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

Conference or Workshop Item

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

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Introduction

Long non-coding RNAs (lncRNAs) are the longest class of ncRNAs (>200 nt) recently characterized as key players in several cancer-associated processes such as tumorigenesis and drug resistance[1-3] . Emerging data indicate that lncRNAs 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 lncRNA ***H19*** is highly upregulated in the context of PCa tumorigenesis and that ***HORAS5*** promotes CRPC drug resistance. Both lncRNAs are also associated with clinical features, showing their translational potential as therapeutic targets for PCa and CRPC patients.

Methods

- RNA sequencing: Illumina and Ion Torrent NGS
- Gene expression analysis: Taqman RT-qPCR with *HPRT1* as normalization control and ΔΔCt method.
- Drug treatment: PCa cells treated with cabazitaxel upon *HORAS5* 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

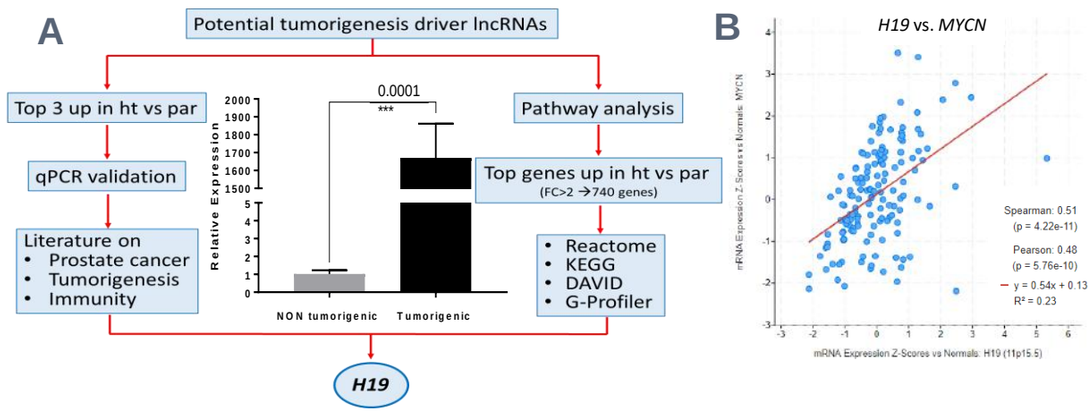

Figure1: A. Flowchart of H19 selection and qPCR validation of RNA-Seq data from sequencing of E006AA (non tumorigenic in nude mice) vs E006AA-ht (highly tumorigenic). B. H19 is positively coexpressed with PCa regulating genes, such as BIRC5 (encoding survivin). Statistics: Student's t-test: p<0.0001

2. *HORAS5* promotes drug resistance in CRPC cells via reduced caspase activity

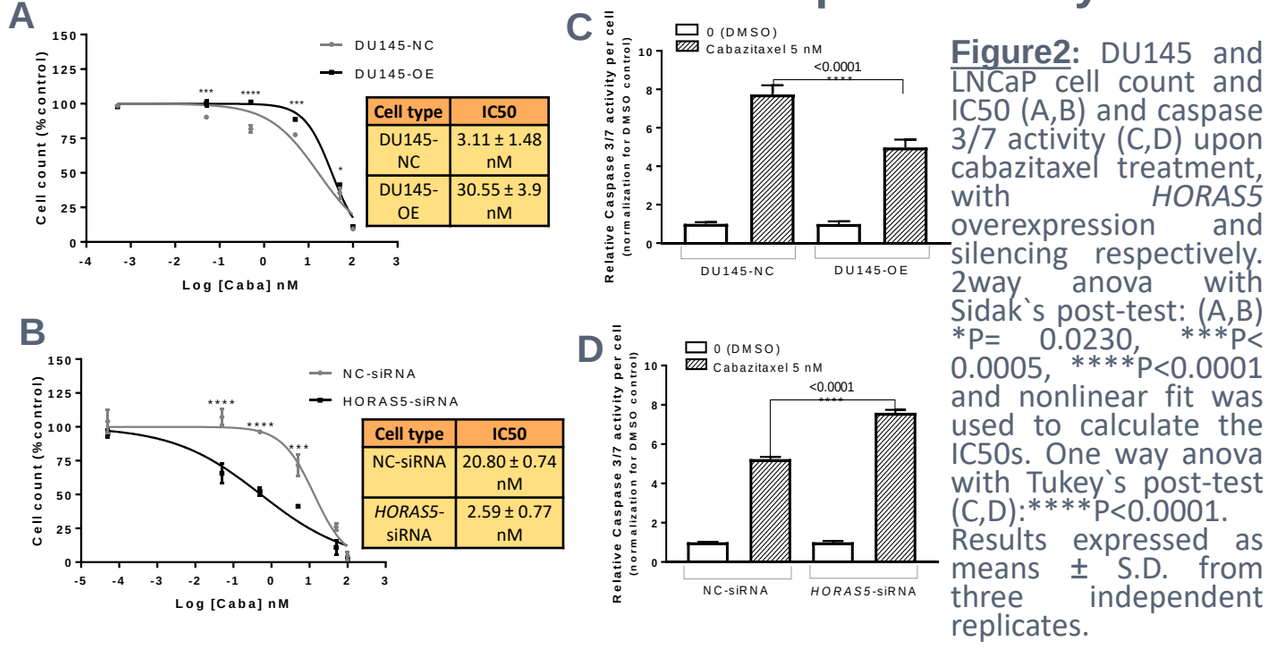

Figure2: DU145 and LNCaP cell count and IC50 (A,B) and caspase 3/7 activity (C,D) upon cabazitaxel treatment, with *HORAS5* overexpression and silencing respectively. 2way anova with Sidak`s post-test: (A,B) *P= 0.0230, ***P< 0.0005, ****P<0.0001 and nonlinear fit was used to calculate the IC50s. One way anova with Tukey`s post-test (C,D):****P<0.0001. Results expressed as means ± S.D. from three independent replicates.

3. HORAS5-BCL2A1: mechanism of action

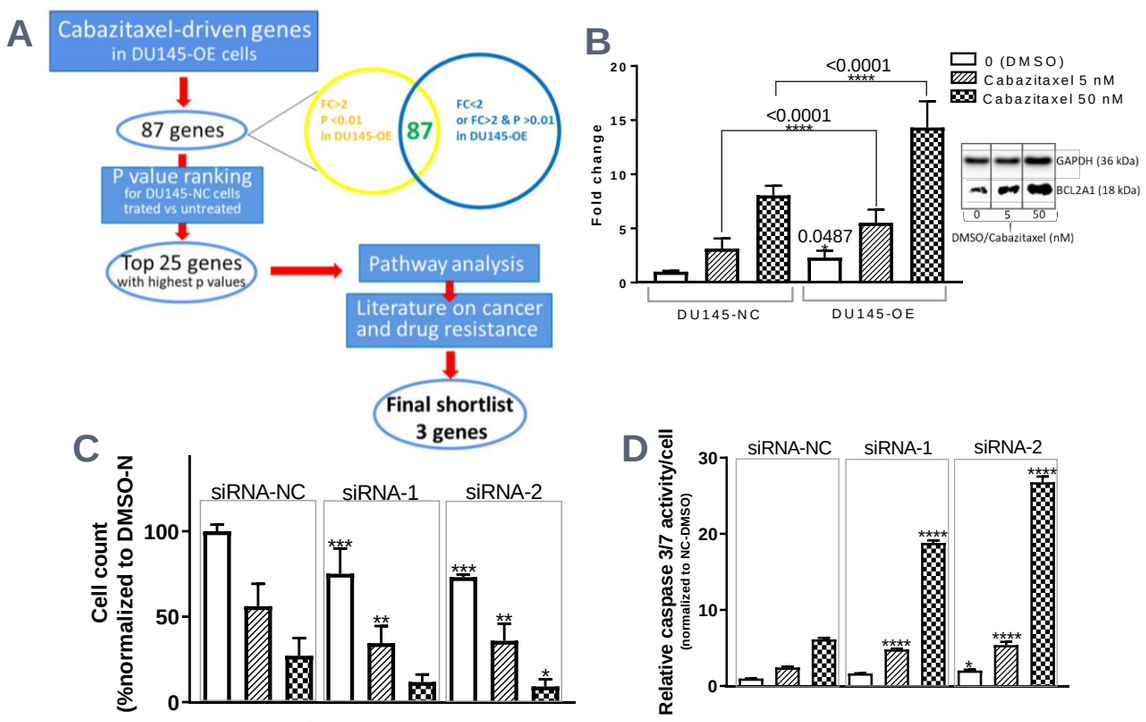

Figure 3: RNA seq flowchart of DU145-OE (*HORAS5* overexpression) cells vs DU145-NC (negative control) (A) and qPCR and protein validation of BCL2A1 (B) . Cell count (C) and apoptosis assay (caspase 3/7, D) upon *BCL2A1* silencing and cabazitaxel treatment. Results as means ± S.D. from three independent replicates. Statistics: One way anova with Tukey`s post-test: *P<0.05,**P<0.01,***P<0.001,****P<0.0001. WB: Syngene Genesys tool.

4. Clinical evidence on H19 and HORAS5 and gene therapy using lncRNA-ASOs

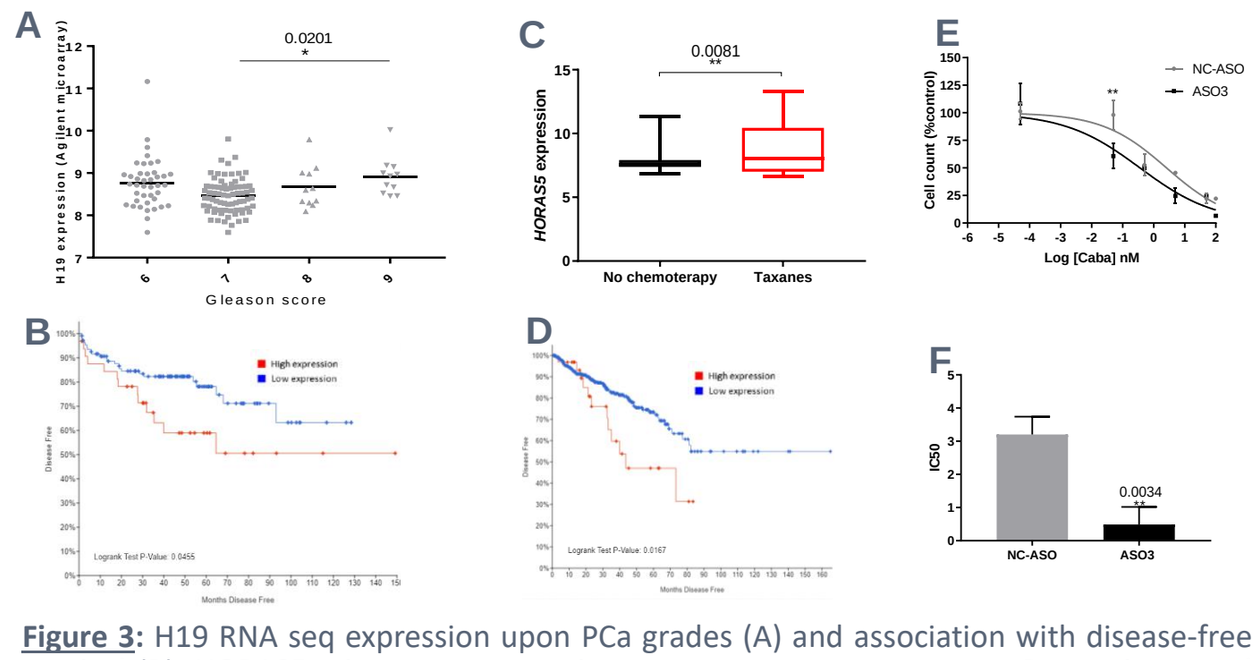

Figure 3: H19 RNA seq expression upon PCa grades (A) and association with disease-free survival (B). HORAS5 microarray expression upon taxanes treatment on Pca patients and association with disease-free survival (D). HORAS5 inhibition with ASO decreases cell count in cabazitaxel treated cells (E) and reduces the IC50 (F). Results as means ± S.D. from three independent replicates. Statistics: One way anova with Tukey`s post-test: *P<0.5 (A). Student's t-test: **P<0.01 (C, F). 2way anova with Sidak`s post-test: **P<0.01 (E).

Conclusions

1. *H19* is upregulated in PCa *in vivo* tumorigenic cells and is co-expressed with PCa associated genes in patients.
2. *HORAS5* overexpression increases CRPC drug resistance (IC50). *HORAS5* silencing favours cabazitaxel-induced CRPC cell death.
3. *HORAS5* inhibits cell death by upregulating the anti-apoptotic protein BCL2A1.
4. *H19* and *HORAS5* 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

1.Zhai, W. et al. Mol. Cancer, 2019.
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3.Tan, D. et al., Nat. Med., 2017.

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Conflict of interest

The authors declare no conflict of interest

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