Role of the long non-coding RNA MALAT-1 in Pancreatic Cancer

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INTRODUCTION

Long non-coding RNAs (LncRNAs) contain > 200 nucleotides and based on limited studies there is evidence that they regulate multiple functions and contribute to cellular homeostasis and diseases including cancer. The lncRNA Metastasis-Associated-in-Lung-Adenocarcinoma-Transcript-1 (MALAT-1) is overexpressed in pancreatic and other cancer cell lines and tumors and is a negative prognostic factor for multiple cancers. Pancreatic cancer is the fourth leading cause of cancer deaths in the US with the lowest five year-survival rate, therefore the development of new therapeutic targets for cancer development and new serum biomarkers for the early detection of cancer is urgent. In this project, the pro-oncogenic role of MALAT-1 in pancreatic cancer cells and transgenic mouse model will be determined. In addition, the mechanism of the action of MALAT-1 will be investigated.

METHODOLOGY

- ❖ Cell culture: Panc1, Miapaca2 pancreatic cancer cell lines
- ❖ MTT assay & cell counting assay
- ❖ Flow cytometry analysis of apoptosis and cell cycle
- ❖ Scratch assay, "ibidi" migration assay, Boyden chamber assay
- ❖ Ingenuity analysis of microarray data
- ❖ RT-PCR and western blot
- ❖ Analysis of MALAT-1 knockout pancreatic cancer mouse model
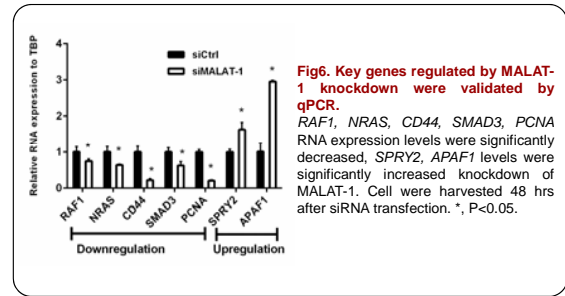
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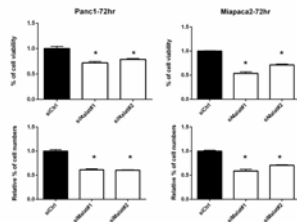
Fig1. Knockdown of MALAT-1 decreased cell viability and cell numbers.
Cells were transfected with control siRNA (siCtrl), MALAT-1 siRNA (siMALAT-1) for 72 hrs, then MTT assay (A) and cell counting assay (B) were used to measure cell viability and proliferation. Knockdown of MALAT-1 significantly reduced cell viability and inhibited cell growth of both cell lines. *, P<0.005.
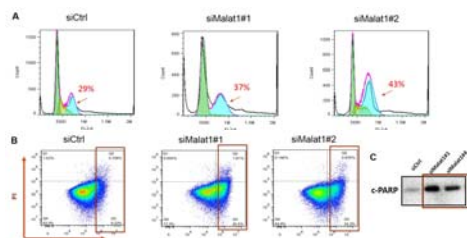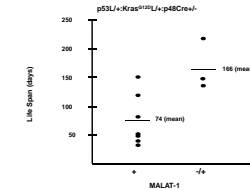

Fig2. Knockdown of MALAT-1 induced G2/M arrest and cell apoptosis.
Panc1 cells were transfected with control siRNA (siCtrl), MALAT-1 siRNA (siMALAT-1) for 48 hrs then harvested for (A) PI staining. PI staining were used to determining cell cycle. Green peak, G1 phase; yellow peak, S phase; blue peak, G2/M phase. Arrow points out G2/M phase. Cells in G2/M phase were increased after MALAT-1 knockdown. (B) Annexin V/ PI staining. Knockdown of MALAT-1 increased percentage of Annexin V positive cells. (C) Western blot analysis. Expression of apoptosis marker cleaved PARP increased after MALAT-1 knockdown. Similar results were observed in Miapaca2 cells.
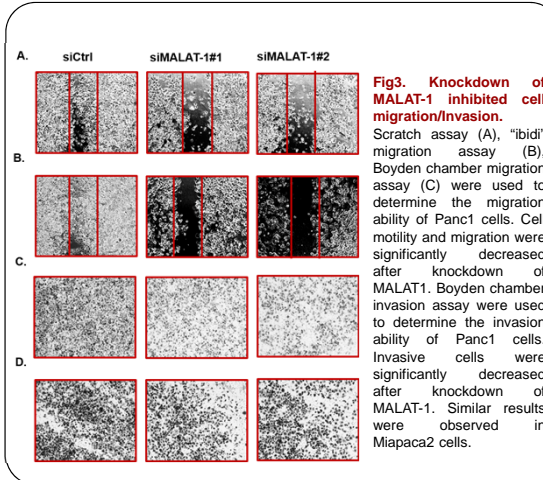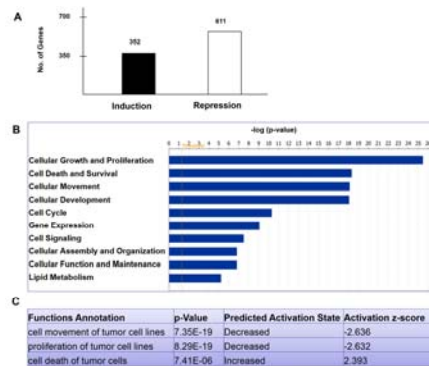

Fig3. Knockdown of MALAT-1 inhibited cell migration/Invasion.
Scratch assay (A), "ibidi" migration assay (B), Boyden chamber migration assay (C) were used to determine the migration ability of Panc1 cells. Cell motility and migration were significantly decreased after knockdown of MALAT1. Boyden chamber invasion assay were used to determine the invasion ability of Panc1 cells. Invasive cells were significantly decreased after knockdown of MALAT-1. Similar results were observed in Miapaca2 cells.


Fig4. Microarray analysis of knockdown of MALAT-1 in Panc1 cell.
(A) MALAT-1 knockdown in Panc1 cells results in the induction of 352 and repression of 611 genes. (B) Ingenuity functional analysis of genes induced or repressed in Panc1 cells transfected with siMALAT-1. (C) MALAT-1 knockdown is predicted to increase apoptosis, decrease cell proliferation/migration based on analysis of differentially expressed genes.
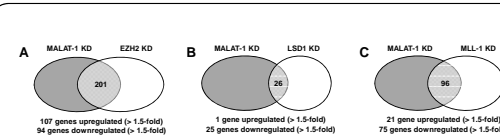
Fig5. Interaction with histone methylation complex.
Common genes regulated by MALAT-1 and EZH2 (A), MALAT-1 and LSD1 (B), and MALAT-1 and MLL-1 (C) by comparing gene arrays after silencing of MALAT-1, EZH2, LSD1 and MLL-1 in Panc1 cells.

Fig6. Key genes regulated by MALAT-1 knockdown were validated by qPCR.
RAF1, NRAS, CD44, SMAD3, PCNA RNA expression levels were significantly decreased, *SPRY2, APAF1* levels were significantly increased knockdown of MALAT-1. Cell were harvested 48 hrs after siRNA transfection. *, P<0.05.

Fig7. Survial of heterozygous floxed p53L/+: Kras[GD12]: p48Cre+/- mice expressing MALAT-1(+) or with loss of MALAT-1(-/+).
MALAT-1 knockout increased life span of the pancreatic cancer mouse model.

CONCLUSION

- ❖ MALAT-1 knockdown decreases pancreatic cancer cell proliferation, migration/Invasion, induces G2/M cell cycle arrest and apoptosis
- ❖ Microarray analysis result is consistent with experimental observation
- ❖ Interaction with histone methylation complex is an possible mechanism
- ❖ MALAT-1 knockout increases life span of a pancreatic cancer mouse model (p53L/+: Kras[GD12])

FUTURE WORK

- Identify genes coregulated by MALAT-1 and histone methylation complex.
- Key genes and pathways will be validated by qPCR and western blot, then further validated in transgenic mice
- Function of MALAT-1 in transgenic mice will be further extensive investigated once enough mice are available

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