51P - A Novel Crosstalk between Pyridoxal 5'-Phosphate (PLP) Dependent Enzymes; CBS & CSE Modulated by MALAT-1/STAT-3 Axis

Abstract #165

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BACKGROUND

Aberrant overexpression of pyridoxal 5'-phosphate (PLP) dependent enzymes: cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), is generally observed in several oncological contexts [1-4] including breast cancer (BC) [5]. Our group has recently recognized MALAT-1 as a pioneer lncRNA that modulates STAT-3 regulated hydrogen sulfide (H_2S) production via CSE in BC, thereby nominating MALAT-1/STAT-3/CSE as a novel pathway that regulates H_2S machinery. Additionally, we elucidated the importance of simultaneous suppression of MALAT-1 and CSE in BC to by-pass the compensatory feedback loop employed by CSE to restore H_2S levels [6].

AIM

Owing to the tightly regulated, and highly resistant protective mechanism employed by CSE, the aim of this study is to identify potential non-coding RNAs (ncRNAs) that can directly and effectively target both H_2S synthesizing enzymes.

SUBJECTS AND METHODS

Sample Collection

Twenty-five Egyptian female BC patients were recruited for this study. Breast tumor biopsies and their normal counterparts were resected; tumor stages and clinic-pathological classifications were determined with the pathologic TNM and immuno-histochemical profiles.

Cell Culture

MDA-MB-231 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4.5g/L Glucose, L-Glutamine, Penicillin/Streptomycin and 10% Fetal Bovine Serum (FBS).

Knockdown of CBS

MDA-MB-231 cells were cultured and transiently transfected with CBS siRNAs using lipofection.

RNA Extraction

Total RNA was extracted using Biozol reagent, reverse transcribed and then quantified using qRT-PCR.

Gene Expression Analysis

All conducted gene expression analyses were normalized to 18s rRNA in tissues and β–actin in MDA-MD-231 cell lines. Values were calculated as Relative Quantification (RQ) and represented as $2^{-\Delta\Delta CT}$.

Statistical Methods:

All statistics were performed using the student's unpaired t-test where $p<0.05$ was considered significant. All results were analyzed using Graphpad prism 8.0.1.

CONCLUSION

This study validates the compensatory mechanism applied by CSE and showcases its resilience against repression attempts to consistently maintain H_2S levels in the cells. Moreover, it categorizes miR-30a-5p as an efficient dual repressor upstream of H_2S synthesizing machinery that is capable of simultaneously targeting CBS and CSE, thereby paving the way towards promising therapeutic approaches in aggressive BC subtypes.

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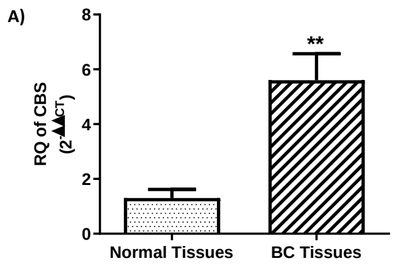
RESULTS

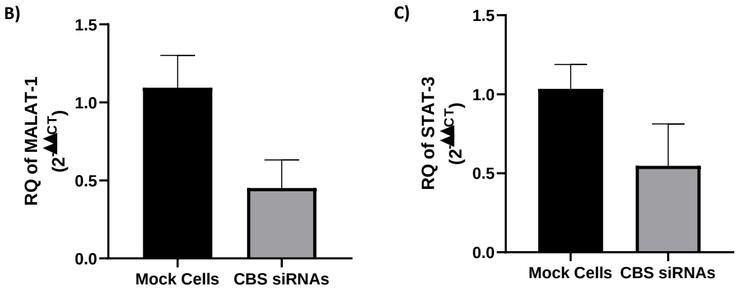
1. Expression Profile of CBS in Breast Cancer Patients

Screening showed a distinct upregulation of CBS in BC tissues by more than 5 folds (P=0.0028, **Figure A**) compared to normal control counterparts.

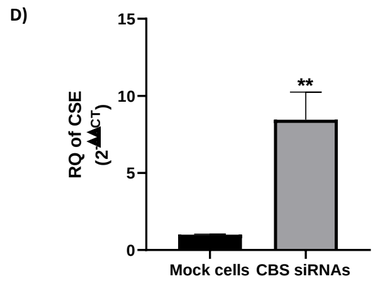


2. Impact of CBS Knockdown on MALAT-1 and STAT-3 Expression

The treatment of MDA-MB-231 cells with CBS siRNAs resulted in a noticeable repression of MALAT-1 (**Figure B**) and STAT-3 (**Figure C**) expression levels by 55% and 46% respectively compared to mock cells.



In contrast, CBS knockdown led to a significant elevation in CSE transcript levels (P=0.0481, **Figure D**) in triple negative BC cell lines. These results underscored the highly resistant compensatory mechanism employed by CSE in response to H_2S repression attempts in the cells.



3. Impact of the Ectopic Expression of miR-30a-5p on CBS and CSE Expression

In-silico analysis showed that miR-30a-5p can simultaneously act on and both CBS and CSE enzymes; this was validated as the forced expression of miR-30a-5p led to a considerably significant reduction in CBS (P<0.0001, **Figure E**) and CSE (P<0.0001, **Figure F**) transcript levels in TNBC cells compared to mock untreated cells.