Ou O, Zhu Y, *et al.*

Selective targeting of KRAS-mutant cells by miR-126 through repression of multiple genes essential for the survival of KRAS-mutant cells. Oncotarget 2014; 5:7635-50.

13. Friedman RC, Farh KKH, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2008; 19:92-105.
14. Lal A, Navarro F, Maher CA, Maliszewski LE, Yan N, O'Day E, *et al*. miR-24 Inhibits cell proliferation by targeting E2F2, MYC, and other cell-cycle genes via binding to "seedless" 3'UTR microRNA recognition elements. Mol Cell 2009; 35:610-25.
15. Bartel DP. MicroRNAs: Target Recognition and Regulatory Functions. Cell 2009; 136:215-33.
16. Eichhorn SW, Guo H, McGeary SE, Rodriguez-Mias RA, Shin C, Baek D, *et al*. mRNA destabilization is the dominant effect of mammalian microRNAs by the time substantial repression ensues. Mol Cell 2014; 56:104-15.
17. Broderick JA, Zamore PD. MicroRNA therapeutics. Gene Ther 2011; 18:1104-10.