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miR-363-3p Mediates Maintenance and Tumorigenicity of Breast Cancer Stem Cells and Provides A Potential Diagnostic and Prognostic Sera-Exosome Biomarker of Early Breast Cancer

Stéphanie Tissot-Renaud[1*#], Athina Stravodimou[2#], Fabrizio Benedetti[3], Valentina Scabia[4], Valérian Dormoy[5], Jacqueline Masternak[1], Cathrin Brisken[4], Jean-Yves Meuwly[6], Laetitia Rossier[7], Maryse Fiche[8], Marie Galmiche[2], Khalil Zaman[2§], and Nicolas Mermod[1§]

Abstract

Purpose: Despite great advances in breast cancer (BC) treatment, 20–30% of patients develop metastatic disease, eventually leading to death. Cancer stem cells (CSC) are key players in disease persistence and recurrence. However, methods for their detection and specific targeting are still lacking. MicroRNAs (miRNAs) are involved in the expression of many genes and biological processes. In this study, we aimed to identify miRNAs specific to BC stem cells (BCSC) that are resistant to treatment.

Methods: By treating BCSC-enriched cells in vitro in mammospheres with chemotherapeutic agents, we selected miRNAs specific to resistant BCSCs. The most promising miRNA was then assessed in a mouse model and in clinical material from healthy women and patients treated with neoadjuvant chemotherapy.

Results: *In vitro* enrichment of chemoresistant BCSC identified five-miRNA. miR-363-3p exhibited the highest expression in various BC cell lines and derived BCSC-enriched populations compared with non-tumorigenic cells. The inhibition of miR-363-3p decreased BCSC maintenance and tumorigenicity *in vitro*, whereas its overexpression increased mammosphere formation. Consistently, miR-363-3p downregulation decreased the growth and metastasis of human BC cells transplanted in mice. In human samples, miRNA-363-3p was overexpressed in cancer tissue before chemotherapy, whereas higher levels of miR363-3p in serum exosomes were correlated with better survival. Furthermore, a 7-exosomal miRNA (exomiRNA) signature, including miR-363-3p, enabled the discrimination of patients with BC from healthy women.

Conclusion: *In vitro*, mouse and clinical models suggest that miR-363-3p and miRNA signatures act as biomarkers of the BCSC phenotype, providing a promising approach for BC detection, follow-up, and BCSC targeting.

Affiliation:

[1]Institute of Biotechnology and Department of Fundamental Microbiology, University of Lausanne, 1015 Lausanne, Switzerland

[2]Breast Center, Department of Oncology, Lausanne University Hospital CHUV, 1011 Lausanne, Switzerland

[3]Ludwig Institute for Cancer Research, Lausanne Branch, University of Lausanne, Lausanne, Switzerland

[4]Swiss Institute for Experimental Cancer Research, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

[5]University of Reims Champagne-Ardenne, Inserm, P3Cell UMR-S1250, SFR CAP-SANTE, 51092 Reims, France

[6]Department of Radiology, University Hospital of Lausanne (CHUV) and University of Lausanne (UNIL), 1011 Lausanne, Switzerland

[7]Department of Oncology, Center of experimental therapeutics, Lausanne University Hospital CHUV, 1011 Lausanne, Switzerland

[8]Institute of Pathology, Lausanne University Hospital CHUV, CH-1011 Lausanne, Switzerland

***Corresponding author:**
Dr Stéphanie Tissot-Renaud, Department of Oncology, Center of experimental therapeutics Lausanne University Hospital, 1011 Lausanne, Switzerland

[#]Considered as co-first author
[§]Considered as co-last author

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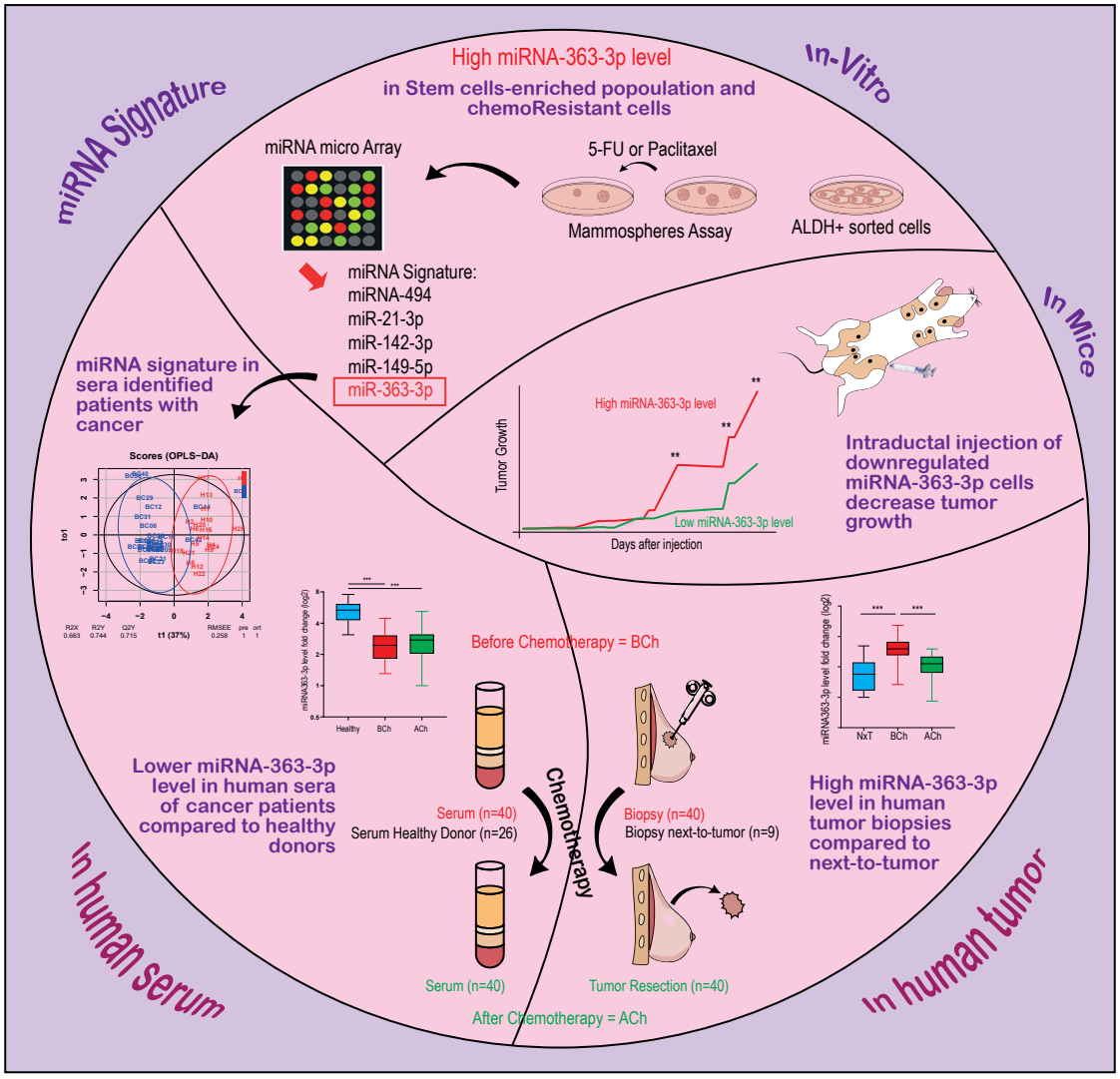

Keywords: Cancer stem cells; miRNA; Chemoresistance; Breast cancer; exomiR.

Introduction

Breast cancer (BC) is the most common malignancy in women worldwide and the leading cause of cancer-related deaths among women in most Western countries [1]. Approximately 20–30% of patients with BC develop metastatic disease, sometimes decades after the initial diagnosis, and eventually die from the disease [1]. Neoadjuvant chemotherapy (NAC) is increasingly being proposed for BC patients not only for cancer shrinkage before surgery, but also as a surrogate marker of outcome [2]. Pathological complete response (pCR) and residual cancer burden after NAC are correlated with long-term survival [3]. Nevertheless, many patients still remain at risk of recurrence after NAC, sometimes despite achieving pCR [1]. More reliable biomarkers are needed to better predict NAC failure and the likelihood of BC recurrence. There is increasing evidence that a subset of cancer cells, called cancer stem cells (CSCs), plays crucial roles in the persistence of the disease, with specific capacities for self-renewal, differentiation, and resistance to chemotherapy and radiotherapy [7, 8]. Biomarkers that allow their detection and specific therapies targeting them are still missing [1]. Mammosphere culture allows for the identification of CSCs *in vitro* and in vivo, aiding their enrichment and propagation [10-12]. BC stem cells (BCSCs) expressing biomarkers such as aldehyde dehydrogenase (ALDH), ESA, and CD44+CD24– exhibit higher tumorigenic potential in NOD/SCID mouse xenografts than non-BCSCs [13-16]. Although BCSCs are implicated in therapy resistance, further investigation is warranted to discern the key regulators of their cellular phenotype and their role in mediating resistance. In the last decade, microRNAs (miRNAs) have emerged as the key players in carcinogenesis. miRNAs are small non-coding RNA molecules that suppress gene expression by interacting with the 3'-untranslated region (3'-UTR) of mRNAs. They regulate

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Table 1: Characteristics of the 38 patients with early breast cancer treated with neoadjuvant chemotherapy

Characteristics	n (%)
Age (years)	
mean (range)	47.4 (31-70)
median	48
Menopausal status	
Premenopausal	25 (66)
postmenopausal	13 (34)
ER	
Negative	17 (45)
Positive (>1% stained cells)	21 (55)
PR	
Negative	29 (76)
Positive (>1% stained cells)	9 (24)
HER2 status	
Negative	30 (79)
Positive	8 (21)
Cancer Subtypes	
Luminal (ER+,PR+ and HER2)	19 (50)
HER2+	8 (21)
Triple Negative (ER-, PR-, HER-)	11 (29)
Axillary lymph node involvement	
Negative	27 (71)
Positive	11 (29)
Histologic grade	
1	2 (5)
2	18 (47.5)
3	18 (47.5)
Pathologic complete response (pCR)	
Yes	16
No	22

ER: Estrogen receptor alpha; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2.

various biological processes, including cell fate and stemness [4, 5]. miRNA dysregulation affects BC occurrence and resistance [6]. Notably, miR-205 and Let-7 are key players in the expansion and proliferation of mouse mammary stem or progenitor cells [7, 8]. The identification of specific miRNAs as potential regulators of BCSCs is a promising strategy for early BC diagnosis and treatment. In this study, we aimed to identify miRNAs that are specifically correlated with BCSCs and their involvement in chemoresistance and metastatic process. To achieve this, we used mammospheres to identify miRNAs in a BCSC-enriched population resistant to several chemotherapeutic agents. Thereafter, the role of the identified miRNAs was assessed *in vitro* and in mouse models before evaluation in a cohort of patients treated with NAC.

Materials and Methods

Cell lines

Human BC cell lines (MCF7, MDA-MB-231, BT549, HCC70, and HCC38) were purchased from the ATCC. The normal-like BC cell line MCF10A was cultured in DMEM/F12 supplemented with horse serum, EGF, hydrocortisone, insulin, and cholera toxin. All cell lines were maintained at 37°C in a 5% CO_2 humidified incubator.

Human samples

Frozen tumor samples and sera were collected prospectively from 40 women with BC treated with neoadjuvant chemotherapy (CHUV, Lausanne, protocol 30/10, Table 1). The eligibility criterion was locoregionally advanced BC requiring neoadjuvant chemotherapy. Morphologically normal tissues adjacent to breast cancers (next-to-tumor samples) were obtained from the frozen tissue bank at the Institute of Pathology of Lausanne University Hospital, independently of the main cohort. As a control arm, serum samples were obtained from healthy female volunteers (HFV) matched for age. Our trial adhered to the Declaration of Helsinki, Good Clinical Practice Guidelines by ICH, and Swiss regulations, and received approval from the Ethical Committee of Research on Humans of the Canton of Vaud, Switzerland. All patients and HFV signed an informed consent form.

Plasmid constructions and transfections

To modulate the level of miR363-3p in cells, the BLOCK-iT™ Inducible Pol II miR RNAi Expression Vector Kit with EmGFP (Invitrogen, Thermo Fisher Scientific) was used. Pre-miRNA sequences for miR363-3p and anti-miR363-3p were cloned into destination vectors (Supplementary Table 1). The miR-negative control vector provided by the manufacturer was used as a control. MCF7 cells were seeded in 12-well plates at a density of 150,000 cells per well and transfected with the vector of interest using JetPRIME reagent according to the manufacturer's instructions (Polyplus-transfection SA). After three days, resistant cells were selected by adding blasticidin and G418 to the culture medium. The expression of miRNA was induced by tetracycline addition.

Mammosphere assay

Cells were grown in ultra-low attachment plates (Corning) and MammoCult culture medium (STEMCELL Technologies). To generate chemoresistant mammospheres, cells were maintained for three days before 48h-treatment with 5-fluorouracil (5-FU) or paclitaxel (Pac).

Anchorage-independent soft agar colony formation

Cells were seeded in 6-well plates at a density of 2500 cells per well in DMEM supplemented with agarose and fetal bovine serum with or without tetracycline as an inducer of

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miRNA expression. After 21 days, colonies were stained with crystal violet and counted.

In vivo experiments

Animal experiments were performed in accordance with the protocols approved by the Service de la Consommation et des Affaires Veterinaries of Canton de Vaud. NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice were purchased from Jackson Laboratories. Female NSG mice were handled according to the Swiss guidelines for animal safety, following a protocol approved by the Swiss Institutional Animal Care and Use Committee. MCF7 cells stably transfected with miR-control or anti-miR363-3p were engineered to express luciferase and grown as mammospheres for three days before being resuspended as single cells in DMEM supplemented with 10% FCS. Mice were anesthetized by intraperitoneal injection of xylazine and ketamine (Graeub AG). A total of 50,000 cells per gland were injected intraductally without surgery as previously described [9]. Paracetamol was added to drinking water one day before surgery. After injection, doxycycline was added to drinking water to induce miRNA expression. Tumor growth was monitored for up to 45 days using the Xenogen IVIS Imaging System 200 (Caliper Life Sciences). At the end of the experiment, no tumors had reached the size limit set at 1 cm^3 or were palpable, and no animals showed signs of sickness or dying. Mice were euthanized via intraperitoneal injection of xylazine and ketamine (Graeub AG), and the mammary glands, lungs, livers, and brains were analyzed.

ALDEFLUOR assay and FACS sorting

The ALDEFLUOR™ fluorescent assay (STEMCELL Technologies) enables the detection and sorting of stem and progenitor cells based on their ALDH activity. Cells grown as adherent monolayers were harvested, resuspended, and subjected to ALDH enzymatic assay according to the manufacturer's protocol.

RNA extraction and RT-qPCR

RNA extraction was performed using TRIzol with an increase in isopropanol volume to 800 µL for better small RNA recovery. miRNAs were extracted from blood serum using the miRCURY™ Exosome Isolation kit and miRCURY™ RNA Isolation kit (Qiagen). cDNA synthesis was performed using miRCURY LNA™ Universal RT microRNA PCR (Qiagen). Real-time PCR was performed using the SYBR Green Master Mix on a Light Cycler 480 instrument (Roche). The LNATM PCR primer sets (Qiagen) targeted various miRNAs. U6 and miR-16p served as normalization controls in tissues/cells and serum exosomes, respectively, using the 2^-dCt method.

miRNA microarray

miRNA expression profiles of mammospheres were evaluated using Agilent miRNA microarrays G4870A (2006 probes for human miRNAs). Fluorescence was scanned with an Agilent G2566AA scanner and analyzed using Feature Extraction 10.7.3.1 software. Data were normalized and analyzed to identify differentially expressed miRNAs between MCF7- and MCF10A-derived mammospheres using the limma package, with false discovery rate adjustments using the Benjamini-Hochberg method. This facilitated the identification of a chemoresistance miRNA signature. Raw data were deposited under accession number GPL22934 in the Gene Expression Omnibus according to the MIAME guidelines.

Statistical and bioinformatics analysis

All quantified data represent the mean ± standard deviation of at least three samples. Statistical significance was determined using Student's t-test or by Mann-Whitney and Wilcoxon tests for nonparametric populations ($p \leq 0.05$).

For exomiR signature analysis, the difference between various categories, including before chemotherapy (BCh), after chemotherapy (ACh), and healthy subjects (H), was assessed using Hotelling's T2 test. Identification of unknown categories of samples was performed using the Mahalanobis distance. This method was tested using unseen data and applied using several metrics: accuracy, precision, and recall. Accuracy = (tp + tn)/ (tp + tn + fp + fn), precision = tp/ (tp + fp), and recall = tp / (tp + fn), where tp is true positive, tn is true negative, fp is false positive, and fn is false negative.

The Receiver Operating Characteristic (ROC) curve visually summarizes the classifier's performance across various thresholds. The Area under the Curve (AUC) quantifies the overall performance, with a value closer to 1 indicating better discrimination between positive and negative cases and a value of 0.5 indicating a random classification. To identify the most critical miRNA in the signature, an OPLS-DA analysis was conducted [10]. Survival data analysis was performed using the survival package (v3.4-0; Therneau, 2022) and the Survminer package (v0.4.9; Kassambara, 2021).

Results

Identification of a miRNA chemoresistance signature in BCSC-enriched cell populations

To identify miRNAs involved in BCSC chemoresistance, MCF7 cancer cells were grown in mammospheres using 5-FU or Pac. RNAs extracted from the resistant cells, or untreated cells used as control, were profiled using microRNA microarrays (Figure 1a). Twelve miRNAs were specific to MCF7 mammosphere cells resistant to 5-FU, and five miRNAs were specific to Pac. Among these miRNAs, three were similarly regulated in both 5-FU- and Pac-resistant mammospheres: miR-1290, miR-363-3p, and miR-494 (Supplementary Figure s1 and Figure 1b). To exclude

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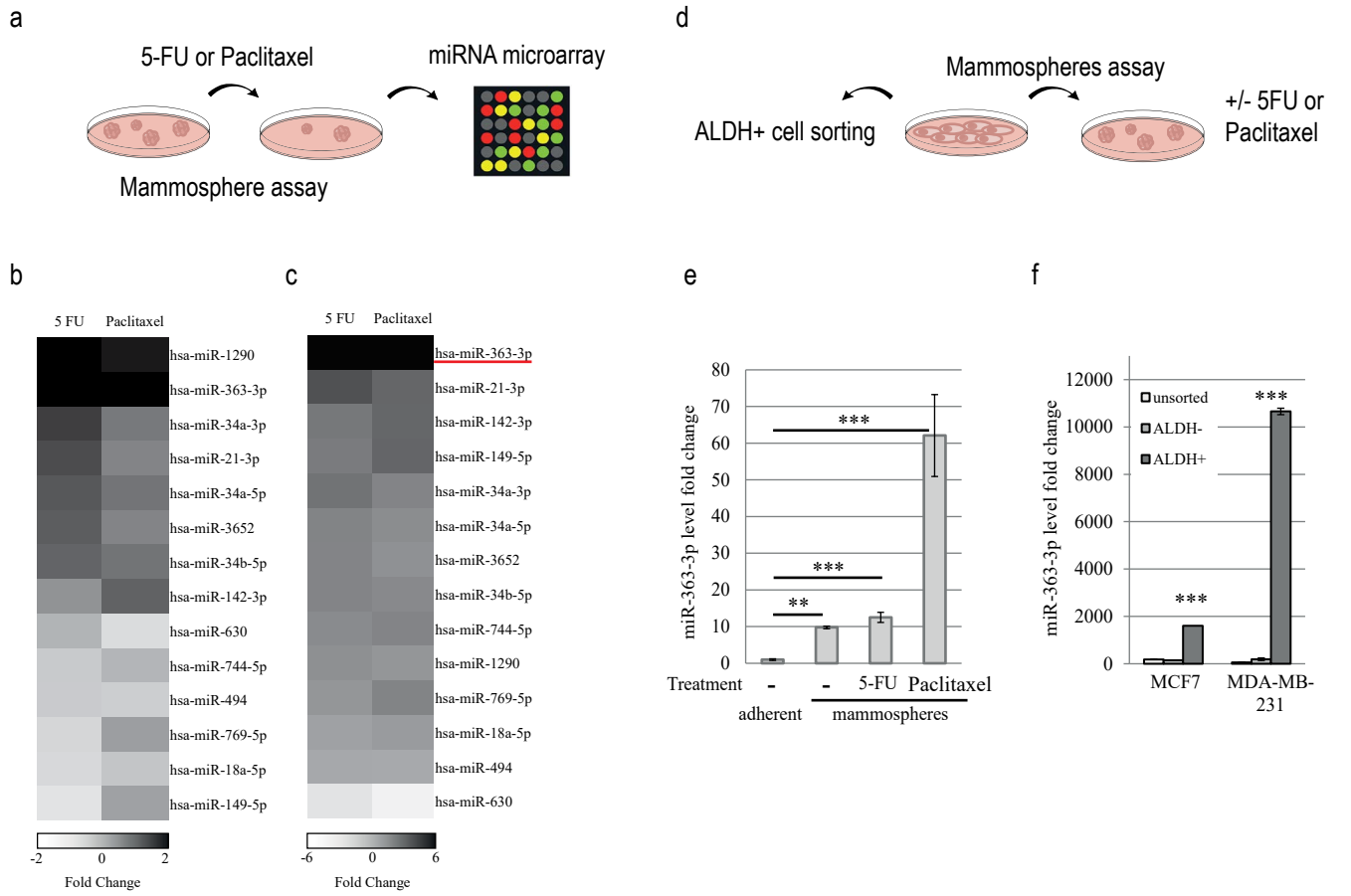
Figure 1: Identification of a miRNA signature of chemoresistance in BCSC-enriched cell population.



(a) Experimental design scheme used for the identification of a miRNA signature after the treatment of BCSC-enriched cells with chemoreagents. **(b)** Fold-change profiling of miRNAs in MCF7 cancer cell-derived mammospheres selected to be resistant to 5-FU or paclitaxel, as compared to unselected MCF7 mammospheres. **(c)** Fold-change profiling of miRNA MCF7 cell mammospheres resistant to 5-FU or paclitaxel, as compared to non-tumorigenic MCF10A mammospheres. **(d)** Experimental design scheme used to identify miRNA-363-3p as a key candidate marker for chemoresistance and stemness of BC cells. **(e)** Relative miRNA-363-3p levels in MCF7 cells from adherent cell cultures, unselected mammospheres and 5-FU- or paclitaxel-resistant mammospheres (n=3; t-test **p≤ 0.01, ***p≤ 0.001). **(f)** Relative miRNA-363-3p levels of MCF7 and MDA-MB-231 total cell populations (unsorted) or sorted by cytofluorometry to be positive or negative for ALDH activity (n=3; t-test, **p≤ 0.01, and ***p≤ 0.001 relative to unsorted populations). All values are represented as fold-changes over the reference population (adherent cells in a and unsorted cells in b), which was assigned a value of 1

miRNAs expressed in normal stem cells, the miRNA profiles of untreated MCF7 mammospheres were compared with those of non-tumorigenic MCF10A cells. Upon 5-FU and Pac treatment, all MCF10A mammosphere cells died, indicating the absence of chemoresistant BCSCs in the non-tumorigenic group. Overall, the levels of five miRNAs were altered in MCF7 mammospheres resistant to both 5-FU and Pac compared to untreated non-tumorigenic MCF10A mammospheres (Figure 1c). miR-494 was downregulated, whereas miR-363-3p, miR-21-3p, miR-142-3p, and miR-149-5p were overexpressed, potentially indicating a BCSC chemoresistance signature. We focused on miR-363-3p because of its notable overexpression in the chemoresistant mammosphere signature across both populations (Figure 1c).

High levels of miR-363-3p linked to chemoresistance and BCSC marker expression

miR-363-3p levels were quantified in BCSC-enriched MCF7 cells using a mammosphere assay or ALDH$^+$ sorting (Figure 1d). miRNA363-3p levels were 10 times higher in CSC-enriched mammospheres than in adherent MCF7 cells (Figure 1e) and 12- and 60-fold higher in mammospheres resistant to 5-FU and Pac, respectively. It was not found in non-tumoral MCF10A cells but was present in all tested BC cell lines (Supplementary Figure s2), indicating its potential as a tumorigenic cell population marker. We further assessed the RT-qPCR miR363-3p levels in ALDH$^+$ and ALDH$^-$ populations FACS-sorted from MCF7 and MDA-MB-231 tumorigenic cells. A 20- and 100-fold increase in miR-363-3p levels was observed in ALDH$^+$ cells relative to ALDH$^-$

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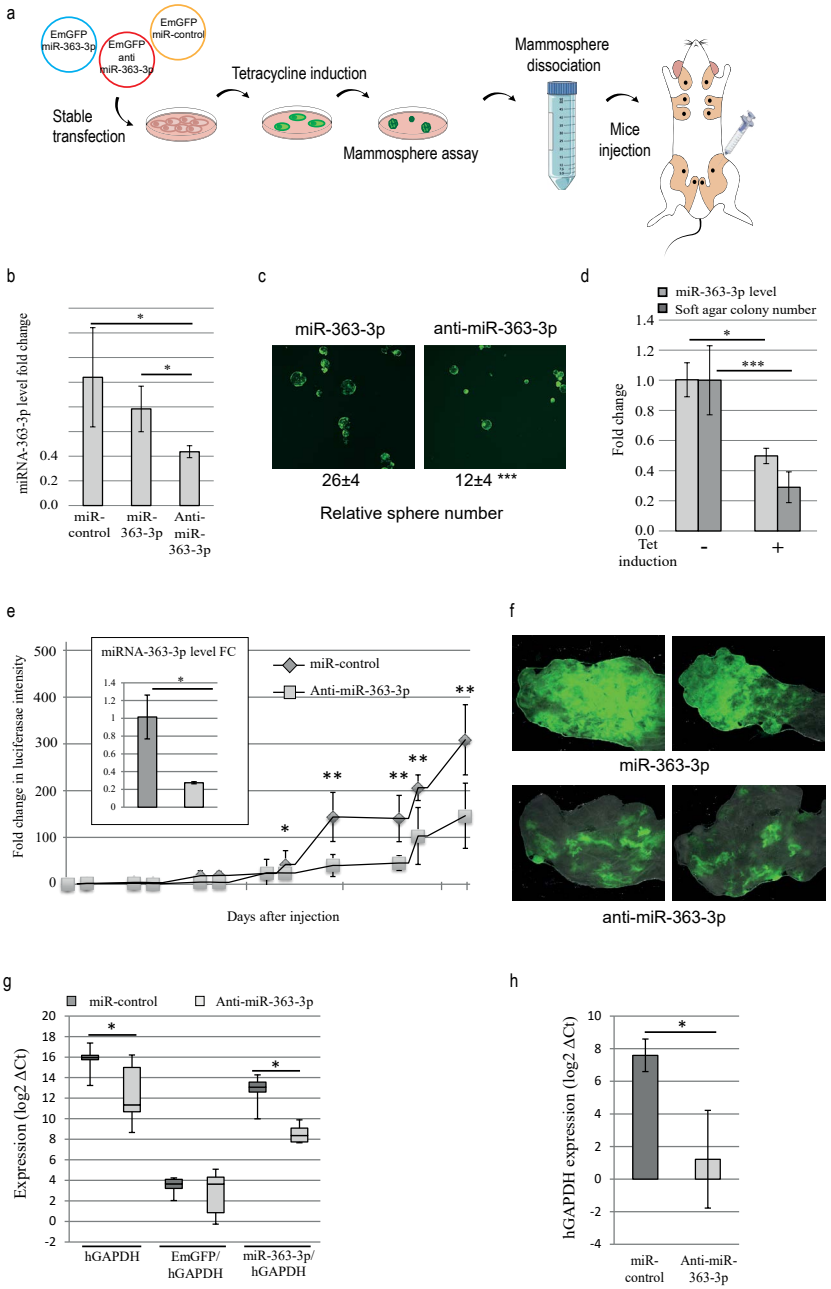
Figure 2: Effect of miRNA-363-3p inhibition in MCF7 cell line ex vivo in culture and in vivo in mice.



(a) Experimental design scheme of MCF7 cell line generation allowing up- or downregulation of miR-363-3p upon tetracycline addition and their use to follow *ex vivo* and *in vivo* effects of miR-363-3p. **(b)** Relative miRNA-363-3p levels in MCF7 cells stably transfected with a tetracycline promoter-driven expression vector for negative control miRNA (miRNA-control), miRNA-363-3p, miRNA-363-3p inhibitor (anti-miRNA-363-3p), and GFP. Cells were cultivated under mammosphere-forming conditions in the presence of tetracycline inducer (n=3; t-test, *p 0.05). **(c)** The effect of miRNA-363-3p upregulation or inhibition on mammosphere size and number was determined using fluorescence microscopy. The average numbers and standard deviation of spheres were determined from miRNA-363-3p and anti-miRNA-363-3 expressing MCF7 cells (n=5; t-test ***p≤ 0.001). **(d)** miRNA-363-3p expression levels and the number of colonies formed in soft agar obtained from MCF7 cells expressing or not expressing anti-miRNA-363-3p (n=3 or 9; t-test, *p≤0.05 and ***p≤0.001). **(e)** Tumor growth in vivo was determined from luciferase signal (nmiRcontrol=4, nmiR-363-3pi=7; t-test, *p≤ 0.05, **p≤ 0.01). The embedded panel displays the fold-change of miRNA-363-3p levels in MCF7 cells stably transfected with miRNA-control or anti-miRNA-363-3p prior to injection (n=3; t-test, *p≤0.05). **(f)** Images of whole mammary glands displaying the fluorescence of injected MCF7 cells from GFP cDNA co-transcribed with the indicated miRNAs. **(g)** RT-qPCR quantification of human GAPDH (hGAPDH) mRNA, EmGFP mRNA, and miR-363-3p in the glands of mice injected with miR-control or anti-miR-363-3p expressing MCF7 cells (n=5 or 4 for mice injected with miR-control or anti-miR363-3p, respectively. Mann-Whitney test, *p≤0.05). **(h)** RT-qPCR assay of hGAPDH mRNA in the lungs of injected mice, representing the invasion of human BC cells into the peripheral organs (n=3 or 6 for mice injected with miR-control or anti-miR363-3p-expressing cells, respectively. Mann-Whitney test, *p≤0.05)

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cells, or when related to the whole MCF7 and MDA-MB-231 populations, respectively (Figure 1f). Additionally, miR363-3p levels were 5-fold higher in ALDH$^+$ cells derived from the triple-negative MDA-MB-231 cell line, a phenotype often associated with more aggressive tumors, than in ALDH$^+$ MCF7 cells (Figure 1f).

Effect of miRNA-363-3p on MCF7 growth *in vitro* in culture and *in vivo* in mice

To assess whether overexpression of miR363-3p could influence the BCSC phenotype, we manipulated its expression levels. We developed MCF7 cell lines with chromosome-integrated vectors carrying co-cistronic sequences for a target miRNA and the EmGFP gene controlled by a tetracycline-inducible promoter. Three constructs were introduced into MCF7 cells: a control miRNA with no biological effect, one for ectopic overexpression of miR-363-3p, and a third containing anti-miR-363-3p to inhibit its function. The impact of miR-363-3p levels on cell growth was evaluated both *in vitro* in mammospheres and *in vivo* following dissociation and intraductal injection into mice (Fig 2a). Under mammosphere-forming conditions, cells transfected with either miR-control or miR-363-3p expression vectors showed no significant difference in miR-363-3p levels (Figure 2b). Time-course analysis post-tetracycline induction revealed a transient rise in miR-363-3p levels, followed by fluctuating returns to baseline, indicating a homeostatic response to ectopic miRNA expression (Supplementary Figure s3). Transfection with the anti-miR-363-3p vector resulted in a consistent 2-fold reduction in miR-363-3p levels, along with a similar decrease in both mammosphere size and number (Figure 2c and Supplementary Figure s4). This implies that high miR-363-3p expression is necessary to sustain the non-adherent growth characteristics of BCSCs. The tumorigenic role of miR-363-3p was assessed using a colony soft agar assay, in which anti-miR363-3p induction led to a 3-fold decrease in the colony number and halved miR-363-3p levels (Figure 2d). High miR-363-3p expression was necessary for efficient mammosphere generation and colony formation, indicating its importance in BCSC propagation and the tumorigenicity of the MCF7 cell line. To evaluate the effect of miR363-3p down-regulation on BC growth *in vivo,* an intraductal human-in-mouse transplantation model was used to mimic the natural microenvironment for BC cell growth and metastasis [9, 11].

MCF7-anti-miR-363-3p and MCF7-miR-c cell lines were transduced with an expression vector for the luciferase reporter to follow tumor growth *in vivo* and grown as mammospheres in the presence of tetracycline to induce expression of the miRNA cassettes. After three days, mammospheres were dissociated and 50,000 cells were injected into mice. RT-qPCR confirmed a 4-fold reduction in miR-363-3p expression in MCF7 cells treated with anti-miR-363-3p compared to the control at the time of injection (Figure 2e, embedded graph). Mice were given doxycycline in their drinking water to sustain the expression of anti-miR363-3p and control miR. Tumor growth was tracked using *in vivo* luciferase imaging (Figure 2e). Cells expressing lower levels of miR-363-3p showed a significant delay in tumor growth, highlighting its involvement in tumor development *in vivo*. After six weeks, mice were euthanized. GFP fluorescence imaging revealed increased colonization in the mammary ducts of glands injected with MCF7-miR-c-expressing cells compared with those injected with anti-miR-363-3p (Figure 2f). Consistently, human GAPDH levels assessed by RT-qPCR were significantly lower in glands injected with MCF7-anti-miR-363-3p than in glands injected with MCF7-miR-c-expressing cells (Figure 2g). No significant differences in normalized EmGFP expression levels indicated maintenance of the miRNA expression cassette *in vivo*. A significant decrease in miR363-3p relative to human GAPDH observed in glands injected with anti-miR-363-3p cells indicated that suppressing miR363-3p reduced MCF7 cell growth in the milk ducts. Human GAPDH was found in the lungs of both mouse groups but not in the brain or liver. All lung fractions from mice injected with MCF7-miRc cells showed human GAPDH expression, in contrast to one lung fraction from mice injected with MCF7-anti-miR363-3p cells. Altogether, the statistically significant difference between the two groups (Figure 2h) highlighted that the depletion of miR-363-3p expression impaired BCSC establishment, growth, and invasion *in vivo*.

Human BC miRNA-363-3p levels in tissues and sera-exosomes are linked to cancer

miR363-3p levels were measured in biopsies from 38 patients treated with NAC (Table 1). BC and serum biopsies were obtained from the same patients and analyzed before (BCh) and after (ACh) chemotherapy. The median follow-up time after surgery was 9.5 years (Figure 3a). miR-363-3p was analyzed in BCh and ACh biopsies and next to tumor tissues (NxT), which served as controls. BCh biopsies exhibited

Table 2: Score of each variable of the miR signature after OPLS-DA analysis.

	miR363-3p	miR145-5p	miR23-3p	miR-149-5p	miR21-3p	miR142-3p	miR-494-3p
Group 1	1.70	1.31	1.27	0.76	0.316	0.0129	0.279
Group 2	1.471	1.463	1.299	0.781	0.397	0.353	0.328

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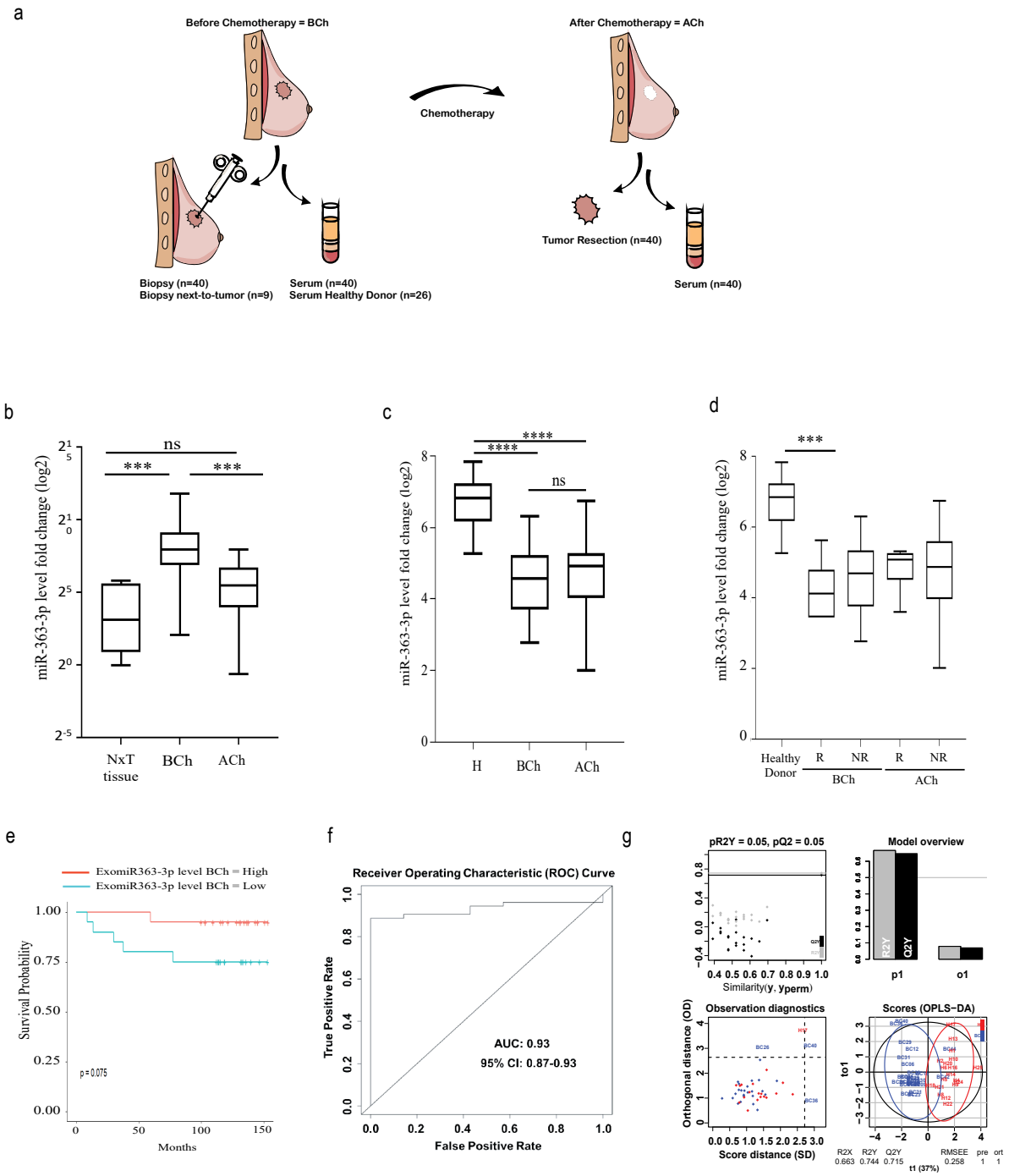
Figure 3: Expression of miRNA-363-3p in human tumor biopsies and blood sera of patients enrolled in a neoadjuvant chemotherapy protocol.



(a) Experimental design scheme summarizing the clinical trial. **(b)** Box plot summary of the distribution of miRNA-363-3p expression levels in breast tumor tissues of 38 patients before or after chemotherapy (BCh and ACh, respectively), as indicated, and in nine next-to-tumor control tissues (NxT tissue) from untreated patients (t-test, ***p≤0.001). **(c)** Box plot summary of the distribution of miRNA-363-3p levels in the sera of the same group of BC patients before or after chemotherapy (BCh and ACh, respectively) and a group of 26 healthy donors (H) (t-test, ****p≤0.0001). **(d)** Box plot summary of the distribution of miRNA-363-3p levels in the sera of the same group of BC patients before or after chemotherapy after separation according to outcome status relapse (R) or no relapse (NR) and a group of 26 healthy donors (t-test, ***p≤0.001). **(e)** Survival analysis relative to exomiR363-3p level before chemotherapy. **(f)** Receiver Operating Characteristic (ROC) curve of H and BC patient data analysis using the 7-exomiRNA signature. The Area under the Curve (AUC) value was 0.93, suggesting good discrimination between H and BC. **(g)** The OPLS-DA model of miRNA using the 7-exomiRNA signature. Top left: significance diagnostic; random permutation on the categories was made on the dataset, and R2Y and Q2Y were calculated; these values were then compared to the original categories. Top right: inertia bar plot; this graphic suggests that one principal component is enough to capture the inertia of the system. Bottom left: outlier diagnostics. Bottom right: x-score plot; the plot reports the various metrics, predictive components (1), and orthogonal components (1)

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significantly higher miR-363-3p levels than the ACh and NxT samples (Figure 3b). The same analysis of exosomes from BCh and ACh sera, compared to 26 healthy donors (HFV), showed no significant difference in exomiR-363-3p levels between BCh and ACh patients after normalization. However, exomiR363-3p levels were significantly lower in patients with BC than in healthy donors (Figure 3c). Further analysis did not reveal any significant differences in exomiR-363-3p levels based on patient outcomes (Figure 3d). Survival analyses indicated a potential correlation between higher exomiR363-3p levels and improved survival. This trend was not statistically significant (p=0.075) in this small population (Figure 3e). We then examined the levels of the five miRNAs identified in our tissue signature and added miR145-5p and miR23-3p, as they were recently identified as key players in BC and mesenchymal stem cell-derived exosomes [12, 13], providing a signature of seven exosomal miRNAs (exomiRNAs). Using Hoteling's T2 test, we evaluated this signature in the HFV, BCh, and ACh samples. When testing various combinations of groups, HFV versus BCh, HFV versus ACh, and BCh versus Ach, we observed p-values of 1.6e-14, 1.2e-11, and 0.0034, respectively, indicating that this signature can discriminate between the three groups. To test whether the signature could classify unknown serum samples, we divided the data into two random groups for separate training. The first group consisted of 13 BCh, seven HFV, and 40 ACh samples, whereas the second group had 27 BCh and 19 HFV. In the first group, no statistical difference was found between BCh and ACh (p=0.54), leading us to combine these categories into a BC group for comparison against the HFV group using Hotelling's T-squared test. A highly significant difference was found between BC and HFV (p-values of 8e-13 and 2.2e-12 for Groups 1 and 2, respectively). To test if the 7-exomiRNA signature also discriminate BC from HFV, we employed the Mahalanobis distance of unseen data to "BC" set and "HFV" set. Testing our method using data from Group 2 following training made with Group 1 and *vice versa*, we achieved 85–94% accuracy, 100% precision, and 83–85% recall in categorizing unseen data. ROC curve analysis showed an AUC of 0.93, effectively distinguishing between HFV and BC with a 7-exomiRNA signature (Figure 3f). Our findings showed that the 7-exomiRNA signature effectively predicts BC from patient serum exosomes. OPLS-DA analysis identified key markers differentiating healthy (HFV) subjects from BC subjects (Figure 3g and Table 2), identifying miR-363-3p as the primary discriminator, along with significant roles of miR-145-5p and miR-23-3p.

Discussion

CSCs play a crucial role in cancer resistance to treatment and recurrence, prompting extensive research on their detection and targeting. miRNAs can regulate genes linked to chemoresistance and metastasis [14-16]. However, no miRNAs have been identified that are specific to BCSC chemoresistance or as diagnostic markers for BC. In this study, we performed miRNA microarray profiling of chemoresistant cell populations from BC cell mammospheres, leading to the identification of a 5-miRNA signature that may be linked to chemoresistant BCSCs. The signature consisted of overexpression of miR-363-3p, miR-21-3p, miR142-5p and miR-149-5p, and downregulation of miR494. Several of these miRNAs have already been linked to pathways involved in cancer. miR-21 was associated with poorer outcomes in many cancers [17-19], while miR-149-5p is emerging as a cancer marker [20]. In glioblastoma, miR494-3p suppression induces cell death [21]. miR142-3p suppresses BC by targeting HMGA2 [22]. This approach helped to identify miRNAs that are important for cancer progression and treatment resistance. Our research focused on miR-363-3p as a potential BCSC fate regulator, showing its role in BCSC chemoresistance *in vitro* and its impact on tumor growth and metastasis *in vivo*. Importantly, miR-363-3p levels were higher in BC biopsies before NAC than in adjacent tissues and tended to decrease after chemotherapy. The patient sample size and low level of events do not allow formal demonstration of the ability of miR363-3p to discriminate or predict treatment response in the clinic. However, a recent study demonstrated a correlation between high miRNA-363-3p expression and chemoresistance in diffuse large B-cell lymphoma [23], supporting our finding that miR363-3p might be a key biomarker of chemoresistance in BC. Interestingly, miR-363-3p has also been proposed to have an anti-proliferative action, inducing apoptosis in retinoblastoma cells *via* the Akt/mTOR signaling pathway [24] or by downregulating S1PR1 in clear cell renal cell carcinoma [25]. This may indicate distinct roles for this miRNA in various cancers and conditions. BC, CSCs, and metastasis were key in the ontology study of miR-363-3p's potential targets like KAT2B, TGIF1, TOB1, and TBLR1 [26]. Krüppel-like factor 4 (KLF4), known for its dual oncogenic and tumor suppressor roles in breast [27, 28] and other cancers [27, 28], is also a potential target of miR-363-3p. This suggests that miR-363-3p can function as either a tumor suppressor or an oncogene, depending on the context.

Recent research highlights the crucial role of exosome-derived miRNAs (exomiRs) in cancer diagnosis and prognosis. [29, 30]. Our study found significantly lower levels of miRNA-363-3p in the exosomes of BC patient sera than those of healthy donors. Our 7-exomiR signature effectively distinguished BC patients from healthy donors, predicting disease status with 85–94% accuracy. Notably, miRNA-363-3p was the most significant contributor in distinguishing BC patients from healthy donors within this signature. The discovery that miR363-3p overexpression supports BCSC maintenance, cancer growth, and metastasis is in contrast to its low levels in exosomes from BC patient sera. This suggests that miR363-3p might remain concentrated in cancer tissues

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and not disperse into the stroma or circulation in BC. The mechanisms underlying miRNA secretion into exosomes and their retention inside tumor cells remain largely unknown. However, miRNA expression levels differ between tissues and the blood circulation [31]. Matamala *et al.* [32] found five miRNAs that were differentially expressed in the plasma of BC patients compared to healthy donors, some of which were regulated in opposite directions to their expression in tumor tissues. The discrepancy in the distinct miRNA expression patterns of exosomes and tissues observed in our study could stem from impaired miRNA transport. Changes in tumor cell expression may signal local tissue alterations, whereas exosome miRNA levels may indicate the systemic status of the disease. Considering our promising results, these biomarkers hold potential for BC screening, patient monitoring, prognosis assessment, and treatment target. Further validation in a larger independent cohort is required to better define the clinical role of these miRNAs. Furthermore, these results suggest new avenues for targeting miRNAs such as miR-363-3p to combat cancer stem cells, a source of resistance to conventional treatments.

List of abbreviations

BCh and ACh: patients before or after chemotherapy

ALDH: aldehyde dehydrogenase

BCSC: Breast cancer stem cells

CSC: Cancer stem cells

ExomiR: Exosomes microRNA

HFV: Healthy female volunteers

miRNA: microRNA

NxT: next-to-tumors tissues

Pac: paclitaxel

5-FU: 5-fluorouracil

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Statements and Declarations

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Competing interests

The authors declare that they have no competing interests.

Author contributions

STR designed the study, generated and interpreted the data, and was the major contributor to writing the manuscript. JM actively contributed to the data generation. AS analysed and interpreted the patient data regarding breast cancer disease status and contributed to writing the manuscript. FB performed all statistical analyses of the miRNA signatures. VS, VD, and CB performed all in vivo mouse experiments. JYM contributed to tumor collection. LR wrote all legal and ethical documents related to the study. MF and MG performed histological examinations of the tumor biopsies and made all pathological reports. KZ and NM were the principal investigators and financing contributors to the project; they actively participated in drafting the project and proofreading the manuscript.

Study conception and design: Initiated by STR, with contributions from NM, AS, KZ, and LR.

Material preparation and data collection: STR, AS, KZ, JM, VS, VD, CB, JYM, LR, MF, and MG.

Analysis: STR, NM, AS, KZ, and FB.

The draft of the manuscript was written by STR, with contributions from NM, AS, and KZ.

All authors commented on the previous versions of the manuscript and read and approved its final version.

Availability of data and materials

MiRNA microarrays raw data were deposited at Gene Expression Omnibus, accession number GPL22934.

Ethics approval

The trial was conducted in accordance with the Declaration of Helsinki, Guidelines for Good Clinical Practice issued by ICH, and the Swiss regulatory authorities' requirements. This study was approved by the Ethics Committee of Research on Humans of the Canton of Vaud, Switzerland. Animal experiments were performed in accordance with the protocols approved by the Service de la Consommation et des Affaires Vétérinaires of Canton de Vaud.

Consent to participate

Written informed consent was obtained from all patients and healthy donors.

Supplementary File Link:

https://cdn.fortunejournals.com/supply/JCSCT12115.docx

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 71 (2021): 209-249.
2. Murphy BL, Day CN, Hoskin TL, et al. Neoadjuvant

Tissot-Renaud S, et al., J Cancer Sci Clin Ther 2025
DOI:10.26502/jcsct.5079264

Chemotherapy Use in Breast Cancer is Greatest in Excellent Responders: Triple-Negative and HER2+ Subtypes. Ann Surg Oncol 25 (2018): 2241-2248.

3. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 384 (2014): 164-172.
4. Bagga S, Bracht J, Hunter S, et al. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 122 (2005): 553-563.
5. Olsen PH, Ambros V. The lin-4 regulatory RNA controls developmental timing in Caenorhabditis elegans by blocking LIN-14 protein synthesis after the initiation of translation. Dev Biol 216 (1999): 671-680.
6. Mulrane L, McGee SF, Gallagher WM, et al. miRNA dysregulation in breast cancer. Cancer Res 73 (2013): 6554-6562.
7. De Cola A, Volpe S, Budani MC, et al. miR-205-5p-mediated downregulation of ErbB/HER receptors in breast cancer stem cells results in targeted therapy resistance. Cell Death Dis 6 (2015): e1823.
8. Yu F, Yao H, Zhu P, et al. let-7 regulates self-renewal and tumorigenicity of breast cancer cells. Cell 131 (2007): 1109-1123.
9. Sflomos G, Dormoy V, Metsalu T, et al. A Preclinical Model for ERalpha-Positive Breast Cancer Points to the Epithelial Microenvironment as Determinant of Luminal Phenotype and Hormone Response. Cancer Cell 29 (2016): 407-422.
10. Thevenot EA, Roux A, Xu Y, et al. Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. J Proteome Res 14 (2015): 3322-3335.
11. Behbod F, Kittrell FS, LaMarca H, et al. An intraductal human-in-mouse transplantation model mimics the subtypes of ductal carcinoma in situ. Breast Cancer Res 11 (2009): R66.
12. Ferguson SW, Wang J, Lee CJ, et al. The microRNA regulatory landscape of MSC-derived exosomes: a systems view. Sci Rep 8 (2018): 1419.
13. Gonzalez-Villasana V, Rashed MH, Gonzalez-Cantu Y, et al. Presence of Circulating miR-145, miR-155, and miR-382 in Exosomes Isolated from Serum of Breast Cancer Patients and Healthy Donors. Dis Markers 2019 (2019): 6852917.
14. Raza U, Zhang JD, Sahin O. MicroRNAs: master regulators of drug resistance, stemness, and metastasis. J Mol Med (Berl) 92 (2014): 321-336.
15. Sun X, Jiao X, Pestell TG, et al. MicroRNAs and cancer stem cells: the sword and the shield. Oncogene 33 (2014): 4967-4977.
16. Wang J, Yang M, Li Y, et al. The Role of MicroRNAs in the Chemoresistance of Breast Cancer. Drug Dev Res 76 (2015): 368-374.
17. Pfeffer SR, Yang CH, Pfeffer LM. The Role of miR-21 in Cancer. Drug Dev Res 76 (2015): 270-277.
18. Yan LX, Huang XF, Shao Q, et al. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 14 (2008): 2348-2360.
19. Yan LX, Wu QN, Zhang Y, et al. Knockdown of miR-21 in human breast cancer cell lines inhibits proliferation, *in vitro* migration and in vivo tumor growth. Breast Cancer Res 13 (2011): R2.
20. Ren FJ, Yao Y, Cai XY, et al. MiR-149-5p: An Important miRNA Regulated by Competing Endogenous RNAs in Diverse Human Cancers. Front Oncol 11 (2021): 743077.
21. Li XT, Wang HZ, Wu ZW, et al. miR-494-3p Regulates Cellular Proliferation, Invasion, Migration, and Apoptosis by PTEN/AKT Signaling in Human Glioblastoma Cells. Cell Mol Neurobiol 35 (2015): 679-687.
22. Mansoori B, Duijf PHG, Mohammadi A, et al. MiR-142-3p targets HMGA2 and suppresses breast cancer malignancy. Life Sci 276 (2021): 119431.
23. Zhou W, Xu Y, Zhang J, et al. MiRNA-363-3p/DUSP10/JNK axis mediates chemoresistance by enhancing DNA damage repair in diffuse large B-cell lymphoma. Leukemia 36 (2022): 1861-1869.
24. Ma X, Jin L, Lei X, et al. MicroRNA-363-3p inhibits cell proliferation and induces apoptosis in retinoblastoma cells via the Akt/mTOR signaling pathway by targeting PIK3CA. Oncol Rep 43 (2020): 1365-1374.
25. Xie Y, Chen L, Gao Y, et al. miR-363 suppresses the proliferation, migration and invasion of clear cell renal cell carcinoma by downregulating S1PR1. Cancer Cell Int 20 (2020): 227.
26. Li Q, Liu Z, Xu M, et al. PCAF inhibits hepatocellular carcinoma metastasis by inhibition of epithelial-mesenchymal transition by targeting Gli-1. Cancer Lett 375 (2016): 190-198.
27. Wang B, Zhao MZ, Cui NP, et al. Kruppel-like factor 4 induces apoptosis and inhibits tumorigenic progression in

Citation: Stéphanie Tissot-Renaud, Athina Stravodimou, Fabrizio Benedetti, Valentina Scabia, Valérian Dormoy, Jacqueline Masternak, Cathrin Brisken, Jean-Yves Meuwly, Laetitia Rossier, Maryse Fiche, Marie Galmiche, Khalil Zaman, and Nicolas Mermod. miR-363-3p Mediates Maintenance and Tumorigenicity of Breast Cancer Stem Cells and Provides A Potential Diagnostic and Prognostic Sera-Exosome Biomarker of Early Breast Cancer. Journal of Cancer Science and Clinical Therapeutics. 9 (2025): 57-68.

Tissot-Renaud S, et al., J Cancer Sci Clin Ther 2025
DOI:10.26502/jcsct.5079264

SK-BR-3 breast cancer cells. FEBS Open Bio 5 (2015): 147-154.

28. Yu F, Li J, Chen H, et al. Kruppel-like factor 4 (KLF4) is required for maintenance of breast cancer stem cells and for cell migration and invasion. Oncogene 30 (2011): 2161-2172.

29. Padda J, Khalid K, Khedr A, et al. Exosome-Derived microRNA: Efficacy in Cancer. Cureus 13 (2021): e17441.

30. Qian X, Xie F, Wei H, et al. Identification of Key Circulating Exosomal microRNAs in Gastric Cancer. Front Oncol 11 (2021): 693360.

31. Pu Q, Huang Y, Lu Y, et al. Tissue-specific and plasma microRNA profiles could be promising biomarkers of histological classification and TNM stage in non-small cell lung cancer. Thorac Cancer 7 (2016): 348-354.

32. Matamala N, Vargas MT, Gonzalez-Campora R, et al. Tumor microRNA expression profiling identifies circulating microRNAs for early breast cancer detection. Clin Chem 61 (2015): 1098-1106.

Citation: Stéphanie Tissot-Renaud, Athina Stravodimou, Fabrizio Benedetti, Valentina Scabia, Valérian Dormoy, Jacqueline Masternak, Cathrin Brisken, Jean-Yves Meuwly, Laetitia Rossier, Maryse Fiche, Marie Galmiche, Khalil Zaman, and Nicolas Mermod. miR-363-3p Mediates Maintenance and Tumorigenicity of Breast Cancer Stem Cells and Provides A Potential Diagnostic and Prognostic Sera-Exosome Biomarker of Early Breast Cancer. Journal of Cancer Science and Clinical Therapeutics. 9 (2025): 57-68.