MALAT-1/miR-30a-5p ceRNA network Releases the Brakes of Immune Surveillance in Breast Cancer through its Quadruple Targets: PD-L1, MIF, IL-10 and TNF-α

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BACKGROUND

Tumor immune escape phenomena is a vicious cycle of smart tactics orchestrated by cancer cells to evade immune recognition [1]. Induction of co-inhibitory molecules such as macrophage migration inhibitory factor (MIF), immune checkpoints such as PD-L1 and immunoinhibitory cytokines in the tumor microenvironment are the most effective strategies cancer cells follow to hold the brakes of the immune surveillance phenomena [2-4]. Immune evasion tactics in breast cancer (BC) patients are still poorly understood.

AIM

The aim of this study is to investigate the involvement of miR-30a-5p in MALAT-1 and its impact of PD-L1, MIF, IL-10 and TNF-α in BC.

SUBJECTS AND METHODS

Sample Collection

Twenty four BC patients were recruited in this study. Tumor stages and clinic-pathological classifications were defined with the pathologic TNM and immuno-histochemical profiles. Furthermore, quantification of Ki-67 (proliferative index) of BC was also assessed.

Cell Culture:

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

Oligonucleotides Transfection:

MDA-MB-231 cell lines were transfected using miR-30a-5p oligonucleotides and MALAT-1 siRNAs using a previously validated lipofection technique.

RNA extraction and Gene expression analysis:

Total RNA was extracted from breast tissue using B Qiagen. cDNA was synthesized, amplified, then quantified using qRT-PCR. Gene expression analysis were normalized to 18sRNA in breast tissues and Total RNA was extracted from breast tissue using BIOZOL. cDNA was synthesized, amplified, then quantified using validated lipofection technique.

RESULTS

1. Expression profile of miR-30a-5p and MALAT-1, IL-10, MIF and TNF-α in BC Patients

- miR-30a-5p was found to be down regulated in BC tissues compared to its normal counterparts (P<0.001, Figure A), while MALAT-1, IL-10, TNF-α and MIF were found to be up-regulated (P<0.001, Figure B, P<0.001, Figure C, P<0.0001, Figure D, P<0.0001, Figure E, respectively). 

2. Efficient transfection of miR-30a-5p mimics and MALAT1 siRNA

- miR-30a-5p mimics were successfully transfected with >185X fold increase in THBC MDA-MB-231 cells (P<0.001, Figure F) and MALAT1 siRNA (P<0.001, Figure G) showed a significant decrease in their expression levels compared to mock.

3. Impact of miR-30a-5p on MALAT-1, IL-10, and TNF-α transcript levels

-敲除型expression of miR-30a-5p oligonucleotides resulted in a significant decrease in MALAT-1 (P<0.001, Figure H), IL-10 (P<0.0001, Figure I) and TNF-α (P<0.0001, Figure J) expression levels compared to mock.

4. Impact of MALAT1 siRNA on PD-L1 and MIF

- MALAT1 siRNAs resulted in a marked reduction of PD-L1 (P=0.0003, Figure K) and MIF(P=0.0331,Figure L) expression levels compared to mock.

CONCLUSION

MALAT-1/miR-30a-5p are vital players releasing the immune surveillance brakes supported by BC cells. Dual targeting of MALAT-1 and miR-30a-5p is novel immune- oncological therapeutic approach in BC.

REFERENCES