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Award Number: W81XWH-18-1-0444

TITLE:
Identification of lncRNAs required for synthetic lethal interactions with mutant KRAS in pancreatic cancer

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REPORT DATE:
October 2019

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2019	Annual	15 Sep 2018-14 Sep 2019

4. TITLE AND SUBTITLE
Identification of lncRNAs Required for Synthetic Lethal Interactions with Mutant KRAS in Pancreatic Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0444

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The major goal of this application is to identify lncRNAs involved in synthetic lethal interaction with KRAS in pancreatic cancer. There are two specific aims. The first aim is to screen for synthetic lethal interactions between lncRNAs and KRAS using the genome-wide CRISPR/Cas9-based lncRNA libraries. The second aim is functional characterization of candidate synthetic lethal lncRNAs in pancreatic cancer. The success of this study will help identify novel biomarkers and develop a better strategy for pancreatic cancer treatment.

15. SUBJECT TERMS
lncRNA, KRAS, synthetic lethality, biomarker

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU		19b. TELEPHONE NUMBER *(include area code)*

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Introduction

In pancreatic cancer, mutant RAS is a major oncogenic driver, yet, there is lack of drugs against this oncogenic RAS. Thus, there is an urgent need for identification of novel therapeutic targets associated with mutant RAS in pancreatic cancer. Although extensive studies have been carried out in the past years to develop drugs against mutant KRAS, to date no effective pharmacological inhibitors targeting mutant RAS have reached the clinic. A major reason for this failure is because expression of mutant RAS function can cause hyperproliferative developmental disorders and cancer. The previous literature evidence has identified a number of protein-coding genes that are essential to the growth of mutant KRAS cancer cells by screen of shRNA library. However, little is known whether long non-coding RNAs (lncRNAs) can also play a role in this aspect. In this application, we aimed to take an alternative indirect approach, i.e., synthetic lethal approach, which seeks targets that are essential for growth of the cells bearing an activated RAS. We will use CRISPR/Cas9-based screening approach for this purpose.

Body

The overall objective was to identify synthetic lethal interactions with mutant KRAS in pancreatic cancer with two specific aims and four major tasks as listed below.

Results

Major Task 1 (complete)
Screen for synthetic lethal interactions between KRAS and lncRNAs using the genome-wide CRISPR/Cas9-based lncRNA libraries.

Task 1 Results: We have generated two complementary lncRNA libraries, i.e., lncRNA KO (dual gRNA) and lncRNA SAM libraries. These libraries have been used for screening experiments

Major Task 2 (partially complete)
Perform library screens and validate potential lncRNAs

Task 2 Results: We have completed the screening the lncRNA KO library and starting to screen the SAM library. From the initial screening results, we identified a number of

Table 1, Candidate lncRNAs

LncRNA	Fold*
LINC01716	87
LINC02078	60
STARD13-IT1	42
MIR4300HG	32
LINC02246	29
LINC01759	15
SMG6-IT1	14
LMCD1-AS1	13
LINC00515	13
DANCR	7

*, negative enrichment

candidate lncRNAs based on the loss of gRNAs (negative selection) after 2 weeks. We listed top 10 candidates in Table 1. Evidently, little literature information is available for most of these ten lncRNAs, suggesting the novel aspect of this screening. Of particular interest is DANCR, although it is listed #10 candidate. Literature suggests that DANCR is an important regulator for cancer stem cells. For example, DANCR was first identified as an anti-differentiation lncRNA and loss of DANCR in progenitor cells causes rapid induction of differentiation genes (1). Later, DANCR was shown to be important in supporting cancer cell stemness in hepatocellular carcinoma (HCC) and osteosarcoma (2,3). Consistent with these findings, DANCR is upregulated in a variety of cancer types including nasopharyngeal carcinoma, bladder cancer, breast cancer, prostate and ovarian cancer (4-7). In addition, DANCR can increase HIF-1α mRNA stability through interacting with the NF90/NF45 complex (4); it activates IL-11-STAT3 signaling and increases cyclin D1 and PLAU expression via guiding leucine-rich pentatricopeptide repeat containing (LRPPRC) to stabilize mRNA (5). DANCR can also upregulate PI3K/AKT signaling through activating serine phosphorylation of RXRA (7). These studies suggest the importance of DANCR in cancer. Therefore, it is critical to determine the role of DANCR in growth of KRAS pancreatic cancer cells.

To determine which lncRNAs are essential to KRAS mutant cell growth, we will also perform screening against the SAM library. We expect that this screening would cross validation for those lncRNA candidates identified from KO library. The advantage of this SAM library, unlike the KO library, is a positive selection procedure. However, this screening system requires more components are (Fig. 1). Thus, this will take a bit longer time before the real screening starts.

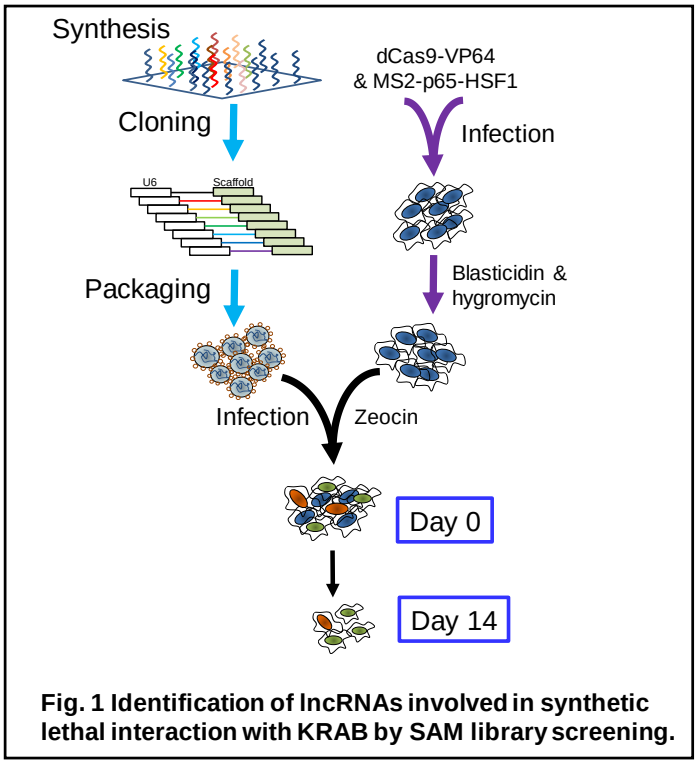

Fig. 1 Identification of lncRNAs involved in synthetic lethal interaction with KRAB by SAM library screening.

Major Task 3 (not started yet)
Determine the role of synthetic lethal action of candidate lncRNA in KRAS-dependent PDAC

Major Task 4 (not started yet)
Dissect molecular mechanism of lncRNA-mediated synthetic lethality in mutant KRAS PDAC

Key Research Accomplishments at this point

- We generated two lncRNA gRNA libraries (KO and SAM).
- We completed KO library screening; SAM library screening is underway.
- We identified a number of lncRNA candidates that may be required for growth of KRAS pancreatic cancer cells; they will be validated in the coming year.

Reportable Outcomes

Not yet

Conclusions

We have completed part of the proposed work and made a reasonable progress. We will continue to work on the rest tasks in the coming year.

References:

1. Kretz M, Webster DE, Flockhart RJ, Lee CS, Zehnder A, Lopez-Pajares V, *et al.* Suppression of progenitor differentiation requires the long noncoding RNA ANCR. Genes Dev **2012**;26:338-43
2. Yuan SX, Wang J, Yang F, Tao QF, Zhang J, Wang LL, *et al.* Long noncoding RNA DANCR increases stemness features of hepatocellular carcinoma by derepression of CTNNB1. Hepatology **2016**;63:499-511
3. Jiang N, Wang X, Xie X, Liao Y, Liu N, Liu J, *et al.* lncRNA DANCR promotes tumor progression and cancer stemness features in osteosarcoma by upregulating AXL via miR-33a-5p inhibition. Cancer Lett **2017**;405:46-55
4. Wen X, Liu X, Mao YP, Yang XJ, Wang YQ, Zhang PP, *et al.* Long non-coding RNA DANCR stabilizes HIF-1alpha and promotes metastasis by interacting with NF90/NF45 complex in nasopharyngeal carcinoma. Theranostics **2018**;8:5676-89
5. Chen Z, Chen X, Xie R, Huang M, Dong W, Han J, *et al.* DANCR Promotes Metastasis and Proliferation in Bladder Cancer Cells by Enhancing IL-11-STAT3 Signaling and CCND1 Expression. Mol Ther **2019**;27:326-41
6. Lu Y, Hu Z, Mangala LS, Stine ZE, Hu X, Jiang D, *et al.* MYC Targeted Long Noncoding RNA DANCR Promotes Cancer in Part by Reducing p21 Levels. Cancer Res **2018**;78:64-74
7. Tang J, Zhong G, Zhang H, Yu B, Wei F, Luo L, *et al.* LncRNA DANCR upregulates PI3K/AKT signaling through activating serine phosphorylation of RXRA. Cell Death Dis **2018**;9:1167