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Especializado em Vida

# LINC00941 is an Oncogenic Long Non-coding RNA Regulated by KRAS in Pancreatic Cancer


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## Introduction and objectives

Oncogenic *KRAS*-driven pancreatic ductal adenocarcinoma (PDAC) is a frequent and very aggressive disease, for which there are currently no effective therapies. Therefore, identification of KRAS targets with therapeutic potential is warranted. Even though long non-coding RNAs (lncRNAs) are important cancer biomarkers and can functionally affect the oncogenic process, lncRNAs involved in oncogenic KRAS-induced PDAC remain unknown. We aimed to identify lncRNAs that play an important role in KRAS-induced PDAC.

## Materials and Methods

- **TCGA Sequencing Data**: Combined RNAseq and exome sequencing data from 145 PDAC patients were obtained from The Cancer Genome Atlas (TCGA) and used to identify differentially expressed lncRNAs according to KRAS status.
- **PDAC Sequencing Data**: Expression of identified lncRNAs was analyzed in RNA-Seq data from 14 paired samples of PDAC tumors versus adjacent normal tissue. These data were obtained from *Paixão et al., 2022*.
- **KRAS Silencing Analysis**: LncRNA regulation by KRAS was validated by KRAS silencing in PDAC cell lines and in isogenic KRAS-mutant and KRAS-wildtype pancreatic cells (HPDE/HPDE-KR) by qPCR.
- **Survival Analysis**: Survival analysis was performed for the identified lncRNAs using PDAC RNA-seq and clinical datasets from the International Cancer Genome Consortium (ICGC; PACA-AU) (90 patients).
- **Single-cell Sequencing Data**: Expression of LINC00941 was analyzed in PDAC tumors using single cell RNA-seq data of 57,530 cells from 24 PDAC and 11 non-tumor pancreatic samples. These data were obtained from *Peng et al., 2019*.
- **Gene Network Analysis**: We used the "Weighted Gene Co-expression Network Analysis (WGCNA)" R package to identify gene networks involved in biological processes that are co-expressed with LINC00941.
- **Functional Analysis**: LINC00941 silencing by siRNA transfection was used in cell-based assays (migration, invasion, DNA repair and chemoresistance) to evaluate functional impact and in qPCR assays to evaluate gene expression changes.

## Results

10 lincRNAs were identified as differentially expressed according to *KRAS* mutation status. Of these, 7 were also enriched in PDAC tumors (green) versus adjacent non-tumor pancreatic tissue (PDAC vs Normal, n=14).

**RNAs selecionados para análise a partir de dados de RNASeq do TCGA (123 amostras *KRAS*$^{Mut}$ vs 22 amostras *KRAS*$^{Wt}$)**

| Nome do gene | Tipo do gene | Base mean | Fold change Log2 (Mut/Wt) | P-valor | P-valor ajustado (padj) |
|---|---|---|---|---|---|
| *RNAs não codificadores longos intergênicos* | | | | | |
| LINC01133 | lincRNA | 702,5 | 1,00 | 1,40E-04 | 7,08E-03 |
| LINC01559 | lincRNA | 548,6 | 1,39 | 8,10E-07 | 3,56E-04 |
| LINC01764 | lincRNA | 248,9 | 1,06 | 6,76E-04 | 1,91E-02 |
| RP3-340N1.2 | lincRNA | 166,1 | 1,77 | 6,63E-08 | 2,09E-05 |
| LINC00941 | lincRNA | 111,8 | 1,23 | 8,65E-07 | 3,68E-04 |
| RP11-400N13.3 | lincRNA | 33,4 | 1,07 | 9,40E-05 | 5,47E-03 |
| AC006262.5 | lincRNA | 77,0 | 1,43 | 1,48E-05 | 1,70E-03 |
| CTA-363E6.2 | lincRNA | 52,9 | 1,30 | 3,42E-06 | 7,47E-04 |
| RP5-894M6.1 | lincRNA | 69,5 | 1,11 | 7,72E-05 | 4,85E-03 |
| MIR3142HG | lincRNA | 41,4 | -1,22 | 1,53E-06 | 5,08E-04 |

KRAS silencing in pancreatic cell lines modulates expression of 4 of the identified lincRNAs.

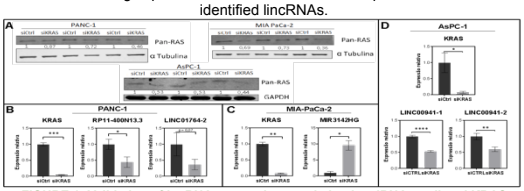

**FIGURE 1: Validation of lncRNA expression regulation by siRNA-mediated KRAS silencing followed by Western blotting (A) or qPCR (B, C and D).**

KRAS modulates expression of 4 of the identified lincRNAs in primary isogenic pancreactic cells expressing wildtype KRAS (HPDE) or mutant KRAS (HPDE-KR).

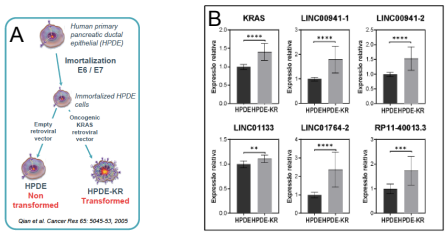

**FIGURE 2. Validation of lncRNA expression regulation by oncogenic KRAS in the HPDE/HPDE-KR cell model. A) schematic representation of HPDE and HPDE-KR isogenic cell lines generation. B) KRAS and lincRNA expression analysis by qPCR.**

LINC00941 is enriched in PDAC malignant cells and high expression of LINC00941 is associated with shorter survival in PDAC.

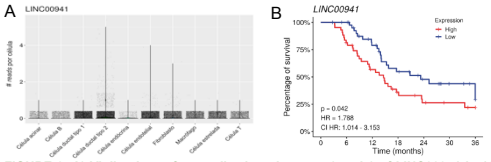

**FIGURE 3: A) Violin chart of normalized read counts (y-axis) of LINC00941 in 10 PDAC cell types (x-axis) by single cell RNA-seq analysis shows enrichment in the malignant type 2 ductal cells found only in PDAC tumors. B) Kaplan-Meyer survival curves of LINC00941 high and low expression groups were generated using the R survival package and compared by the log-rank test.**

LINC00941 silencing reduces PDAC cell migration and invasion.

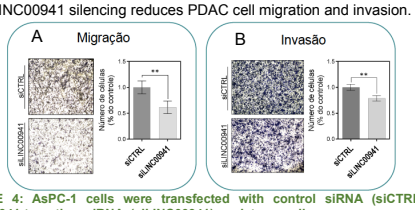

**FIGURE 4: AsPC-1 cells were transfected with control siRNA (siCTRL) or LINC00941-targeting siRNA (siLINC00941) and transwell assays were used to evaluate cell migration (A) or invasion (B).**

LINC00941 is co-expressed with DNA repair genes and its silencing inhibits DNA repair.

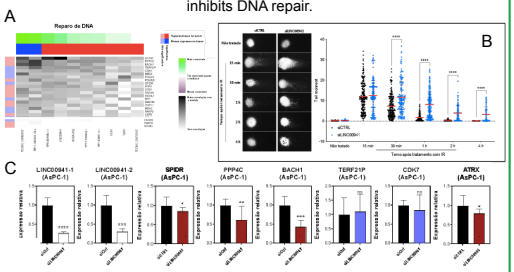

**FIGURE 5: a) WGCNA analysis shows LINC00941 is co-expressed with DNA repair genes. B and C) AsPC-1 cells were transfected with control siRNA (siCTRL) or LINC00941-targeting siRNA (siLINC00941) and DNA repair was analyzed by Comet assay (B) and DNA repair gene expression by RT-qPCR (C).**

LINC00941 silencing reduces chemoresistance to Gemcitabine.

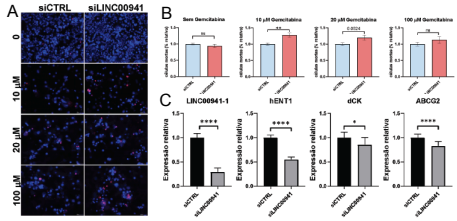

**FIGURE 6: AsPC-1 cells were transfected with control siRNA (siCTRL) or LINC00941-targeting siRNA (siLINC00941). A and B) Transfected cells were treated with the indicated concentrations of Gemcitabine and cell death was measured after ?h by PI and Hoechst staining. C) Expression of chemoresistance genes was analuzed by RT-qPCR .**

## Conclusões

Oncogenic *KRAS* promotes LINC00941 expression in PDAC. LINC00941 has prognostic and functional value and may represent a new PDAC biomarker or therapeutic target.

## Referências


PAIXÃO *et al*. **Cellular Oncology**, 2022. PMID: 35567709

PENG *et al*. **Cell Research**, 2019. PMID: 31273297


## Contato


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