Expression Signature of Let-7a, miR-34a and miR-486-5p in Young Triple Negative Breast Cancer Patients Overexpressing PD-L1: A Step towards Precision Immuno-oncology

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Background

In an era of immunotherapeutic approaches, immune checkpoint blockers (ICBs) had interposed the oncology market. Yet, most patients do not benefit [1]. The new direction is shifted towards identifying novel predictive biomarkers in the field of the precision immuno-oncology [2]. Thus, the development of a multifactorial synergistic predictive-model has become a pressing need. Triple Negative Breast Cancer (TNBC) and especially at younger age (<40 years) tends to exhibit an aggressive phenotype evading immune-surveillance mediated by immune cells [3]. This occurs by overexpressing PD-L1 and shedding of CD155 [4, 5]. Our group has recently identified sONE, a tumor suppressor lncRNA that is absent in young TNBC patients [6]. sONE functional activity was found to be directly correlated to other immunomodulatory microRNAs. However, their expression signature in young TNBC patients has never been investigated.

Aim

To identify an immunomodulatory-related mRNA-signature for young TNBC patients.

Methods

TNBC patients: TNBC patients (n=28) were recruited. Median age at the time of diagnosis was 39 years old (range 22-70). Lymph node metastasis was evident in 60.7%. 71% showed Ki-67>14%. 53.6% of patients had stage 3 diagnosis. 93% of tumors were IDC and the rest was ILC. 68% had a tumor size >5 cm.

Cell culture and transfection: MDA-MB-231 were cultured and transfected with miR-486-5p, let-7a and miR-34a mimics using a validated oligonucleotide delivery system.

miRNA expression profile: RNA was extracted from tissues and serum, reverse transcribed and quantified using q-RT-PCR. TNBC cell lines were cultured, transfected using oligonucleotides using lipofection.

Statistical methods: all statistics were performed using one-way anova where P<0.05 was considered significant. All results were analyzed using Graphpad prism 8.0.

Results

Screening PD-L1 and CD155 in young TNBC patients

An elevated expression pattern for PD-L1 (Figure A) in young TNBC patients and a marked repression of CD155 (Figure B) compared to the older group were observed.

Screening Let-7a, miR-34a and miR-486-5p in young TNBC patients

Ectopic expression of let-7a (Figure F) and miR-34a(Figure G) resulted in a significant repression of PD-L1 while miR-486-5p (Figure H) mimics resulted in an induction of CD155 levels.

Impact of Let-7a, miR-34a and miR-486-5p on PD-L1 and CD155 expression in TNBC cell lines

Marked repression of Let-7a (Figure C), miR-34a (Figure D) and miR-486-5p (Figure E) in young TNBC patients compared to the older group were observed.

Clinical correlation analysis between Let-7a, miR-34a, miR-486-5p and PD-L1 with different disease parameters

immuno-modulatory miRNAs Let-7a (Figure I), miR-34a (Figure J) and miR-486-5p (Figure K) expression pattern was inversely correlated to Ki-67 in TNBC patients. Yet, PD-L1 was directly associated with lymphnode metastasis and stage of the disease (Figure L).

Conclusion

This study identified a panel of 3 immunomodulatory miRNAs as a signature among young TNBC patients over-expressing PDL1 and under-expressing CD155.

References

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