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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. In the past year, we completed the SAM library screening and identified LINC00901 as a potential lncRNA that might be involved in synthetic lethal interaction with KRAS.

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

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1. 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.

2. Keywords

Synthetic lethality, KRAS, lncRNA, CRISPR screening

3. Accomplishments (Results)

Major Task 1 (completed)
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 (completed)
Perform library screens and validate potential lncRNAs

Task 2 Results: We have completed the screening the lncRNA KO library (reported last year) and the SAM library (this reporting period).

From the KO library, we identified top 10 lncRNA. Among them was DANCR which was our focused lncRNA. We found that DANCR promotes cell proliferation and stemness-like properties (Fig 1). For example, overexpression of

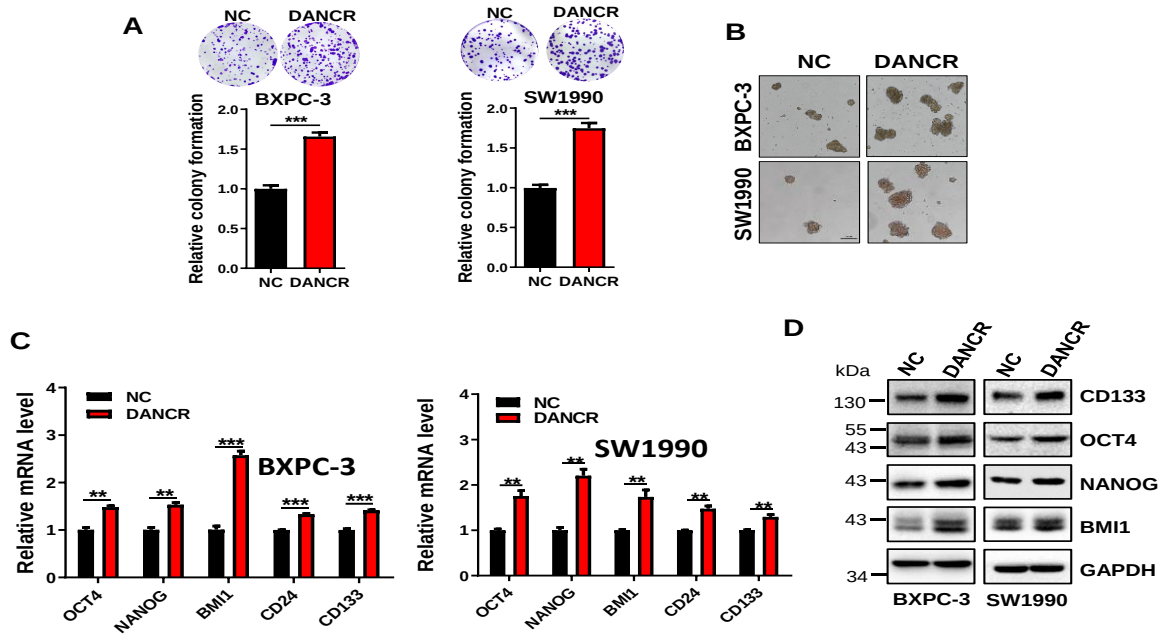

Fig. 1. DANCR promotes cell proliferation and stemness-like properties. (A) DANCR promotes colony formation. (B) Ectopic expression of DANCR increases sphere formation in number and size. (C) DANCR-overexpression promotes expression of stemness-related genes (OCT4, NANOG, BMI 1, CD24 and CD133) in pancreatic cancer cells, as determined by qRT-PCR and at the protein level (D). Values are mean ± SEM. *, P<0.05; **, P<0.01; ***, P<0.001.

DANCR increased colony formation in both BXPC-3 and SW1990 cells (Fig. 1A). Furthermore, it increased the number sphare formation (Fig. 1B). Experiments with xenograft models revealed that while ectopic expression of DANCR promotes, DANCR KO suppresses tumor growth (Fig. 2). Mechanistically, DANCR is modified at N6-methyladenosine (m6A) and mutagenesis assay identified that adenosine at 664 of DANCER is critical to the interaction between IGF2BP2 and DANCR where IGF2BP2 serves a reader for m6A modified DANCR and stabilizes DANCR RNA. Together, these results suggest that DANCR is a novel

Table 1, Top 10 enriched lncRNAs

LncRNA	Fold
LINC00901	8.2
LINC00961	7.3
Lnc-DC	6.5
LINC00266	6.1
HOXB-AS1	5.3
DANCR	4.3
HOXB-AS3	4.2
LINC00346	3.6
LINC-RoR	3.1
UCA1	3.0

target for IGF2BP2 through m6A modification, and IGF2BP2 and DANCR work together to promote cancer stemness-like properties and pancreatic cancer pathogenesis. Of interest, screening the SAM library also identified DANCR as a candidate lncRNA (Table 1), further supporting the importance of DANCR.

In addition to DANCR, this screening identified LINC00901 as a top candidate from the SAM library (Table 1), and this LINC00901 was also enriched in the KO library, though at a slightly low level of enrichment. Thus, LINC00901 will be our next lncRNA which will be characterized in details in the coming year.

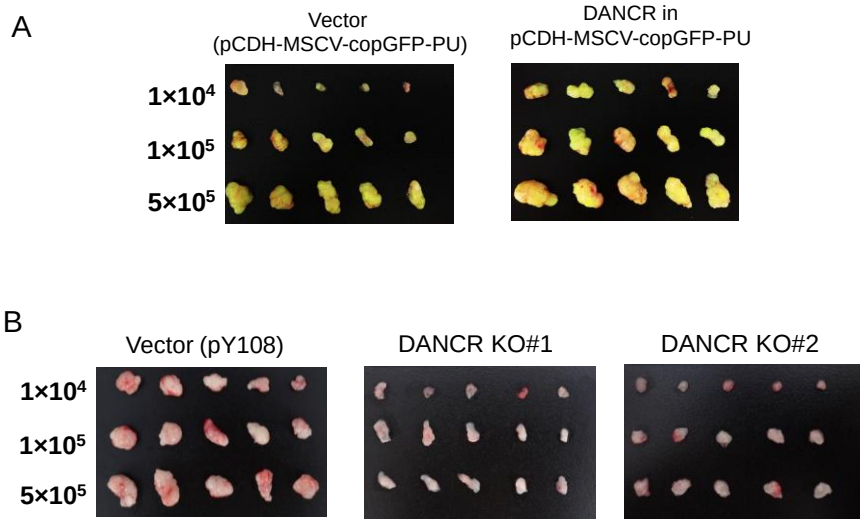

Fig. 2. DANCR overexpression promotes, whereas DANCR KO suppresses tumor progression. (A) DANCR promotes the tumor growth of BXPC-3 cells. DANCR overexpression (DANCR) and vector control (pCDH-MSCV-copGFP-T2A-Pu) cells were subcutaneously injected into the nude mice with 1×10^4, 1×10^5, 5×10^5 cells per mouse. (B) DANCR KO significantly suppresses the tumor growth rate of BXPC-3 cells. DANCR KO and vector control (pY108) cells were subcutaneously injected into the nude mice with 1×10^4, 1×10^5, 5×10^5 cells per mouse.

Major Task 3 (Underway)

Determine the role of synthetic lethal action of candidate lncRNA in KRAS-dependent PDAC. We have been working both DANCR and LINC00901 for this aspect.

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 and SAM library screening.
- We identified a number of lncRNA candidates, including DANCR and LINC00901, that may have synthetic lethal interactions with KRAS in pancreatic cancer cells.

4. Impact

We identified DANCR and LINC00901 as potential lncRNAs that may have synthetic lethal interaction with KRAS through screening both KO and SAM libraries. Characterization experiments suggested that DANCR plays an oncogenic role in pancreatic cancer. Thus, DANCR may serve as a potential target for therapeutic purpose in KRAS pancreatic cancer.

5. Changes/Problems

Our progress was severely impacted by Covid-19. Especially, our school froze hiring last year. In addition, no qualified person can be found even after the school removed the freezing policy. However, there is no change for the original SOW.

6. Products

No products for this year

7. Participants & Other Collaborating Organizations

N/A

8. Special Reporting Requirements

N/A

9. Appendices

N/A