# HEIH: A novel Immunomodulatory LncRNA Tweaking NK cells and TME in Triple Negative Breast Cancer patients

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## Background and Aim

Immune-related long non-coding RNAs (lncRNAs) have been acknowledged as potential modulators in immune surveillance process [1]. Given the scarcity of effective targeted therapies along with high immunogenicity of TNBC, immunotherapy was presented [2]. In such context, immune checkpoint inhibitors (ICIs) were put through clinical trials however, prominent side effects and resistance prevailed [3]. Thus, a promising approach would be targeting the innate arm of the immunity and sacking the immune suppressive tumor microenvironment (TME) [4]. LncRNAs were found to have a pivotal role in regulating Natural killer (NK) cells and tuning the TME [5]. Hepatocellular carcinoma up-regulated EZH2-associated (HEIH) is a novel lncRNA that has been seldom examined in TNBC. So, our aim is to unravel the expression profile of the lncRNA HEIH in TNBC tissues, evaluate its oncological actions in TNBC cells and evaluate its unprecedented immunomodulatory role on NKG2D ligands and TME of TNBC.

## Subjects, Materials and Methods

**BC Patients:** BC tissues from 40 BC patients were recruited.

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

**Transfection Experiments:** TNBC cell lines were transfected using different oligonucleotides using a validated oligonucleotide delivery system [4,5]

**mRNA Quantification:** Total RNA was extracted from breast tissues, MDA-MB-231 using Biozol. cDNA was synthesized 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 one-way anova where $p<0.05$ were considered significant .

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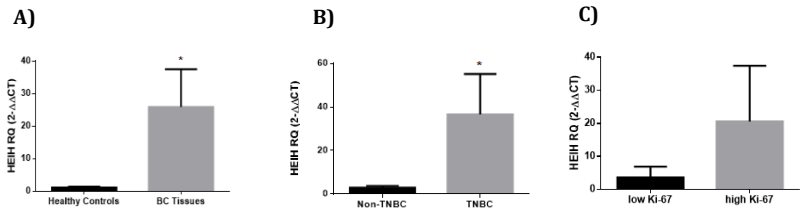
## Results

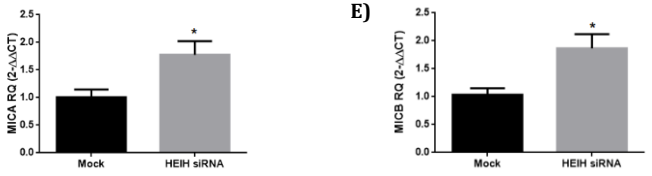
❖ **Screening of lncRNA HEIH in BC patients**

LncRNA HEIH was up-regulated in BC patients compared to healthy patients (Figure A). TNBC Patients showed a significant increase in HEIH transcript levels compared to non-TNBC counterparts (Figure B). Increased HEIH levels were encountered in patients with high Ki-67 (proliferation marker) (Figure C).



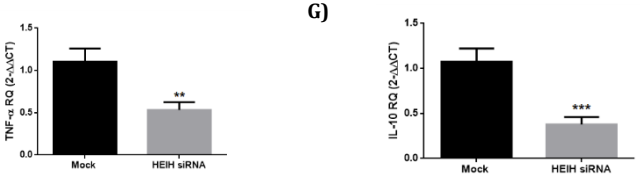

❖ **Impact of HEIH knockdown on NKG2D ligands**

HEIH knockdown in MDA-MB-231 cells resulted in a significant elevation of the immune ligands MICA (Figure D) and MICB (Figure E ) expression levels.

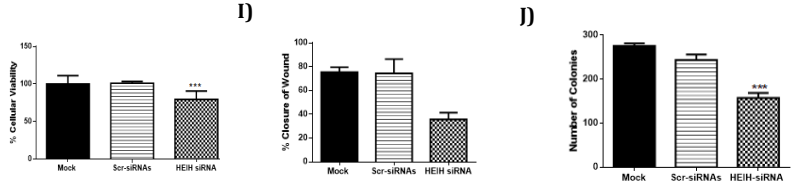



❖ **Impact of HEIH knockdown on tumorigenic cytokines**

Knockdown of HEIH in MDA-MB-231 potentially decreased TNF-α (Figure F) and IL-10 (Figure G) levels .




❖ **Impact of HEIH knockdown on BC hallmarks**




## Conclusion

The present study categorized HEIH as an oncogenic lncRNA that is solitarily expressed in TNBC patients and cell lines. Moreover, it constitutes the first major advancement in unraveling the immunomodulatory role of HEIH in boosting NK cells cytotoxicity and trimming TME in favor of TNBC eradication, thus proposing HEIH as a novel therapeutic target in TNBC.

## References

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