Triple-negative breast cancer and DNA repair capacity: identification of epigenetic markers using an *in vitro* model

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Background

- Breast cancer (BC) is the leading type of cancer diagnosed in women worldwide.
- BC is the second leading cause of cancer-related mortality in women in the US and the **first** in Puerto Rican women.
- Puerto Rico (PR) Cancer Registry data (2018) shows that BC is the leading cancer type in terms of incidence (**28.9%** of all cancer cases) and mortality (**18.9%** of all cancer deaths).

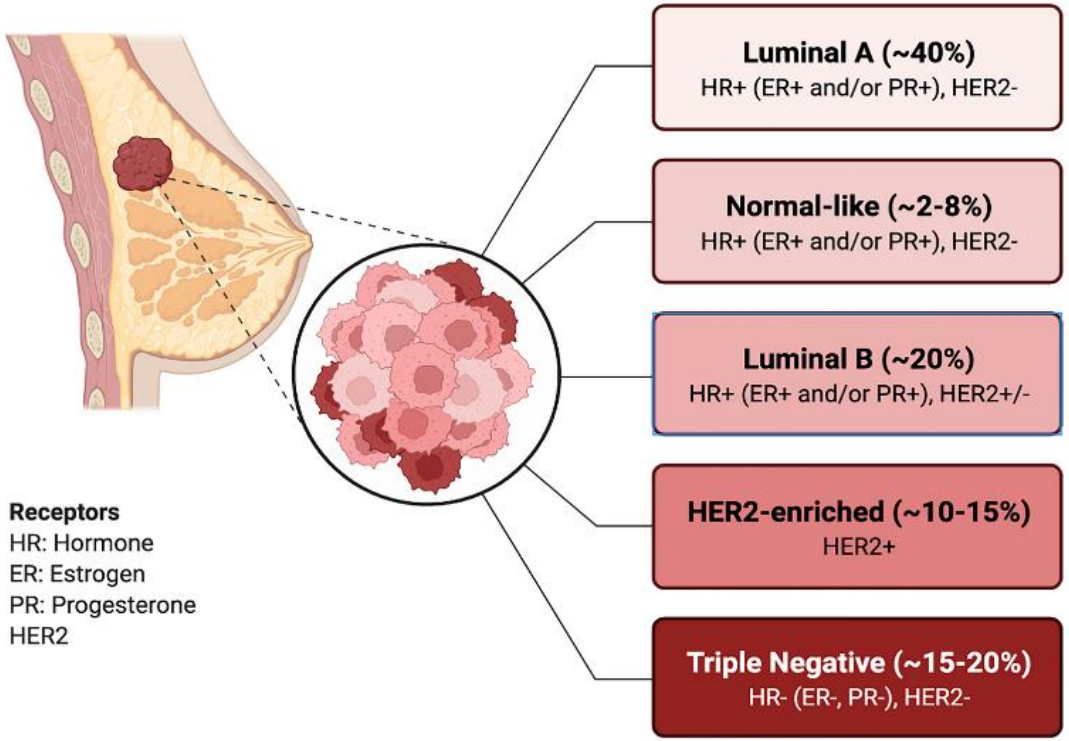
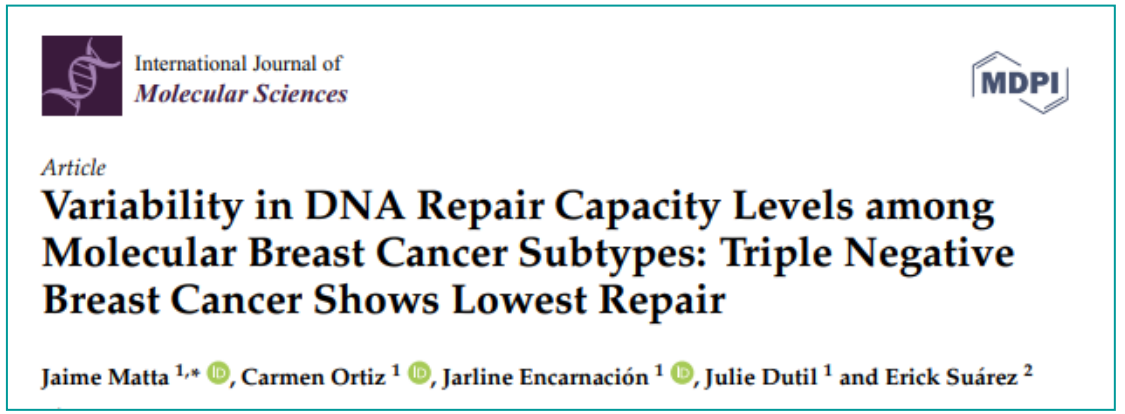


Figure 1. Schematic figure summarizing breast cancer subtypes based on their expression of hormone receptors, Ki-67, and the receptor tyrosine kinase HER2. Source: Rizzo et al. 2022

Background

- DNA repair capacity (DRC) is defined as the ability of the cell to repair any damage to the DNA by endogenous or exogenous sources.
- DRC is an important factor contributing to the inter-individual variability in response to carcinogens and cancer susceptibility in the general population.
- Regarding BC, studies have shown that having a low DNA repair capacity (DRC) measured in lymphocytes, has been associated with an increased risk of developing the disease.
- Moreover, TNBC patients have shown to have the lowest DRC levels when compared with patients with other molecular subtypes.



Background

- miRNAs, as epigenetic modulators, affect the protein levels of the target mRNAs without modifying the gene sequences.

- Exo-miRNAs: small non-coding RNAs that regulate gene expression at a post-transcriptional level packed in exosomes.

- Exo-miRs function as cellular communication centers involved in genetic exchange between cells, and their preservation in body fluids make them a good target for a liquid biopsy.

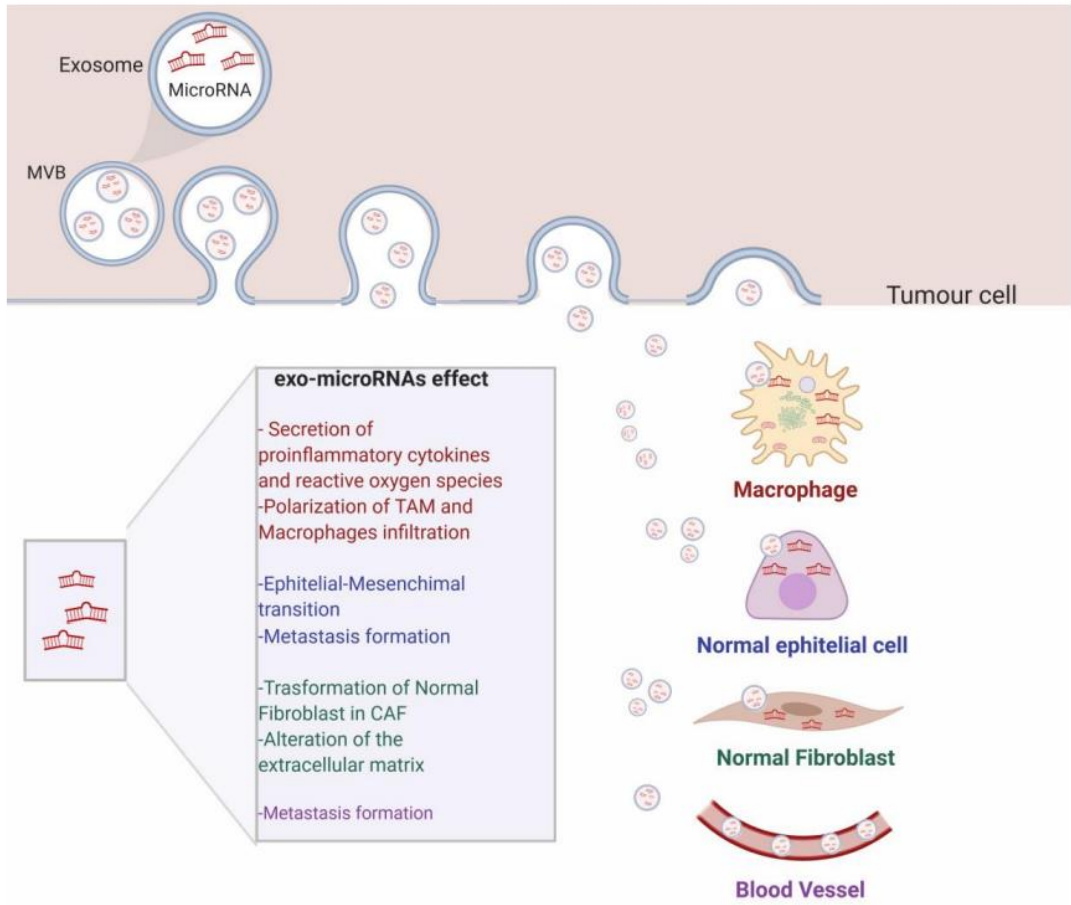


Figure 2. Schematic representation of the roles of exo-miRNAs on distant cells and in the cancer microenvironment

Although some studies have identified exo-miRNA candidates in TNBC using clinical samples, there is a gap regarding exo-miRNAs related to DRC levels in TNBC.

Objective

- To identify the exo-miRNAs involved on the interaction between TNBC cells and lymphocytes with different DRC levels using an *in vitro* model through coculture systems.

Hypothesis

- We expect to detect significant variations on the exo-miRNA expression profiles in cocultures resulting from the interaction between MDA-MB-231 cells and lymphocytes with different DRC levels.

Methods

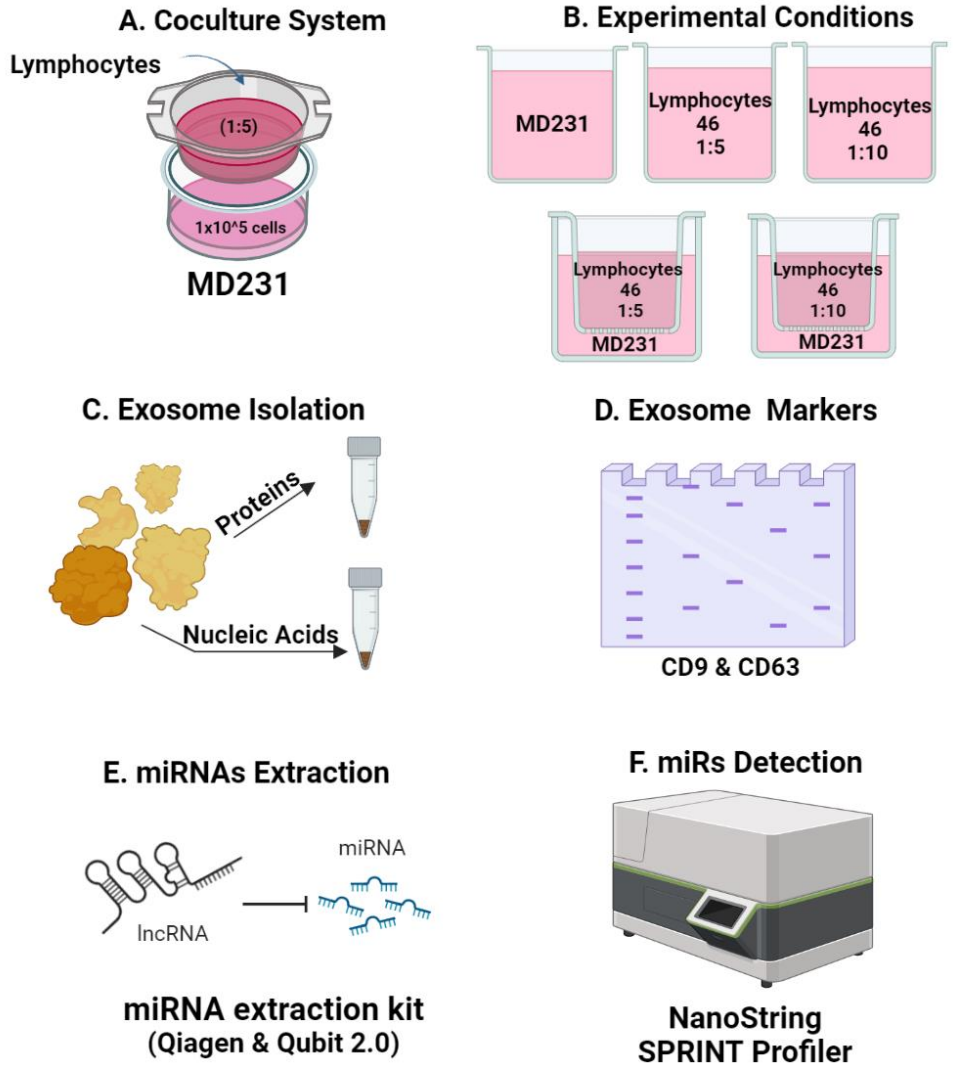


Table 1. Cell lines used in coculture systems

Cell line	Cell type	Model
MDA-MB-231	Breast adenocarcinoma	TN BC
GM02246	B-Lymphocyte	Medium DRC (*XPC*-KD)
GM02253	B-Lymphocyte	Low DRC (*XPD*-KD)

Results

Western blot for exosome markers

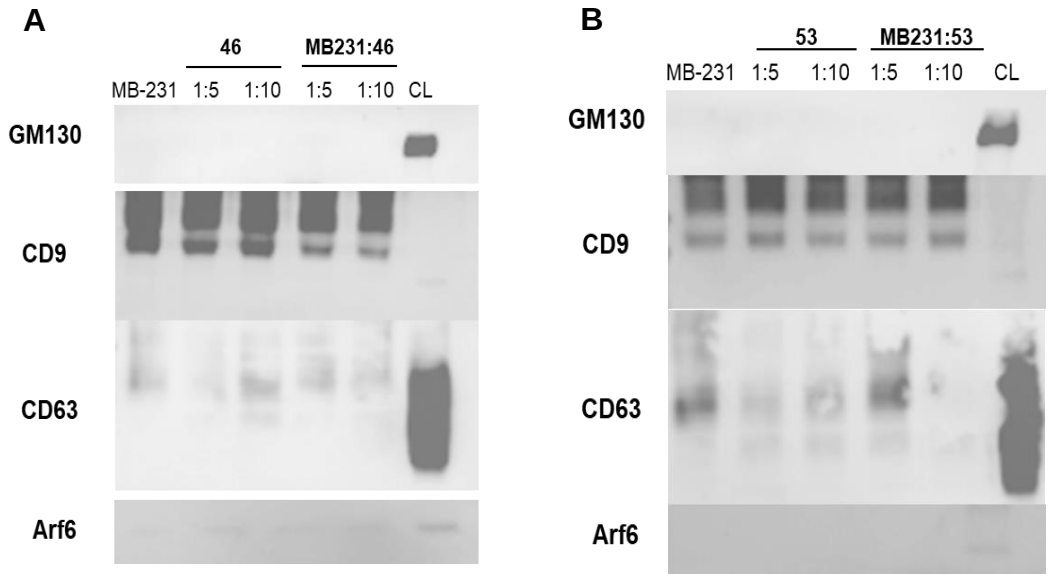


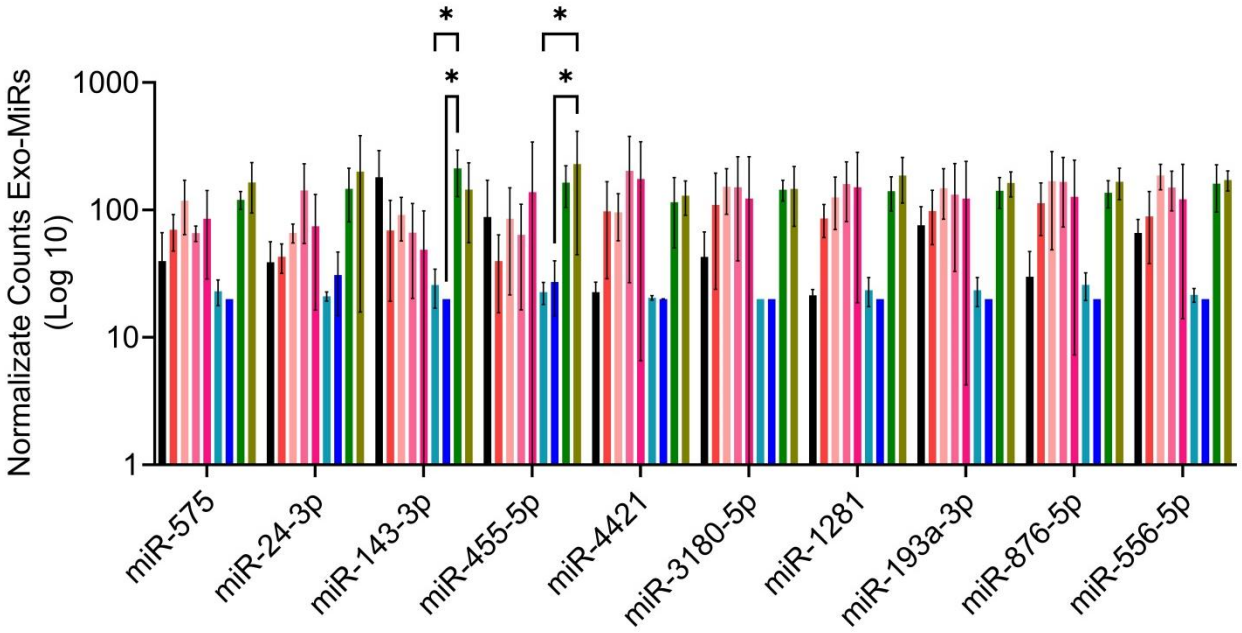
Figure 3. Expression of exosomal markers in coculture systems of MDA-MB-231 and **(A)** GM02246 and **(B)** GM02253 cell lines at two seeding ratios (1:5 and 1:10). GM130 and Arf6 were used as markers for cellular contamination. Cell lysate (CL) was included for detection of cellular contamination markers.

Results

798 exo-miR candidates

20 significant exo-miR candidates

Top 5 exo-miRs



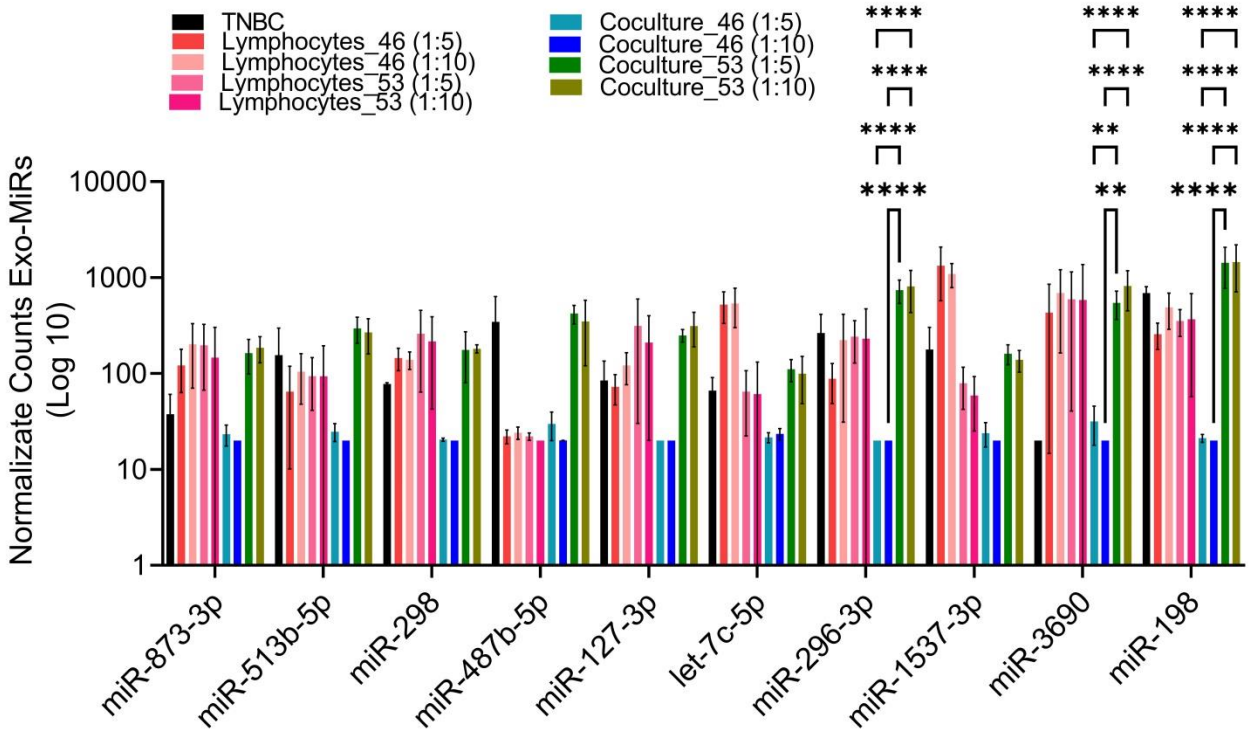


Figure 6. Differentially expressed exo-miRs among TNBC and lymphocytic cell line co-culture systems. Bars represent mean of the normalized exo-miRs counts ± SD of three independent experiments, ****p < 0.001, **p < 0.01, and *p < 0.05.

Results

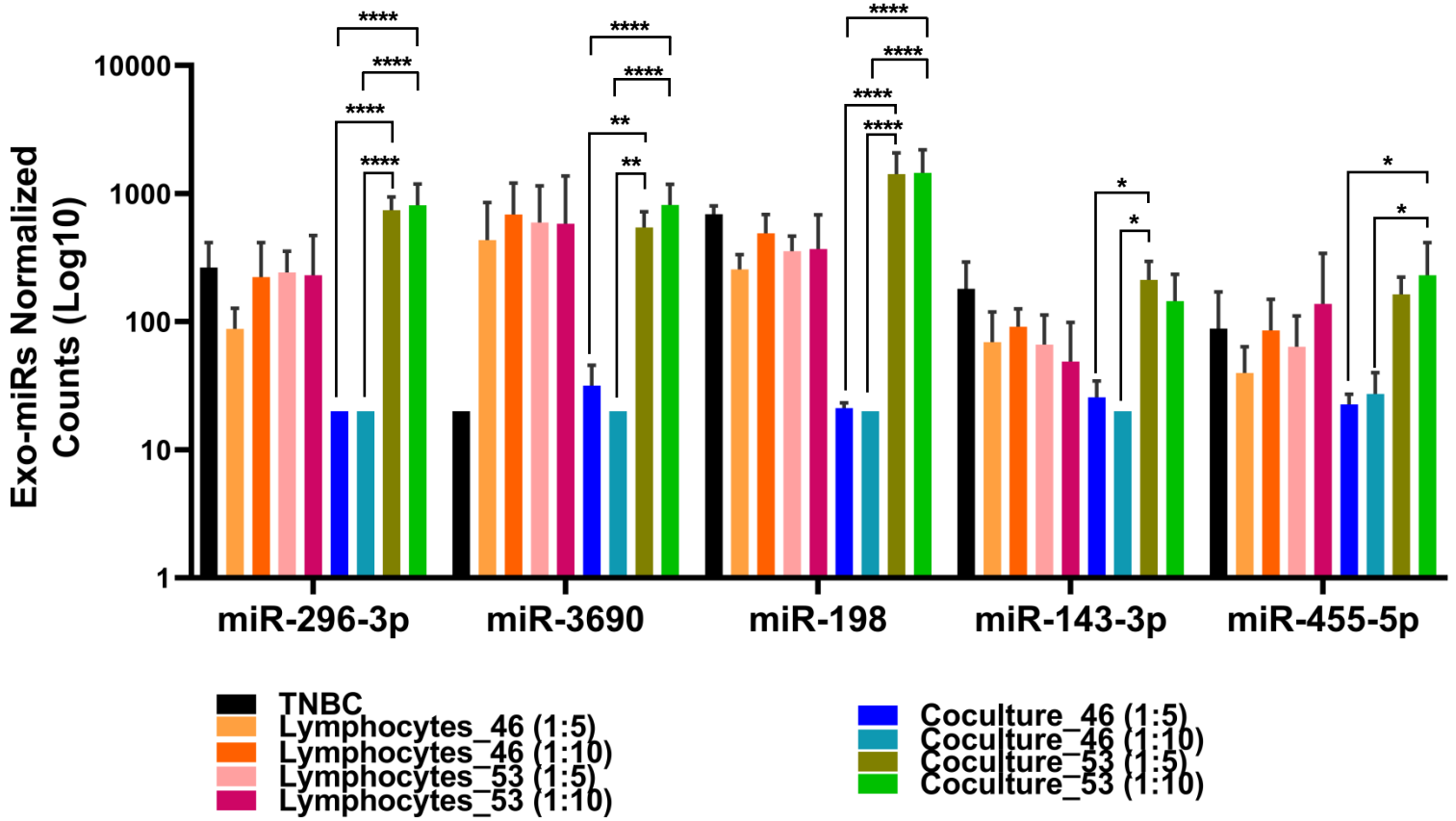


Figure 7. Top 5 differentially expressed exo-miRs among TNBC and lymphocytic cell line co-culture systems. Bars represent mean of the normalized exo-miRs counts ± SD of three independent experiments, ****p < 0.001, **p < 0.01, and *p < 0.05.

Conclusions

- Variations on exo-miRNA profile were found when comparing monocultures with cocultures with the different cell lines, in terms of exo-miR secretion.
- In terms of TNBC monocultures, two exo-miRs were observed in high expression **miR-487b-5p** and **miR-143-3p** when compared with the monocultures of any of the cell lines.
- For the top 5 candidates (**miR-296-3p**, **miR-3690**, **miR-198**, **miR-143-3p**, and **miR-455-5p**), an increased expression was observed in coculture systems with the low DRC cell model (MDA-MB-231:GM02253) at any of the two seeding ratios.
- Our results shows that the TNBC cells can differentially secret exo-miRNAs. The results also suggest that these secretions might be associated by the DRC levels of the lymphocytes.
- We believe that our results will allow for the understanding of the role of exo-miRs in TNBC and their use as a potential tool to improve BC diagnosis.

Future Directions

- Perform the validation of the selected candidates (miR-296-3p , miR-3690, miR-198, miR-143-3p, and miR-455-5p).

- As a secondary aim, the expression of these candidates will also be evaluated in plasma samples from TNBC patients to test the potential of these exo-miRs to identify TNBC and any other tumor feature (i.e. tumor grade, metastasis, among other).

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