MicroRNA 362-3p enhances cisplatin sensitivity in BRCA mutant and HER2-negative breast cancer

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Background and Aim

- Breast cancer is the second most common cancer in women.
- Therapy response prediction in breast cancer patients remains a key obstacle in personalized treatment.
- We leverage the two-arm, randomized phase II INFORM trial (NCT01670500) to identify differentially expressed pre-treatment plasma miRNAs associated with a) complete pathological response (pCR; i.e., cancer free) and b) residual cancer burden (RCB) 0/1 when treated with an anthracycline- or a platinum-containing neoadjuvant regimen (cisplatin)[1].

Hypothesis

- Pre-treatment plasma miRNAs were associated with pCR.

Materials & Methods

Subjects of the INFORM trial [1]:

- 118 patients were enrolled over a 7 year period from 2012 to 2019 from 13 US-hospitals
  - Eligibility was germline *BRCA1/2^{mut}* , tumor was HER2 negative, tumor at least 1.5 cm or node positive.
  - Randomized by estrogen receptor (ER) status and hospital site to receive either neoadjuvant cisplatin monotherapy or doxorubicin plus cyclophosphamide (AC).
  - 1 to 2 tumor core biopsies were obtained for correlative studies, and EDTA blood samples was collected before starting treatment (Fig 1).
- After 4 cycles of protocol-assigned chemotherapy, participants underwent breast surgery (mastectomy or lumpectomy), and sentinel lymph node biopsy or axillary dissection.
- Excised tumor and axillary nodes were assessed for residual cancer.
- Primary outcome of the trial was whether or not the tumor achieved pCR. pCR is defined as the absence of residual invasive breast disease with or without ductal carcinoma *in situ.*
- Secondary outcome was assessing RCB 0/1 versus RCB 2/3. RCB scores of 0, 1, 2, and 3 correspond to absence, minimal, moderate, and extensive residual disease.

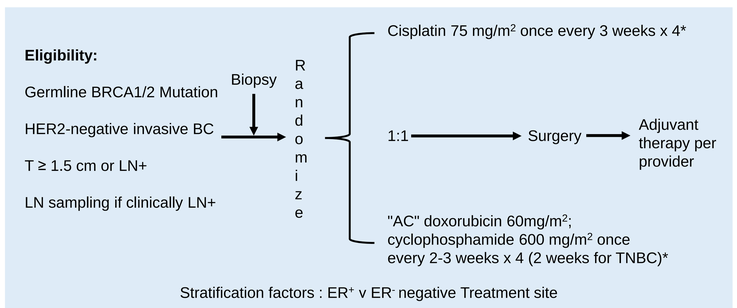

Fig 1. Schematic design of the INFORM trial. Pre-treatment EDTA blood sample was obtained at biopsy. AC, doxorubicin plus cyclophosphamide; LN, lymph node; T, tumor size

MiRNA screening of pre-treatment EDTA blood samples:

- 99 out of 118 patients completed 4 full cycles of therapy and had pre-treatment EDTA blood samples collected.
- Plasma (200 uL) underwent RNA extraction, and screened for 352 miRNAs using the ID3EAL™ Cancer miRNA Knowledge Panel (Mirxes Pte Ltd, Singapore).
- Raw miRNA Ct values were pre-processed prior to differential analysis (Fig 2).

Reference

1. Tung N, et al. J Clin Oncol. 2020 May 10;38(14):1539-1548.

Materials & Methods

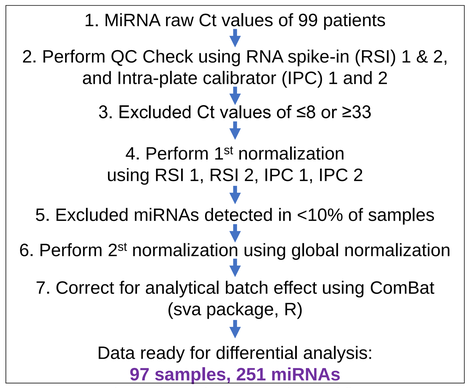

Fig 2. MiRNA data processing workflow

Data analysis:

- Stratified analysis by treatment was performed using crude and adjusted binary logistic regression analyses.
- Confounders include age at diagnosis, year of enrollment, clinical grade, stage, triple negative receptor status, and degree of tumor infiltrating lymphocytes.
- No miRNA achieved a false discovery rate (FDR) of <0.05.
- We report miRNAs that achieved p<0.01 and FDR<0.25

Results

Plasma miR-362-3p was associated with outcomes only in cisplatin arm.

- Of the 97 subjects, 53 received cisplatin and 44 received AC. As INFORM was a randomized control trial, the clinical characteristics of patients were similar in both treatment arms.
- No miRNA was associated with pCR or RCB 0/1 among patients that received AC at a combined threshold of p<0.01 and FDR<0.25.
- Among patients that received cisplatin, 3 miRNAs were associated with pCR and 10 miRNAs associated with RCB 0/1. MiR-362-3p was common between the two analysis (Fig 3).
- MiR-362-3p expression was 1.8- and 1.7-fold higher in patients who achieved pCR or RCB 0/1, respectively (Fig 3).

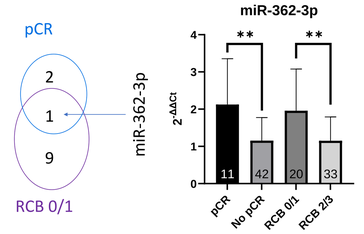

Fig 3. MiR-362-3p was associated with both pCR and RCB 0/1. Tumors that achieved pCR or RCB 0/1 have significantly higher miR-362-3p expression than no pCR or RCB 2/3.

Paired tumor biopsies express miR-362-3p

- 79 out of the 97 patients had paired pre-treatment tumor smallRNASeq data.
- miR-362-3p expression was detected in all 79 tumors.
- Tumor miR-362-3p expression was not associated with pCR (p>0.05; Mann-Whitney U test). There was also no correlation between tumor and plasma miR-362-3p expression (Figs 4A-B).

MiR-362-3p in TCGA breast cancer cases

- In TCGA, miR-362-3p expression in primary and metastatic tumors was 1.9- and 3.4-fold significantly higher compared to adjacent normal breast tissue, respectively (Fig 4C).
- Within primary tumors, miR-362-3p expression was 1.6-fold significantly higher in triple negative breast cancer cases versus ER+/PR+ cases (Fig 4D).

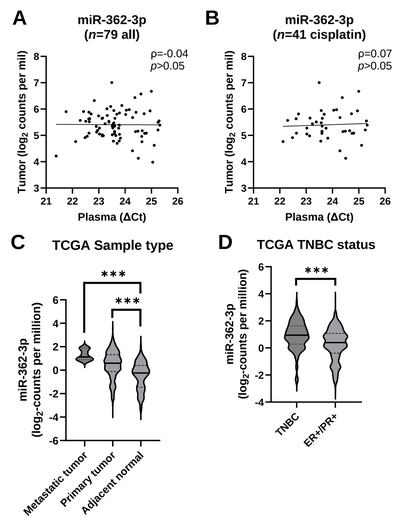

Fig 4. No correlation between plasma and tumor miR-362-3p expression in all 79 patients (**A**) or among 41 patients who received cisplatin (**B**). In TCGA, miR-362-3p expression was higher in primary and metastatic tumors compared to adjacent normal breast tissue (**C**). Triple negative breast tumors have higher miR-362-3p expression than ER+/PR+ tumors.

Results

Breast cancer cells express and secret miR-362-3p

- We performed *in vitro* experiments to better understand the relationship between miR-362-3p and cisplatin sensitivity.
- Breast cancer cell lines express and secrete miR-362-3p (Figs 5A-B). Figs 5C and 5D show their baseline sensitivity to cisplatin.
- Follow-on experiments were carried out using MDA-MB-231 and 436.

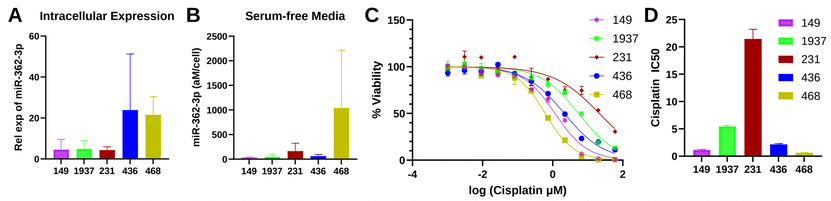

Fig 5. **A**. Intracellular miR-362-3p expression in three *BRCA1^{mut}* cell lines (SUM 149PT, HCC 1937, and MDA-MB 436) and two *BRCA1^{wt}* cell lines (MDA-MB 468 and MDA-MB 231). **B**. All cell lines secrete miR-362-3p. **C**. The cell lines demonstrate varying degrees of cisplatin sensitivity; IC50 is shown in **D.**

- We established miR-362-3p overexpression (OE) and knockdown (KD) 231 and 436 cells lines via lentiviral transduction. (Fig 6). We confirmed the upregulation of miR-362-3p expression in overexpressing cells, and that miR-362-3p was secreted into the extracellular medium.

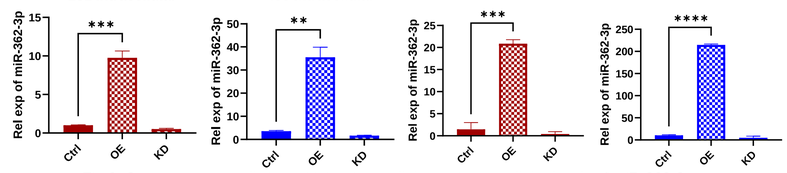

Fig 6. Overexpressed cells have higher intracellular and extracellular levels of miR-362-3p.

MiR-362-3p inhibits tumor growth and sensitizes tumor cells to cisplatin

- Cisplatin *resistant* 231 cells (Fig 5D) with miR-362-3p overexpression have lower cellular proliferation and are now *sensitive* to cisplatin (Fig 7A, C).
- Cisplatin *sensitive* 436 cells (Fig 5D) with miR-362-3p knockdown shows higher cellular proliferation and are now *resistant* to cisplatin (Fig 7B, D).
- We screened selected downstream target genes of miR-362-3p linked to cisplatin resistance.

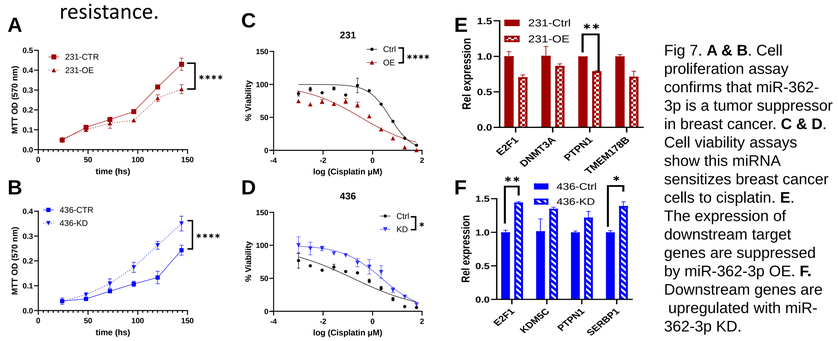

Fig 7. **A & B**. Cell proliferation assay confirms that miR-362-3p is a tumor suppressor in breast cancer. **C & D**. Cell viability assays show this miRNA sensitizes breast cancer cells to cisplatin. **E**. The expression of downstream target genes are suppressed by miR-362-3p OE. **F.** Downstream genes are upregulated with miR-362-3p KD.

Conclusion

- *BRCA*-related breast cancer patients with higher plasma miR-362-3p expression had better response to cisplatin.
- MiR-362-3p is expressed in breast tumors *in vivo* and *in vitro*, and functions as a tumor suppressor.
- Cell lines with high expression of miR-362-3p are sensitive to cisplatin *in vitro*. We are validating this *in vivo.*

Acknowledgments

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