Preclinical Imaging Evaluation of MiRNAs Delivery and Effects in Breast Cancer Mouse Models: A Systematic Review

Francesca Maria Orlandella  
IRCCS SDN

Luigi Auletta  
Institute of Biostructure and Bioimaging National Research Council: Istituto di Biostrutture e Bioimmagini Consiglio Nazionale delle Ricerche

Adelaide Greco (Adegreco@unina.it)  
University of Naples Federico II: Universita degli Studi di Napoli Federico II

Antonella Zannetti  
Institute of Biostructure and Bioimaging National Research Council: Istituto di Biostrutture e Bioimmagini Consiglio Nazionale delle Ricerche

Giuliana Salvatore  
Universita degli Studi di Napoli Parthenope: Universita degli Studi di Napoli Parthenope

Research

Keywords: breast cancer, miRNAs, mice models, preclinical imaging

Posted Date: September 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-895673/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

**Background:** miRNAs have been defined as a tumor suppressor or oncogene (oncomiR) in several human cancers. We have conducted a systematic review highlighting and specifically focusing in the advancements in preclinical molecular imaging to study *in vivo* the delivery and the therapeutic efficacy of miRNAs in mouse models of breast cancer.

**Methods:** A systematic review of English articles published in peer-reviewed journals using PubMed® (including MEDLINE®), EMBASE, BIOSIS™, Scopus was performed. Search terms included breast cancer, mouse, mice, microRNA(s) and miRNA(s). The search was focused on the last five years (2015-2021). All studies using miRNA in breast cancer models which included a preclinical imaging evaluation, both *in vivo* or *ex vivo* were analyzed.

**Result:** From a total of 2,073 records, 1,221 papers were assessed for full text eligibility, but excluding all those in which there was no use of mouse models of breast cancer, there was not *in vivo* imaging or *ex vivo* on whole organs, and without a clear link to a miRNA, our final data extraction was made on a total of 114 manuscripts. The murine genetic background most used in miRNA studies have been resulted to be the Balb/C (46,7%). Regarding cell lines, MDA-MB-231 parental and derived cells were used in most experiments (62,5%). The most used model was the *i.v.* metastatic model (46,8%), which was obtained via intravenous injection (68,9%) in the tail vein. The modulation of miRNA was obtained mainly by stable transfection with specific lentiviral plasmid or DNA constructs in luciferase-labelled BC cells (54,4%). Bioluminescence resulted the most used tool (64%) and was used as a surrogate of tumor growth for efficacy treatment or for the evaluation of tumorigenicity in miRNA transfected cells (29,9%); for tracking, evaluation of engraftment and for response to therapy in metastatic models (50,6%).

**Conclusion:** this review provides a systematic and focused analysis of all the information currently available and related to the imaging protocols to test miRNA therapy in *in vivo* mice model of BC and has the purpose to provide an important tool to suggest the best pre-clinical imaging protocol on currently available evidences.

**Background**

As recently estimated, breast cancer (BC) alone accounts for ~ 30% of all new diagnoses in women(1). Although the improvements in BC's early diagnostic strategies and therapy have increased survival rates, this malignant tumor remains one of the most frequent causes of cancer-related mortality among females worldwide(1). To date, it is well known that BC is a complex and heterogeneous disease that could be classified into several subtypes based on histological and genetic characteristics. Through the combinations of molecular markers expression in the cancer cells, such as Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2), it is possible to define the principal intrinsic BC subtypes: Luminal A, Luminal B, HER2-enriched, Basal-like/Triple Negative
and Normal-like, which are characterized by different pathophysiology, prognosis and sensitivity to treatments (2, 3).

Recent studies reported that these different BC molecular subtypes are also associated with alterations in microRNAs’ expression and function (4–6). Micro-RNAs (miRNAs) are small non-coding molecules (18–22 nucleotides) that act on gene expression at the post-transcriptional level contributing to the regulation of several biological functions. Indeed, through targeting the sequences in the 3’ Untranslated Region (UTR) of specific target mRNAs, miRNAs can induce the inhibition of translation or the degradation of their targets (7). Consequently, based on target mRNAs’ activity, the miRNAs have been defined as a tumor suppressor or oncogene (oncomiR) (8). Several studies have highlighted the prognostic and therapeutic roles of specific miRNAs in BC cells, and have also suggested their important role in the modulation of drug response or resistance (9).

In BC, miRNAs’ dysregulation has been demonstrated to promote malignant hallmarks such as proliferation, genome instability, cell invasion, drug resistance and metastasis. Thus, the restoration of these molecules’ expression using miRNA mimic or inhibitory sequences could become an essential point for the future development of novel therapeutic tools (10).

However, major drawbacks in using miRNAs as a therapy are the presence of nucleases in body fluids, which prevents the existence of any intact RNA free in the extracellular space, their rapid blood clearance, immunotoxicity and low tissue diffusion (11, 12). Indeed, it has been proven that miRNAs exist both intracellularly, and when they are secreted extracellularly, they are included in small vesicles called exosomes (11). Thus, it is clear that miRNAs cannot be directly injected into the organism to be treated and, hence, the need for miRNAs’ delivering systems development. Some of the most used systems for delivering miRNAs into target cells are inorganic nano-materials (such as nanoparticles, NPs), lipid-based delivery systems or viral vectors (12, 13). The availability of these novel local and systemic delivery systems has allowed exploiting the miRNAs in clinical trials by restoring the expression of tumor suppressor miRNAs or by inhibiting the activity of oncomiR (9).

Among the miRNA based therapeutic strategies being tested in ongoing phase 1 trials there are mimic specific for the tumor suppressor miR-16 (MesomiR-1) for the treatment of mesothelioma (14) and the anti miR-155 (MRG-106 Cobomarsen), currently in phase 1 and 2 clinical trials for the treatment of lymphoma and leukemia (15). While the first clinical trial with MRX34, a mimic of miR-34 encapsulated in a liposomal NPs, was discontinued due to severe adverse events. Thus, still to date, major obstacles into fully translating miRNAs in clinic are effective delivery and off-target effects.

Parallel to the therapeutic miRNA development procedure, preclinical imaging advances, using mouse model, for evaluating miRNAs deliveries have happened in recent years.

Mouse models still represent an essential step in translating results from cell biology into the target species. The use of different preclinical molecular imaging techniques, in particular optical imaging, has significantly contributed in investigating the crucial role of miRNAs in BC progression and in evaluating
miRNAs delivery to tumors and their therapeutic effects. Molecular imaging (MI) allows studying non-invasively in vivo, in real-time and over-time, in a quantitative way, at sub-cellular and molecular levels the main altered cancer pathways(16). Using MI, it is possible to continuously obtain numerous information by the same animal, i.e., each animal acting as its own control, thus reducing the biological variability, the number of animals required and the costs for a particular study. The multi-modality imaging approach provides anatomical and physiological complementary data that can improve the development of new anticancer drugs and more easily translate preclinical evidence into clinics(17). Many studies have been performed using bioluminescence or fluorescence imaging integrated, in some cases, with morphological CT or MRI to assess the function and effects of specific miRNAs.

The most innovative strategy in this field is the use of the theranostic NPs that by integrating targeting, imaging, and therapeutic abilities into one single nano-formulation allow to monitor drug accumulation in a real-time manner, allow precise disease diagnosis and allow to evaluate treatment efficiency(18). These multifunctional nano-theranostic platforms permit the visualization of miRNAs specific targeting to the tumor and evaluation of their effects on tumor growth and metastases formation.

Within this frame, despite the growing interest and the promising finding related to the potential of miRNAs in the public health, still to date, in literature to our knowledge, there isn't an updated overview concerning recent advances of miRNAs as diagnostic and therapeutic agents using molecular imaging in preclinical mouse model of BC that could help the researchers to have a more detailed and comprehensive knowledge regarding this aspect.

Thus, in this systematic review, we wanted to explore the advancements, by updating previous reviews on the subject, and by applying preclinical molecular imaging to study miRNAs in mouse models of BC. Due to the versatility of MI, we looked for all possible imaging techniques' applications, i.e., tracking the delivery and studying the efficacy of miRNAs as potential anticancer agents.

**Methods**

**Literature search strategy**

This systematic review was prepared according to both PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) and SYRCLE (Systematic Review Protocol for Animal Intervention Studies) guidelines and checklists (19, 20).

Studies were searched on PubMed® (including MEDLINE®), EMBASE, BIOSIS™ and Scopus, using the following keywords: "Breast cancer" (and) "microRNA" (and) "mouse".

A total of eight search strings were applied in each database; indeed, searches were repeated using both singular and plural and using both "miRNA" and "microRNA", “mouse” and “mice”. A PRISMA flow diagram (21) is reported in Figure 1.
All the studies published in the last five years (2015-2021), which reported preclinical molecular and diagnostic imaging results \textit{in vivo} or \textit{ex vivo} in murine models of BC, were included in this systematic review. Reference lists from relevant reviews identified in the databases search were manually searched to identify other eventual studies. Searches were concluded on May 1\textsuperscript{st}, 2021.

**Study selection and eligibility criteria**

An electronic spreadsheet was prepared to report, for each study, the title, the authors' list, the date of publication, and the language; moreover, whenever indicated, it was reported if the study was a review, an extenso research paper, an abstract or a letter/editorial.

All non-English language papers were excluded for the authors' and readers' convenience. After removing all duplicates, congress abstracts and posters, letters to Editors or editorials, each author was asked to screen the studies based on title and abstract. Exclusion criteria in the screening phase were (i) article's titles referring not to cancer, (ii) article's titles identifying cancers other than BC, (iii) article's abstract not including miRNAs.

Eligibility assessment was performed by all authors independently by screening the full texts. Inclusion criteria were (i) the use of \textit{in vivo} or \textit{ex vivo} molecular preclinical or diagnostic imaging, (ii) mouse models of BC, both xenografts, orthotopic and metastatic, (iii) miRNAs involvement in the molecular processes studied apparent. Both murine and human BC cell lines were included. Studies were considered not eligible if (i) \textit{ex vivo} imaging was not performed on whole organs, but on histological samples, e.g., immunohistochemistry, as well as fluorescent confocal microscopy on tissues, (ii) models, were produced in species other than mice (Mus musculus) or they were other than BC (iii) the study was on molecules other than miRNAs, e.g., long non-coding RNAs, small interfering RNAs, etc., or their effect on miRNAs could not be identified by reading the study. Since all five authors worked independently, the majority dictated if an article should have been included or not. Whenever an author identified an interesting article that was excluded, consensus for eventual inclusion was reached by discussion between all authors.

**Data extraction**

A.G., L.A. and A.Z. independently extracted from the selected studies (i) the mouse strain, (ii) the cell line used, and all its peculiar characteristics, i.e., all eventual genetic modification of the original cell line, (iii) the model generated with the cell line, i.e., orthotopic, subcutaneous xenograft, or metastatic, (iv) the miRNAs studied and their administration route, (v) the imaging modality or the multimodal approach used, (vi) the outcome measure, i.e., tumor volume reduction, changes in pathophysiologic aspects. Two other authors (G.S. and F.M.O.) independently extracted from the selected studies (i) the cell line used and all its peculiar characteristics, i.e., all eventual genetic modification of the original cell line, (ii) the model generated with the cell line, i.e., orthotopic, subcutaneous xenograft, or metastatic, (iii) the miRNAs studied, their administration route and the presumed effect. L.A. and F.M.O. reviewed and summarized all
the information retrieved and discussed with all authors whenever discrepancies were detected, and a consensus was needed.

Results

Literature Search

As reported in Figure 1, the search strategy using the eight research strings identified 6,860 scientific manuscripts on PubMed® (MEDLINE®), 4,294 on EMBASE, 2,631 on BIOSIS™, and 5,120 on Scopus. The eight lists were compared within each search engine deleting all duplicates hence the final number of manuscripts was 875 for PubMed® (MEDLINE®), 1,441 for EMBASE, 844 for BIOSIS™ and 1,447 for Scopus. At this point the four lists were merged together, erasing again duplicates, with a definitive list of 2,073 records. Such list was individually screened by each author, relying on title and abstract, and excluding all non-English papers, congress abstracts and posters, letter to editors, clear reference to cancers other than BC or to other pathologies at all, and reviews. Reviews were however searched for other relevant references, but no other eligible papers were detected. In this phase 852 records were excluded, and 1,221 papers were assessed for full text eligibility, excluding all those in which there was no use of mouse models of BC, there was not in vivo imaging or ex vivo on whole organs, i.e., were excluded imaging techniques applied to histological samples. At this point, 170 papers were studied to prepare the quali-quantitative synthesis, further excluding all manuscript in which the animal models were used to study long non-coding, small interfering or other RNAs, as well as genes or other signaling molecules, without a clear link to a miRNA. The final data extraction was made on a total of 114 manuscripts.

Mouse models of breast cancer

Various factors play a role in the study of preclinical models, in particular the mouse strain, the cell line and the engraftment route. A summary of the murine strain used in the articles analyzed and the relative references are shown in Table 1, while Figure 2 shows the absolute number of experiments for each strain. The murine genetic background most used in miRNA studies have been resulted to be the Balb/C (46,7%), followed by different strains of athymic and/or nude mice (23,3%) and non-obese diabetic, severely immunocompromised strains (NOD/SCID) (14,2%) and SCID strains (9,2%). Only few experiments were performed on NOD/SCID gamma strains (NSG) (3,3%) and only one on NOD mice (0,8%). In two papers it was not possible to identify the murine strain used (1,7%) (22, 23). To be noticed, only one was a transgenic model, the vascular-endothelial growth factor receptor 2 (VEGFR2) -luc mouse. This model was generated, in the studied report, from an FVN/B strain and it harbors the luciferase gene downstream the VEGFR2 promoter region. In brief, anytime the VEGFR2 is transcriptionally activated, luciferase is transcribed as well, hence this model allows the direct, non-invasive and quantitative monitoring of VEGFR2 via bioluminescence imaging (BLI) (24).
Regarding cell lines, in most of the experiments human derived cell lines were used, and only few used syngeneic, i.e., mouse derived cell lines of BC. Figure 3 shows the absolute number of experiments for each cell line, and Table 2 shows the different specific modification to each cell line and the relative references. In details, MDA-MB-231 were used in most experiments (62,5%), followed by MCF- (16,7%). SKBR3 and SUM-derived cells were used in two experiments each (1,4% each), whereas R2N1d – labeled with green fluorescent protein (GFP) and transfected with miR–, BT549– transfected with miR–, and T47D-TR (tamoxifen resistant) cell lines were used in one experiment each (0,7% each). Two experiments (1,4%) used breast cancer stem cells (BrCSCs): in one experiment, the BrCSCs were obtained after induction of differentiation of BC cells purified from fresh tissues from patients’ mastectomies and then transduced with GFP via lentivirus infection, prior to be used in an orthotopic model (25), in the second experiment BrCSCs were obtained from both patients tissues and from MDA-MB-231 and MCF-7 cell lines (26). One experiment (0,7%) used patients derived xenograft (PDX) labeled with luciferin and modulated for miR precursor expression (27) . The only syngeneic cell line used was 4T1 (13,8%).

Regarding the murine model, the three models for cellular engrafting, i.e., subcutaneous and orthotopic xenografts as well as metastatic model obtained by intravenous injection (i.v.) of cancer cells, were all represented (Table 3). The most used model was the i.v. metastatic model (46,8%), which was obtained either via intravenous injection (68,9%) – in one paper it was indicated as intraarterial (28) – in the tail vein, or in the left ventricle (9,8%). Direct intratibial injection to study osseous metastasis was applied in few experiments (6,6%) as well as the direct intrapulmonary injection (1,6%). Finally, the development of spontaneous metastasis after orthotopic injection was obtained either after surgical resection of the primary nodule (4,9%) - with lymph node (29) or pulmonary metastasization (30, 31) - or with the primary orthotopic implant on site (8,2%). In two papers, it was not specified how the metastatic model was obtained (32, 33).

The orthotopic model, with injection in the second or forth mammary gland or fat pad, trans-cutaneously, after surgical exposure, or intra nipple, was the second most used model (29,2%). Subcutaneous xenograft, implanted in various sites, i.e., on the shoulder, the armpit, the flank and thigh, was used in 24% of the experiments.

When interpreting tables and results, it should be noted that various reports performed multiple experiments using different cell lines and/or multiple models and/or multiple mouse strains, for all of which imaging was applied (27, 30, 31, 34-55).

**Mode and route of therapy administration**

In this paragraph we systematically reported the different in vivo modality and route of miRNA therapy administration. In Figure 4 are showed the absolute number of experiments done for each delivery system, while in Table 4 are reported the miRNAs used, the specific formulation of vehicle system and the relative references.
The most of pre-clinical mice models (54.4%) were generated by injecting luciferase (Luc)-labelled BC cells transfected with DNA or lentiviral plasmids. In detail, a lentiviral vector was used to modulate the expression of miR: -206 (27), -1 (32), -124 (39), -211-5p (42), -494 (44), -1204 (46), -133b (47) (37), -101 (53), -630 (56), -150 (57), -133a-3p (58), -452 (59), -543 (60), -96 (61), -29a (62), -455-3p (63), -30a (64), -100 (25), -548j (45), -940 (65), -429 (43), -442a (66), -30a (64), -138 (72), -27b (73), -454-3p (52), -23a (74), -218-5p (75), and of miR-30 family members (miR-30a-b-c-d-e) (28). A lentiviral vector was also generated to express a circular inhibitor miRNA (CimiRs) specific to silence the expression of miR-223 and miR-21 (76).

Moreover, BC cell line were transfected with DNA constructs encoding for the following miRNAs precursor and/or inhibitors: let-7a-5p (77), miR-196a (78), -205 (79), -361-5p (50), -590-3p (80), -567 (81), -106b-5p (82), -497 (24), -135/-203 (55), -29/-30 (83), 14q32-encoded miRNAs (84), miR-191/425 cluster (34). The effect of miR-1 overexpression was studied both in mice injected with MDA-MB-231-Luc cells stably transfected with miR-1 precursor and in tumor-bearing mice treated with the synthetic miR-1 mimic (85).

In 8 studies BC cells were transfected with different type of plasmid (lentiviral or DNA) encoding mimic and/or inhibitor specific for miR-200 family members (miR-200a, -200b, -200c, -141, -429) (86) (35) (87) (88) (48) (40, 41).

In 1 experiment a doxycycline inducible vector was used to over-express miR-301a-3p (90).

Nanoparticles (NPs)-based delivery represent a promising strategy for BC treatment preventing miRNA degradation in bloodstream and improving the miRNA delivery in tissue-specific targeting. Indeed, we found that 29 experiments (25.4%) were conducted using different formulation of NPs including natural based lipid NPs (LNPs) and synthetic NPs composed by inorganic materials such as silica (SiO$_2$), gold (Au) or polymer (e,i. polyamidoamine -PAMAM- dendrimers) (91) (Table 4).

Organic LNPs were generated to encapsulate: miR-203 mimic (92), AgomiR-143 (93), AgomiR-186-3p (94) and the "edited" form of miR-379-5p (95). AntagomiR-214-3p was loaded into the osteoclast-targeting delivery system (D-Asp8-liposome) (96).

Inorganic synthetic NPs was engineered to encapsulate miR-145 using PAMAM dendrimers modified with a thioaptamer (TA), a protein that binds CD44-receptors highly expressed on BC cells (97). Poly(ethylene glycol)–polyethylenimine (mPEG–PEI) was complexed with Molecular Beacon (MB) to detect miR-34a in BC (23).

Gold nanoparticles (AuNPs) were used to deliver miR-708 (31) and miR-96/-182 (98) mimics; other AuNPs were formulated with a photoacoustic (PA) nanoprobe that released a PA signal in the presence of the oncogenic miR-155 (99). Magnetic (MN) NPs were engineered to the recognition of specific oncomiR in BC tissue (100, 101) or conjugated with locked nucleic acid (LNA) to inhibit the activity of miR-10b (29, 102). SuperparaMN iron oxide NPs (SPIONs) conjugated with Argonaute-2 protein (AGO2) were formulated to deliver miR-376B mimic in BC tissue (103).
In 5 independent studies, the activity of the tumor suppressor miR-34a was replenished using: i) hTERT promoter-driven VISA liposomal NPs(26); ii) polymeric hybrid nanomicelles simultaneously delivering Doxorubicin (Dox)(104); iii) Dextrin-PEI-CM nanoplex (DPC) delivering also cyclam monomer (a CXCR antagonist)(105); iv) silica dioxide NPs (SiO$_2$NPs)(106), v) lipid core-shell nanocarrier coated with cationic albumin co-delivering docetaxel(107).

In 5 studies, miR-21 inhibition was obtained in vivo using: i) a core of phi29 pRNA- three-way junction motif (3WJ) harboring the RNA aptamer for EGFR (3WJ/EGFRapt/anti-miR21)(108), ii) a core of 3WJ harboring the aptamer binding to CD133 receptor (3WJ/CD133apt/anti-miR21)(109), iii) a polydopamine (PDA)-based NPs(110) , iv) tumor-extracellular vesicles complexed with gold-iron oxide NPs (TEV-GIONs) (111), v) RNA nanospheres into nanopompons(112).

In few studies, multiple miRNAs were simultaneously co-delivered using polymeric NPs triggered in BC tissue by the urokinase plasminogen activator peptide (uPA)(54), by ultrasound(113) or by RNA-triple-helix hydrogel scaffolds(114).

The combined delivery of miRNA and a chemotherapeutic drug into tumor sites was obtained using polymeric hybrid NPs (Dox + miR-34a)(104), polydopamine (PDA)-based NPs (Dox + antisense-miR-21) (110), magnetic NPs (Dox + miR-10b)(29), calcium/phosphate lipid NPs (Paclitaxel + miR-124)(115) and lipid nanocarrier coated by cationic albumin (Docetaxel + miRNA-34a)(107). Interestingly, specific NPs were developed to co-deliver photosensitizer indocyanine green (ICG) and the inhibitor of miR-21(116).

In 12 studied (10,5%) we found that to enhance the systemic delivery efficacy of mimic/inhibitor miRNA, in absence of a protective vehicle, synthetic small molecules or chemical modifications are added to miRNA increasing their stability in blood system. “CMM489” is a chemically modified mimic in which Uracil in the guide strand of miR-489 tumor suppressor was reply with 5-fluorouracil (5-FU)(117). Single-strand miRNA inhibitor (“AntagomiR”) and double-stranded mimic (“AgomiR”) are RNA harboring bases chemically modified to overcome the RNA instability. In this context, mice were treated with AgomiR-338-3p(118) or with AntagomiR-16-1-3p(119) or with AntagomiR-100(120). Additionally, FolamiR-34a is a modified mimic in which a folate group was attached to miR-34a sequence to directly bind the BC cells over-expressing the folate receptor(121). Another example of artificially synthesized nucleic acid is represented by the peptide nucleic acid (PNA) labelled with [$^{99}$Tc] that recognize in vivo the presence of the oncomiR-155(122). Finally, the inhibition in the activity of miR-21(123-125), miR-210 ("Targapremir-210") (126), miR-544(127) and miR-10b ("Linifanib") (128) was obtained using small molecules compounds.

Exosomes are small extracellular vesicles (EVs) of 30–150 nm in diameter, which are released by cancer cells in tumor microenvironment to intercellular communication. In 6 studies (5,3%), researchers have exploited the possibility to use exosomes to encapsulate the following miRNAs: let-7(129), miR-210(130), -335(131), -159(132), -4443(38) and Anti-miR-21(111).
Recently, the anticancer activity of miRNAs derived from marine invertebrate *marsupenaeus japonicus* shrimp was analyzed in 2 experiments in which tumor bearing mice were fed with shrimp fed mja-miR-35-expressing bacteria(133) or treated with synthesized shrimp miR-34(134).

### Therapy effect and efficacy

The potential role of miRNAs could be categorized based on their mode of action and of therapeutic efficacy established in pre-clinical BC mouse models. The number of experiments and references regarding the therapy effect and efficacy are summarized in Figure 5 and in Table 5.

Among the biological effects reported in mice, tumor growth alone (30.7%) or in combination with tumor metastasis (34.2%) are resulted to be the effects most studied.

Indeed, tumor growth inhibition occurred in tumor-bearing mice intravenous injected with several miRNAs (let-7, miR-145, -335, -34a, -203, -376B, -205/Anti miR-221, -379-5p, Anti miR-21) delivered using different approaches such as NPs(92, 95, 97, 101, 103, 106, 108-110, 113, 114), and extracellular vehicles(111, 129, 131, 132). The inhibition in tumor growth occurred in mice injected with BC cells transfected with miR-442a(66), -100(25), -27b(73), -567(81), -455-3p(63), -301a-3p(90), AntagomiR-138 (72), cirBulg21/223(76) compared to mice injected with BC cells transfected with a control plasmid. On the contrary, miR-196a over-expression in MDA-MB-231 -Luc cells promoted this capability (78).

Tumor growth was impaired in tumor bearing mice treated with: Linifanib(128), TargapremiR-210(126), small molecule “1” (specific for miR-544)(127), FolamiR-34a(121) , Trichostatin A (an inhibitor of histone deacetylase that up-regulates miR-125a-5p)(70), “CMM489” (a chemically modified miR-489)(117), or with Shrimp miR-34(134).

Interestingly, the injection of lipid vehicles loaded with AgomiR-186-3p(94) and with AgomiR-143(93) inhibited tumor growth and reduced the uptake of [18F]-fluoro-deoxyglucose ([18F]-FDG).

Regarding the effects of miRNA delivery on either tumor growth and lung metastasis, we found that luciferase expressing BC cells transfected with: miR-101(53), -141(89), -361-5p(50), -30a-5p(64), -125a-5p(71), -1(85), -211-5p(42), -190(68), -206(27), -33b(30), -33a(49), -96(61), -133b(47), -1(32), -494(44), -29b/30d(83), Anti miR-1204(46), miR-191/-425 sponge(34) exerted antitumor and metastatic activity compared to BC cells transfected with an empty vector. Silencing of let-7a-5p(77) and of miR-16-1-3p(119) in MDA-MB-231 and of miR-338-3p(118) in 4T1 cells influenced tumorigenesis and lung metastasis after implantation in nude mice.

The effects of miR-122 on glucose metabolism, tumor growth and metastasis were evaluated in different animal models using luciferase-labelled BC transfected cells or EV containing miR-122(36). Antitumor and antimetastatic effects was evaluated after injection of NPs loaded with specific miRNAs (-34a(105), -96/-182(98), -708(31), AntimiR-21/-10b(54), AntagomiR-10b(102)) or following the treatment with AC1MMYR2 (a specific small-molecule inhibitor of miR-21)(125), with AntagomiR-100(135) or with the
antioxidant Pterostilbene(136). A novel approach was reported by Wu and colleagues in which the co-delivery of miR-21 inhibitor and indocyanine green (ICG) exerted anticancer activity photokilling MDA-MB-231 cells(116).

Twenty animal models (17.5%) were done studying the effects of miRNA delivery on lung metastasis, and only few experiments were performed analyzing bone (7.9%), brain (1.7%) and liver metastasis (0.87%).

Lung metastasis were suppressed when BC-Luc cells were transfected with the following miRNAs: miR-630(56), -452(59), -590-3p(80), -150(57), -543(60), -133a-3p(58), -133b(37), 14q32 microRNA cluster (84), or transfected with the inhibitors for miR-106b-5p(82), -23a(74), -454-3p(52) or when mice were injected with Shrimp miR-35(133), with a small molecule that bind the precursor of miR-21 activating its destruction(124). On the contrary, an increased incidence of metastasis was established in mice injected with BC cells over-expressing miR-29a(62), -373(51). miR-548j overexpression increased the metastatic potential of BC cells without affecting tumor growth (45). Five studies reported that miR-200 family members (miR-200a, miR-200b, miR-200c, miR-429, miR-141) play an important role in the primary tumor formation and in the metastatic phenotype of BC (35, 40, 41, 86, 88).

The co-delivery of miRNA and small-molecule chemotherapy drugs in tumor site represents a promising strategy to fight the cancer progression in mice. In this context, the co-delivery of Dox with miR-34a(104) or with miR-159(132) in cancer site suppressed tumor growth. A regression of lung metastasis disease was established by the cotreatment of miR-10b and Dox(29). The combination treatment of Taxol and AC1MMYR2 (a small molecule that reduce miR-21 expression)(123), or of miRNA-34a and docetaxel (107) impaired tumor growth and metastasis. Paclitaxel and miR-124 coloaded in lipid nanosystem impaired lung metastasis formation in orthotopic mice(115). Co-delivery of miR-96/-182 with cisplatin, using NPs, reduced primary tumour and prevented lung metastasis formation(98).

Two experiments reported that brain metastasis formation was affected in vivo by the modulation of miR-509(67) and of miR-141(48). In only 1 study liver metastasis was impaired by the administration of EV carrying miR-4443 inhibitor(38). Bone metastasis was impaired by the over-expression in BC cells of miR-124(39), -429(43), -205(79), -940(65), -125b(33), -30 family members(28) or by the inhibition of miR-218-5p (75) or by intratumorally injection of synthetic miR-135/-203 mimics(55) or of the osteoclast-targeting AntagomiR-214-3p using (D-Asp)8-liposome(96).

Currently, the detection of miRNAs in cancer tissues could help to monitor the progression of cancer. From our research, biodistribution studies were found in 6 articles (5.3%). miR-155 expression was monitored in 2 different studies by intravenously injection of PA nanoprobe(99) and by the synthesized peptide nucleic acid (PNA) mimic loaded with $^{99m}$Tc(122). Molecular beacon (MB) circuit was developed to monitor the expression of miR-34a in BC tissue with high sensitivity(23). A nanosensor conjugated with a MN-NPs allowed to discriminate BC cells from non-tumoral cells based on miR-10b expression(101). Monitoring the expression of miR-200c (87) and of miR-14/-21/-9(100) in tumor bearing mice was useful to determine the therapeutic approach. Finally, tumor angiogenesis was evaluated in 3
studies reporting that miR-497 exhibited anti-angiogenesis and anti-tumor effects targeting VEGFR2(24), miR-210 promoted angiogenesis(130), while miR-125a-5p affected tumorigenesis, metastasis, and angiogenesis in vivo(71).

**Molecular Imaging**

Most of the known preclinical imaging techniques have been applied in studying miRNAs delivery and/or efficacy. Figure 6 shows the absolute number of experiments for each imaging modality, and Table 6 shows the number of experiments for each specific modality and aim, and the relative references.

Bioluminescence resulted the most used tool (64%); this technique was used as a surrogate of tumor growth for efficacy treatment or for the evaluation of tumorigenicity in miRNA transfected cells (29,9%); for tracking, evaluation of engraftment and response to therapy in metastatic models (50,6%); for both the afore mentioned aims in the same experiment, evaluating metastasis either in vivo or ex vivo on whole organs (16,1%). As already reported, in one experiment (1,1%), a transgenic VEGFR2-luc mouse was used to evaluate the expression of VEGFR by non-invasive bioluminescence, and to evaluates the effect of miRNA-mimic treatment as anti-angiogenetic therapy(24). Bioluminescence was also used for vector uptake and intercellular target repression (2,3%), although most of these experiments were performed by fluorescence imaging.

Fluorescence imaging was the second most used technique (21,3%) and was used primarily to trace vector biodistribution (73,2%) by using different strategies, e.g., by directly conjugating the miR to the fluorophore, or simply uploading the fluorophore within the vector. In one interesting report, the vector was neither a NP nor an extracellular vesicle nor a liposome, but the vector was a folate, directly linked to the miR as well as to a near-infrared (NIR) fluorophore for fluorescent detection(121). Fluorescence was rarely used for tumor growth evaluation (11,5%), analysis of tumor persistence, after direct intratumoral injection, of the miR labeled with fluorophore(116) or within fluorescent SiO$_2$ NPs(106) (7,7%), and for cell tracking (3,8%). One interesting experiment (3,8%) showed the ability of a molecular beacon to detect and image endogenous miRNAs with a high level of specificity in vivo(23). Besides these two mostly used imaging techniques, other tools were used to study biodistribution or different aspects of miRNAs treatment efficacy. Micro Computed Tomography (µCT) was used to analyze in vivo or ex vivo osteolytic lesions in metastatic bone models or to identify pulmonary metastases (5,2%). The former evaluation was performed with standard radiography (1,5%) in two other experiments(28, 39). Magnetic Resonance Imaging (MRI) was used in 2,9% of the experiments, mainly for detection of magnetic NPs biodistribution, and only in one experiment for the evaluation of invasiveness of adjacent tissue(38). Positron Emission Tomography (PET)/CT was applied with $[18F] -$ FDG administration to evaluate tumor growth, in term of tumor glucose metabolism, or for detection of pulmonary metastases (2,2%). High Frequency Ultrasonography (HFUS) was performed to evaluate tumor growth or microbubbles-mediated nanoparticles delivery as therapeutic intervention (1,5%). Photoacoustic (PA) (0,7%) imaging was used to determinate the ability of self-assembling nanoprobes to identify specific miRNA. In brief, in presence of
the specific miRNA, aurum aggregation from the nanoprobe, via a hybridization chain reaction, allowed identification of the PA signal\(^{99}\). Finally, single photon emission computed tomography (SPECT) (0.7\%) was used to label and track molecular probe, and to evaluate both the specificity in detecting the selected miR and both for biodistribution purposes\(^{122}\).

In addition to what has been already stated a multimodal imaging approach, i.e., the use of multiple imaging technologies to evaluate different aspects or models within the same manuscript, was used and evaluated in 19 papers (16.7\%) \(^{28, 29, 31, 33, 38, 39, 43, 75, 84, 93, 96, 98, 101, 102, 104, 108, 111, 130, 137}\). Finally, it is important to highlights that in in some manuscripts in which multiple animal model are developed, the primary tumor growth was evaluated exclusively by tumor caliper measurement or tumor weighting \textit{ex vivo}, whereas imaging (\textit{in vivo} or \textit{ex vivo}) was applied only for metastasis evaluation\(^{28, 32, 34, 35, 42, 44, 46, 47, 50, 61, 64, 69, 77, 83, 85, 89, 118, 119, 134}\). In other studies, the therapeutic effects of miRNA delivery were evaluated independently from their biodistribution visualization obtained with preclinical imaging \(^{92, 103-107, 109, 110, 112, 115, 121, 129}\).

**Discussion**

We systematically analyzed the most recent studies using pre-clinical imaging technologies to investigate the potential of specific miRNAs as therapeutic and diagnostic tools in BC. Although several systematic reviews focused on the crucial role played by miRNA in BC biology and as therapeutics \(^{138}\) to our knowledge this review is the first systematic review that specifically focus of the use of pre-clinical molecular imaging for evaluation of miRNAs delivery and effects in BC. Numerous are the advantages offered by application of different imaging techniques to study animal model of cancer\(^{16}\). First, the possibility to perform in real time non-invasive longitudinal studies of the same mice. This allows to reduce the number of animal to be analyzed in accordance with Directive 2010/63/EU, the principle of the 3Rs (Replacement, Reduction and Refinement) and animal welfare considerations. Noteworthy, it is the translational aspect of pre-clinical imaging that may be considered as a bridge from basic to clinical research. In this context, this systematic review documented all investigations that used different imaging technologies dedicated to small animals such as Optical Imaging (OI), HFUS, MRI, CT and PET/CT, to evaluate and validate miRNAs as anti-cancer agents and shed lights on molecular mechanisms.

Most of the studies included in this systematic review were performed on miR-10b (5 studies), on miR-21 (11 studies), on miR-34 family (8 studies) and on miR-200 family (10 studies). High level of miR-10b indicates poor prognosis in BC, correlating with angiogenesis and metastatic behaviors (increased tumor size, lymph node positivity and the high Ki-67 score)\(^{139-141}\). Importantly, in NOD-SCID mice, the high miR-10b level led to distant metastasis, while in the 4T1 mouse mammary tumor metastasis model, the delivery of AntagomiR specific for the silencing of miR-10b suppressed the distant metastasis\(^{142, 143}\). Over-expression of miR-21 one of the most studied oncomiR in BC, is associated with lymph node metastasis, resistance to anticancer agents and poor prognosis\(^{144-146}\). Up-regulation of miR-21 in this cancer induces silencing of several tumor suppressor genes such as Programmed Cell death 4 (PDCD4)\(^{147}\) and Leucine zipper transcription factor-like 1 (LZTFL1)\(^{148}\). A tumorigenicity assay was
recently performed in Balb/c-nude mice, which were inoculated with BC cells silenced for miR-21 using specific peptide nucleic acids (PNA). In vivo, functional studies showed that PNA-AntimiR-21 inhibits tumor growth in vivo(149). Another important family correlated to cancer is the miR-34 family; it comprises miR-34a, miR-34b and miR-34c. They exert a tumor suppressor role in various cancers and are regulated by p53(150). In BC, miR-34a plays a crucial role in proliferation, motility and stemness(151). Identified targets of miR-34a are SIRT1 and BCL2(152). Another example of oncomiR is miR-200a that promotes epithelial-mesenchymal transition (EMT), drug resistance and metastasis by targeting Tumor Protein P53 Inducible Nuclear Protein 1 (TP53INP1) and Yes-associated protein 1 (YAP1) in human BC(153, 154). Importantly, miR-200a belongs to the miR-200 family that appears to be crucial for BC progression. In particular, the miR-200 family is composed of five members (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) that are reported to be involved in EMT and angiogenesis of BC cells(155–157). Besides, it is reported that deregulated levels of miR-200a and miR-200c occurred in the tamoxifen resistance BC model, where they induced a reduction of the mRNA of c-MYB(157).

A major problem in the clinical use of miRNAs is the delivery method. This is due to several reasons as: the destabilization of the RNA in circulation due to serum ribonucleases, an ineffective targeting to the tumor cells because of the tumor microenvironment, and a poor uptake of the miRNA. Several delivery methods have been tested: as the lentiviral- and liposomal-mediated delivery of the tumor-suppressive miRNA miRNA-34a (miR-34a) reduces tumor burden in non–small cell lung cancer (NSCLC) mouse models(158). In addition to vehicle- and viral-mediated miRNA delivery, systemic injection of vehicle-free oligonucleotides has also been tested. However, this approach has proven problematic because of the pharmacokinetic and stability limitation associated with intravenous delivery, and thus either relies on local delivery or necessitates achieving a high oligonucleotide concentration that is often only seen in kidneys and liver. Although local delivery is an option, achieving delivery beyond sites that are accessible to local delivery, such as to micrometastatic lesions, is not achievable.

Most of the imaging studies reported in this review used optical imaging (OI) to analyze miRNAs effects on cancer murine models, in particular bioluminescence (BLI) (64%) and fluorescence (21,3%). One of the most common applications of OI is to monitor tumor growth and metastasis formation in orthotopic xenograft models and transgenic animal models(159). Furthermore, it is a highly sensitive technique and allows non-invasive monitoring of disease-relevant processes and permits tracking of cells(160). The main advantages of OI compared to other imaging platforms are the low cost and the absence of ionizing radiation, as well as the possibility to more easily translate the observations obtained in vitro on the corresponding cell line injected in animals. Fluorescence shows some disadvantages due to background signals and autofluorescence which are absent in the BLI which in turn has brightness and low spatial-temporal resolution(159). Among the numerous studies analyzed in this review regarding BLI with luciferase to monitor tumor growth and/or metastatic spread it is worthy of note the investigation that used VEGFR2-luc transgenic mice to monitor the effect of miR-497 mimic not only on tumor growth but also on tumor angiogenesis(24). The results demonstrated that overexpression of miR-497 showed inhibitory effects on VEGFR2 activation(24). The limits of fluorescence can be overcome by using near infrared fluorophores that penetrate deeper into tissues and exhibit very low autofluorescence. An
interesting study conjugated microRNAs to folate (FolamiR) for delivering them into cells that overexpress the folate receptor. In particular, the tumor-suppressive FolamiR, FolamiR-34a, was labeled with NIR fluorophore and its delivery to TNBC xenografts was evaluated by OI (121). Furthermore, Tu et al. reported a novel strategy for miRNA detection through enzyme-free signal amplification by self-circulation of the hybridization between the miRNAs and molecular beacon (MB) circuits. This approach allowed to detect miRNA in the BC xenografts by amplifying the fluorescence signal and contributing to improvement in detection sensitivity(23).

HFUS is the most suitable technique to monitor tumor growth, due to the capability of this technique to perform an accurate morphologic imaging. Most interesting, HFUS has been used also to deliver directly therapeutic microRNAs (AmiR-21 and miR-100) and TK-p53-NTR triple therapeutic gene, co-loaded in PLGA-PEG-PEI polymer NPs to tumor models of TNBC(137). As our research group, demonstrated in past experiments, Ultrasound mediated therapy, enhance vascular permeability and microbubbles cavitation improving drug delivery directly into tumor sites(161). PET also have been used to evaluate the response to miRNA therapy in a tumor model of TNBC by targeting tumor glycolysis(93) as well as to assess metastasis in an in vivo mice model of TNBC. The limitation of this technique is related to the high-cost relative to the radiotracer and/or to the necessity of having a cyclotron close to the animal facility, plus the necessity of using radiations. Then, a specific and long training is necessary to have personal able to perform experiments of nuclear medicine. An emergent methodology is certain high field MRI that combines the possibility of performing a morphologic analysis of the primary tumor and to follow the spread of metastasis, plus the possibility of therapeutic delivery of miRNA or miRNA combined with chemotherapeutic agents with magnetic NPs. MRI has the advantage of avoiding the use of ionizing radiation but has the disadvantages of requiring long time acquisition to obtain high quality diagnostic images. A good anesthetic protocol and a continuous monitoring of the mice model could compensate the last issue. Finally, what mainly emerges from the cited papers, is the advantage that we could obtain having a multimodal imaging approach to diagnose both the mice model of BC and to perform an efficient therapy. The last issue could also be better addressed through targeted nanosystem directed delivered against molecular marker of breast cancer.

**Conclusion**

The studies reported and discussed in this systemic review highlights the utility of preclinical molecular imaging focused on the development of novel therapeutic strategies miRNAs based in the breast cancer management. To date, although the multiple advances in imaging technology, this extensive and focused literature review shows that optical imaging remains the most widely used method in preclinical investigations, probably due to its low cost and ease of use. In fact, only few of the papers we cited demonstrated the advantages that we could obtain having a multimodal imaging approach to diagnose both the mice model of BC and both to perform an efficient therapy. Therefore, given the large amount of information that can be extrapolated from multimodal imaging and its strong translational power to the clinic, future studies using multiple imaging modalities are desirable. Finally, the development of NPs engineered to encapsulate miRNAs alone or in combination with other drugs and its delivery to specific
targets will provide deeper knowledge in this research field and will be certainly one of the fields that will be improved in the future.

We aimed to highlight the role of preclinical imaging and its potentiality to test new experimental therapy for breast cancer patients to aim the translation from in vitro study to the clinic. Preclinical imaging is a continuously evolving field, new nanoprobes could represent the novel systems for personalized therapy in the future.

**Abbreviations**

AuNPs: gold nanoparticles; BC: breast cancer; BLI: Bioluminescence Imaging; BrCSCs: Breast cancer stem cells; Dox: Doxorubicin; FDG: fluorodeoxyglucose; GFP: green fluorescent protein; HER2: human epidermal growth factor receptor 2; HFUS: High Frequency Ultrasonography; NPs: nanoparticles; µCT: Micro Computed Tomography; MI: molecular imaging; MRI: Magnetic Resonance Imaging; OI: Optical Imaging; PA: Photoacoustic; PET: Positron Emission Tomography; PDX: patients derived xenograft; PNA: peptide nucleic acid; SiO$_2$NPs: silica dioxide NPs; SPECT: Single Photon Emission Computed Tomography; TR: tamoxifen resistant; UTR: untranslated region; VEGFR2: vascular-endothelial growth factor receptor 2.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Not applicable.

**Competing interests**

The authors declare that they have no conflict of interests.

**Fundings**
This study was supported in part by: Ministero dell’Istruzione, dell’Università e della Ricerca, Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN)-Bando 2017-grant 2017MHJJ55; Ricerca Corrente, Italian Ministry of Health; MIUR-PON “Ricerca e Innovazione” 2014-2020 (grant MOLIMONCOBRAIN LAB).

Authors’ contributions
A.G., L.A., G.S., F.M.O. and A.Z. collected the papers and each author independently extracted the information from the selected studies. L.A. and F.M.O. reviewed and summarized all the information retrieved and draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements
Not applicable

References
1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics. 2021. CA Cancer J Clin. 2021;71(1):7–33.
2. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, et al. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. 2018;5(2):77–106.
3. Barba D, León-Sosa A, Lugo P, Suquillo D, Torres F, Surrén F, et al. Breast cancer, screening and diagnostic tools: All you need to know. Crit Rev Oncol Hematol. 2021;157:103174.
4. Bertoli G, Cava C, Castiglioni I. MicroRNAs. New Biomarkers for Diagnosis, Prognosis, Therapy Prediction and Therapeutic Tools for Breast Cancer. Theranostics. 2015;5(10):1122–43.
5. Kurozumi S, Yamaguchi Y, Kurosumi M, Ohira M, Matsumoto H, Horiguchi J. Recent trends in microRNA research into breast cancer with particular focus on the associations between microRNAs and intrinsic subtypes. J Hum Genet. 2017;62(1):15–24.
6. Zelli V, Compagnoni C, Capelli R, Cannita K, Sidoni T, Ficorella C, et al. Circulating MicroRNAs as Prognostic and Therapeutic Biomarkers in Breast Cancer Molecular Subtypes. J Pers Med. 2020;10(3).
7. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215–33.
8. Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nat Rev Cancer. 2015;15(6):321–33.
9. Romano G, Acunzo M, Nana-Sinkam P. microRNAs as Novel Therapeutics in Cancer. Cancers (Basel). 2021;13(7).
10. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov. 2017;16(3):203–22.
11. Ranganathan K, Sivasankar V. MicroRNAs - Biology and clinical applications. J Oral Maxillofac Pathol. 2014;18(2):229–34.
12. Dasgupta I, Chatterjee A. Recent Advances in miRNA Delivery Systems. Methods Protoc. 2021;4(1).
13. Chakraborty C, Sharma AR, Sharma G, Lee SS. Therapeutic advances of miRNAs: A preclinical and clinical update. J Adv Res. 2021;28:127–38.
14. van Zandwijk N, Pavlakis N, Kao SC, Linton A, Boyer MJ, Clarke S, et al. Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study. Lancet Oncol. 2017;18(10):1386–96.
15. Witten L, Slack FJ. miR-155 as a novel clinical target for hematological malignancies. Carcinogenesis. 2020;41(1):2–7.
16. Serkova NJ, Glunde K, Haney CR, Farhoud M, De Lille A, Redente EF, et al. Preclinical Applications of Multi-Platform Imaging in Animal Models of Cancer. Cancer Res. 2021;81(5):1189–200.
17. Fang H, Cavaliere A, Li Z, Huang Y, Marquez-Nostra B. Preclinical Advances in Theranostics for the Different Molecular Subtypes of Breast Cancer. Front Pharmacol. 2021;12:627693.
18. Li X, Wang X, Zhao C, Shao L, Lu J, Tong Y, et al. From one to all: self-assembled theranostic nanoparticles for tumor-targeted imaging and programmed photoactive therapy. J Nanobiotechnology. 2019;17(1):23.
19. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009;6(7):e1000100.
20. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE’s risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14:43.
21. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.
22. Zhao J, Zou H, Han C, Ma J, Tang J. Circulair. RNA BARD1 (Hsa_circ_0001098) overexpression in breast cancer cells with TCDD treatment could promote cell apoptosis via miR-3942/BARD1 axis. Cell Cycle. 2018;17(24):2731–44.
23. Guk K, Hwang SG, Lim J, Son HY, Choi Y, Huh YM, et al. Fluorescence amplified sensing platforms enabling miRNA detection by self-circulation of a molecular beacon circuit. Chem Commun (Camb). 2019;55(24):3457–60.
24. Tu Y, Liu L, Zhao D, Liu Y, Ma X, Fan Y, et al. Overexpression of miRNA-497 inhibits tumor angiogenesis by targeting VEGFR2. Sci Rep. 2015;5:13827.
25. Petrelli A, Carollo R, Cargnelutti M, Iovino F, Callari M, Cimino D, et al. By promoting cell differentiation, miR-100 sensitzes basal-like breast cancer stem cells to hormonal therapy. Oncotarget. 2015;6(4):2315–30.
26. Lin X, Chen W, Wei F, Zhou BP, Hung MC, Xie X. Nanoparticle Delivery of miR-34a Eradicates Long-term-cultured Breast Cancer Stem Cells via Targeting C22ORF28 Directly. Theranostics. 2017;7(19):4805–24.
27. Samaekia R, Adorno-Cruz V, Bockhorn J, Chang YF, Huang S, Prat A, et al. miR-206 Inhibits Stemness and Metastasis of Breast Cancer by Targeting MKL1/IL11 Pathway. Clin Cancer Res. 2017;23(4):1091–103.
28. Croset M, Pantano F, Kan CWS, Bonnelye E, Descotes F, Alix-Panabières C, et al. miRNA-30 Family Members Inhibit Breast Cancer Invasion, Osteomimicry, and Bone Destruction by Directly Targeting Multiple Bone Metastasis-Associated Genes. Cancer Res. 2018;78(18):5259–73.
29. Yoo B, Kavishwar A, Ross A, Wang P, Tabassum DP, Polyak K, et al. Combining miR-10b-Targeted Nanotherapy with Low-Dose Doxorubicin Elicits Durable Regressions of Metastatic Breast Cancer. Cancer Res. 2015;75(20):4407–15.
30. Lin Y, Liu AY, Fan C, Zheng H, Li Y, Zhang C, et al. MicroRNA-33b Inhibits Breast Cancer Metastasis by Targeting HMGA2, SALL4 and Twist1. Sci Rep. 2015;5:9995.
31. Ramchandani D, Lee SK, Yomtoubian S, Han MS, Tung CH, Mittal V. Nanoparticle Delivery of miR-708 Mimetic Impairs Breast Cancer Metastasis. Mol Cancer Ther. 2019;18(3):579–91.
32. Liu R, Li J, Lai Y, Liao Y, Qiu W. Hsa-miR-1 suppresses breast cancer development by down-regulating K-ras and long non-coding RNA MALAT1. Int J Biol Macromol. 2015;81:491–7.
33. Maroni P, Bendinelli P, Matteucci E, Desiderio MA. The therapeutic effect of miR-125b is enhanced by the prostaglandin endoperoxide synthase 2/cyclooxygenase 2 blockade and hampers ETS1 in the context of the microenvironment of bone metastasis. Cell Death Dis. 2018;9(5):472.
34. Zhang X, Wu M, Chong QY, Zhang W, Qian P, Yan H, et al. Amplification of hsa-miR-191/425 locus promotes breast cancer proliferation and metastasis by targeting DICER1. Carcinogenesis. 2018;39(12):1506–16.
35. Humphries B, Wang Z, Li Y, Jhan JR, Jiang Y, Yang C. ARHGAP18 Downregulation by miR-200b Suppresses Metastasis of Triple-Negative Breast Cancer by Enhancing Activation of RhoA. Cancer Res. 2017;77(15):4051–64.
36. Fong MY, Zhou W, Liu L, Alontaga AY, Chandra M, Ashby J, et al. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. Nat Cell Biol. 2015;17(2):183–94.
37. Li X, Deng S, Pang X, Song Y, Luo S, Jin L, et al. LncRNA NEAT1 Silenced miR-133b Promotes Migration and Invasion of Breast Cancer Cells. Int J Mol Sci. 2019;20(15).
38. Wang J, Zhang Q, Wang D, Yang S, Zhou S, Xu H, et al. Microenvironment-induced TIMP2 loss by cancer-secreted exosomal miR-4443 promotes liver metastasis of breast cancer. J Cell Physiol. 2020;235(7–8):5722–35.
39. Cai WL, Huang WD, Li B, Chen TR, Li ZX, Zhao CL, et al. microRNA-124 inhibits bone metastasis of breast cancer by repressing Interleukin-11. Mol Cancer. 2018;17(1):9.
40. Sánchez-Cid L, Pons M, Lozano JJ, Rubio N, Guerra-Rebollo M, Soriano A, et al. MicroRNA-200, associated with metastatic breast cancer, promotes traits of mammary luminal progenitor cells. Oncotarget. 2017;8(48):83384–406.
41. Jin T, Suk Kim H, Ki Choi S, Hye Hwang E, Woo J, Suk Ryu H, et al. microRNA-200c/141 upregulates SerpinB2 to promote breast cancer cell metastasis and reduce patient survival. Oncotarget. 2017;8(20):32769–82.
42. Chen LL, Zhang ZZ, Yi ZB, Li JJ. MicroRNA-211-5p suppresses tumour cell proliferation, invasion, migration and metastasis in triple-negative breast cancer by directly targeting SETBP1. Br J Cancer. 2017;117(1):78–88.
43. Zhang X, Yu X, Zhao Z, Yuan Z, Ma P, Ye Z, et al. MicroRNA-429 inhibits bone metastasis in breast cancer by regulating CrkL and MMP-9. Bone. 2020;130:115139.
44. Zhan MN, Yu XT, Tang J, Zhou CX, Wang CL, Yin QQ, et al. MicroRNA-494 inhibits breast cancer progression by directly targeting PAK1. Cell Death Dis. 2017;8(1):e2529.
45. Zhan Y, Liang X, Li L, Wang B, Ding F, Li Y, et al. MicroRNA-548j functions as a metastasis promoter in human breast cancer by targeting Tensin1. Mol Oncol. 2016;10(6):838–49.
46. Liu X, Bi L, Wang Q, Wen M, Li C, Ren Y, et al. miR-1204 targets VDR to promotes epithelial-mesenchymal transition and metastasis in breast cancer. Oncogene. 2018;37(25):3426–39.
47. Wang QY, Zhou CX, Zhan MN, Tang J, Wang CL, Ma CN, et al. MiR-133b targets Sox9 to control pathogenesis and metastasis of breast cancer. Cell Death Dis. 2018;9(7):752.
48. Debeb BG, Lacerda L, Anfossi S, Diagaradjane P, Chu K, Bambhroliya A, et al. miR-141-Mediated Regulation of Brain Metastasis From Breast Cancer. J Natl Cancer Inst. 2016;108(8).
49. Zhang C, Zhang Y, Ding W, Lin Y, Huang Z, Luo Q. MiR-33a suppresses breast cancer cell proliferation and metastasis by targeting ADAM9 and ROS1. Protein Cell. 2015;6(12):881–9.
50. Ma F, Zhang L, Ma L, Zhang Y, Zhang J, Guo B. MiR-361-5p inhibits glycolytic metabolism, proliferation and invasion of breast cancer by targeting FGFR1 and MMP-1. J Exp Clin Cancer Res. 2017;36(1):158.
51. Chen D, Dang BL, Huang JZ, Chen M, Wu D, Xu ML, et al. MiR-373 drives the epithelial-to-mesenchymal transition and metastasis via the miR-373-TXNIP-HIF1α-TWIST signaling axis in breast cancer. Oncotarget. 2015;6(32):32701–12.
52. Ren L, Chen H, Song J, Chen X, Lin C, Zhang X, et al. MiR-454-3p-Mediated Wnt/β-catenin Signaling Antagonists Suppression Promotes Breast Cancer Metastasis. Theranostics. 2019;9(2):449–65.
53. Li JT, Jia LT, Liu NN, Zhu XS, Liu QQ, Wang XL, et al. MiRNA-101 inhibits breast cancer growth and metastasis by targeting CX chemokine receptor 7. Oncotarget. 2015;6(31):30818–30.
54. Devulapally R, Sekar NM, Sekar TV, Foygel K, Massoud TF, Willmann JK, et al. Polymer nanoparticles mediated codelivery of antimiR-10b and antimiR-21 for achieving triple negative breast cancer therapy. ACS Nano. 2015;9(3):2290–302.
55. Taipaleenmäki H, Browne G, Akech J, Zustin J, van Wijnen AJ, Stein JL, et al. Targeting of Runx2 by miR-135 and miR-203 Impairs Progression of Breast Cancer and Metastatic Bone Disease. Cancer Res. 2015;75(7):1433–44.
56. Zhou CX, Wang CL, Yu AL, Wang QY, Zhan MN, Tang J, et al. MiR-630 suppresses breast cancer progression by targeting metadherin. Oncotarget. 2016;7(2):1288–99.

57. Tang W, Xu P, Wang H, Niu Z, Zhu D, Lin Q, et al. suppresses triple-negative breast cancer metastasis through targeting HMGA2. Onco Targets Ther. 2018;11:2319–32.

58. Shi W, Tang T, Li X, Deng S, Li R, Wang Y, et al. Methylation-mediated silencing of miR-133a-3p promotes breast cancer cell migration and stemness via miR-133a-3p/MAML1/DNMT3A positive feedback loop. J Exp Clin Cancer Res. 2019;38(1):429.

59. Kim M, Jang K, Miller P, Picon-Ruiz M, Yeasky TM, El-Ashry D, et al. VEGFA links self-renewal and metastasis by inducing Sox2 to repress miR-452, driving Slug. Oncogene. 2017;36(36):5199–211.

60. Ji W, Mu Q, Liu XY, Cao XC, Yu Y. ZNF281-miR-543 Feedback Loop Regulates Transforming Growth Factor-β-Induced Breast Cancer Metastasis. Mol Ther Nucleic Acids. 2020;21:98–107.

61. Pillar N, Polsky AL, Weissglas-Volkov D, Shomron N. Comparison of breast cancer metastasis models reveals a possible mechanism of tumor aggressiveness. Cell Death Dis. 2018;9(10):1040.

62. Wu Y, Shi W, Tang T, Wang Y, Yin X, Chen Y, et al. miR-29a contributes to breast cancer cells epithelial-mesenchymal transition, migration, and invasion via down-regulating histone H4K20 trimethylation through directly targeting SUV420H2. Cell Death Dis. 2019;10(3):176.

63. Zeng Y, Gao T, Huang W, Yang Y, Qiu R, Hou Y, et al. MicroRNA-455-3p mediates GATA3 tumor suppression in mammary epithelial cells by inhibiting TGF-β signaling. J Biol Chem. 2019;294(43):15808–25.

64. Li L, Kang L, Zhao W, Feng Y, Liu W, Wang T, et al. miR-30a-5p suppresses breast tumor growth and metastasis through inhibition of LDHA-mediated Warburg effect. Cancer Lett. 2017;400:89–98.

65. Hashimoto K, Ochi H, Sunamura S, Kosaka N, Mabuchi Y, Fukuda T, et al. Cancer-secreted hsa-miR-940 induces an osteoblastic phenotype in the bone metastatic microenvironment via targeting ARHGAP1 and FAM134A. Proc Natl Acad Sci U S A. 2018;115(9):2204–9.

66. Zou Y, Chen Y, Yao S, Deng G, Liu D, Yuan X, et al. MiR-422a weakened breast cancer stem cells properties by targeting PLP2. Cancer Biol Ther. 2018;19(5):436–44.

67. Xing F, Sharma S, Liu Y, Mo YY, Wu K, Zhang YY, et al. miR-509 suppresses brain metastasis of breast cancer cells by modulating RhoC and TNF-α. Oncogene. 2015;34(37):4890–900.

68. Yu Y, Luo W, Yang ZJ, Chi JR, Li YR, Ding Y, et al. miR-190 suppresses breast cancer metastasis by regulation of TGF-β-induced epithelial-mesenchymal transition. Mol Cancer. 2018;17(1):70.

69. Dong H, Hu J, Zou K, Ye M, Chen Y, Wu C, et al. Activation of LncRNA TINCR by H3K27 acetylation promotes Trastuzumab resistance and epithelial-mesenchymal transition by targeting MicroRNA-125b in breast Cancer. Mol Cancer. 2019;18(1):3.

70. Hsieh TH, Hsu CY, Tsai CF, Long CY, Wu CH, Wu DC, et al. HDAC inhibitors target HDAC5, upregulate microRNA-125a-5p, and induce apoptosis in breast cancer cells. Mol Ther. 2015;23(4):656–66.

71. Hsieh TH, Hsu CY, Tsai CF, Long CY, Chai CY, Hou MF, et al. miR-125a-5p is a prognostic biomarker that targets HDAC4 to suppress breast tumorigenesis. Oncotarget. 2015;6(1):494–509.
72. Nama S, Muhuri M, Di Pascale F, Quah S, Aswad L, Fullwood M, et al. MicroRNA-138 is a Prognostic Biomarker for Triple-Negative Breast Cancer and Promotes Tumorigenesis via TUSC2 repression. Sci Rep. 2019;9(1):12718.

73. Takahashi RU, Miyazaki H, Takeshita F, Yamamoto Y, Minoura K, Ono M, et al. Loss of microRNA-27b contributes to breast cancer stem cell generation by activating ENPP1. Nat Commun. 2015;6:7318.

74. Ma F, Li W, Liu C, Yu H, Lei B, Ren Y, et al. MiR-23a promotes TGF-β1-induced EMT and tumor metastasis in breast cancer cells by directly targeting CDH1 and activating Wnt/β-catenin signaling. Oncotarget. 2017;8(41):69538–50.

75. Taipaleenmäki H, Farina NH, van Wijnen AJ, Stein JL, Hesse E, Stein GS, et al. Antagonizing miR-218-5p attenuates Wnt signaling and reduces metastatic bone disease of triple negative breast cancer cells. Oncotarget. 2016;7(48):79032–46.

76. Shu Y, Wu K, Zeng Z, Huang S, Ji X, Yuan C, et al. A Simplified System to Express Circularized Inhibitors of miRNA for Stable and Potent Suppression of miRNA Functions. Mol Ther Nucleic Acids. 2018;13:556–67.

77. Shi Y, Zhang Y, Ran F, Liu J, Lin J, Hao X, et al. Let-7a-5p inhibits triple-negative breast tumor growth and metastasis through GLUT12-mediated warburg effect. Cancer Lett. 2020;495:53–65.

78. Yuan Y, Anbalagan D, Lee LH, Samy RP, Shanmugam MK, Kumar AP, et al. ANXA1 inhibits miRNA-196a in a negative feedback loop through NF-kB and c-Myc to reduce breast cancer proliferation. Oncotarget. 2016;7(19):27007–20.

79. Seo S, Moon Y, Choi J, Yoon S, Jung KH, Cheon J, et al. The GTP binding activity of transglutaminase 2 promotes bone metastasis of breast cancer cells by downregulating microRNA-205. Am J Cancer Res. 2019;9(3):597–607.

80. Yan M, Ye L, Feng X, Shi R, Sun Z, Li Z, et al. MicroRNA-590-3p inhibits invasion and metastasis in triple-negative breast cancer by targeting Slug. Am J Cancer Res. 2020;10(3):965–74.

81. Bertoli G, Cava C, Diceglie C, Martelli C, Rizzo G, Piccotti F, et al. MicroRNA-567 dysregulation contributes to carcinogenesis of breast cancer, targeting tumor cell proliferation, and migration. Breast Cancer Res Treat. 2017;161(3):605–16.

82. Wang Z, Li TE, Chen M, Pan JJ, Shen KW. miR-106b-5p contributes to the lung metastasis of breast cancer via targeting CNN1 and regulating Rho/ROCK1 pathway. Aging. 2020;12(2):1867–87.

83. Yin H, Wang Y, Wu Y, Zhang X, Liu J, Wang T, et al. EZH2-mediated Epigenetic Silencing of miR-29/miR-30 targets LOXL4 and contributes to Tumorigenesis, Metastasis, and Immune Microenvironment Remodeling in Breast Cancer. Theranostics. 2020;10(19):8494–512.

84. Uppal A, Wightman SC, Mallon S, Oshima G, Pitroda SP, Zhang Q, et al. 14q32-encoded microRNAs mediate an oligometastatic phenotype. Oncotarget. 2015;6(6):3540–52.

85. Liu C, Zhang S, Wang Q, Zhang X. Tumor suppressor miR-1 inhibits tumor growth and metastasis by simultaneously targeting multiple genes. Oncotarget. 2017;8(26):42043–60.

86. Kim HK, Park JD, Choi SH, Shin DJ, Hwang S, Jung HY, et al. Functional Link between miR-200a and ELK3 Regulates the Metastatic Nature of Breast Cancer. Cancers (Basel). 2020;12(5).
87. Liu J, Shen JX, He, Zhang GJ. Bioluminescence Imaging for Monitoring miR-200c Expression in Breast Cancer Cells and its Effects on Epithelial-Mesenchymal Transition Progress in Living Animals. Mol Imaging Biol. 2018;20(5):761–70.

88. Meng Z, Zhang R, Wang Y, Zhu G, Jin T, Li C, et al. miR-200c/PAI-2 promotes the progression of triple negative breast cancer via M1/M2 polarization induction of macrophage. Int Immunopharmacol. 2020;81:106028.

89. Li T, Lu H, Mukherjee D, Lahiri SK, Shen C, Yu L, et al. Identification of epidermal growth factor receptor and its inhibitory microRNA141 as novel targets of Krüppel-like factor 8 in breast cancer. Oncotarget. 2015;6(25):21428–42.

90. Lettlova S, Brynychova V, Blecha J, Vrana D, Vondrusova M, Soucek P, et al. MiR-301a-3p Suppresses Estrogen Signaling by Directly Inhibiting ESR1 in ERα Positive Breast Cancer. Cell Physiol Biochem. 2018;46(6):2601–15.

91. Revia RA, Stephen ZR, Zhang M. Theranostic Nanoparticles for RNA-Based Cancer Treatment. Acc Chem Res. 2019;52(6):1496–506.

92. Yan Y, Li XQ, Duan JL, Bao CJ, Cui YN, Su ZB, et al. Nanosized functional miRNA liposomes and application in the treatment of TNBC by silencing Slug gene. Int J Nanomedicine. 2019;14:3645–67.

93. Miao Y, Zhang LF, Guo R, Liang S, Zhang M, Shi S, et al. (18)F-FDG PET/CT for Monitoring the Response of Breast Cancer to miR-143-Based Therapeutics by Targeting Tumor Glycolysis. Mol Ther Nucleic Acids. 2016;5(8):e357.

94. He M, Jin Q, Chen C, Liu Y, Ye X, Jiang Y, et al. The miR-186-3p/EREG axis orchestrates tamoxifen resistance and aerobic glycolysis in breast cancer cells. Oncogene. 2019;38(28):5551–65.

95. Xu X, Wang Y, Mojumdar K, Zhou Z, Jeong KJ, Mangala LS, et al. A-to-I-edited miRNA-379-5p inhibits cancer cell proliferation through CD97-induced apoptosis. J Clin Invest. 2019;129(12):5343–56.

96. Liu J, Li D, Dang L, Liang C, Guo B, Lu C, et al. Osteoclastic miR-214 targets TRAF3 to contribute to osteolytic bone metastasis of breast cancer. Sci Rep. 2017;7:40487.

97. Fan W, Wang X, Ding B, Cai H, Fan Y, Li Y, et al. Thioaptamer-conjugated CD44-targeted delivery system for the treatment of breast cancer in vitro and in vivo. J Drug Target. 2016;24(4):359–71.

98. Gilam A, Conde J, Weissglas-Volkov D, Oliva N, Friedman E, Artzi N, et al. Local microRNA delivery targets Palladin and prevents metastatic breast cancer. Nat Commun. 2016;7:12868.

99. Cao W, Gao W, Liu Z, Hao W, Li X, Sun Y, et al. Visualizing miR-155 To Monitor Breast Tumorigenesis and Response to Chemotherapeutic Drugs by a Self-Assembled Photoacoustic Nanoprobe. Anal Chem. 2018;90(15):9125–31.

100. Yu Y, Yao Y, Yan H, Wang R, Zhang Z, Sun X, et al. A Tumor-specific MicroRNA Recognition System Facilitates the Accurate Targeting to Tumor Cells by Magnetic Nanoparticles. Mol Ther Nucleic Acids. 2016;5:e318.

101. Yoo B, Kavishwar A, Ross A, Pantazopoulos P, Moore A, Medarova Z. In Vivo Detection of miRNA Expression in Tumors Using an Activatable Nanosensor. Mol Imaging Biol. 2016;18(1):70–8.
102. Yoo B, Kavishwar A, Wang P, Ross A, Pantazopoulos P, Dudley M, et al. Therapy targeted to the metastatic niche is effective in a model of stage IV breast cancer. Sci Rep. 2017;7:45060.

103. Unal O, Akkoc Y, Kocak M, Nalbat E, Dogan-Ekici Al, Yagci Acar H, et al. Treatment of breast cancer with autophagy inhibitory microRNAs carried by AGO2-conjugated nanoparticles. J Nanobiotechnology. 2020;18(1):65.

104. Xie X, Chen Y, Chen Z, Feng Y, Wang J, Li T, et al. Polymeric Hybrid Nanomicelles for Cancer Theraanostics: An Efficient and Precise Anticancer Strategy for the Codelivery of Doxorubicin/miR-34a and Magnetic Resonance Imaging. ACS Appl Mater Interfaces. 2019;11(47):43865–78.

105. Yang X, Gao F, Zhang W, Li H, Huang X, Wei J, et al. "Star" miR-34a and CXCR4 antagonist based nanoplex for binary cooperative migration treatment against metastatic breast cancer. J Control Release. 2020;326:615–27.

106. Panebianco F, Climent M, Malvindi MA, Pompa PP, Bonetti P, Nicassio F. Delivery of biologically active miR-34a in normal and cancer mammary epithelial cells by synthetic nanoparticles. Nanomedicine. 2019;19:95–105.

107. Zhang L, Yang X, Lv Y, Xin X, Qin C, Han X, et al. Cytosolic co-delivery of miRNA-34a and docetaxel with core-shell nanocarriers via caveolae-mediated pathway for the treatment of metastatic breast cancer. Sci Rep. 2017;7:46186.

108. Shu D, Li H, Shu Y, Xiong G, Carson WE, Haque F, et al. Systemic Delivery of Anti-miRNA for Suppression of Triple Negative Breast Cancer Utilizing RNA Nanotechnology. ACS Nano. 2015;9(10):9731–40.

109. Yin H, Xiong G, Guo S, Xu C, Xu R, Guo P, et al. Delivery of Anti-miRNA for Triple-Negative Breast Cancer Therapy Using RNA Nanoparticles Targeting Stem Cell Marker CD133. Mol Ther. 2019;27(7):1252–61.

110. Mao W, Hu C, Zheng H, Xie J, Shi X, Du Y, et al. A Functionalized Polydopamine Theranostic Nanoprobe for Efficient Imaging of miRNA-21 and In Vivo Synergetic Cancer Therapy. Mol Ther Nucleic Acids. 2020;22:27–37.

111. Bose RJC, Uday Kumar S, Zeng Y, Afjei R, Robinson E, Lau K, et al. Tumor Cell-Derived Extracellular Vesicle-Coated Nanocarriers: An Efficient Theranostic Platform for the Cancer-Specific Delivery of Anti-miR-21 and Imaging Agents. ACS Nano. 2018;12(11):10817–32.

112. Guo Q, Li C, Zhou W, Chen X, Zhang Y, Lu Y, et al. GLUT1-mediated effective anti-miRNA21 pompon for cancer therapy. Acta Pharm Sin B. 2019;9(4):832–42.

113. Kumar SU, Wang H, Telichko AV, Natarajan A, Bettinger T, Cherkaoui S, et al. Ultrasound Triggered Co-Delivery of Therapeutic MicroRNAs and a Triple Suicide Gene Therapy Vector by Using Biocompatible Polymer Nanoparticles for Improved Cancer Therapy in Mouse Models. Advanced Therapeutics. 2021;4(5):2000197.

114. Conde J, Oliva N, Atilano M, Song HS, Artzi N. Self-assembled RNA-triple-helix hydrogel scaffold for microRNA modulation in the tumour microenvironment. Nat Mater. 2016;15(3):353–63.
115. Chen C, Shen M, Liao H, Guo Q, Fu H, Yu J, et al. A paclitaxel and microRNA-124 coloaded stepped cleavable nanosystem against triple negative breast cancer. J Nanobiotechnology. 2021;19(1):55.

116. Wu C, Tian Y, Zhang Y, Xu J, Wang Y, Guan X, et al. Acid-Triggered Charge-Convertible Graphene-Based All-in-One Nanocomplex for Enhanced Genetic Phototherapy of Triple-Negative Breast Cancer. Adv Healthc Mater. 2020;9(1):e1901187.

117. Soung YH, Chung H, Yan C, Fesler A, Kim H, Oh ES, et al. Therapeutic Potential of Chemically Modified miR-489 in Triple-Negative Breast Cancers. Cancers (Basel). 2020;12(8).

118. Liang Y, Xu X, Wang T, Li Y, You W, Fu J, et al. The EGFR/miR-338-3p/EYA2 axis controls breast tumor growth and lung metastasis. Cell Death Dis. 2017;8(7):e2928.

119. Ye T, Liang Y, Zhang D, Zhang X. MicroRNA-16-1-3p Represses Breast Tumor Growth and Metastasis by Inhibiting PGK1-Mediated Warburg Effect. Front Cell Dev Biol. 2020;8:615154.

120. Wang W, Liu Y, Guo J, He H, Mi X, Chen C, et al. miR-100 maintains phenotype of tumor-associated macrophages by targeting mTOR to promote tumor metastasis via Stat5a/IL-1ra pathway in mouse breast cancer. Oncogenesis. 2018;7(12):97.

121. Orellana EA, Tenneti S, Rangasamy L, Lyle LT, Low PS, Kasinski AL. FolamiRs. Ligand-targeted, vehicle-free delivery of microRNAs for the treatment of cancer. Sci Transl Med. 2017;9(401).

122. Jiang Y, Gai Y, Long Y, Liu Q, Liu C, Zhang Y, et al. Application Evaluation of Mol Imaging. 2020;19:1536012120916124.

123. Ren Y, Zhou X, Liu X, Jia HH, Zhao XH, Wang QX, et al. Reprogramming carcinoma associated fibroblasts by AC1MMYR2 impedes tumor metastasis and improves chemotherapy efficacy. Cancer Lett. 2016;374(1):96–106.

124. Costales MG, Aikawa H, Li Y, Childs-Disney JL, Abegg D, Hoch DG, et al. Small-molecule targeted recruitment of a nuclease to cleave an oncogenic RNA in a mouse model of metastatic cancer. Proc Natl Acad Sci U S A. 2020;117(5):2406–11.

125. Ren Y, Zhou X, Yang JJ, Liu X, Zhao XH, Wang QX, et al. AC1MMYR2 impairs high dose paclitaxel-induced tumor metastasis by targeting miR-21/CDK5 axis. Cancer Lett. 2015;362(2):174–82.

126. Costales MG, Haga CL, Velagapudi SP, Childs-Disney JL, Phinney DG, Disney MD. Small Molecule Inhibition of microRNA-210 Reprograms an Oncogenic Hypoxic Circuit. J Am Chem Soc. 2017;139(9):3446–55.

127. Haga CL, Velagapudi SP, Strivelli JR, Yang WY, Disney MD, Phinney DG. Small Molecule Inhibition of miR-544 Biogenesis Disrupts Adaptive Responses to Hypoxia by Modulating ATM-mTOR Signaling. ACS Chem Biol. 2015;10(10):2267–76.

128. Monroig-Bosque PDC, Shah MY, Fu X, Fuentes-Mattei E, Ling H, Ivan C, et al. OncomiR-10b hijacks the small molecule inhibitor linifanib in human cancers. Sci Rep. 2018;8(1):13106.

129. Wang Y, Chen X, Tian B, Liu J, Yang L, Zeng L, et al. Nucleolin-targeted Extracellular Vesicles as a Versatile Platform for Biologics Delivery to Breast Cancer. Theranostics. 2017;7(5):1360–72.
130. Jung KO, Youn H, Lee CH, Kang KW, Chung JK. Visualization of exosome-mediated miR-210 transfer from hypoxic tumor cells. Oncotarget. 2017;8(6):9899–910.

131. Almanza G, Rodvold JJ, Tsui B, Jepsen K, Carter H, Zanetti M. Extracellular vesicles produced in B cells deliver tumor suppressor miR-335 to breast cancer cells disrupting oncogenic programming in vitro and in vivo. Sci Rep. 2018;8(1):17581.

132. Gong C, Tian J, Wang Z, Gao Y, Wu X, Ding X, et al. Functional exosome-mediated co-delivery of doxorubicin and hydrophobically modified microRNA 159 for triple-negative breast cancer therapy. J Nanobiotechnology. 2019;17(1):93.

133. Chen Y, Zhang S, Cao J, Zhang X. Shrimp Antiviral mja-miR-35 Targets. Front Immunol. 2018;9:2071.

134. Cui Y, Yang X, Zhang X. Shrimp miR-34 from Shrimp Stress Response to Virus Infection Suppresses Tumorigenesis of Breast Cancer. Mol Ther Nucleic Acids. 2017;9:387–98.

135. Jayarangaiah A, Sidhu G, Brown J, Barrett-Campbell O, Bahtiyar G, Youssef I, et al. Therapeutic options for advanced thyroid cancer. Int J Clin Endocrinol Metab. 2019;5(1):26–34.

136. Su CM, Lee WH, Wu AT, Lin YK, Wang LS, Wu CH, et al. Pterostilbene inhibits triple-negative breast cancer metastasis via inducing microRNA-205 expression and negatively modulates epithelial-to-mesenchymal transition. J Nutr Biochem. 2015;26(6):675–85.

137. Kumar SU, Wang H, Telichko AV, Natarajan A, Bettinger T, Cherkaoui S, et al. Ultrasound Triggered Co-Delivery of Therapeutic MicroRNAs and a Triple Suicide Gene Therapy Vector by Using Biocompatible Polymer Nanoparticles for Improved Cancer Therapy in Mouse Models. Advanced Therapeutics. 2021;4(5):2000197.

138. Grimaldi AM, Salvatore M, Incoronato M. miRNA-Based Therapeutics in Breast Cancer: A Systematic Review. Front Oncol. 2021;11:668464.

139. Wang N, Chen P, Huang LP, Wang TZ. Prognostic significance of microRNA-10b overexpression in breast cancer: a meta-analysis. Genet Mol Res. 2016;15(2).

140. Liu X, Guan Y, Wang L, Niu Y. MicroRNA-10b expression in node-negative breast cancer-correlation with metastasis and angiogenesis. Oncol Lett. 2017;14(5):5845–52.

141. Zhang J, Yang J, Zhang X, Xu J, Sun Y, Zhang P. MicroRNA-10b expression in breast cancer and its clinical association. PLoS One. 2018;13(2):e0192509.

142. Ma L. Role of miR-10b in breast cancer metastasis. Breast Cancer Res. 2010;12(5):210.

143. Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson EG, et al. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. Nat Biotechnol. 2010;28(4):341–7.

144. Gong C, Yao Y, Wang Y, Liu B, Wu W, Chen J, et al. Up-regulation of miR-21 mediates resistance to trastuzumab therapy for breast cancer. J Biol Chem. 2011;286(21):19127–37.

145. Petrović N, Mandušić V, Stanojević B, Lukić S, Todorović L, Roganović J, et al. The difference in miR-21 expression levels between invasive and non-invasive breast cancers emphasizes its role in breast cancer invasion. Med Oncol. 2014;31(3):867.
146. Najjary S, Mohammadzadeh R, Mokhtarzadeh A, Mohammadi A, Kojabadi AB, Baradaran B. Role of miR-21 as an authentic oncogene in mediating drug resistance in breast cancer. Gene. 2020;738:144453.

147. Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem. 2008;283(2):1026–33.

148. Wang H, Tan Z, Hu H, Liu H, Wu T, Zheng C, et al. microRNA-21 promotes breast cancer proliferation and metastasis by targeting LZTFL1. BMC Cancer. 2019;19(1):738.

149. Yan LX, Wu QN, Zhang Y, Li YY, Liao DZ, Hou JH, 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. 2011;13(1):R2.

150. Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell. 2007;26(5):731–43.

151. Li WJ, Wang Y, Liu R, Kasinski AL, Shen H, Slack FJ, et al. MicroRNA-34a: Potent Tumor Suppressor, Cancer Stem Cell Inhibitor, and Potential Anticancer Therapeutic. Front Cell Dev Biol. 2021;9:640587.

152. Li L, Yuan L, Luo J, Gao J, Guo J, Xie X. MiR-34a inhibits proliferation and migration of breast cancer through down-regulation of Bcl-2 and SIRT1. Clin Exp Med. 2013;13(2):109–17.

153. Yu SJ, Hu JY, Kuang XY, Luo JM, Hou YF, Di GH, et al. MicroRNA-200a promotes anoikis resistance and metastasis by targeting YAP1 in human breast cancer. Clin Cancer Res. 2013;19(6):1389–99.

154. Yu SJ, Yang L, Hong Q, Kuang XY, Di GH, Shao ZM. MicroRNA-200a confers chemoresistance by antagonizing TP53INP1 and YAP1 in human breast cancer. BMC Cancer. 2018;18(1):74.

155. Lim YY, Wright JA, Attema JL, Gregory PA, Bert AG, Smith E, et al. Epigenetic modulation of the miR-200 family is associated with transition to a breast cancer stem-cell-like state. J Cell Sci. 2013;126(Pt 10):2256–66.

156. Manavalan TT, Teng Y, Litchfield LM, Muluhngwi P, Al-Rayyan N, Klinge CM. Reduced expression of miR-200 family members contributes to antiestrogen resistance in LY2 human breast cancer cells. PLoS One. 2013;8(4):e62334.

157. Gao Y, Zhang W, Liu C, Li G. miR-200 affects tamoxifen resistance in breast cancer cells through regulation of MYB. Sci Rep. 2019;9(1):18844.

158. Zhang L, Liao Y, Tang L. MicroRNA-34 family: a potential tumor suppressor and therapeutic candidate in cancer. J Exp Clin Cancer Res. 2019;38(1):53.

159. Pirovano G, Roberts S, Kossatz S, Reiner T. Optical Imaging Modalities: Principles and Applications in Preclinical Research and Clinical Settings. J Nucl Med. 2020;61(10):1419–27.

160. Camorani S, Hill BS, Collina F, Gargiulo S, Napolitano M, Cantile M, et al. Targeted imaging and inhibition of triple-negative breast cancer metastases by a PDGFRβ aptamer. Theranostics. 2018;8(18):5178–99.
161. Greco A, Di Benedetto A, Howard CM, Kelly S, Nande R, Dementieva Y, et al. Eradication of therapy-resistant human prostate tumors using an ultrasound-guided site-specific cancer terminator virus delivery approach. Mol Ther. 2010;18(2):295–306.

162. Lu M, Wu Y, Zeng B, Sun J, Li Y, Luo J, et al. CircEHMT1 inhibits metastatic potential of breast cancer cells by modulating miR-1233-3p/KLF4/MMP2 axis. Biochem Biophys Res Commun. 2020;526(2):306–13.

163. Drasin DJ, Guarnieri AL, Neelakantan D, Kim J, Cabrera JH, Wang CA, et al. TWIST1-Induced miR-424 Reversibly Drives Mesenchymal Programming while Inhibiting Tumor Initiation. Cancer Res. 2015;75(9):1908–21.

164. 164. !!! INVALID CITATION !!! [25, 27, 28, 30, 32, 33, 35, 37, 39–49, 51–53, 56–75, 87, 88].

165. Shu L, Cheung KL, Khor TO, Chen C, Kong AN. Phytochemicals: cancer chemoprevention and suppression of tumor onset and metastasis. Cancer Metastasis Rev. 2010;29(3):483–502.

Tables

Table 1

Murine strains used in miRNA experiments.
| Background | Strain | No. of experiments | References |
|------------|--------|--------------------|------------|
| Balb/C     | As it  | 12                 | (30, 53, 61, 77, 83, 99, 102, 104, 113, 118-120) |
|            | /J     | 1                  | (31)       |
| cAnNCr     |        | 1                  | (98)       |
| athymic nude |       | 3                  | (93, 94, 97) |
| Nude       |        | 31                 | (26, 28, 34, 37, 41, 43, 46, 47, 50, 52, 58, 59, 62, 69, 78, 82, 83, 92, 100, 105, 112, 115, 116, 119, 122, 123, 125, 129, 132, 133, 162) |
| -nu        |        | 1                  | (110)      |
| -nu/nu     |        | 1                  | (130)      |
| -nu/nu athymic |     | 1                  | (32)       |
| cAJcl-nu/nu |       | 1                  | (65)       |
| cAnN.Cg-Foxn1nu/Crl-Narl | | 2                  | (70, 71)   |
| cJNju-Foxn1nu/Nju |  | 1                  | (38)       |
| nude athymic CAnN.Cg-Foxn1nu/Crl | | 1                  | (90)       |
| SCID       | As it  | 7                  | (44, 50, 55, 60, 63, 68) |
| Beige      |        | 2                  | (47, 48)   |
| CB17.Cg-PrkdcscidHrhr/IcrCrl | | 2                  | (31, 114)  |
| NOD        | -Prkdc_{em26}I/2rg_{em26}/Nju | 1                  | (104)      |
| NOD/SCID   | As it  | 14                 | (25, 27, 40, 45, 51, 55, 56, 66, 74, 84, 85, 124, 134, 136) |
| NOD.CB17-Prkdcs{scid}/J | | 1                  | (114)      |
| B6.CB17-Prkdcs{scid}/Sz | | 2                  | (126, 127) |
| NSG        | NOD/SCID/IL2Ry-null | 1                  | (36)       |
| NSG        | NOD.Cg-Prkdcs{scid}II2_{rgtm1WJL}/SzJ | 2                  | (72, 106)  |
| NSG        | NOD scid gamma | 1                  | (131)      |
| Athymic /  | Nude (Nu/Nu) | 8                  | (33, 35, 54, 57, 87, 111, 117, 128) |
| Nude                     | Count | Reference |
|-------------------------|-------|-----------|
| Athymic nude            | 2     | (86, 101) |
| Athymic nu/nu           | 1     | (101)     |
| Nude (NIH III nude)     | 1     | (29)      |
| NCrnu/nu                | 1     | (109)     |
| Athymic NCrnu/nu        | 1     | (79)      |
| Nu/Nu (NU-Foxn1nu)      | 1     | (121)     |
| Athymic Nude-Foxn1nu nude (NCI) | 1 | (89) |
| Athymic Nude-Foxn1nu/nu | 1     | (81)      |
| Nude (mice not furtherly identified) | 10 | (30, 34, 44, 46, 49, 67, 76, 77, 80, 103) |
| J:NU (outbred athymic nude) | 1   | (95)      |

**NOD**: Non-obese diabetic; **SCID**: Severe combined immunodeficient mice; **NSG**: NOD scid gamma mouse.

**Table 2**

Cell lines used in miRNA experiments.
| Cell line       | Derived     | Labeling      | Transfection | No. of experiments | References                                                                 |
|-----------------|-------------|---------------|--------------|--------------------|-----------------------------------------------------------------------------|
| MDA-MB-231      | parental    |               |              | 17                 | (38, 64, 74, 83, 92, 93, 102, 104, 105, 112, 115, 116, 122, 127, 129, 132, 134) |
|                 | parental    | miR           |              | 14                 | (30, 37, 41-43, 46, 50, 57, 60, 65, 77, 85, 88, 119)                        |
|                 | parental    | GFP           |              | 1                  | (162)                                                                      |
|                 | parental    | GFP - luciferase |              | 3                  | (54, 128, 136)                                                            |
|                 | parental    | GFP - luciferase | miR         | 3                  | (58, 84, 86)                                                              |
|                 | parental    | GFP           | miR         | 1                  | (35)                                                                       |
|                 | parental    | luciferase    | miR/AntimiR  | 17                 | (39, 44, 45, 47, 49, 55, 59, 64, 68, 72, 75, 77, 78, 80, 81, 87, 119)       |
|                 | parental    | luciferase    |              | 15                 | (34, 83, 85, 95, 108, 114, 117, 121, 123-127, 133, 134)                    |
|                 | HM          |               |              | 1                  | (36)                                                                       |
| (Meningeal      |             |               |              |                    |                                                                             |
| metastasis)     |             |               |              |                    |                                                                             |
|                 | D3H2LN      | luciferase    |              | 5                  | (29, 47, 63, 96, 101)                                                     |
| (pleural        |             | luciferase    | miR         | 1                  | (56)                                                                       |
| effusion)       |             |               |              |                    |                                                                             |
|                 | B02         |               | miR         | 1                  | (28)                                                                       |
| (pleural        |             |               |              |                    |                                                                             |
| effusion)       |             |               |              |                    |                                                                             |
|                 | BrM (brain  | luciferase    | miR         | 1                  | (67)                                                                       |
| metastasis)     |             |               |              |                    |                                                                             |
|                 | 1833/TGL    |               |              | 1                  | (33)                                                                       |
| (metastatic     |             |               |              |                    |                                                                             |
| bone)           |             |               |              |                    |                                                                             |
|                 | IBC3        | GFP           | miR KD      | 1                  | (48)                                                                       |
|                 | 4175 LM2    | luciferase    |              | 3                  | (31, 35, 131)                                                             |
|                 | K8ikd       |               | miR         | 1                  | (89)                                                                       |
| MCF-7           | DCIS        | luciferase    |              | 1                  | (36)                                                                       |
|                 | parental    |               |              | 4                  | (32, 100, 101, 122)                                                       |
| Models used in miRNA experiments. |
|-----------------------------------|

|                      |          |          |          |
|----------------------|----------|----------|----------|
| MCF-10CA1h parental  | GFP - luciferase | miR      | 1        | (40)     |
| SKBR3 parental       | luciferase | Anti/miR sponge | 1        | (76)     |
|                       | TR (Trastuzumab resistant) | miR sponge | 1        | (69)     |
| SUM-149              | GFP      | miR KD   | 1        | (48)     |
| 159pt                |          |          | 1        | (106)    |

**Table 3**

Models used in miRNA experiments.
| Model                                      | No. of Experiments | References                                                                 |
|--------------------------------------------|--------------------|---------------------------------------------------------------------------|
| Metastatic                                 | 42                 | (27, 28, 30, 32, 34, 37, 40, 41, 44-48, 50, 51, 53, 56-58, 60-62, 64, 69, 74, 77, 80, 82-84, 86, 88, 89, 105, 115, 117-119, 124, 133, 134, 162) |
| – tail vein intravenous                     |                    |                                                                           |
| – intrarterial                              |                    |                                                                           |
| Metastatic                                 | 6                  | (36, 39, 43, 67, 79, 96)                                                |
| – left ventricle                            |                    |                                                                           |
| Metastatic                                 | 4                  | (39, 43, 65, 75)                                                        |
| – intratibial                               |                    |                                                                           |
| Metastatic                                 | 1                  | (71)                                                                      |
| – intrapulmonary                            |                    |                                                                           |
| Metastatic                                 | 5                  | (35, 42, 52, 68, 107)                                                   |
| – spontaneous after orthotopic              |                    |                                                                           |
| Metastatic                                 | 3                  | (29-31)                                                                  |
| – spontaneous after orthotopic with primary mass removed | |                                                                           |
| Orthotopic                                 | 40                 | (23, 25-27, 36, 40, 41, 44, 45, 47, 53, 55, 61, 63, 66, 68, 72, 73, 78, 81, 89, 95, 98, 99, 102, 105, 106, 108, 109, 112, 114, 115, 118-120, 123, 125, 126, 128, 131) |
| Xenograft (subcutaneous)                    | 33                 | (22, 24, 32, 34, 46, 50, 58, 69-71, 76, 77, 83, 85, 87, 90, 93, 100, 101, 103, 104, 110, 111, 116, 121, 122, 127, 130, 132, 134, 136, 137, 163) |

**Table 4**

miRNA delivery system in mice.

**Table 5**

Therapy effects in mice models following miRNA delivery.
| Vehicle     | Formulation | miRNA                                    | No. of experiments | References                          |
|-------------|-------------|------------------------------------------|--------------------|-------------------------------------|
| NP (n = 29) | Lipid (LNP) | miR-34a, -124, -143, 186-3p, -203,       | 8                  | (26, 92-96, 107, 115)              |
|             |             | -214-3p, -379-5p                          |                    |                                     |
|             | Gold (Au)   | miR-155, -708, -96/-182                   | 3                  | (31, 98, 99)                        |
|             | Silico (SiO2) | miR-34a                                 | 1                  | (106)                               |
|             | Magnetic (MN) | miR-10b, -376B, 21/-145/-9               | 5                  | (29, 100-103)                       |
|             | Polymers    | miR-21, -34a, -145, -21/10b              | 8                  | (23, 54, 97, 104, 105, 110, 113, 116) |
|             | RNA         | miR-21, -205/-221                        | 4                  | (108, 109, 112, 114)               |
| miRNA chemically modified (n = 12) | Mimic       | miR-489 (CMM489), miR-34a (FolamiR)      | 2                  | (117, 121)                          |
|             | AgomiR      | miR-16-1-3p, -100, -338-3p               | 3                  | (118-120)                           |
|             | AntagomiR   | miR-10b (“Linifanib”), miR-21 (“AC1MMYR2”), miR-210 (“TargapremiR”), -544 | 6                  | (123-128)                           |
|             | Small Molecules inhibitors | miR-155                               | 1                  | (122)                               |
| EV (n = 6)  | Exosome     | miR-21, -159, -210, -335, -4443, let-7   | 6                  | (38, 111, 129-132)                 |
| Plasmid (n=62) | Lentiviral | miR-1, 23a, -27b, -29a, -33a, -33b, -96, -100, -101, -124, -125a, -125b, -133a-3p, -133b, -138, -150, -190, -206, -211-5p, -218-5p, -373, -429, -442a, -452, -454-3p, -455-3p, -494, -509, -543, -548j, -630, -940, -1204, -200 family, -30 family | 44 | (164) |
|             | DNA         | miR-1; -29/-30, 106b-5p, -135/203, -196a, -205, 361-5p, -497, -590-3p, -567, let-7a-5p, -14q32-encoded miRNAs, -191/425, -200 family | 16 | (24, 34, 50, 53, 55, 77-87) |
|             | Circular inhibitor | miR-21/-223 | 1 | (76) |
|                          |                      |     |     |
|--------------------------|----------------------|-----|-----|
| Inducible plasmid        | miR-301a-3p          | 1   | (90)|
| **Other (n = 5)**        |                      |     |     |
| Antiviral miRNA          | mja miR-34, -35      | 2   | (133, 134)|
| Circular RNA             | miR-1233-3p, -3942   | 2   | (22, 39)|
| Pterostilbene            | miR-105              | 1   | (136)|

NPs: Nanoparticles, EVs: extracellular vesicles.
| Therapy effects                      | Vehicles | miRNAs studied                                                                 | No. of experiments | References                  |
|-------------------------------------|----------|---------------------------------------------------------------------------------|--------------------|------------------------------|
| **Tumor Growth**                    | NP       | miR-203, 143, -145, -186-3p, -379, -376B 5p, -34a, -21, -205/-221               | 16                 | (26, 92-95, 97, 103, 104, 106, 108, 109, 112, 114, 116, 137, 165) |
| *(n = 35)*                          |          |                                                                                  |                    |                              |
| EV                                  | miR-335, -159, -21, let-7                                                        | 4                  | (111, 129, 131, 132)        |
| miRNA chemically modified           | Linifanib (miR-10b), “Small mol.1” (miR-544), FolaramiR-34a                    | 4                  | (121, 126-128)              |
| Plasmid                             | miR-455-3p, -100, -442a, -125a-5p, -138, -27b, 196a, -567, cirBulg21/223, -301a-3p | 10                 | (25, 63, 66, 70, 72, 73, 76, 78, 81, 90) |
| Other                               | Shrimp miR-34                                                                     | 1                  | (134)                       |
| **Tumor Growth & Lung Metastasis**  | Plasmid  | miR-101, -1, -211-5p, -96, -494, -1204, -133b, -206, -30a-5p, -548j, -141, -190, -125b, -33a, -33b, -29/30, -361-5p, let-7a, -191/-425, -200 family | 24                 | (27, 30, 32, 34, 36, 40-42, 44-47, 49, 50, 53, 61, 64, 68, 69, 71, 77, 83, 85, 89) |
| *(n = 39)*                          |          |                                                                                  |                    |                              |
| NP                                  | -708, -96/-182, -34a, 10b; -124, -21/10b                                         | 7                  | (31, 54, 98, 102, 105, 107, 115) |
| miRNA chemically modified           | CMM489 (miR-489), miR-338-3p, AntagomiR-100; AntagomiR-16-1-3p, AC1MMYR2 (miR-21 inhibitor) | 6                  | (117-120, 123, 125)        |
| Other                               | Pterostilbene, CircularRNA                                                        | 2                  | (22, 136)                   |
| **Lung Metastasis**                 | Plasmid  | miR-630, -150, -133b, -133a-3p, -10b, -452, -543, -29a, -373, -23a, -454-3p, -590-3p, -106b-5p, -200 family members, 14q32-encoded miRNAs | 16                 | (35, 37, 51, 52, 56-60, 62, 74, 80, 82, 84, 86, 88) |
| *(n = 20)*                          |          |                                                                                  |                    |                              |
| NP                                  | miR-10b                                         | 1                  | (29)                        |
| Other                               | miR-35; -1233                                           | 2                  | (133, 162)                  |
| miRNA chemically modified           | miR-21                                           | 1                  | (124)                       |
| **Bone**                            | Plasmid  | miRNA-124, -125b, -135/203; 429,                                               | 8                  | (28, 33, 39, 82)            |
| Metastasis (n =9) | -940, -205, -218-5p, -30 family members | 43, 55, 65, 75, 79 |
|------------------|------------------------------------------|------------------|
| NP               | miR-214-3p                               | 1                |
| Liver metastasis (n =1) | EV  miR-4443,                           | (38)             |
| Brain metastasis (n =2) | Plasmid  miR-141, -509                     | 2                |
| Biodistribution (n =6) | miR-200c, -34a, -155, -10b               | 6                |
| Angiogenesis (n =3) | miR-497, -210, -125-5p                    | 2                |

**NP**: Nanoparticle, **EV**: extracellular vesicle.

**Table 6**

Number of experiments for each specific modality and aim.
| Imaging       | Aim                                           | No. of experiments | References                                                                 |
|--------------|-----------------------------------------------|-------------------|---------------------------------------------------------------------------|
| Bioluminescence | Metastasis engraftment and growth            | 44                | (26, 28-30, 32, 34, 35, 37, 39, 41, 42, 44, 46-48, 50, 55-60, 62, 64, 67-69, 74, 77, 79, 80, 82-86, 88, 89, 96, 119, 124, 133, 134, 162) |
|               | Tumor engraftment and growth                 | 26                | (25, 38, 41, 55, 61, 63, 66, 72, 73, 75, 76, 78, 81, 87, 95, 101, 102, 108, 111, 114, 126-128, 130, 131, 136) |
|               | Tumor growth & metastasis                    | 14                | (27, 31, 33, 35, 40, 45, 49, 52-54, 71, 120, 123, 125)                     |
|               | Vector uptake and intracellular target repression | 2               | (38, 121)                                                                    |
|               | VEGFR2 transcription in transgenic mice      | 1                 | (24)                                                                        |
| Fluorescence  | Vector biodistribution                       | 19                | (29, 31, 92, 93, 97, 101-105, 107-111, 115, 129, 130, 132)                   |
|               | Tumor growth                                 | 3                 | (22, 70, 98)                                                                 |
|               | Vector persistence after intratumoral injection | 2               | (106, 116)                                                                   |
|               | Cell tracking                                | 1                 | (84)                                                                        |
|               | Molecular beacon for specific miR detection  | 1                 | (23)                                                                        |
| µCT           | Evaluation of osteolytic lesions             | 5                 | (33, 43, 65, 75, 96)                                                        |
|               | Pulmonary metastasis                         | 2                 | (61, 98)                                                                    |
| MRI           | Nanoparticles biodistribution                | 3                 | (100, 104, 111)                                                             |
|               | Adjacent tissues invasion from primary mass  | 1                 | (38)                                                                        |
| PET/CT – [18F]-FDG | Tumor growth and metabolism                 | 2                 | (93, 94)                                                                    |
|               | Pulmonary metastasis                         | 1                 | (43)                                                                        |
| Radiography   | Osseous                                      | 2                 | (28, 39)                                                                    |
| Method | Application | Count | Reference |
|--------|-------------|-------|-----------|
| HFUS   | Tumor growth | 1     | (116)     |
|        | Therapy delivery micro bubbles-mediated | 1 | (137) |
| PA     | Specific identification of miR | 1 | (99) |
| SPECT  | Specific identification of miR | 1 | (122) |

FDG: fluorodeoxyglucose; HFUS: high frequency ultrasonography; µCT: micro computed tomography; MRI: magnetic resonance imaging; PA: photoacoustic; PET/CT: Positron Emission Tomography/Computed Tomography; SPECT: single photon emission computed tomography; VEGFR2: vascular-endothelial growth factor receptor 2.

**Figures**
Figure 1

Flowchart for the strategy searches.
Figure 2

Number of experiments regarding the murine strain used in the articles analyzed. NOD: Non-obese diabetic; SCID: Severe combined immunodeficient mice; NSG: NOD scid gamma mouse.
Figure 3

The absolute number of experiments regarding the cell lines, BrCSCs and PDX used to generate animal mice model. BrCSCs: breast cancer stem cells; PDX: patients derived xenograft; T47D-TR: tamoxifen resistant.
Figure 4

The absolute number of experiments done for each miRNA delivery system.

Image not available with this version

Figure 5
The absolute number of experiments regarding the therapy effect and efficacy are shown.

Figure 6

The absolute number of experiments for each imaging modality used to analyze the biodistribution and the therapeutic effect of miRNAs delivery in mice. CT: Computed Tomography; MRI: Magnetic Resonance Imaging; PET: Positron Emission Tomography; HFUS: High Frequency Ultrasonography; PA: Photoacoustic; SPECT: Single Photon Emission Computed Tomography.