Immunoregulatory loop between let-7a and CCAT1 lncRNA coordinated by c-Myc underlies the PD-1/PD-L1 Immunoresistance in Triple negative breast cancer

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Abstract #32

Background and Aim

PD-1/PD-L1 blockade therapeutic strategy has revolutionized the oncological treatment landscape [1]. Patients with chemo-resistant immunogenic triple negative breast cancer (TNBC) tumors were among the top listed applicable candidates for PD-1/PD-L1 inhibitors [2]. Yet, TNBC patients developing innate and adaptive immune-resistance have started to appear in the clinics [3]. Thus it became essential to identify novel predictive biomarkers to discriminate between resistant and responsive TNBC patients in a precision immune-oncological approach [3]. Our research group is currently focusing on molecular engines controlling PD-L1 in TNBC patients and cell lines. CCAT1 long noncoding RNA and let-7a microRNA are among the recently identified regulators of PD-L1 [4]. The aim of this study is to investigate the synchronization between CCAT1 and let-7a in regulating PD-L1 in TNBC patients and cell lines.

Subjects, Materials and Methods

BC Patients: BC and normal tissues from 17 BC patients were resected.

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

Transfection Experiments: TNBC cell lines were cultured and transfected with let-7a oligonucleotides and lncRNA CCAT1 siRNAs using Hiperfect Transfection Reagent.

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).

Functional characterization: BC hallmarks were assessed using MTT, colony forming assay and migration experiments.

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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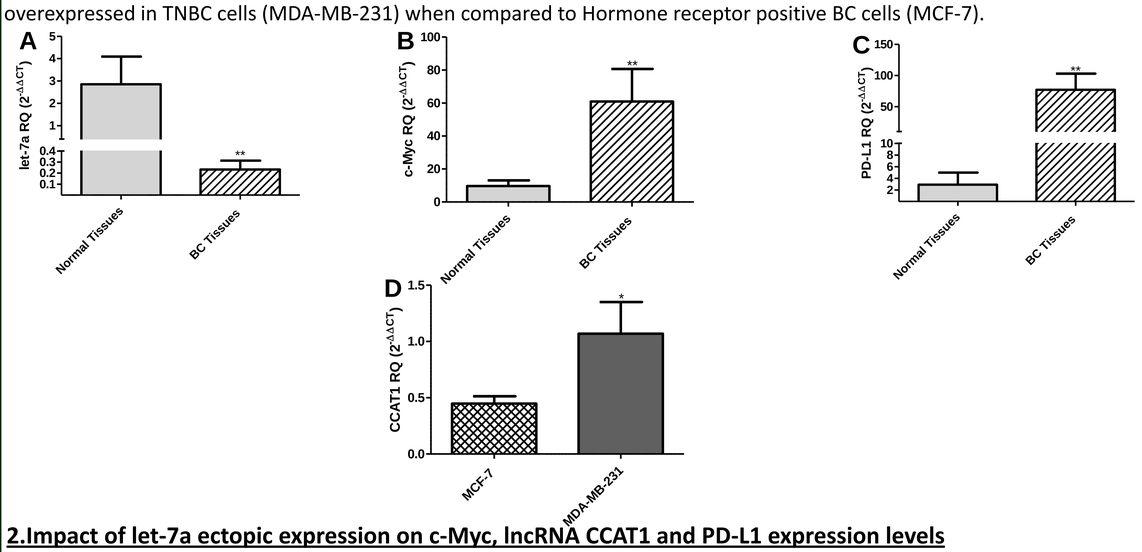
Results

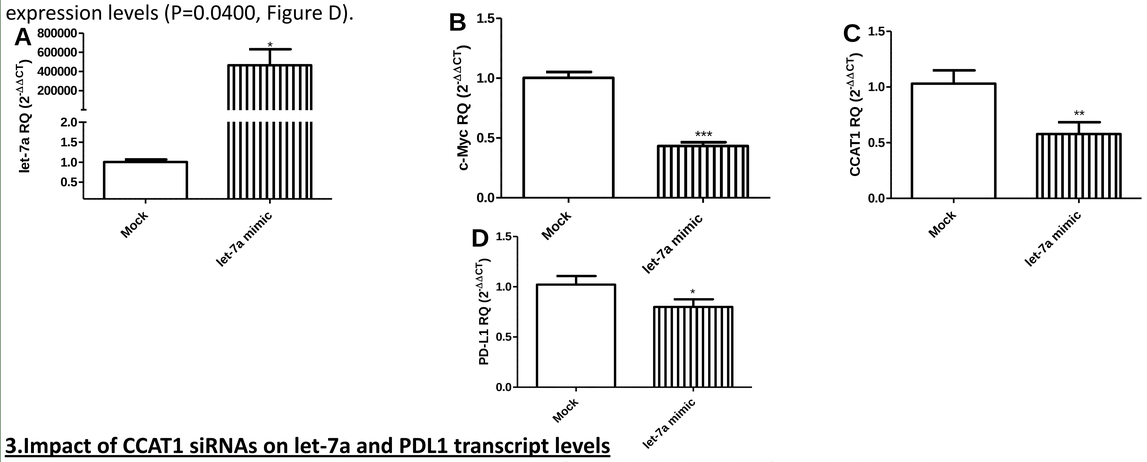
1.Screening of let-7a, c-Myc, PD-L1 in BC patients and CCAT1 in BC cell lines

Let-7a (P=0.0019, Figure A) was found to be down-regulated, while c-Myc (P=0.0085, Figure B) and PD-L1 (P=0.0094, Figure C) were found to be up-regulated in BC tissues compared to their normal counterparts. CCAT1 (P=0.0490, Figure D) was overexpressed in TNBC cells (MDA-MB-231) when compared to Hormone receptor positive BC cells (MCF-7).



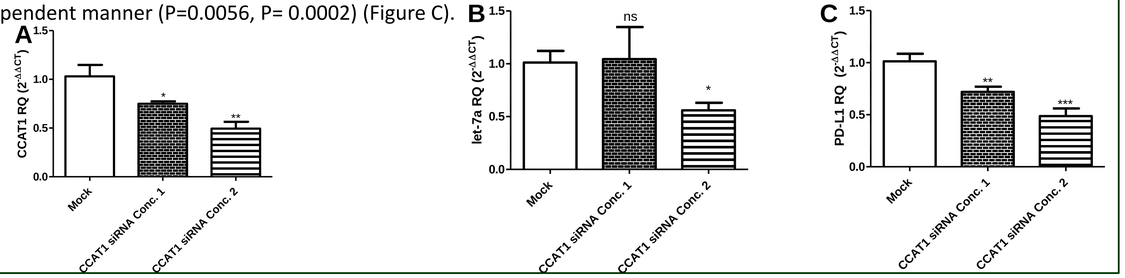
2.Impact of let-7a ectopic expression on c-Myc, lncRNA CCAT1 and PD-L1 expression levels

Efficient transfection of let-7a oligonucleotides was confirmed (P= 0.0250 > 400,000 fold increase, Figure A). Ectopic expression of let-7a resulted in a prominent reduction of c-Myc (P< 0.0001, Figure B), CCAT1 (P= 0.0088, Figure C) and PD-L1 expression levels (P=0.0400, Figure D).



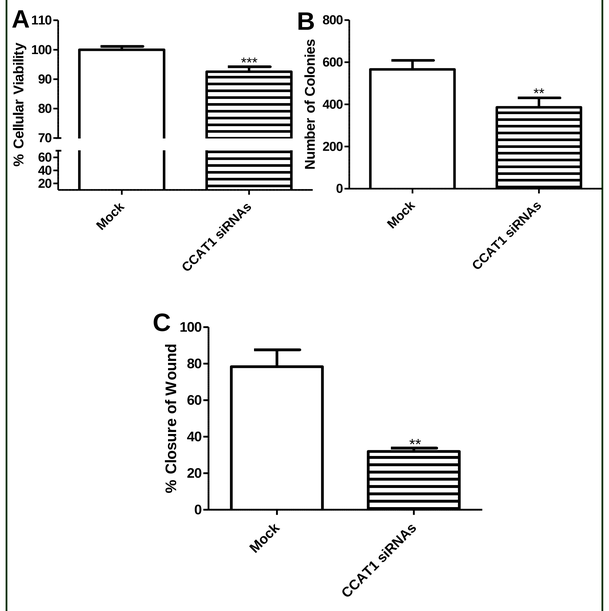
3.Impact of CCAT1 siRNAs on let-7a and PDL1 transcript levels

Efficient knockdown of lncRNA CCAT1 was confirmed (10 ng/µl, P= 0.0470), (20 ng/µl, P= 0.0014) (Figure A). Knocking down of CCAT1 led to a significant reduction of let-7a (P= 0.0131) (Figure B), and PD-L1 transcript levels in a concentration dependent manner (P=0.0056, P= 0.0002) (Figure C).



4.Impact of CCAT1 on TNBC hallmarks

CCAT1 siRNAs significantly decreased cellular viability (P=0.0009 (Figure A), clonogenicity (P=0.0045) (Figure B) and migration capacity (P=0.0039) (Figure C) of TNBC cells.



Conclusion

This study highlights Let-7a/c-Myc/CCAT1 as a novel immunoregulatory loop for PD-L1 in TNBC patients and cell lines. Nonetheless, This study underlines the significance of let-7a and CCAT1 are novel predicative immunomodulatory biomarkers in PD-L1 overexpressing TNBC patients.

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