Knocking Down of Cystathionine-γ-lyase (CSE) in Breast Cancer alters PD-L1 Expression Pattern through Tuning CCAT1/let-7a ceRNAs circuit

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Abstract #149

Background and Aim

Recently, our research group has shed the light onto the indisputable role of Hydrogen Sulphide (H_2S) in BC. In parallel, immunotherapy has emerged a novel therapeutic approach acting by altering the immunogenic profile of BC tumors [1]. Overexpression of Programmed death -ligand 1 (PD-L1) has become one of the most acceptable tactics for tumor cells to escape the immune surveillance phenomena [1]. PD-L1 is highly expressed in BC tumors and correlated with its aggressiveness [1]. Thus immune checkpoint blockades (ICBs) such as PD-L1 antibodies had reached the clinics, yet patients response is variable and resistance cases started to appear [2]. So, the molecular engines regulating PD-L1 are needed to be investigated [3]. Recently, our group showed that H_2S postulates a powerful immunomodulatory role through tuning non-coding RNAs (ncRNAs) [4]. CCAT1 is an oncogenic long ncRNA which can act as a sponge for the direct regulator of PD-L1. Let-7a [5]. The aim of this study is to investigate the impact of H_2S on a novel ceRNA circuit and to probe ncRNAs linking H_2S with PD-L1 in BC cells.

Subjects, Materials and Methods

BC Patients: Tumor tissues as well as its normal counterparts has been resected from 30 BC patients.

Cell Culture: MDA-MB-231 were cultured in DMEM supplemented with 1% L-glutamine, 1% penicillin/streptomycin and 10% FBS

Transfection Experiments: MDA-MB-231 cells were cultured and transfected with CSE siRNAs using Hiperfect Transfection Reagent.

H_2S Levels Detection : H_2S levels Were measured using H_2S detection kit.

mRNA Quantification: Total RNA was extracted from breast tissues and MDA-MB-231 cells using Biozol Reagent. Reverse transcribed then amplified and quantified using qRT-PCR. Values were calculated as Relative Quantitation (RQ)

Statistical Methods: All statistics were performed using student t-test where $p<0.05$ were considered significant. All results were analyzed using Graphpad prism 5.0.

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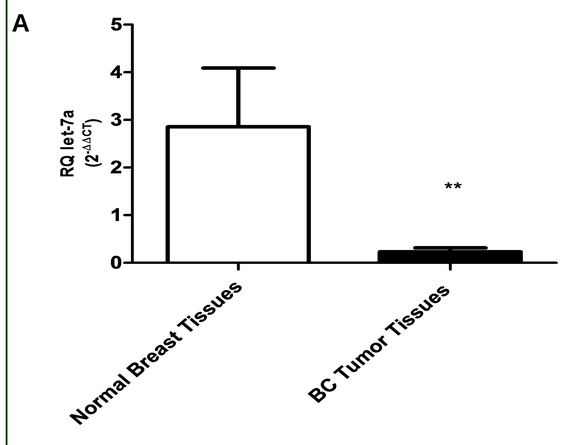
Results

1.Screening of miR-Let-7a and PD-L1 in BC patients

miR-Let-7a (P=0.0039, Figure A) was found to be down-regulated, while PD-L1 (P=0.0388, Figure B) was found to be up-regulated in BC tissues compared to their normal counterparts.



2. Bioinformatics analysis of miR-Let-7a with target genes

Using microRNA.org it was revealed that Let-7a targets PD-L1 with high binding score (Figure A)

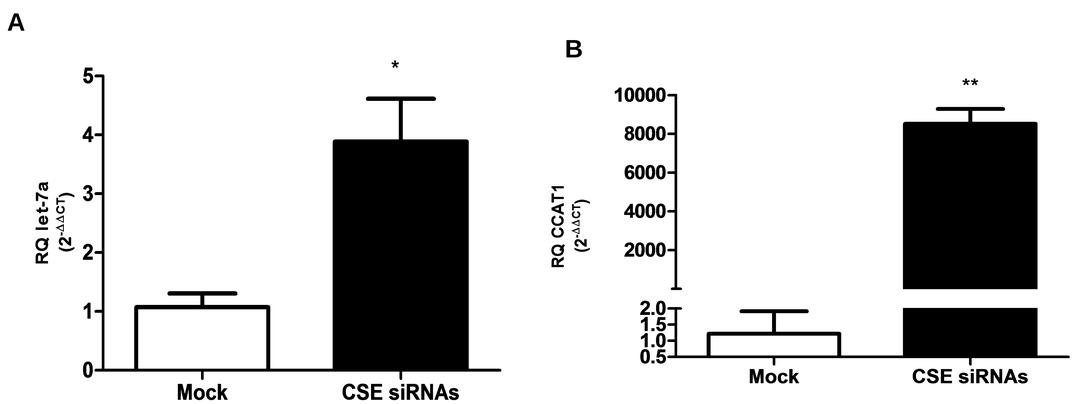
hsa-let-7a*/CD274 Alignment

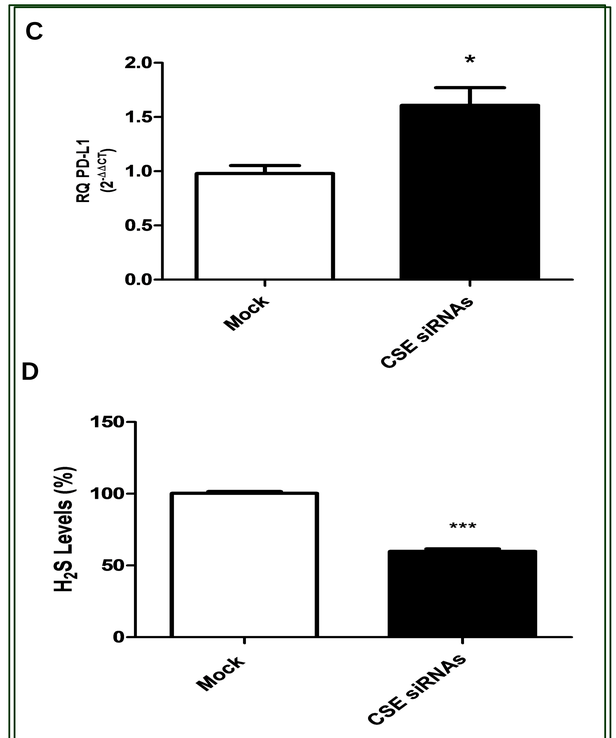
```
    3' cuuuCUGUCAUCUAACAU-AUc 5' hsa-let-7a*
          || : || |||||| ||
516:5' uuuuGAAGAUAUAUUGUAGUAg 3' CD274
```

mirSVR score: -1.1978
PhastCons score: 0.5955

3.Impact of CSE siRNAs on Let-7a, lncRNA CCAT1, PD-L1 and H_2S expression levels

Knocking down of CSE-induced H_2S production resulted in a marked induction of Let-7a (P= 0.0103 >4 folds Figure A) , CCAT1 (P=0.0080 >4000 folds, Figure B) and an elevation of PD-L1 (P= 0.0128, Figure C) expression levels. Repression of H_2S levels in MDA-MB-231 cells following CSE siRNA was confirmed (P<0.0001, Figure D).





Conclusion

This study highlights a novel ceRNA circuit responsible for evading immune surveillance in BC patients. It showed that CCAT1 oncogenic lncRNA overrides the tumor suppressor activity of let-7a in regulating PD-L1 expression.

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