Long non-coding RNAs are key players in Prostate cancer tumorigenesis and drug resistance

P. Pucci1,2, E. Venalainen3, I. Alborelli4, L. Quagliata6, M. Bootman1, S. Rigas1, I. Romero1, Y. Wang3,6, F. Crea1,3
1School of Life Health and Chemical Sciences, The Open University, Milton Keynes, UK. 2Present: Division of Cellular and Molecular Pathology, Department of Pathology, University of Cambridge, UK. 3Experimental Therapeutics, BC Cancer Agency Cancer Research Centre, Vancouver, BC, Canada. 4Institute of Pathology, University Hospitals Basel, Basel 4031, Switzerland. 5Global Head of Medical Affairs, Clinical NGS & Oncology Division, Thermo Fisher Scientific, Baar, Switzerland. 6Vancouver Prostate Centre, University of British Columbia, Vancouver, Canada.

Introduction

Long non-coding RNAs (IncRNAs) are the longest class of ncRNAs (>200 nt) recently characterized as key players in several cancer-associated processes such as tumorigenesis and drug resistance1-3. Emerging data indicate that IncRNAs affect the progression of prostate cancer (PCA) and promotes the formation of its aggressive and incurable forms, such as castration resistant PCA (CRPC).

In the present study we show that IncRNA H19 is highly upregulated in the context of PCA tumorigenesis and that HORASS promotes CRPC drug resistance. Both IncRNAs are also associated with clinical features, showing their translational potential as therapeutic targets for PCAs and CRPC patients.

Methods

• RNA sequencing: Illumina and Ion Torrent NGS
• Gene expression analysis: Taqman RT-qPCR with HPR7 as normalization control and ΔΔCt method.
• Drug treatment: PCA cells treated with cabazitaxel upon HORASS RNAi or lentiviral-mediated overexpression.
• Trypan blue-based cell count and caspase 3/7 assay to determine cell proliferation and apoptosis, respectively.
• Western blot (WB) with anti-BCL2A1 and anti-GAPDH antibodies and Syngene Gbox with GeneTools software.
• Transfection with DsiRNAs (2nM) and ASOs (75nM) was performed using RNAiMax.

Results

1. LncRNA H19 is highly upregulated in tumorigenic PCA cells

HORASS5 promotes drug resistance in CRPC cells via reduced caspase activity

Figure 1: A. Flowchart of H19 selection and qPCR validation of RNA-Seq data from sequencing of E006AA (non tumorigenic in nude mice) vs E006AA-Ht (highly tumorigenic). B. H19 is positively coexpressed with PCa regulating genes, such as BIRC5 (encoding survivin). Statistics: Student’s t-test: p<0.0001

Figure 2: DU145 and LNCaP cell count and IC50 (A,B) and caspase 3/7 activity (C,D) upon cabazitaxel treatment, with HORASS overexpression and silencing respectively. 2Way anova with Sidak’s post-test. (A) *P<0.0230, **P<0.0005, ***P<0.0001 and nonlinear fit was used to calculate the IC50. One way anova with Tukey’s post-test (C,D).**P<0.0001. Results expressed as means ± S.D. from three independent replicates.

2. HORASS5 is a mechanism of action

Figure 3: H19 RNA seq expression upon PCa grades (A) and association with disease-free survival (B). HORASS5 microarray expression upon taxanes treatment on PCA patients and association with disease-free survival (D). HORASS inhibition with ASO decreases cell count in cabazitaxel treated cells (E) and reduces the IC50 (F). Results as means ± S.D. from three independent replicates. Statistics: One way anova with Tukey’s post-test: **P<0.05, ***P<0.001, ****P<0.0001. (D) Student’s t-test: **P<0.01, (F) F2-way anova with Sidak’s post-test: **P<0.01 (E).

4. Clinical evidence on H19 and HORASS5 and gene therapy using IncRNA-ASOs

Conclusions

1. H19 is upregulated in PCa in vivo tumorigenic cells and is co-expressed with PCa associated genes in patients.
2. HORASS overexpression increases CRPC drug resistance (IC50). HORASS silencing favours cabazitaxel-induced CRPC cell death.
3. HORASS5 inhibits cell death by upregulating the anti-apoptotic protein BCL2A1.
4. H19 and HORASS are associated with PCA clinical features (tumor grade and drug treatment, respectively) and patient poor prognosis. LncRNA-targeting antisense gene therapy works in PCa cells and is a promising novel therapeutic approach in cancer.

References


Funding

• The Open University
• Cancer Research UK:22592

Conflict of interest

The authors declare no conflict of interest