Life Sciences 217 (2019) 57–69

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

MicroRNA-22 inhibits proliferation, invasion and metastasis of breast cancer cells through targeting truncated neurokinin-1 receptor and ERα

Xiaobin Liu[a,b,1], Lufang Zhang[a,1], Yingna Tong[a,c,1], Man Yu[d], Wang Meng[e], Dong Dong[a], Jie Shao[a], Fei Zhang[f], Ruifang Niu[f], Yunli Zhou[a,*]

[a] Department of Clinical Laboratory, Key Laboratory of Cancer Prevention and Therapy, Tianjin Cancer Institute and Hospital, Tianjin Medical University, Tianjin, Tianjin's Clinical Research Center for Cancer, China
[b] Department of Clinical Laboratory, Beijing Huaxin Hospital, First Hospital of TsingHua University, Beijing, China
[c] Department of Clinical Laboratory, Tianjin Children's Hospital, Tianjin, China
[d] Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada
[e] Department of Clinical Laboratory, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing Maternal and Child Health Care Hospital, Beijing, China
[f] Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, The Key Laboratory of Breast Cancer Prevention and Therapy, Ministry of Education, Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

ARTICLE INFO

Keywords:
microRNA-22
Neurokinin-1 receptor
ERα
Breast cancer
Proliferation
Invasion

ABSTRACT

Heading aims: This topic aims to clarify whether miR-22 directly targets and downregulates the expression of ERα and NK1R-Tr to inhibit the malignant behaviors of breast cancer cells.
Materials and methods: RT-PCR and Western Blotting were used to detect the expression profile of miR-22, NK1R-Tr and ERα. Luciferase reporter assay and CHIP experiment were conducted to investigate the regulation network between miR-22, NK1R-Tr and ERα. MCF-7-ERαI and MDA-MB-231-ERα cell lines were constructed to study the biological behaviors. The SP-NK1R-ERK1/2 signaling pathway was analyzed using Western Blotting. The subcutaneous and metastases tumor models were employed to study the effects of miR-22 on cell proliferation and metastasis of breast cancer cells in vivo.
Key findings: MiR-22 expression level was significantly lower in breast cancerous tissues and cell lines than the adjacent normality, while that of NK1R-Tr increased. The ERα could positively regulate NK1R-Tr expression at DNA level. The descent degree of NK1R-Tr in MCF-7-ERαI cells was far less than that in wild MCF-7 cells, while the findings in MDA-MB-231-ERα cells was more apparent than wild MDA-MB-231 cells. The malignant phenotype was decreased in miR-22 overexpressing cells compared with the wild type. The peak of ERK1/2 phosphorylation was delayed and weakened in miR-22 overexpressing MCF-7 cells, which was agreed with the findings using NK1R-Tr antagonist. The size and number of metastatic tumors declined compared to the controls.
Significance: MiR-22 downregulated the expression of NK1R-Tr and ERα to delay and weaken phosphorylation of ERK1/2 to inhibit proliferation and metastasis of breast cancer cells.

1. Introduction

Substance P (SP), the first member of tachykinin family [1], exerts its biological effects by connecting to the preferential ligand neurokinin-1 receptor (NK1R), and this ligand-receptor compound was regarded as an independent anticancer target in humans [2–4]. Previous studies reported two natural forms of NK1R mediate the effects of SP: full-length receptor (NK1R-FL) and truncated receptor (NK1R-Tr) [5–8], and the expression of NK1R–Tr increased with the malignant advancement. Moreover, NK1R antagonist has used for clinical for chemotherapy-induced side effects [9–13]. Even so, there are only a few reports on the responses mediated by NK1R-Tr in human breast cancer.

Breast cancer is one of the most common malignancies in women [14], and usually classified into estrogen receptor alpha (ERα) positive or negative subtypes. Evidence showed overexpression of ERα was observed in approximately 70% of all breast cancer patients [15,16]. However, 20% to 40% of the patients relapsed in distant apparatuses

* Corresponding author.
E-mail address: zhouyunli@tjmuch.com (Y. Zhou).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.lfs.2018.11.057
Received 29 August 2018; Received in revised form 15 November 2018; Accepted 27 November 2018
Available online 28 November 2018
0024-3205/

after dormant for years [17–19]. Multiple ERα mechanisms have been proposed to explain how breast cancer cells survive and remain in dormancy [20]. For example, ERα, as a transcription factor, regulates diverse genes, which makes it an attractive drug target [21,22]. At molecular level, coupling with its ligand estradiol (E2), ERα forms a homodimer and translocates into the nucleus to regulate ERα-mediated transcription [16,17,23].

MicroRNAs (miRNAs), a cluster of endogenous short (~22 nucleotides) noncoding RNAs, have the capacity to suppress the expression of protein-coding genes by binding to the 3′-untranslated region (3′UTR) [24–26]. So far, miRNA-22 (miR-22) has been reported involved in a variety of cancers [27–29] including breast cancer [24], and several target prediction algorithms have revealed that 3′UTR of both NK1R-Tr and ERα contain the putative miR-22 binding sites, but the direct interaction between them has not been confirmed.

As an important target of endocrine therapy for breast cancer, ERα has been recognized by the whole society, which means that the endocrine system represented by ERα plays a crucial role in anti-tumor therapy. SP is one of the most important neurotransmitters in the nervous system, and its most sensitive receptor, NK1R, is widely distributed in various parts of the brain, including the striatum, the hippocampus, and the brainstem monoamine nerve nuclei. It can be said that SP-NK1R regulatory axis is one of the important components of the body's neural regulation. When SP activates NK1R, the latter can participate in the proliferation, invasion and metastasis of tumors through direct or indirect methods, which has been proved by many studies. According to the prediction, the 3′UTR region of mRNA of ERα and NK1R-Tr both contained the binding site of miR-22.Therefore, the researchers hypothesized that whether mir-22 could act on both of them simultaneously, so that the endocrine system represented by ERα and the neural regulatory network represented by NK1R could be combined to participate in the occurrence and development of breast cancer. This is the first study to link ERα with NK1R, if confirmed, it will provide new possibilities for the diagnosis and treatment of breast cancer.

2. Methods and materials

2.1. Clinical specimens

A total of 50 frozen breast tumor tissues and their adjacent normal tissues were obtained from the Tissue Bank of Tianjin Cancer Hospital. Both tumor and normal tissues were histologically affirmed by hematoxylin and eosin (H&E) staining. None of patients from whom the tissues were obtained received radiotherapy or chemotherapy prior to surgery. According to the 2009 International Union Against Cancer (UICC) TNM staging system, our cohort consists of 14 cases of stage I breast cancer, 21 cases of stage II, 15 cases of stage III, 0 case of stage IV, and includes 46 cases invasive ductal carcinoma and 4 intraductal carcinoma. Informed consent was obtained from all individual participants and the collection of specimens received the approval from the Institutional Research Ethics Committee and had been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

2.2. Cell culture

Human breast cancer cell line MDA-MB-231, MCF-7, T47D and SK-BR-3 were obtained from the American Type Culture Collection (Manassas, Virginia, USA) and cultured in RPMI-1640 medium. The normal mammary epithelial cell line HBL-100 was purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences and cultured in the DMEM/F12 (Hyclone, USA) medium. miR-22 overexpressing MDA-MB-231 cells were constructed by the YueYan biological technology co., LTD (Tianjin, China) and cultured in RPMI-1640 medium. All mediums were supplemented with 10% fetal bovine serum (Gibco, USA), and cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere.

2.3. mRNA/miRNA isolation and quantitative real-time PCR

Total RNA from tissue samples and cultured cells were extracted using TRIzol®Reagent (Invitrogen, USA), and quantitative PCR was performed as described according to the manufacturer's instructions. The expression of miR-22 was quantified using Taqman microRNA assay from Applied Biosystems (RuiBo, China). Briefly, 1 μg of total RNA was used to synthesize the complementary DNA (cDNA) through reverse transcription process with the existence of specific stem-loop RT primers, and quantitative PCR was performed on an Applied Biosystems 7900 Real-time PCR System. miRNA expression in each sample was normalized to U6 as an internal control consistently. Primers sequences were as follows: NK1R-Tr (NM015727) forward: 5′-GACCATCTACATA CACAGTGGC-3′; NK1R-Tr reverse: 5′-GGCTGAGTTGTGTGATGAT AAG-3′. The RT-qPCR results were expressed with the threshold cycle (Ct) and were converted to fold changes ($2^{-\Delta\Delta Ct}$), in which $\Delta\Delta Ct = (Ct_{miRNA}\text{-}Ct_{U6})_{target}\text{-}(Ct_{miRNA}\text{-}Ct_{U6})_{control}$.

2.4. Western blotting

In brief, cells were lysed in lysis buffer (1 × SDS sample buffer) with the protease inhibitor cocktail and the phosphatase inhibitor. Approximately 100 μg of total protein was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred onto a PVDF membrane. The PVDF membrane was blocked with 5% milk in Tris-buffered saline with Tween 20 (TBST) for 1 h before incubation with the following primary antibodies at 4 °C overnight: anti-N-terminus-polyclonal (1:500, R&D, Cat. MAB6687) specific for NK1R-Tr, anti-ERα (1:1000, Cell Signaling Technology, Cat. #8644), anti-Erk1/2 (1:800, Cell Signaling Technology, Cat. #4695), anti-phosphorylated Erk1/2 (1:800, Cell Signaling Technology, Cat. #9101), anti-β-actin (1:1000, Cell Signaling Technology, Cat. #3700s). Subsequently, the PVDF membrane was incubated with a HRP-conjugated secondary antibody (1:5000 Goat Anti-Mouse IgG (H + L), Proteintech, Cat. SA00001-1; 1:5000 Goat Anti-Rabbit IgG (H + L), Proteintech, Cat. SA00001-2), and detected using an ECL kit (Millipore, Germany).

2.5. Luciferase reporter assay

The cDNAs of NK1R-Tr and ERα were amplified by regular PCR. The wild type (WT) and mutant type (MUT) 3′UTR sequences of NK1R-Tr and ERα were respectively cloned into the pMIR-REPORT luciferase reporter vector (Ambion, USA) to generate Luc-NK1R-Tr/ERα-3′-UTR-WT and Luc-NK1R-Tr/ERα-3′-UTR-MUT constructions. HEK-293 cells cultured in 6-well plates were co-transfected with NK1R-Tr/ERα-3′-UTR-WT or NK1R-Tr/ERα-3′-UTR-MUT, and miR-22 mimic or mimic control. The miR-22 mimic or mimic control was transfected at a final concentration of 50 nM/mL using a Lipofectamine™2000 reagent kit (Invitrogen, USA) according to the manufacturer's instructions. The cell lysates were analyzed 48 h after transfection on a luciferase reporter assay system (Promega, USA). All the experiments were repeated at least three times independently.

2.6. ChIP

MCF-7 and T47D cells were treated with and without 1 nmol/L E2 separately in RPMI 1640 medium (without phenol red) for 30 min. A ChIP assay was performed using a ChIP chromatin immunoprecipitation kit (Cell Signaling Technology, USA) following the manufacturer's instructions. Briefly, cells were cross-linked with 1% formaldehyde, and the cross-linking reaction was stopped by glycine solution. Cells were then lysed with the Micrococcal Nuclease at 37 °C for 20 min to cut DNAs into 200 to 1000-bp fragments, and later the lysates were

sonicated to shear the cytomembrane and karyolemma.

50 μl chromatin extract of each sample was added into 450 μl dilution buffer, and 2% of the mixture was set aside as "input" and the rest was divided into 3 groups. Corresponding antibodies were added to each group and incubated with rotation at 4 °C overnight. The protein G magnetic beads were added the next day and incubated with rotation at 4 °C for 1 h, then washed the beads four times on the Magnetic Particle Concentrator (Dynal Biotech ASA, Norway). Then, the beads were digested with proteinase K and NaCl at 65 °C for 2 h. Finally, quantitative PCR (qPCR) was performed with the purified DNA. The ChIP data were presented as fold changes ($2^{-\Delta\Delta Ct}$), in which ΔΔCt was calculated using the formula ΔΔCt = ΔCt of anti-Era (IP)-ΔCt of IgG (IP), ΔCt (cycle threshold) = Ct of DNA from immunoprecipitate (IP)-Ct of DNA input.

2.7. Transient transfection of miRNA mimics, inhibitors, siRNA and plasmids

The miR-22 mimic, mimic control (MC), antisense miR-22 (anti-miR-22) and inhibitor control (IC) were synthesized by RuiBo Company (Guangzhou, China). Cells were seeded in 6 cm Petri dishes and incubated overnight, followed by transfection with miR-22 mimic, MC, anti-miR-22 and IC using LipofectamineTM 2000 (Invitrogen, USA) according to the manufacturer's instructions.

The siRNA of ERα and NK1R-Tr, ERα and NK1R-Tr expression plasmids were all synthesized by Life Technologies Company (USA), and each siRNA had three sequences. Cells were seeded into 6 cm Petri dishes, incubated with serum-free medium overnight, and then transiently transfected with the specific siRNA using Lipofectamine ™ 2000 (Invitrogen, USA) according to the manufacturer's instructions. The ERα and NK1R-Tr expression plasmids were transfected with the same method.

2.8. Cell counting kit-8 assay

Cell proliferation assay was conducted using the Cell Counting Kit-8 (Dojindo, Gaithersburg, MD) according to the manufacturer's protocol. Logarithmic growth phase MCF-7 and T47D cells stably overexpressing miR-22 or mimic control were planted in 96-well plates at a concentration of 2×10^3 cells per well, and the absorbance was detected by a micro-ELISA reader 3 h after adding the color agent at 37 °C as the first time point, and then 5 consecutive time points (24,48,72,96 and 120 h) were also detected. The experiment was carried out six replicates for each time, and repeated at least three times.

2.9. Colony formation assay

A total of 1000 MCF-7 or T47D wild-type and transfected cells were seeded in 6 cm Petri dishes and cultured for 7–14 days at 37 °C with 5% CO_2. The colonies were fixed with ice-cold methanol at 4 °C, stained with a three-Step Stain Set kit (Thermo Scientific, USA), and then photographed and counted.

2.10. Wound healing assay

For the wound healing assay, MCF-7 and T47D cells were cultured in complete growth medium until 90% confluency. Three 1.5 mm wide parallel wounds were introduced across each plate. Cell migration was observed under microscopy at 24 h, 48 h and 72 h after scratching and analyzed objectively.

2.11. Transwell migration and invasion assays

The invasion assay was performed with the Transwell chambers (8 μm MilliCell chambers, Millipore, USA), and each well was filled with 50 μL mixture of serum-free RPMI-1640 medium and Matrigel (BD Biosciences, USA). A total of 8×10^4 cells were seeded into the upper chambers, and 500 μL RPMI-1640 medium (contains 10% fetal bovine serum) was added to the nether chambers. After a 24-hour incubation, cells at the upper surface of the chamber membrane were scrubbed off. Then the invasive cells were fixed with ice-cold methanol, stained with a three-Step Stain Set kit and counted under the Leica Microsystem (200×, three random fields per well).

2.12. Tumor formation and metastasis assay in vivo

Four to six-week-old female scid mice, weighing about 20 g, were purchased from the Shanghai Institute of Material Medical, Chinese Academy of Sciences (Shanghai, China). All experiments were performed in accordance with the NIH "Guide for the Care and Use of Laboratory Animals". The study protocols were approved by the Shanghai Medical Experimental Animal Care Committee. MDA-MB-231 and T47D miR-22 overexpressing and mimic control cells were injected under the groin skin or into the tail vein of the scid mice. For the subcutaneous tumor formation experiment, we measured the size of the tumor every other day after injection. For the pulmonary metastasis assay, the lung tissues of each mouse were dissected and fixed in formalin for 24 h. The number of metastasis of per lung was analyzed both macroscopically and microscopically. Same treatment was operated on all experimental groups. Paraffin-embedded sections of each lung tissue were stained with H&E. The histopathological examination was conducted with a microscopy (OLYMPUS, Japan) and the metastatic nodules were recorded.

2.13. Statistical analysis

All statistical analyses were performed using the SPSS statistical software package (version 17.0). Each in vitro quantitative test was independently replicated, and all data are presented as means ± SD. One-way ANOVA was used to compare the expression levels of luciferase activities, migrated and invaded cell numbers. Two-way ANOVA and Bonferroni tests were used to measure the proliferation curves in vitro. All the statistical tests were two sided, and $p < 0.05$ was considered with statistical significance.

3. Results

3.1. NK1R-Tr is upregulated and miR-22 is downregulated in primary breast cancer tissues

To identify the potential roles of NK1R-Tr and miR-22 in the progression of breast cancer, we analyzed the expression levels of NK1R-Tr and miR-22 in 50 pairs of primay breast tumorous tissues and adjacent normal tissues using qRT-PCR. According to the median expression score of NK1R-Tr or miR-22, the 50 patients were assigned to either a positive or a negative expression group. As shown in Table 1, NK1R-Tr exhibited higher expression ($p < 0.05$) and miR-22 had lower expression in ERα-positive breast cancer tissues than that in ERα-negative cancer ($p < 0.001$). According to this grouping method, the expression profile of miR-22 in breast cancer tissues was negatively associated with that of NK1R-Tr (Supplementary Fig. 1, $R = -0.342$, $p < 0.05$). Our qRT-PCR analysis also demonstrated that the expression of miR-22 was lower, whereas that of NK1R-Tr was higher compared with their non-malignant counterparts (Fig. 1A, $p < 0.05$; B, $p < 0.05$).

We next studied the expression of NK1R-Tr and miR-22 in normal breast epithelial cell line (HBL-100) and four breast cancer cell lines (MDA-MB-231, T47D, MCF-7, SK-BR-3). As expected, NK1R-Tr was upregulated and miR-22 was downregulated in breast cancer cells (MDA-MB-231, T47D, MCF-7, SK-BR-3) compared with breast epithelial cell (HBL-100) at the mRNA level (Fig. 1C, D). At protein level, NK1R-Tr was upregulated in breast cancer cell lines compared with HBL-100 (Fig. 1E). Moreover, since NK1R-Tr, miR-22 and ERα were all expressed in MCF-7 and T47D cells (Fig. 1E, F), these two cell lines were selected

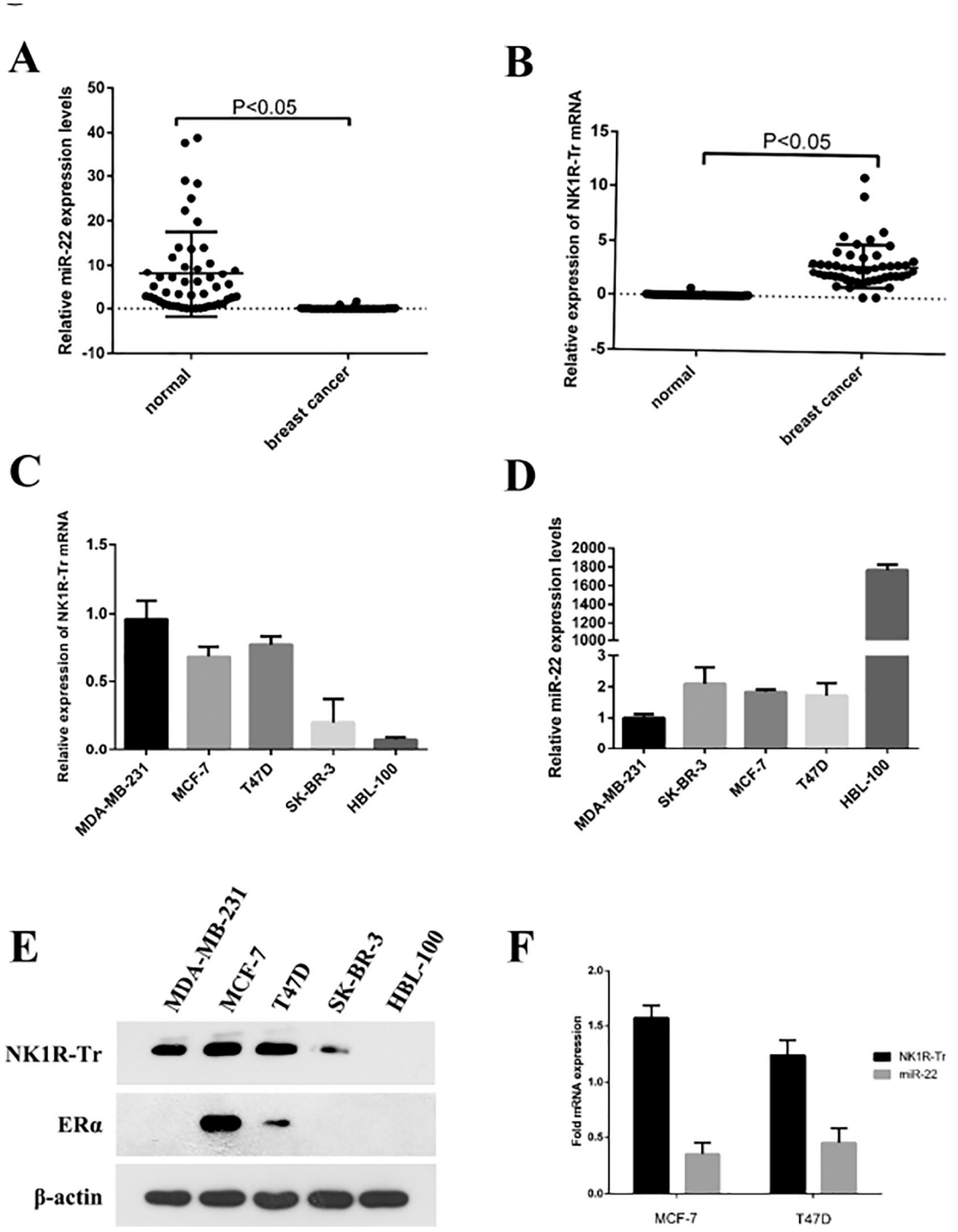


Fig. 1. The expression levels of NK1R-Tr and miR-22 in human breast tissues and cell lines. **A**, **B** Average expression levels of miR-22 and NK1R-Tr in human breast tumor tissues (n = 50) and adjacent normal tissues (n = 50). **C**, **D** Quantitative real-time PCR analysis of NK1R-Tr and miR-22 in human breast cancer cell lines (metastatic, MDA-MB-231; nonmetastatic, T47D/MCF-7; HER2-positive, SK-BR-3) and non-tumorigenic epithelial cells, HBL-100. **E** The expression at protein level of NK1R-Tr and ERα in human breast cancer cell lines (metastatic, MDA-MB-231; nonmetastatic, T47D/MCF-7; HER2-positive, SK-BR-3) and nontumorigeni epithelial cells, HBL-100. **F** The negative correlation of the expression levels of NK1R-Tr and miR-22 in ERα positive human breast cancer cell lines T47D and MCF-7.Each experiment was repeated three times independently. The loading control of Western Blot is 20 μg protein from the cell lysate. And the antibody is against β-actin.

as the appropriate cell models for studying the regulatory network between ERα and NK1R-Tr.

3.2. miR-22 downregulates ERα and NK1R-Tr expression and ERα upregulates NK1R-Tr expression

Despite of an inverse expression profile of ERα, NK1R-Tr and miR-22 in breast cancer tissues and cells, the underlying molecular mechanisms still remain unclear. The prediction algorithmic engine TargetScan showed that the 3′UTR noncoding regions of both NK1R-Tr and ERα contained putative miR-22 target sites (Fig. 2A). To determine whether NK1R-Tr and ERα were the direct targets of miR-22, wild-type and mutant 3′ untranslated regions (3′UTRs) of NK1R-Tr and ERα were cloned into the upstream of the firefly luciferase coding region in a pGL3-basic luciferase reporter vector. The vectors were co-transfected with miR-22 and mimic control into HEK293T cells to observe the luciferase activity. The relative luciferase activity was reduced by 53% in cells carrying miR-22 and pGL3-basic with ERα wild-type 3′UTRs

Table 1
Relationship between NK1R-Tr, miRNA-22 expression and clinicopathological features in breast cancer patients.

Pathology		Cases	NK1R-Tr positive (%)		miRNA-22 positive (%)	
Histological type	Invasive ductal carcinoma	68	66.2	< 0.05*	27.94	< 0.05*
	Intraductal carcinoma	14	21.4		66.67	
Histological grade	I	17	50.0	< 0.05*	67.32	< 0.05*
	II	50	56.0		43.67	
	III	15	85.7		18.94	
ERα	Negative	20	36.0	< 0.01**	60.34	< 0.01**
	Positive	62	85.0		8.75	

* $p < 0.05$.
** $p < 0.01$.

(Fig. 2B, C), while the relative luciferase activity was reduced by 62% in cells transfected with miR-22 and pGL3-basic with NK1R-Tr, but not in those with respective mutant 3′UTRs (Fig. 2C, $p < 0.01$). To further explore whether miR-22 could decrease endogenous NK1R-Tr and ERα expression, we transfected miR-22 mimics and control in MCF-7 and T47D cells. Anti-miR-22 and inhibitor control were also transfected into these cells for the purpose of comparison. Interestingly, we found that overexpression of miR-22 resulted in the significant reduction of NK1R-Tr (Fig. 2E, $p < 0.01$, NK1R-Tr in MCF-7; Fig. 2F, $p < 0.01$, NK1R-Tr in T47D) and ERα (Fig. 2E, $p < 0.01$, ERα in MCF-7; Fig. 2F, $p < 0.01$, ERα in T47D), while downregulation of miR-22 increased the expression of NK1R-Tr and ERα at both protein and mRNA levels (Fig. 2E, $p < 0.01$, ERα in MCF-7, NK1R-Tr in MCF-7; Fig. 2F, $p < 0.01$, ERα in T47D; NK1R-Tr in T47D).

Because ERα is an important transcription factor in breast cancer, we attempted to verify the possible relationship between ERα and NK1R-Tr. CHIP assay was employed to detect whether ERα was recruited to the ERE of NK1R-Tr promoter. As shown in Fig. 2G, we found that ERα was able to bound to the NK1R-Tr gene promoter with the presence of E2 (Fig. 2G). Moreover, we conducted a luciferase reporter assay between ERα and NK1R-Tr promoter. Wild-type and mutant promoter of NK1R-Tr were cloned into the upstream of the firefly luciferase coding region in a pGL3-basic luciferase reporter vector. Then the report vector with NK1R-Tr wild-type or mutant type was co-transfected with the ERα expression plasmid pcDNA3.0 into HEK293T cells to observe the luciferase activity. Our results showed that the relative luciferase activity was dramatically increased compared to the control groups (Fig. 2H, $p < 0.05$). These findings suggest that miR-22 has the ability to directly downregulate ERα and NK1R-Tr expressions through binding to their mRNA 3′UTR seed regions; meanwhile, ERα could upregulate NK1R-Tr expression.

3.3. ERα is involved in the regulation of miR-22 and NK1R-Tr

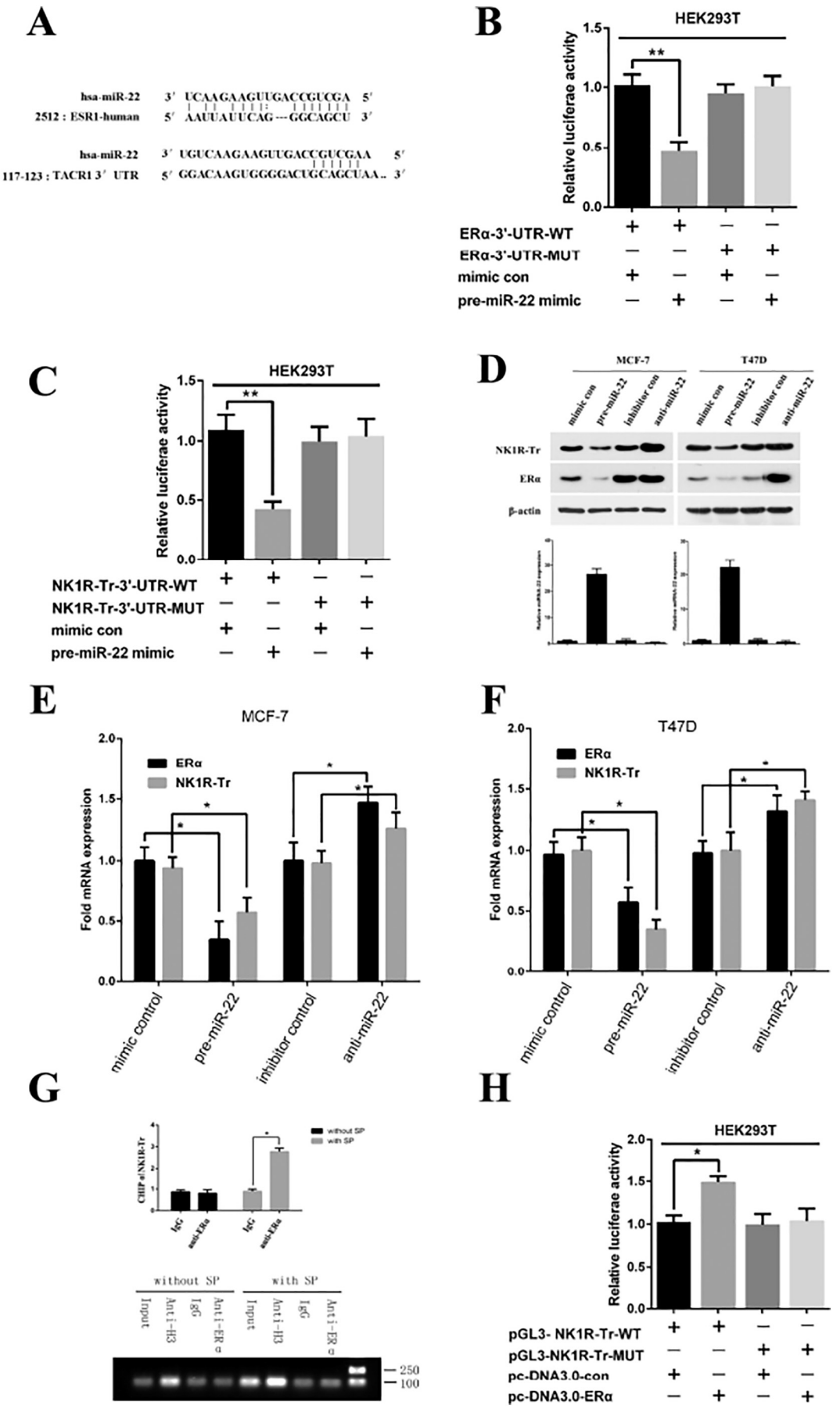
In order to confirm whether ERα acts as an intermediate link in the regulation between miR-22 and NK1R-Tr, we next overexpressed ERα and knocked down its expression in MDA-MB-231 and MCF-7 cells, respectively. The ERα expression plasmid pcDNA3.0 was transfected into MDA-MB-231 cells and ERα overexpressing clones were selected. Meanwhile, we transfected the plasmid PLKO.1 that connected to the shRNA of ERα in MCF-7cells to knockdown its expression. Western blotting results showed that there was a significantly increased expression of ERα in stable transfectants of MDA-MB-231-ERα cells and ERα expression had been effectively suppressed in ERα knockdown MCF-7 (MCF7-ERαI) cells compared with control cells (Fig. 3A). When overexpressing miR-22, the decreased degree of NK1R-Tr expression in ERα knockdown cells (MCF7-ERαI) was much lower than that in control cells, while when downregulating miR-22 with anti-miR-22, the increased degree of NK1R-Tr expression was also minimal (Fig. 3B). Compared to wild-type MDA-MB-231 cells, the action that miR-22 downregulated NK1R-Tr was more apparent in MDA-MB-231-ERα cells. Furthermore, when downregulating miR-22 with anti-miR-22, the increased degree of NK1R-Tr expression was also more outstanding (Fig. 3C). The results at the mRNA level were consistent with our western blot data (Fig. 3D, $p < 0.05$ pre-miR-22, $p < 0.05$ anti-miR-22; Fig. 3E, $p < 0.01$ pre-miR-22, $p < 0.05$ anti-miR-22). Overall, these works indicate that miR-22 is likely to have the priority to negatively regulate NK1R-Tr via ERα.

3.4. miRNA-22 inhibited the proliferation, migration, and invasion of breast cancer cells in vitro

To better understand the exact role of miR-22 in breast tumorigenesis, we empolyed miR-22 overexpression assays in both MCF-7 and MDA-MB-231 cells. Overexpression of miR-22 was confirmed by qRT-PCR (Fig. 4A). To determine the role of miR-22 in the proliferation of breast cancer cells in vitro, CCK-8 and colony-formation assay were performed with the presence of SP. The original proliferation ability of MCF-7 and MDA-MB-231 cells was weakened by overexpressing miR-22 compared with the control groups (Fig. 4B, $p < 0.05$ day 2, $p < 0.01$ day 3, $p < 0.01$ day 4, $p < 0.01$ day 5, $p < 0.01$ day 6; Fig. 4C, $p < 0.05$ day 2, $p < 0.01$ day 3, $p < 0.01$ day 4, $p < 0.01$ day 5, $p < 0.01$ day 6; Fig. 4D). Consistently, the migration ability of miR-22 overexpressing MCF-7 and MDA-MB-231 cells was both declined apparently (by 17% and 21% respectively) with the stimulation of SP in the wound healing test (Fig. 4E). The results of transwell assays demonstrated that miR-22 overexpression in MCF-7 and MDA-MB-231 cells considerably inhibited the invasive ability with the stimulation of SP compared to wild-type cells (Fig. 4F). The above three results were not statistically processed, and the significance of the difference could be seen qualitatively. Collectively, these results demonstrated that ectopic miR-22 depressed cell proliferation, migration and invasion in vitro notably.

3.5. Ectopic expression of NK1R-Tr restored the inhibitory effects of miR-22 on cell proliferation and invasion in breast cancer cells in vitro

To confirm whether downregulation of NK1R-Tr by miR-22 results in inhibition of proliferation, migration and invasion of breast cancer cells, we knocked down the endogenous expression of NK1R-Tr by small interfering RNAs (siRNAs) to simulate the effect of miR-22 overexpression. When the mRNA and protein levels of NK1R-Tr were significantly reduced by siRNA-mediated silencing in MCF-7 and MDA-MB-231 cells (Fig. 5A, $p < 0.01$ siNK1R-Tr#1, $p < 0.01$ siNK1R-Tr#2, in MCF-7; $p < 0.01$ siNK1R-Tr#1, $p < 0.01$ siNK1R-Tr#2, in MDA-MB-231; Fig. 5B), the number of colony and invasive cells were correspondingly reduced remarkably (Fig. 5C, $p < 0.01$ siNK1R-Tr#1, $p < 0.01$ siNK1R-Tr#2, in MCF-7; $p < 0.01$ siNK1R-Tr#1, $p < 0.01$ siNK1R-Tr#2, in MDA-MB-231; Fig. 5D, $p < 0.01$ siNK1R-Tr#1, $p < 0.01$ siNK1R-Tr#2, in MCF-7; $p < 0.01$ siNK1R-Tr#1, $p < 0.01$ siNK1R-Tr#2, in MDA-MB-231), suggesting that the inhibitory effects of miR-22 on cells proliferation, migration and invasion could, at least partially, act through its inhibition on NK1R-Tr. Meanwhile, we evaluated the effects of overexpression of NK1R-Tr protein by a pcDNA3.0-



(caption on next page)

Fig. 2. miR-22 downregulated NK1R-Tr and ERα expressions and ERα upregulated the expression of NK1R-Tr. **A** Discovery of potential miR-22 Target Sites in the 3′-UTR of ERα and NK1R-Tr according to bioinformatics analysis. **B**, **C** Relative luciferase activity in HEK-293 cells. **D** Protein levels of NK1R-Tr and ERα in MCF-7 and T47D cells transfected with pre-miR-22 mimic or mimic control, anti-miR-22 or inhibitor control. **E**, **F** Expression of NK1R-Tr and ERα at mRNA level in MCF-7 and T47D cells transfected with pre-miR-22 mimic or mimic control, anti-miR-22 or inhibitor control; **G** CHIP assay between ERα and NK1R-Tr promoter region. **H** Relative luciferase activity of pGL3-NK1R-Tr-WT and pGL3-NK1R-Tr-MUT in HEK-293 cells co-transfected with pcDNA3.0-ERα. $*p < 0.05$ vs. normal control, $**p < 0.01$ vs. normal control. Each experiment was repeated three times independently. The loading control of Western Blot is 20 μg protein from the cell lysate. And the antibody is against β-actin.

NK1R-Tr vector. Our data showed that pcDNA3.0-NK1R-Tr was able to significantly increase the expression of NK1R-Tr at both mRNA and protein levels (Fig. 5E, $p < 0.01$ pcDNA3.0-NK1R-Tr, in MCF-7; $p < 0.01$ pcDNA3.0-NK1R-Tr, in MDA-MB-231; Fig. 5F), and also ectopic expression of NK1R-Tr could promote cell proliferation and invasion (Fig. 5G, $p < 0.01$ pcDNA3.0-NK1R-Tr, in MCF-7; $p < 0.01$ pcDNA3.0-NK1R-Tr, in MDA-MB-231; Fig. 5H, $p < 0.01$ pcDNA3.0-NK1R-Tr, in MCF-7; $p < 0.01$ pcDNA3.0-NK1R-Tr, in MDA-MB-231).

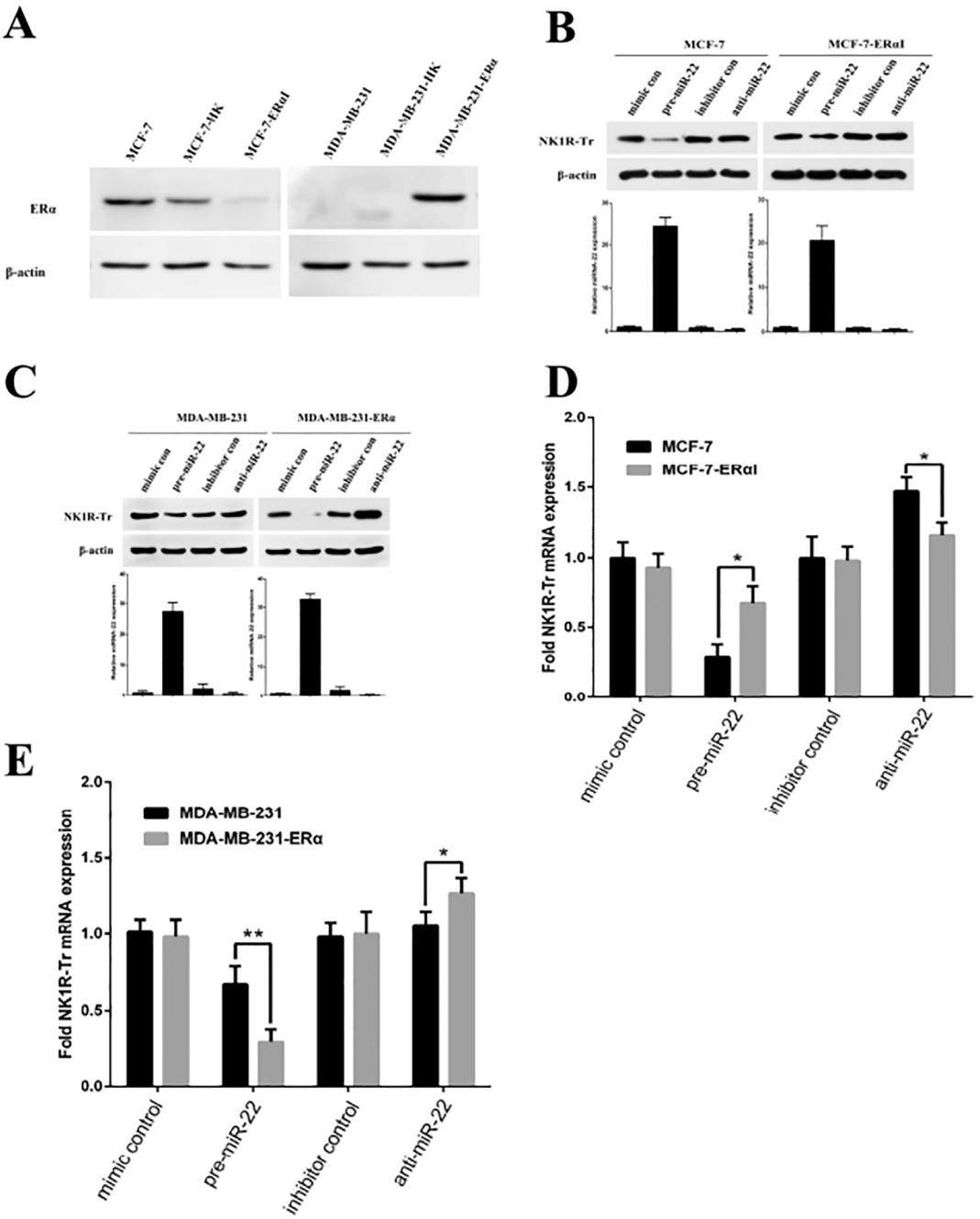


Fig. 3. Transfection and identification of MCF-7 and MDA-MB-231 cells, ERα enhanced the downregulated effect of pre-miR-22 on NK1R-Tr. **A** Western blotting for ERα in MCF-7 and MDA-MB-231 cells. **B**, **C** Western blotting assay for NK1R-Tr in MCF-7 cells and MDA-MB-231 cells. **D**, **E** Expression of NK1R-Tr at mRNA level, assessed by real-time PCR in the MCF-7 cells that were untreated or knocked out ERα and the MDA-MB-231 cells that were transfected or untreated ERα. $*p < 0.05$ vs. normal control. Each experiment was repeated three times independently. The loading control of Western Blot is 20 μg protein from the cell lysate. And the antibody is against β-actin.

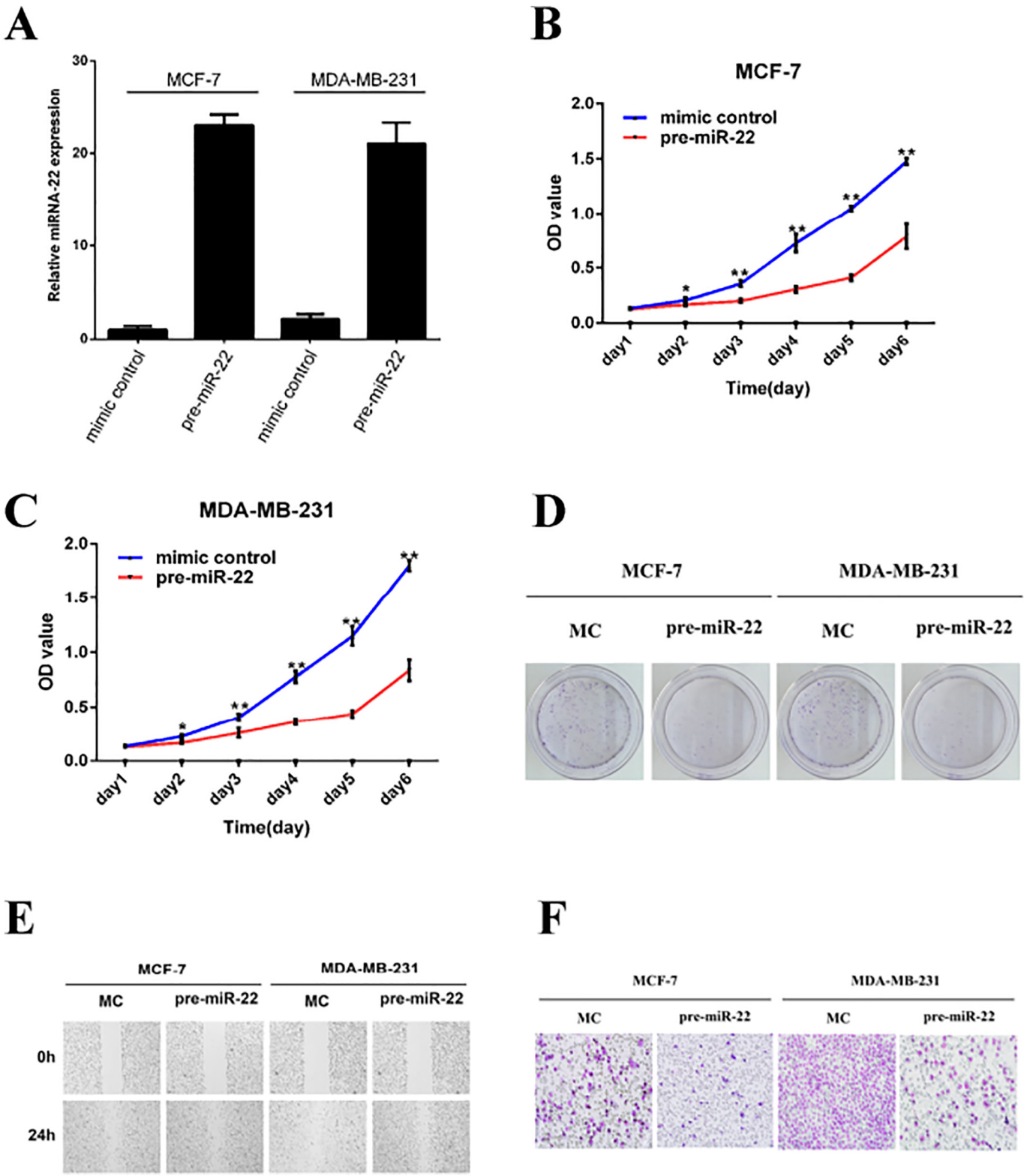
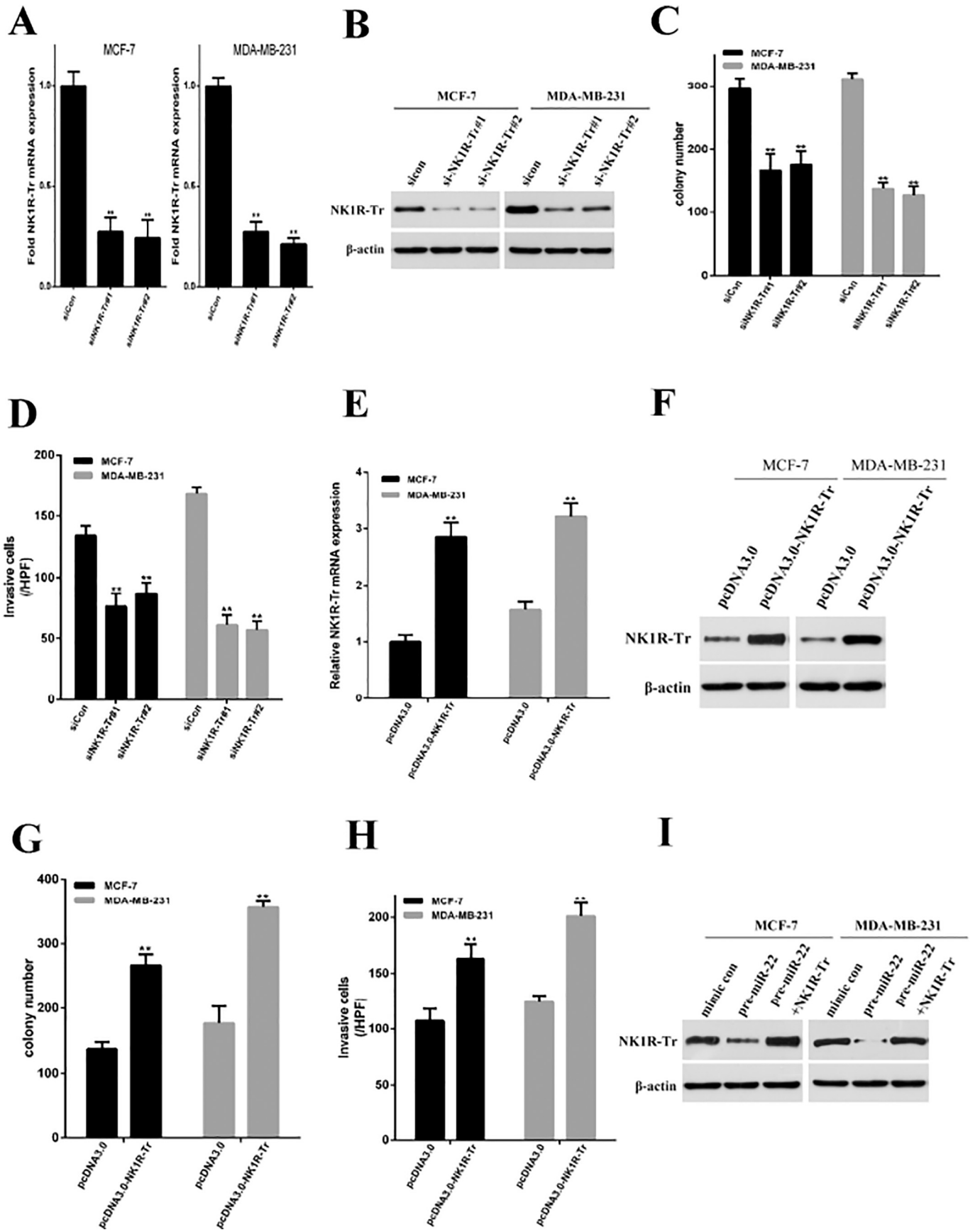


Fig. 4. miR-22 inhibited the proliferation, migration, and invasion of breast cancer cells in vitro. **A** High expression of miR-22 in MCF-7 and MDA-MB-231cells. **B**, **C** The cell counting kit-8 assay was performed in MCF-7 and MDA-MB-231 cells. **D** Representative photomicrographs of colony formation assay of MCF-7cells and MDA-MB-231cells. **E** Wound healing assay operated on MCF-7 and MDA-MB-231cells. **F** Transwell assay in MCF-7cells and MDA-MB-231cells. Data are presented as means ± SD from three independent experiments. $*p < 0.05$ vs. normal control, $**p < 0.01$ vs. normal control.

Moreover, we co-transfected miR-22 and NK1R-Tr into MCF-7 and MDA-MB-231 cells to test whether the overexpression of NK1R-Tr could reverse the inhibitory effects of miR-22 on migration and invasion of breast cancer cells. As predicted, NK1R-Tr expression was markedly decreased in breast cancer cells after the transfection of miR-22, and was restored when cells were co-transfected with pcDNA3.0- NK1R-Tr and miR-22 mimics (Fig. 5I). These findings demonstrate that miR-22 inhibits migration and invasion of breast cancer via the miR-22/NK1R-



(caption on next page)

Fig. 5. Ectopic expression of NK1R-Tr restored the inhibitory effects of miR-22 on cell proliferation and invasion in breast cancer cells in vitro. **A**, **B** The low expression of NK1R-Tr at mRNA and protein levels in both MCF-7 cells and MDA-MB-231 cells. **C**, **D** Inhibition of cell proliferation and invasion by knockdown of NK1R-Tr. **E**, **F** The high expression of NK1R-Tr at mRNA and protein levels in both MCF-7 cells and MDA-MB-231 cells. **G**, **H** Promotion of cell proliferation and invasion by overexpression of NK1R-Tr. **I** Protein expression of NK1R-Tr in MCF-7 and MDA-MB-231 cell lines transfected with miR-MC, pre-miR-22, or co-transfected with pre-miR-22 and pcDNA3.0-NK1R-Tr. Data are presented as means ± SD from three independent experiments. $^{**}p < 0.01$ vs. normal control. Each experiment was repeated three times independently. The loading control of Western Blot is 20 μg protein from the cell lysate. And the antibody is against β-actin.

Tr signaling axis.

3.6. NK1R-Tr transmitted its regulatory signals via the classic ERK1/2 pathway

Our previous study revealed that 5 min after the NK1R-Tr activation with the stimulation of SP, phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) began to increase gradually until 120 min. To study whether miR-22 regulates the biological behaviors of breast cancer cells in the same manner via ERK1/2 signal pathway by targeting NK1R-Tr, we examined the expression level of phosphorylation ERK1/2 in MCF-7 and MDA-MB-231 cells. In both wild types, the phosphorylation of ERK1/2 was detected approximately 30 min after the stimulation of SP, and this signal increased continuously and had no sign of declining before 150 min after the stimulation (Fig. 6A, C). Next, we treated both cell lines with the NK1R antagonist GR73632 for 30 min to explore whether the SP-dependent increase of phosphorylation level of ERK1/2 was specifically mediated by NK1R receptor. Expectedly, the SP-dependent increase in phosphorylation of ERK1/2 was prevented in both cell lines after treatment with GR73632, with no effect on the total cellular ERK1/2 levels (Fig. 6A, C). In addition, in MCF-7 cells transfected with pre-miR-22, a noticeable delay and reduction of phosphorylation of ERK1/2 was observed compared to the control group after the stimulation of SP. The similar result was also found in MDA-MB-231 cells (Fig. 6B, D). These results suggest that NK1R-Tr may promote cancer cell proliferation by sustainedly activating ERK1/2, while miR-22, as a tumor suppressor, may inhibit this process by targeting NK1R-Tr.

3.7. miR-22 inhibited the growth of MDA-MB-231 and MCF-7 engrafted tumors and repressed the distal pulmonary metastases in vivo

To further demonstrate the contribution of miR-22 in the NK1R-Tr mediated breast tumorigenesis and metastasis, we transplanted miR-22 overexpressing MDA-MB-231 and MCF-7 cells into scid mice via either subcutaneous injection or tail vein injection, and monitored the physical state and tumor growth in the xenograft. The tumor xenograft studies revealed that the volume of tumors resulting from miR-22-MDA-MB-231 and miR-22-MCF-7 injection were significantly smaller than those resulting from the mimic control groups (Fig. 7A). As for distant metastases, mice injected with miR-22-MDA-MB-231 and miR-22-MCF-7 cells exhibited much less distant lung metastases compared to mimic control groups (Fig. 7B), which was further confirmed by the H&E stained sections (Fig. 7C, D, $p < 0.01$ for MDA-MB-231, $p < 0.01$ for MCF-7). Together, these data suggest that miR-22 inhibits tumorigenesis of MDA-MB-231 and MCF-7 cells and represses the distal pulmonary metastases in vivo, and NK1R-Tr may be an important target involved in this process.

4. Discussion

NK1R-Tr coupling to SP plays an imperative role as an autocrine/paracrine growth factor in many cancers, and ERα plays a fatal role in tumor occurrence and progression. Thus far, it remains ambiguous whether these two tumor control factors are functionally relevant for co-regulating tumor formation. miRNAs have multiple expression forms in various cell types, and it is well accepted that miRNAs regulate many

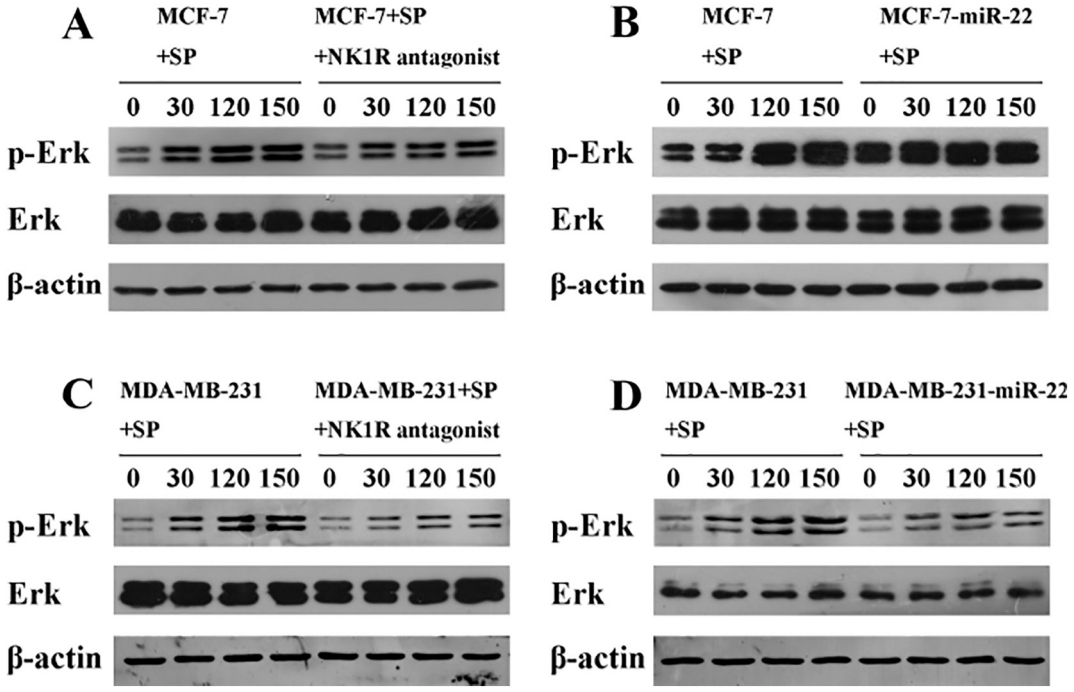


Fig. 6. NK1R-Tr transmitted its regulational signals via the classic ERK1/2 passway. **A**, **C** The MCF-7 and MDA-MB-231 cells were treated with SP for different time intervals with and without incubation of NK1R antagonist. **B**, **D** The MCF-7 and MDA-MB-231 cells were treated with SP for different time intervals with and without the co-transfection of pre-miR-22. The results were representative of three independent western blot assays. Each experiment was repeated three times independently. The loading control of Western Blot is 20 μg protein from the cell lysate. And the antibody is against β-actin.

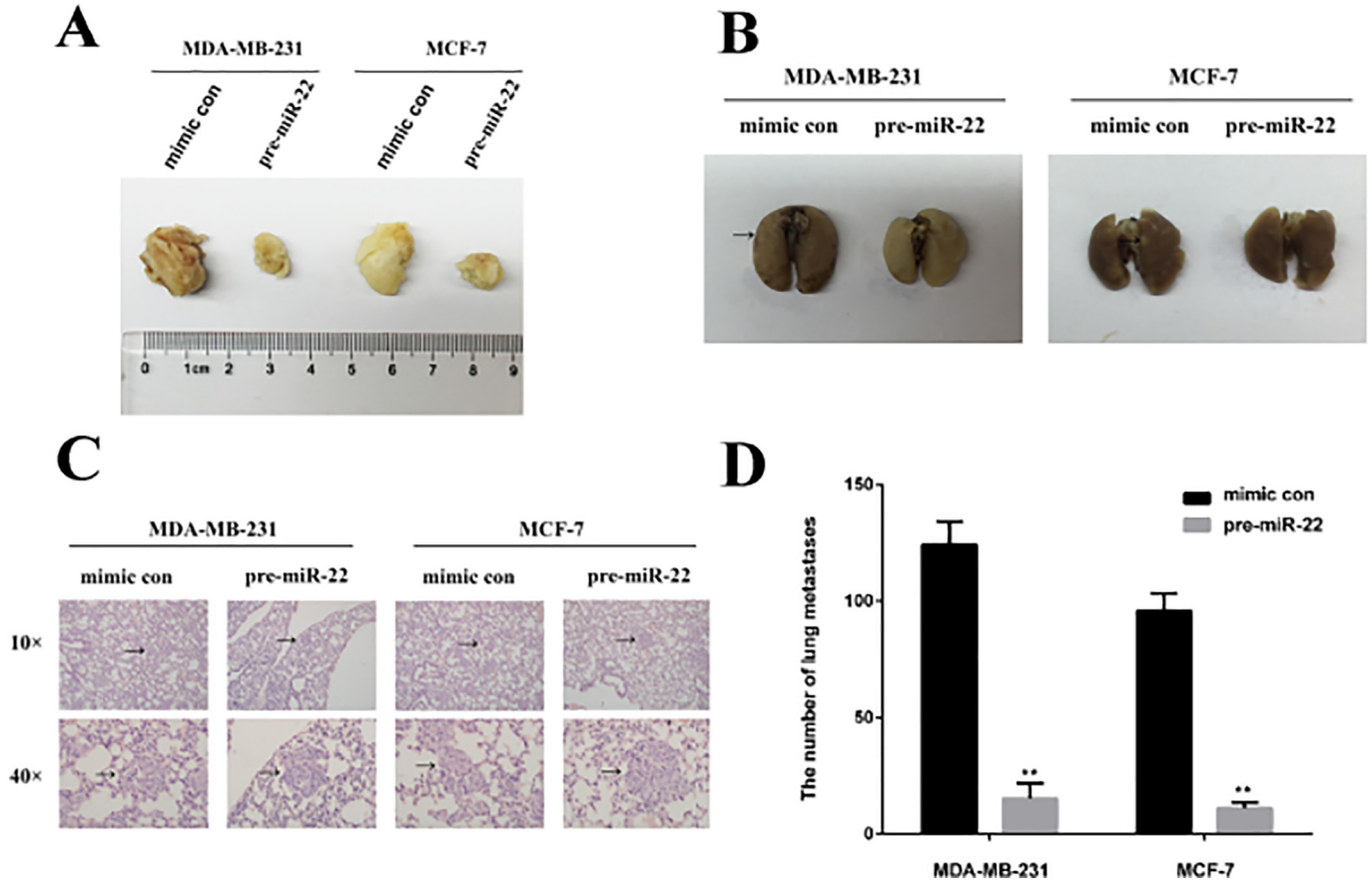


Fig. 7. miR-22 inhibited the tumor growth of MDA-MB-231 and MCF-7 engrafted and repressed the distal pulmonary metastases in vivo. **A** Photographs of tumors harvested from mice injected with MDA-MB-231 and MCF-7cells transfected with mimic control or miR-22 mimics. **B** Representative tumor nodules in the lungs of scid mice injected miR-22-MDA-MB-231 and miR-22- MCF-7 cells and the respective control group of each. **C** H&E staining of lung tissues isolated from the scid mice (n = 6), The metastasis nodules are indicated by arrows. **D** The statistical results of the metastases in mice. Data are presented as means ± SD from three independent experiments. $^{**}p < 0.01$ vs. normal control.

pathological and physiological processes. Therefore, clarifying the regulatory network between these three aspects will provide theoretical and practical guidance for the next step of breast cancer treatment.

Previous studies have demonstrated that miR-22, both as a tumor suppressor and promoter, functioned in multiple cellular processes, including proliferation, differentiation, senescence and apoptosis, and its deregulation is a hallmark of human cancers [30]. In all the earlier observations, Kong, L.M., et al. [31] demonstrated the loss-of-function analyses by silencing the miR-22 in MCF-7 cells resulted in the enhanced cell invasion and migration compared with control cells ($p < 0.05$). Their wound healing assay also indicated that miR-22 downexpression can significantly promote cell motility compared with control group. miR-22 inhibitor treated MCF-7 cells showed higher proliferative capacities than control cells by MTT assay ($p < 0.001$). As a contrast, when they overexpressed miR-22 in MDA-MB-231cells, the protein expression of CD147 was reduced, and the cell migration was also inhibited compared to the control group. In vivo experiment, their mice injected with miR-22 mimics exhibited little increase in the GFP fluorescence signal of primary tumor during the same observation period. The results were in accordance with ours. Yang et al. [32] showed that miR-22 had a tumor-suppressive effect by repressing cyclin A2 expression. Their data exhibited the reduced miR-22 level was along with the increased protein level of CCNA2, which were of hepatic and colonic origin. Ling et al. [33] reported that miR-22 suppressed lung cancer cell progression via post-transcriptional regulation of epidermal growth factor receptor 3. Their data showed that ErbB3 is a direct target of miR-22, and at 48 h after miR-22 transfection, the over-expression of miR-22 could significantly inhibit the cellular numbers by 70% for A549 cells and 60% for H1229 cells, respectively. And the apoptosis is an important cause for the reduction of cell number.

In contrast, it was documented that miR-22 suppressed DNA repair and promoted genomic instability by targeting MDC1, leading to detrimental chromosomal abnormalities and establishing a cellular environment fostering tumorigenesis and cancer progression in colon and liver cancer [34]. Jung-Hee Lee Let al. found that MDC1 is crucial component of the DNA damage response (DDR) machinery and ensures assembly of the DDR protein at the DNA damage sites, and therefore loss of MDC1 results in genomic instability and tumorigenicity. They made the further confirmation that miR-22 can downregulate MDC1 at both mRNA level and protein levels. Their study adds to the polyhedral nature of miR-22 in tumor regulatory networks.

Most importantly, our results suggest that the inverse regulation between miR-22 and NK1R-Tr was more significant with the existence of ERα. Our CHIP assay supported this point because the activated ERα can promote the transcription and expression of NK1R-Tr by combining to its ERE in the form of homodimers or heterodimers. This regulation indicates that there is a cross-talk between the endocrine system and the neural control system represented by ERα and NK1R, respectively. Similarly, ERα upregulates c-Myc expression by binding to its ERE, and the high-level expression of c-Myc would then downregulate ERα via a negative autoregulatory feedback loop [35]. Our previous study showed that high NK1R-Tr expression has a positive correlation with the advanced malignancy of breast cancer cells, the reason for which may be due to the impairment of its desensitization and internalization [36–38]. Our existing results show that NK1R-Tr promotes cancer progression by extending and enhancing the activation of ERK and that

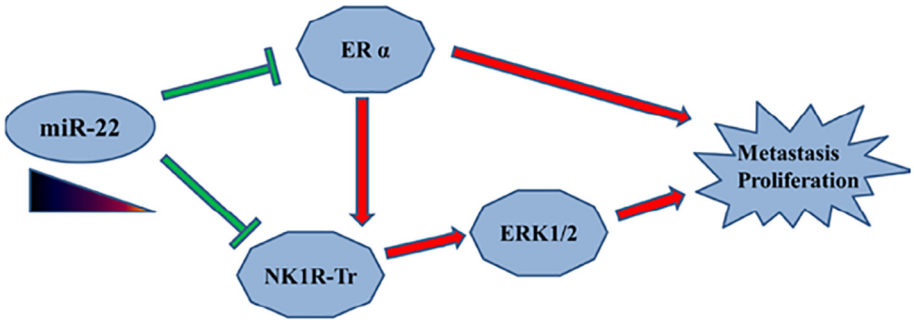


Fig. 8. A schematic model depicting miR-22 downregulation promoted proliferation and metastasis in breast cancer cells.

miR-22 inhibits this process. Further, NK1R-Tr is involved in other signaling pathways, such as the epidermal growth factor receptor (EGFR) and the mitogen-activated protein kinases (MAPK), in which NK1R-Tr regulates DNA synthesis [1,39]. Moreover, the anti-apoptotic effect of NK1R-Tr involves the activation of the anti-apoptotic molecule Akt (protein kinase B) mediated by the Janus kinase 2 (JAK-2) and phosphoinositide 3-kinase (PI3K). According to the most recent report, NK1R could mediate migration by increasing the expression of MMP-2 and MMP-14 in breast cancer cells and MMP-2 and MT1-MMP in melanoma cells.

In summary, we showed that miR-22 is a potential tumor suppressor in breast cancer and that miR-22 downregulation promotes breast cancer cell invasion and metastasis by upregulating ERα and NK1R-Tr. This will delay and weaken the activation of ERK1/2 and then induce malignant behavior (Fig. 8). To our best knowledge, this is the first study to demonstrate that the miR-22/ERα/NK1R-Tr axis regulates the proliferation, migration and invasion of breast cancer cells. These findings provide a better understanding of the development and progression of breast cancer and may have significant implications for the future therapy of breast cancer.

4.1. Statement of human rights

The studies have been approved by the appropriate institutional research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

4.2. Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2018.11.057.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (81201653) to Yunli Zhou, the National Natural Science Foundation of China (81502519) to DongDong, the National Natural Science Foundation of China (3150109) to JieShao, the Natural Science Foundation of Tianjin (16JCYBJC26000) to Yunli Zhou.

Competing interest

The authors have declared that no competing interest exists.

References

[1] Alireza Mashaghi, Anna Marmalidou, Mohsen Tehrani, et al., Neuropeptide substance P and the immune response, Cell. Mol. Life Sci. 73 (22) (2016) 1–16.
[2] M.S. Steinhoff, M.B. Von, P. Geppetti, et al., Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease, Physiol. Rev. 94 (1) (2014) 265–301.
[3] M. Miguel, C. Rafael, Neurokinin-1 receptor antagonists as antitumor drugs in gastrointestinal cancer: a new approach, Saudi J. Gastroenterol. 22 (4) (2016) 260–268.
[4] M. Ilmer, A. Garnier, J. Vykoukal, E. Alt, D. von Schweinitz, R. Kappler, et al., Targeting the neurokinin-1 receptor compromises canonical Wnt signaling in hepatoblastoma, Mol. Cancer Ther. 14 (12) (2015) 2712–2721, https://doi.org/10.1158/1535-7163.MCT-15-0206.
[5] S. Spitsin, V. Pappa, S.D. Douglas, Truncation of neurokinin-1 receptor—negative regulation of substance P signaling, J. Leukoc. Biol. 103 (2018) 1043–1051.
[6] M.S. Steinhoff, M.B. Von, P. Geppetti, et al., Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease, Physiol. Rev. 94 (1) (2014) 265–301.
[7] S. Garciarecio, P. Gascón, Biological and pharmacological aspects of the NK1-receptor, Biomed. Res. Int. 2015 (1) (2015) 495704.
[8] L. Caberlotto, Y.L. Hurd, P. Murdock, et al., Neurokinin 1 receptor and relative abundance of the short and long isoforms in the human brain, Eur. J. Neurosci. 17 (9) (2015) 1736–1746.
[9] A. Garnier, M. Ilmer, K. Becker, B. Haberle, D. Von Schweinitz, R. Kappler, et al., Truncated neurokinin-1 receptor is an ubiquitous antitumor target in hepatoblastoma, and its expression is independent of tumor biology and stage, Oncol. Lett. 11 (1) (2016) 870–878, https://doi.org/10.3892/ol.2015.3951.
[10] B.K. Liu, X.W. Jin, H.Z. Lu, et al., The effects of neurokinin-1 receptor antagonist in an experimental autoimmune cystitis model resembling bladder pain syndrome/interstitial cystitis, Inflammation (2018) 1–9.
[11] H. Zhang, A.K. Kanduluru, P. Desai, et al., Synthesis and evaluation of a novel $^{64}Cu/^{67}Ga$-labeled neurokinin 1 receptor antagonist for in vivo targeting of NK1R-positive tumor xenografts, Bioconjug. Chem. 29 (4) (2018) 1319.
[12] Y. Yu, W. Zhu, Q. Liang, et al., Tropisetron attenuates lipopolysaccharide induced neuroinflammation by inhibiting NF-κB and SP/NK1R signaling pathway, J. Neuroimmunol. 320 (2018) 80–86.
[13] M. Munoz, M. Berger, M. Rosso, A. Gonzalez-Ortega, A. Carranza, R. Covenas, Antitumor activity of neurokinin-1 receptor antagonists in MG-63 human osteosarcoma xenografts, Int. J. Oncol. 44 (1) (2014) 137–146, https://doi.org/10.3892/ijo.2013.2164.
[14] U. Hamann, C. Ankel, Breast cancer: diagnostics and therapy - the most important facts for internists, Dtsch. Med. Wochenschr. 143 (4) (2018) 267.
[15] J.Y. Ho, F.W. Chang, F.S. Huang, J.M. Liu, Y.P. Liu, S.P. Chen, et al., Estrogen enhances the cell viability and motility of breast cancer cells through the ERα-ΔNp63-integrin β4 signaling pathway, PLoS One 11 (2) (2016) e0148301, , https://doi.org/10.1371/journal.pone.0148301.
[16] U.H. Park, M.R. Kang, E.J. Kim, Y.S. Kwon, W. Hur, S.K. Yoon, et al., ASXL2 promotes proliferation of breast cancer cells by linking ERalpha to histone methylation, Oncogene (2015), https://doi.org/10.1038/onc.2015.443.
[17] S. Tang, Q. Yin, J. Su, et al., Inhibition of metastasis and growth of breast cancer by pH-sensitive poly (β-amino ester) nanoparticles co-delivering two siRNA and paclitaxel[J], Biomaterials 48 (2015) 1–15.
[18] S.H. Wang, S.Y. Lin, Tumor dormancy: potential therapeutic target in tumor recurrence and metastasis prevention, Exp. Hematol. Oncol. 2 (1) (2013) (2013-10-16). (2 (1): 29);
X.H. Zhang, M. Giuliano, M.V. Trivedi, R. Schiff, C.K. Osborne, Metastasis dormancy in estrogen receptor-positive breast cancer, Clin. Cancer Res. 19 (23) (2013) 6389–6397, https://doi.org/10.1158/1078-0432.CCR-13-0838.
[19] R. Xiong, H.K. Patel, L.M. Gutgesell, J. Zhao, L. Delgado-Rivera, T.N. Pham, et al., Selective human estrogen receptor partial agonists (ShERPAs) for tamoxifen-resistant breast cancer, J. Med. Chem. 59 (1) (2016) 219–237, https://doi.org/10.1021/acs.jmedchem.5b01276.
[20] J. Liu, G.R. Sareddy, M. Zhou, et al., Differential effects of estrogen receptor beta isoforms on glioblastoma progression, Cancer Res. 78 (2018) 3176–3189 (canres.3470.2017).
[21] H.Y. Wu, P. Zheng, G. Jiang, et al., A modulator based regulatory network for ERα signaling pathway, BMC Genomics 13 (S6) (2012) S6.
[22] J. Xiong, D. Yu, N. Wei, H. Fu, T. Cai, Y. Huang, et al., An estrogen receptor alpha suppressor, microRNA-22, is downregulated in estrogen receptor alpha-positive

human breast cancer cell lines and clinical samples, FEBS J. 277 (7) (2010) 1684–1694, https://doi.org/10.1111/j.1742-4658.2010. 07594.x.
[23] M.A. Bermisheva, Z.R. Takhirova, I.R. Gilyazova, et al., MicroRNA biogenesis pathway gene polymorphisms are associated with breast cancer risk, Russ. J. Genet. 54 (5) (2018) 568–575.
[24] H. Liu, X. Huang, T. Ye, MiR-22 down-regulates the proto-oncogene ATP citrate lyase to inhibit the growth and metastasis of breast cancer, Am. J. Transl. Res. 10 (3) (2018) 659.
[25] J.K. Parrish, M. Sechler, R.A. Winn, P. Jedlicka, The histone demethylase KDM3A is a microRNA-22-regulated tumor promoter in Ewing Sarcoma, Oncogene 34 (2) (2015) 257–262, https://doi.org/10.1038/onc.2013.541.
[26] Y.Y. Tang, X.P. Liu, B. Su, Z.W. Zhang, X. Zeng, Y.P. Lei, et al., microRNA-22 acts as a metastasis suppressor by targeting metadherin in gastric cancer, Mol. Med. Rep. 11 (1) (2015) 454–460, https://doi.org/10.3892/mmr.2014.2682.
[27] M.L. Slattery, L.E. Mullany, L. Sakoda, et al., The NF-κB signalling pathway in colorectal cancer: associations between dysregulated gene and miRNA expression, J. Cancer Res. Clin. Oncol. 144 (2) (2018) 269–283.
[28] S.N. Lone, R. Maqbool, F.Q. Parray, et al., Triose-phosphate isomerase is a novel target of miR-22 and miR-28, with implications in tumorigenesis, J. Cell. Physiol. 233 (11) (2018) 8919–8929.
[29] X. Zhang, Y. Li, D. Wang, et al., miR-22 suppresses tumorigenesis and improves radiosensitivity of breast cancer cells by targeting Sirt1, Biol. Res. 50 (1) (2017) 27.
[30] F. Yang, Y. Hu, H.X. Liu, Y.J. Wan, MiR-22-silenced cyclin A expression in colon and liver cancer cells is regulated by bile acid receptor, J. Biol. Chem. 290 (10) (2015) 6507–6515, https://doi.org/10.1074/jbc.M114.620369.
[31] B. Ling, G.X. Wang, G. Long, J.H. Qiu, Z.L. Hu, Tumor suppressor miR-22 suppresses lung cancer cell progression through post-transcriptional regulation of ErbB3, J. Cancer Res. Clin. Oncol. 138 (8) (2012) 1355–1361, https://doi.org/10.1007/s00432-012-1194-2.
[32] J.H. Lee, S.J. Park, S.Y. Jeongl, M.J. Kim, S. Jun, H.S. Lee, et al., MicroRNA-22 suppresses DNA repair and promotes genomic instability through targeting of MDC1, Cancer Res. 75 (7) (2015) 1298–1310, https://doi.org/10.1158/0008-5472.CAN-14-2783.
[33] L. Castellano, G. Giamas, J. Jacob, R.C. Coombes, W. Lucchesi, P. Thiruchelvam, et al., The estrogen receptor-alpha-induced microRNA signature regulates itself and its transcriptional response, Proc. Natl. Acad. Sci. U. S. A. 106 (37) (2009) 15732–15737, https://doi.org/10.1073/pnas.0906947106.
[34] Y. Zhou, L. Zhao, T. Xiong, X. Chen, Y. Zhang, M. Yu, et al., Roles of full-length and truncated neurokinin-1 receptors on tumor progression and distant metastasis in human breast cancer, Breast Cancer Res. Treat. 140 (1) (2013) 49–61, https://doi.org/10.1007/s10549-013-2599-6.
[35] H. Li, S.E. Leeman, B.E. Slack, G. Hauser, W.S. Saltsman, J.E. Krause, et al., A substance P (neurokinin-1) receptor mutant carboxyl-terminally truncated to resemble a naturally occurring receptor isoform displays enhanced responsiveness and resistance to desensitization, Proc. Natl. Acad. Sci. U. S. A. 94 (17) (1997) 9475–9480.
[36] J.P. Lai, W.Z. Ho, L.E. Kilpatrick, X. Wang, F. Tuluc, H.M. Korchak, et al., Full-length and truncated neurokinin-1 receptor expression and function during monocyte/macrophage differentiation, Proc. Natl. Acad. Sci. U. S. A. 103 (20) (2006) 7771–7776, https://doi.org/10.1073/pnas.0602563103.
[37] I. Castagliuolo, L. Valenick, J. Liu, C. Pothoulakis, Epidermal growth factor receptor transactivation mediates substance P-induced mitogenic responses in U-373 MG cells, J. Biol. Chem. 275 (34) (2000) 26545–26550, https://doi.org/10.1074/jbc.M003990200.
[38] H.W. Koon, D.Z. Zhao, X. Na, M.P. Moyer, C. Pothoulakis, Metalloproteinases and transforming growth factor-alpha mediate substance P-induced mitogen-activated protein kinase activation and proliferation in human colonocytes, J. Biol. Chem. 279 (44) (2004) 45519–45527, https://doi.org/10.1074/jbc.M408523200.
[39] H.W. Koon, D.Z. Zhao, Y.N. Zhan, M.P. Moyer, C. Pothoulakis, Substance P mediates antiapoptotic responses in human colonocytes by Akt activation, Proc. Natl. Acad. Sci. U. S. A. 104 (6) (2007) 2013–2018, https://doi.org/10.1073/pnas.0610664104.