



# LncRNA XIST interacts with miR-454 to inhibit cells proliferation, epithelial mesenchymal transition and induces apoptosis in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is a one of the subtypes of breast cancer which accounts for approximately 10–20% of all breast cancers. LncRNA XIST (XIST) is reported to be dysfunctional in numerous tumor types and is involved in the key pathways of cancer initiation, progression and metastasis. Thus, in the present study, we explored the detailed molecular mechanism of XIST in TNBC. XIST was down-regulated in TNBC tissues and cell lines. Overexpressed XIST inhibited cell proliferation, epithelial mesenchymal transition (EMT) and induced apoptosis *in vitro* as well as suppressed TNBC tumor growth *in vivo*. MicroRNA (miR)-454 was up-regulated in TNBC tissues and cell lines. Knockdown of miR-454 inhibited TNBC progression by suppressing cell proliferation, EMT and inducing cell apoptosis. Moreover, miR-454 was predicted and confirmed to be a target of XIST, and rescue assay indicated that overexpressed miR-454 could reverse XIST restoration mediated-anti-tumor effects on TNBC cells. In conclusion, XIST interacts with miR-454 to inhibit cells proliferation, EMT and induce apoptosis in TNBC, indicating a promising treatment strategy for TNBC patients.

**Keywords.** Apoptosis; EMT; miR-454; TNBC; XIST

**Abbreviations:** TNBC, triple-negative breast cancer; qRT-PCR, quantitative real-time polymerase chain reaction; EMT, epithelial mesenchymal transition; ER, estrogen receptor; HER2, human epidermal growth factor-2; lncRNA, long non-coding RNA; XIST, inactivate-specific transcript; FBS, fetal bovine serum; NC, negative control

## 1. Introduction

Triple-negative breast cancer (TNBC) is a one of the subtypes of breast cancer, defined by negative for the estrogen receptor (ER), progesterone receptor (PR) and excess human epidermal growth factor-2 (HER2) protein, accounting for approximately 10–20% of all breast cancers (Savci-Heijink *et al.* 2015; Li *et al.* 2017). The typical features of TNBC are : it is more aggressive, has poorer prognosis and higher grade, and is more likely to spread and have recurrence compared to other subtypes of breast cancer (Wang *et al.* 2015; Navratil *et al.* 2015; Kumar and Aggarwal 2016). Due

to the tumor cells lacking the necessary receptors, common treatments like hormone therapy and drugs that target estrogen, progesterone, and HER-2 are ineffective. Although some medicines are used to successfully treat TNBC, finding certain medications that can interfere with the processes that cause TNBC to grow is still urgent and is of intense interest.

Long non-coding RNA (lncRNA) is a type of RNA molecule with a transcript length of more than 200 nucleotides (Liu and Ding 2017). It does not encode proteins, but it regulates the expression of the gene such as epigenetic regulation, transcriptional regulation, and post-transcriptional regulation (Jarroux *et al.*

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2017). lncRNAs are reported to be dysfunctional in numerous tumor types, such as lung cancer, prostate cancer, breast cancer and TNBC, and are involved in the key pathways of cancer initiation, progression and metastasis, which can play an oncogenic or tumor suppressor role (Peng *et al.* 2017), and the idea has been consolidated that the expression of lncRNAs have linked to the activation of factors such as p53, c-MYC, E2F, estrogen receptor or androgen receptor (Huarte 2015). In TNBC, several lncRNAs are identified as tumor-driving oncogenic lncRNAs and a few are identified as tumor suppressive lncRNAs: they are all particularly involved in cancer progression (Liu *et al.* 2015). The X inactivate-specific transcript (XIST), the 19 Kb long lncRNA (17 kb in mouse), is vital for the X-chromosome inactivation in embryogenic development (Penny *et al.* 1996). XIST has been elucidated the tumor suppressor role in breast cancer (Liu *et al.* 2015). However, the regulatory functions and molecular mechanisms of XIST on TNBC remain unclear.

MicroRNAs (miRNAs) is a class of small endogenous non-coding RNAs molecules (approximately 22 nt), stimulating translational repression or degradation and playing a vital role in the progress of gene expression (Zhou *et al.* 2016). Among several candidate miRNAs regulating cell progression, numerous evidences have reported that microRNA-454 (miR-454) expression was associated with tumor growth and dissemination in many cancers (Fan *et al.* 2017; Fu *et al.* 2018). In TNBC, miR-454 is involved in poor prognosis and may function as an oncogene via targeting AKT in TNBC (Li *et al.* 2017a, b; Cao *et al.* 2016). While, how miR-454 works remains indistinct yet.

In the present study, we found XIST was down-regulated in TNBC, and overexpressed XIST inhibited TNBC cell proliferation, epithelial mesenchymal transition (EMT) and induced apoptosis *in vitro* and suppressed tumor growth *in vivo*. Moreover, XIST directly bound to miR-454; XIST exerted anti-tumor effects via direct interaction with miR-454. Our study firstly identified the regulatory network of XIST/miR-454 axis in TNBC progression, which may shed new light on the metastasis and therapies in TNBC.

## 2. Materials and methods

### 2.1 Patients and specimens

A total of 35 primary TNBC tissues and adjacent normal tissues were obtained from the Suzhou

Wuzhong People's Hospital between September 2017 and January 2018. All specimens were confirmed by two independent pathologists and did not receive any medical treatment before surgery. Specimens were immediately stored at  $-80^{\circ}\text{C}$  until experiment. The hospitals and all patients had signed informed consents and the project was approved by the Ethics Committee of the Suzhou Wuzhong People's Hospital.

### 2.2 Cell culture and transfection

All human TNBC cell lines MDA-MB-468 and MDA-MB-231 were obtained from American Type Culture Collection (Manassas, VA, USA). The normal breast epithelial cell line MCF-10 A was purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). MDA-MB-468 and MDA-MB-231 cells were cultured in DMEM (Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS) (Gibco), 100  $\mu\text{g}/\text{ml}$  streptomycin and 100 U/ml penicillin, and MCF-10A cells in DMEM/F12 medium (Gibco) with 10% FBS (Gibco). All cells were incubated at  $37^{\circ}\text{C}$  with the 5%  $\text{CO}_2$ .

pcDNA3.1-XIST overexpression vector (XIST), pcDNA3.1 empty vector (vector), small interfering RNA (siRNA) against XIST (si-XIST), siRNA negative control (scramble), miR-454 mimic (miR-454), mimic negative control (NC), miR-454 inhibitor (anti-miR-454), inhibitor negative control (anti-NC) were obtained from Genepharma (Shanghai, China). Transfection was conducted by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After 48 h of transfection, the cells were used for subsequent analysis.

### 2.3 Quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA was extracted from TNBC tissues and cells using TRIzol reagent (Invitrogen). For cell RNA extraction, cells were transfer into a centrifuge tube at a concentration of  $3 \times 10^7$  and then incubated with 1 mL of Trizol reagent for 5 min at room temperature. As to tissues RNA extraction, 100 mg of tissues were added into a centrifuge tube, followed by adding with 1 ml of Trizol reagent for 5 min at room temperature. Next, each centrifuge tube was added with 0.2 mL of chloroform for 2 min and then centrifuged at 120,000g for 15 min at  $4^{\circ}\text{C}$ . Then the supernatant was collected and interacted with 0.5 mL isopropanol for 10 min,

followed by centrifuging at 120,000g for 10 min at 4°C. Immediately, after removing the supernatant, the precipitate was retained and washed with 1 mL of 75% ethanol, and centrifuged at 7500g for 5 min at 4°C. After removing the supernatant and drying the RNA precipitate. RNA precipitate was add with 50 µL DEPC H<sub>2</sub>O for 5–10 min at 55–60°C. Finally, RNA concentration was qualified using NanoDrop (Thermo Fisher, Waltham, MA, USA).

After that, cDNAs were synthesized from with reverse transcriptase (TermoFisher), qRT-PCR was performed with SYBR Green Real-Time PCR kit (Thermo Fisher). U6 or GAPDH was as an endogenous control and all data were analyzed using the  $2^{-\Delta\Delta Ct}$  method. The special primers for miR-454 or U6 were purchased from GeneCopoeia and primers for XIST or GAPDH were listed as follows: XIST, forward 5'-GCATAACTCGGCTTAGGGCT-3', reverse 5'-TCCTCTGCCTGACCTGCTAT-3'; GAPDH, forward 5'-TCTTTTTCGCT- CGCCAGCCGAG-3', reverse 5'-TGACCAGGCGCCCAATACGAC-3'.

#### 2.4 Cell proliferation and apoptosis

The proliferative capacity of TNBC cells was examined using cell Counting Kit-8 (CCK-8) (Sigma, St. Louis, MO, USA). All cells were digested and seeded into 96-well plates after transfection at a density of 3000/well and maintained at 37°C with 5% CO<sub>2</sub> for 24 h. Then CCK-8 reagents were added to the plates and incubated for 2 h. Finally, the absorbance was measured at 450 nm by a microplate reader.

Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, San Jose, CA, USA) was used to analysis apoptosis rate in according to the manufacturer's protocol. Finally, flow cytometry (BD Biosciences) was performed to detect cell apoptotic rate.

#### 2.5 Western blot

Cells were lysed by RIPA buffer (Thermo Scientific), then a total of 50 µg lysates were separated on 10% SDS-PAGE gel and transferred them onto polyvinylidene difluoride (PVDF) membranes (Sigma), and blocked with 5% nonfat milk for 1 h at room temperature. After incubation with primary antibodies at 4°C overnight, the blots were interacted with HRP-conjugated secondary antibody (Abcam, Cambridge, MA, USA) for 2 h at room temperature after washed with TBST for four times. ECL substrates were used to

visualize signals. GAPDH was used as an endogenous protein for normalization.

#### 2.6 Luciferase reporter assay

The binding sequences of XIST and miR-454 were predicted by StarBase software. The wild-type XIST (XIST-wt) or XIST mutant (XIST-mut) containing miR-454 putative target sites were amplified and clone into pGL3 vectors (Promega, Madison, WI, USA) to generate XIST-wt or XIST-mut luciferase reporter vector (pGL3-XIST-wt or -mut), respectively. Then the luciferase reporter vector and miR-454 or miR-NC was co-transfected into MDA-MB-468 and MDA-MB-231 cells using Lipofectamine 2000 (Invitrogen). After 48 h of transfection, cells were lysed and luciferase activity was analyzed though a dual-luciferase assay kit (Promega).

#### 2.7 RNA immunoprecipitation

RNA immunoprecipitation (RIP) assay was conducted by Magna RNA immunoprecipitation kit (Millipore, Billerica, MA, USA). MDA-MB-468 and MDA-MB-231 cells transfected with miR-454 or NC were lysed by RIP buffer and then cell lysis was incubated with magnetic beads coated with anti-Ago2 or IgG antibody (Abcam) at 4°C. Finally, the immunoprecipitated RNA was separated and analyzed by qRT-PCR.

#### 2.8 Pull-down

MiR-454 and miR-NC were biotinylated to be Bio-miR-454, Bio-NC by GenePharma Company (Shanghai, China) and then were transfected into MDA-MB-468 and MDA-MB-231 cells, respectively for 48 h. Sunsequently, cells were collected and lysed and cell lysis was incubated with streptavidin-coated magnetic beads (Invitrogen) for 10 min and washed with buffer. Subsequently, the biotin-coupled RNA complex was pulled down and analyzed by qRT-PCR.

#### 2.9 Murine xenograft assay

BALB/c nude mice (female, four-week-old) were purchased from Vital River Laboratory Animal Technology (Beijing, China) and randomly divided into two groups (6 mice per group). A total of  $3 \times 10^6$  cells

transfected with the lentivirus containing XIST or negative control (vector) were injected into the flanks of the BALB/c nude mice. The tumor size was regularly monitored and measured. After 40 days, the mice were sacrificed and the tumors samples were weighted and used for further analyzed. The animal experiments were approved by the Animal Research Committee of the Suzhou Wuzhong People'S Hospital and performed in accordance with the guidelines of the National Animal Care and Ethics Institution.

### 2.10 Statistical analysis

All data were expressed as the mean $\pm$ SD from at least 3 independent experiments. The differences between two groups were analyzed by student's *t* test and multiple comparisons were conducted by one-way analysis of variance (ANOVA). All statistical analyses were performed using GraphPad Prism 7 (GraphPad Inc., San Diego, CA, USA). *P* value less than 0.05 was considered statistically significant.

## 3. Results

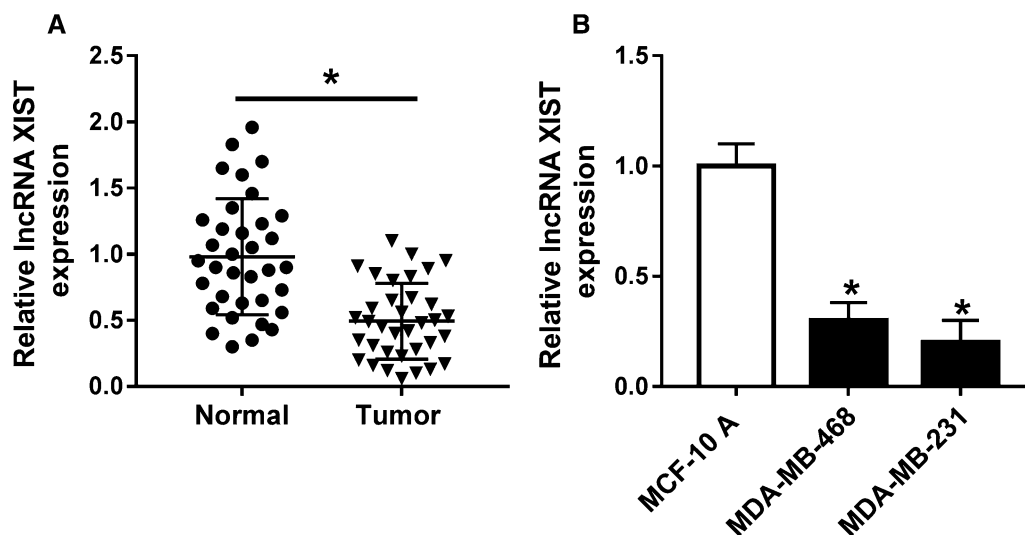
### 3.1 XIST is down-regulated in TNBC tissues and cell lines

Firstly, XIST expression in tissues and cells were measured using qRT-PCR. The level of XIST was significantly decreased in TNBC tissues compared with

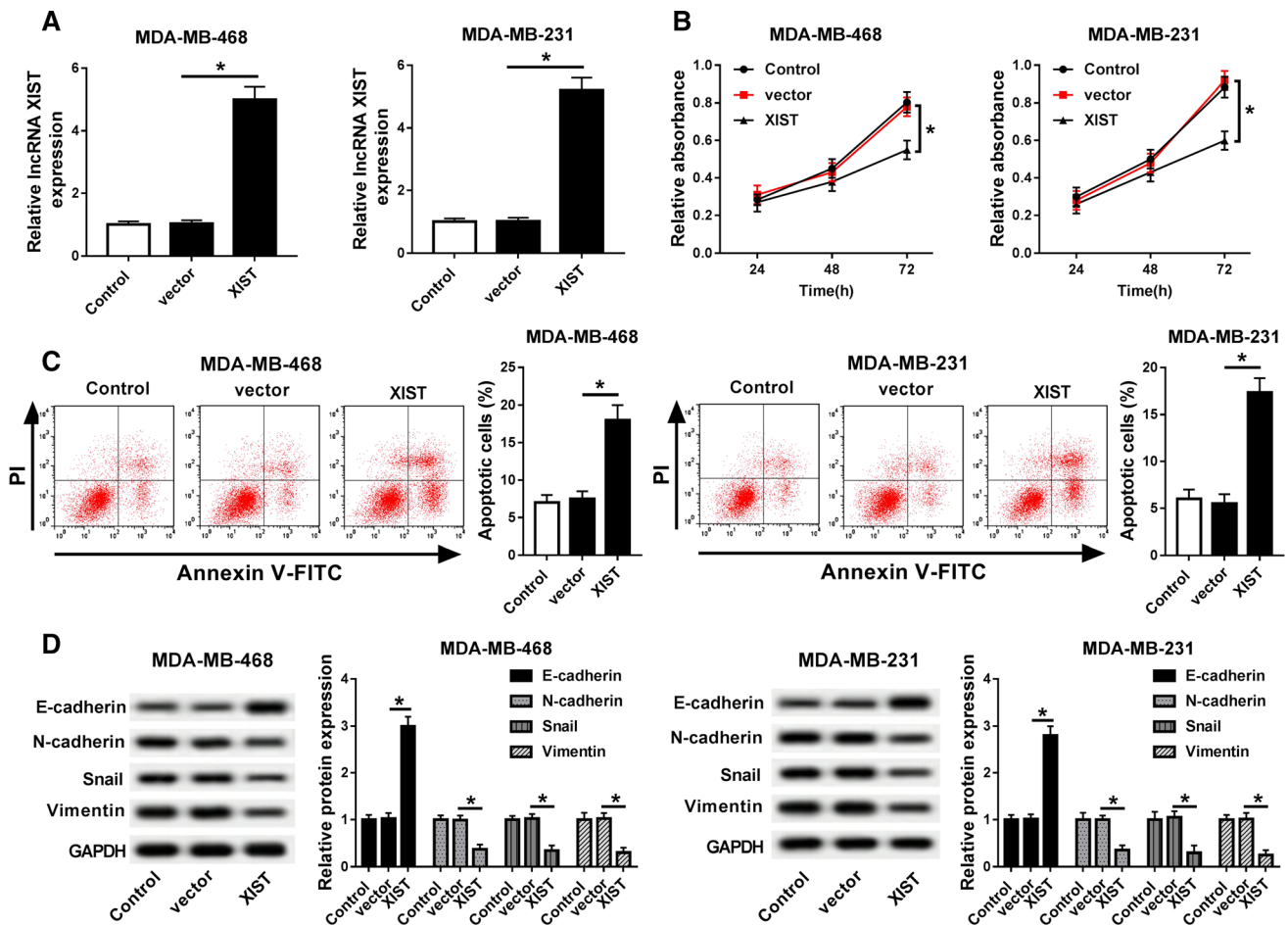
that in adjacent non-tumor tissues (fold change 0.5) ( $P<0.05$ , figure 1A). Moreover, the expression of XIST was clearly down-regulated in MDA-MB-468 (fold change 0.3) and MDA-MB-231 (fold change 0.2) cells compared to in the normal breast epithelial cell line MCF-10 A ( $P<0.05$ , figure 1B).

### 3.2 XIST restoration inhibits TNBC cells proliferation, EMT and induces apoptosis in vitro

To elucidate the potential effects of XIST on TNBC cells, MDA-MB-468 and MDA-MB-231 cells were transfected with XIST or vector for 48 h, then over-expressed XIST was observed in MDA-MB-468 (fold change 5.0) and MDAMB-231 (fold change 5.2) cells transfected with XIST ( $P<0.05$ , figure 2A). Cell proliferation was detected using CCK-8 assays and results showed XIST overexpression inhibited cell proliferation in TNBC ( $P<0.05$ , figure 2B). Furthermore, flow cytometry results indicated XIST restoration induced apoptosis of MDA-MB-468 and MDA-MB-231 cells ( $P<0.05$ , figure 2C). Subsequently, EMT-related proteins (including E-cadherin, N-cadherin, Snail and Vimentin) were examined via western bolt and the results suggested overexpressed XIST facilitated the level of E-cadherin but suppressed the expression of N-cadherin, Snail and Vimentin both in MDA-MB-468 and MDA-MB-231 cells ( $P<0.05$ , figure 2D). These results indicated overexpressed XIST inhibited the proliferation, EMT and induce apoptosis of TNBC cells *in vitro*.



**Figure 1.** LncRNA XIST was significantly down-regulated in TNBC tissues and cells. The expression of XIST was measured in TNBC tissues (A) and cell lines (B) by qRT-PCR. \* $P<0.05$ .



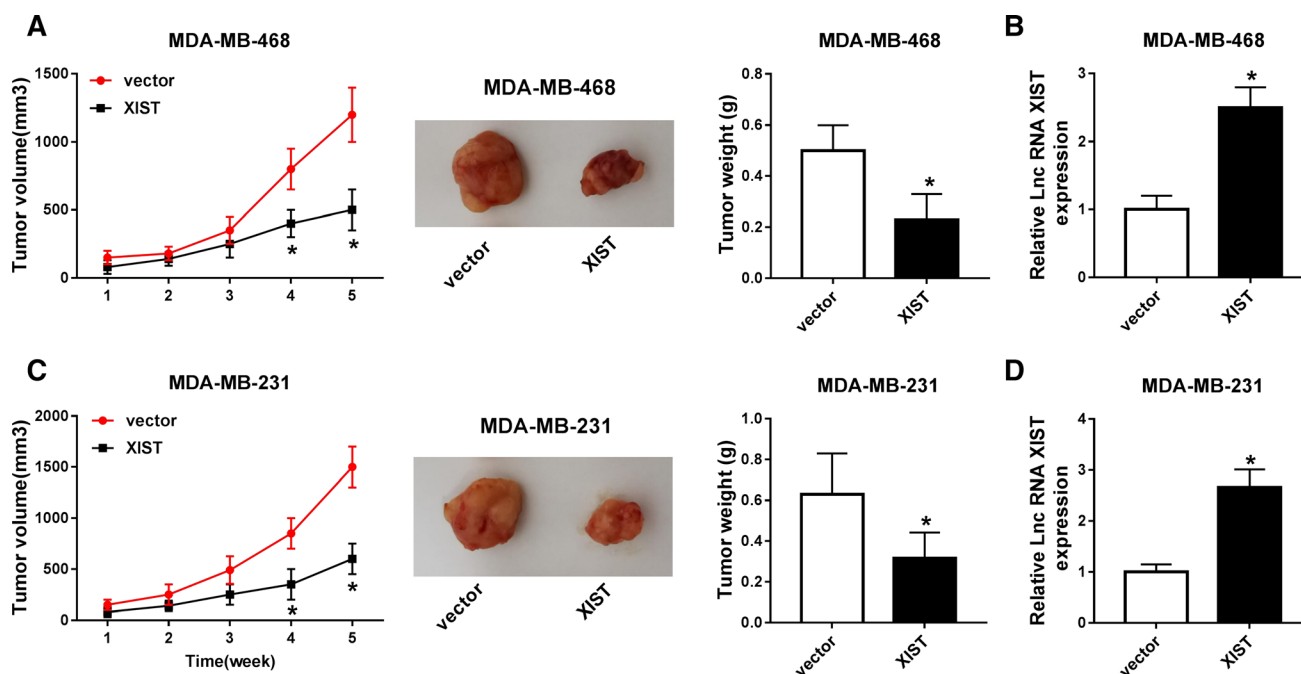
**Figure 2.** Biological functions of XIST in TNBC cells. (A) Transfection efficiency of XIST mimic was confirmed by qRT-PCR. Cells proliferation ability (B) and apoptosis rate (C) were detected by CCK-8 assay and Flow cytometry in MDA-MB-468 and MDA-MB-231 cells, respectively. (D) EMT-related proteins (including E-cadherin, N-cadherin, Snail and Vimentin) were examined via western blot both in MDA-MB-468 and MDA-MB-231 cells. \* $P < 0.05$ .

### 3.3 Overexpressed XIST inhibits tumor growth of TNBC *in vivo*

To further analyze the biological roles of XIST in TNBC tumorigenesis *in vivo*, MDA-MB-468 and MDA-MB-231 cells stably transfected with XIST or vector were used to establish xenograft model *in vivo*, respectively. After 5 weeks following the cell inoculation, the results of tumor volume and weight exhibited XIST restoration markedly reduced tumor growth in mice ( $P < 0.05$ , figure 3A, C). Subsequently, molecular analyses were applied in harvested tumor tissues. The expression of XIST was greatly increased in XIST restoration group compared to control group ( $P < 0.05$ , figure 3B, D). Therefore, we confirmed that XIST overexpression inhibited TNBC tumorigenesis *in vivo*.

### 3.4 Knockdown of miR-454 restrains TNBC cells proliferation, EMT and induces apoptosis

The expression of miR-454 was detected and results suggested miR-454 was notably increased in TNBC tissues (fold change 2.5) and cell lines (3.23 with MDA-MB-468 cell; 4.23 with MDA-MB-231 cell) ( $P < 0.05$ , figure 4A, B). After that, loss-of-function was performed, MDA-MB-468 and MDA-MB-231 cells were transfected with anti-miR-454 or anti-NC for 48 h and a significantly reduction of miR-454 was observed in MDA-MB-468 (fold change 0.31) and MDAMB-231 (fold change 0.26) cells ( $P < 0.05$ , figure 4C). Subsequently, functional analyses indicated that knockdown of miR-454 induced the inhibition of TNBC cells proliferation and the enhancement of TNBC cells apoptosis ( $P < 0.05$ , figure 4D, E). Furthermore, western blot



**Figure 3.** The effect of XIST interference on tumor growth *in vivo*. MDA-MB-468 and MDA-MB-231 cells stably transfected with XIST or vector were used to establish xenograft model *in vivo*, respectively. (A, C) Tumor volume was calculated every week and tumor weight was analyzed in each group after killing mice. (B, D) The expression of XIST was detected in each group at end point using qRT-PCR. \* $P < 0.05$

results indicated miR-454 inhibition led to increased E-cadherin expression and a decreased levels of N-cadherin, Snail and Vimentin both in MDA-MB-468 and MDA-MB-231 cells ( $P < 0.05$ , figure 4F). These data suggested that knockdown of miR-454 could suppressed TNBC cells progression.

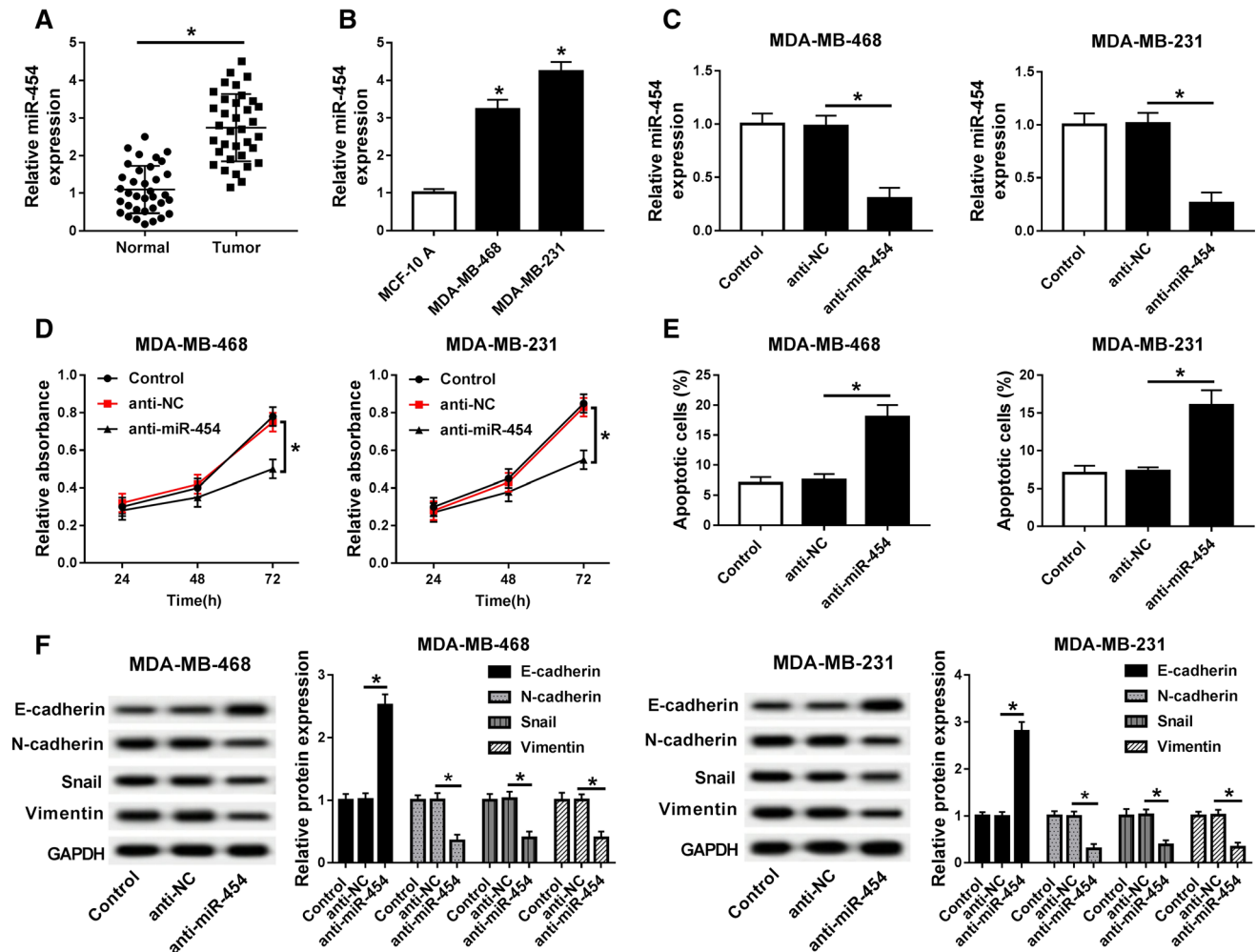
### 3.5 XIST directly binds to miR-454

To explore the underlying molecular mechanism of XIST involved in TNBC progression, the potential targets were explored by bioinformatics analysis via StarBase software. MiR-454 was predicted to be a target of XIST with a putative binding site (figure 5A). Subsequently, the luciferase reporter assay results indicated that luciferase activity was significantly reduced in MDA-MB-468 (reduce from 1 to 0.26) and MDA-MB-231 (reduce from 1 to 0.25) cells co-transfected with XIST-wt and miR-454 compared with the NC control, while the inhibition effects of miR-454 mimic were abolished in XIST-mut group ( $P < 0.05$ , figure 5B). Then RIP assay was used and the data suggested abundance of miR-454 engendered obvious increase of enrichment of XIST after Ago2 RIP in MDA-MB-468 (fold change 4.5) and MDA-MB-231 (fold change 4) cells, whereas its effect was lost in

response to IgG RIP ( $P < 0.05$ , figure 5C). Moreover, bio-miR-454-WT was found could enrich more XIST using RNA pull-down assay in MDA-MB-468 (fold change 6.5) and MDA-MB-231 (fold change 6) cells ( $P < 0.05$ , figure 5D), indicating the directly interaction between miR-454 and XIST. Subsequently, we found that the level of miR-454 was clearly inhibited by overexpressed XIST, but obviously increased by XIST deletion in MDA-MB-468 (fold change 0.29 and 2.73, respectively) and MDA-MB-231 (fold change 0.35 and 2.57, respectively) cells ( $P < 0.05$ , figure 5E). Besides that, we also confirmed that mut-XIST had no effects on miR-454 levels ( $P < 0.05$ , supplementary figure 1), further suggesting XIST regulated miR-454 expression. Taken together, XIST directly bound to miR-454 and negatively modulated miR-454 expression.

### 3.6 Overexpressed XIST exerts anti-tumor effects via regulating miR-454

To verify whether miR-454 involved in XIST mediated anti-tumor effects on TNBC cells, MDA-MB-468 and MDA-MB-231 cells were transfected with XIST, vector, XIST + miR-NC, or XIST + miR-454 for 48 h. After transfection, we found XIST restoration repressed the level of miR-454 (fold change 0.35 in MDA-MB-468,



**Figure 4.** The biological effects of miR-454 on TNBC cells. The expression of miR-454 was examined in TNBC tissues (A) and cell lines (B) using qRT-PCR. (C) Transfection efficiency of XIST was confirmed by qRT-PCR. Cells proliferation ability (D) and apoptosis rate (E) were detected by CCK-8 assay and Flow cytometry in MDA-MB-468 and MDA-MB-231 cells, respectively. (F) EMT-related proteins (including E-cadherin, N-cadherin, Snail and Vimentin) were analyzed by western bolt both in MDA-MB-468 and MDA-MB-231 cells.  $*P < 0.05$ .

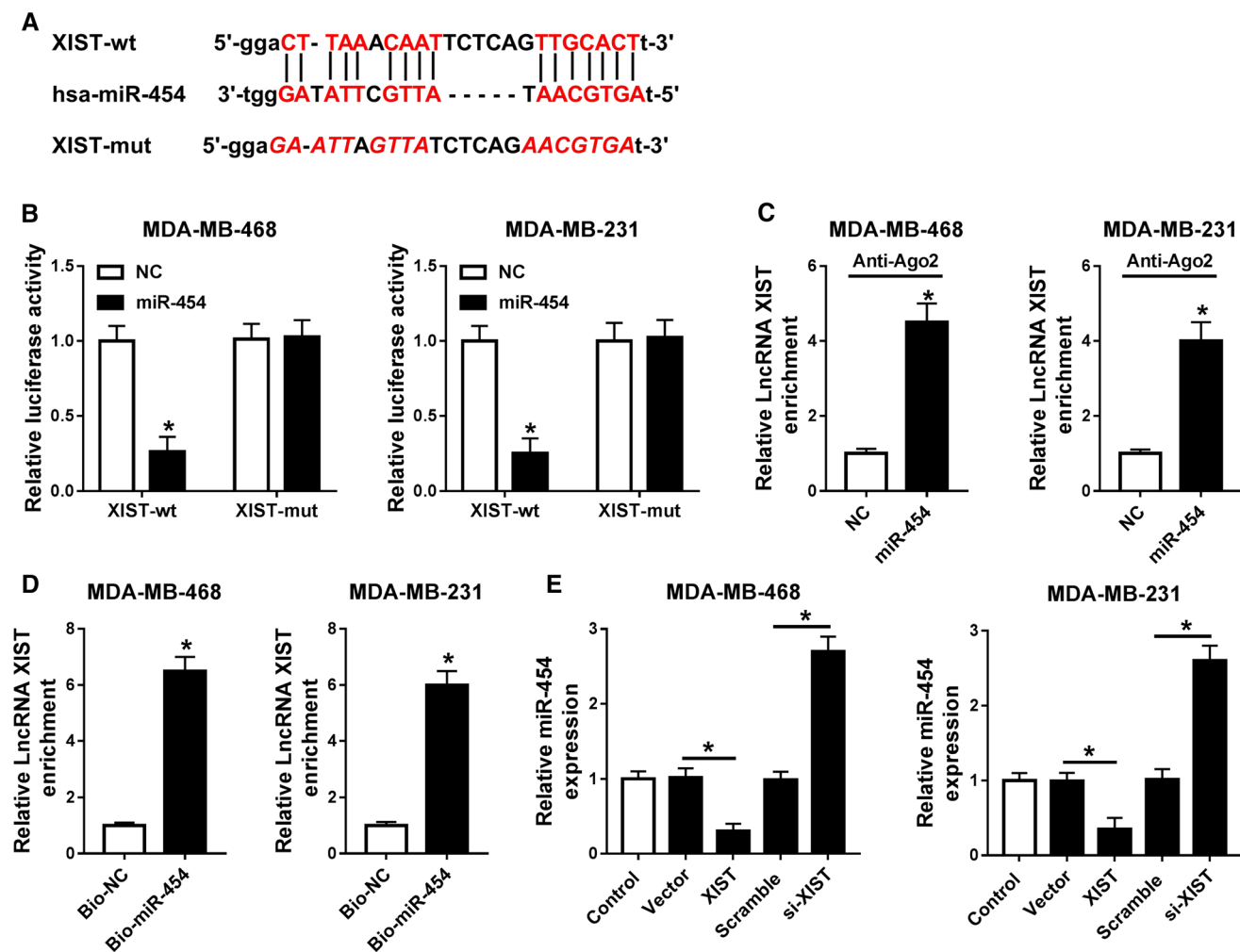
0.3 in MDA-MB-231, respectively), but this suppression was rescued by miR-454 mimic (fold change 2.42 in MDA-MB-468, 2.59 in MDA-MB-231, respectively) ( $P < 0.05$ , figure 6A). Afterwards, rescue assay indicated overexpressed miR-454 reversed XIST restoration mediated inhibition on proliferation, promotion on apoptosis and stimulation on EMT in MDA-MB-468 and MDA-MB-231 cells ( $P < 0.05$ , figure 6B–D). Altogether, these data illustrated that XIST exerted anti-tumor effects by directly regulating miR-454 in TNBC.

#### 4. Discussion

LncRNAs are emerging stars in cancer diagnosis and therapy. Emerging evidence has revealed that aberrant expression, mutations and SNPs of lncRNAs are

associated with tumorigenesis and metastasis, affecting the proliferation, apoptosis, and EMT of cancer cells (Huarte 2015; Zhang and Tang 2018). Increasing studies have reported that lncRNA expression was significantly altered in breast cancer tissue including TNBC while the effects of lncRNA are controversial. For example, lncRNA MALAT1, LINP1 and NEAT1 increased in TNBC tissues and cell lines, and blocking of them inhibited cell progression (Zhang *et al.* 2017a; Zhang *et al.* 2016; Zuo *et al.* 2017), which act as oncogenes. However, lncRNA GAS5 was found to be down-regulated in breast cancer and acted as a tumor suppressor.

XIST is a non-coding RNA which takes part in the beginning of X chromosome inactivation during early embryogenesis. It was first identified as an oncogene in



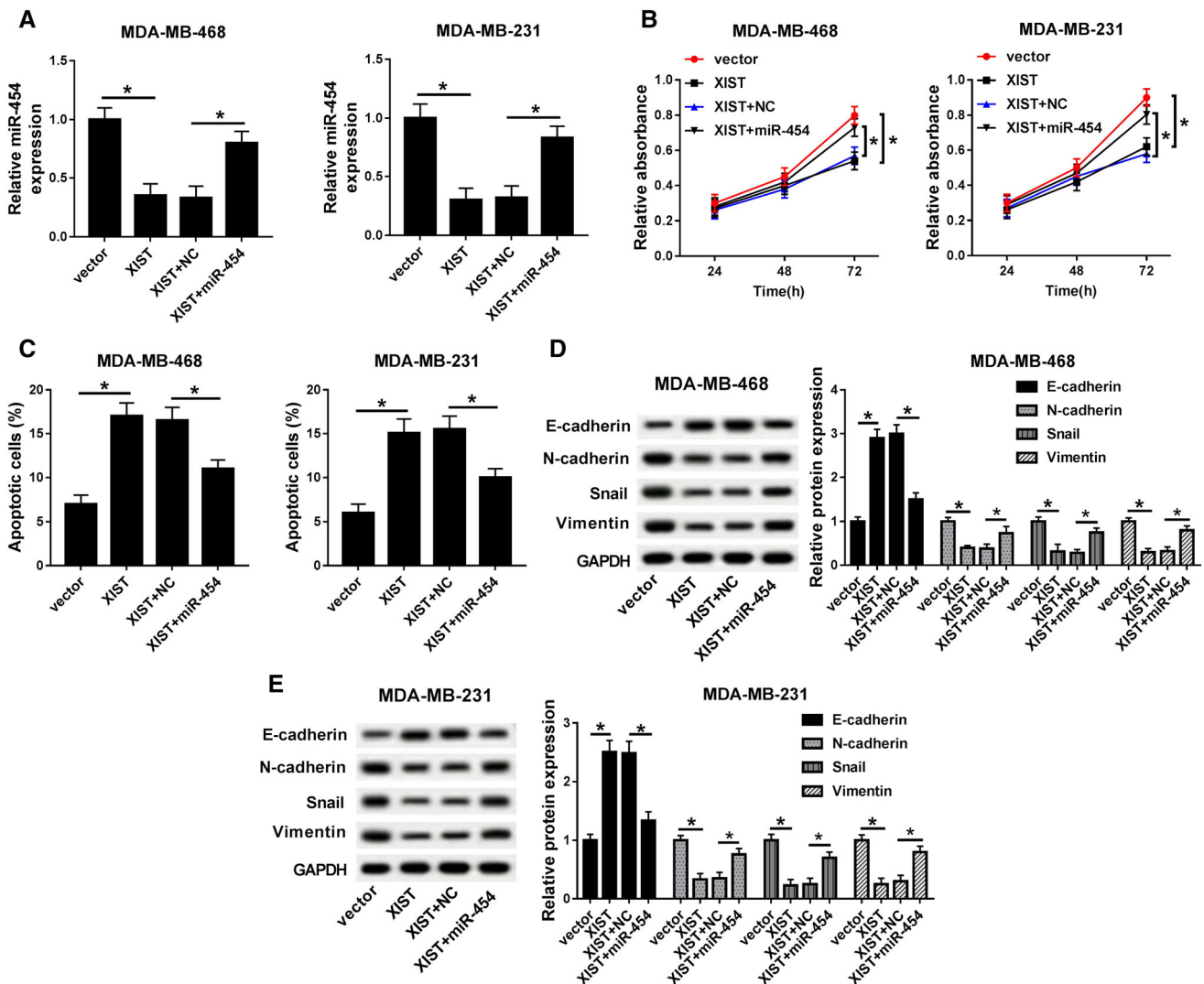
**Figure 5.** XIST directly bound to miR-454. (A) The potential binding sites between XIST and miR-454. Luciferase reporter assay (B), RIP assay (C) and Pull-down assay (D) were used to demonstrate the interaction between XIST and miR-454. (E) The expression of miR-454 was detected by qRT-PCR in MDA-MB-468 and MDA-MB-231 cells transfected with XIST, vector, si-XIST, or scramble. \* $P < 0.05$ .

human glioblastoma stem cells and played important roles in cell progression in glioblastoma (Yao *et al.* 2015). Studies have been performed to explore the role of XIST in cancers and previous reports have indicated that XIST was highly expressed and exerted as tumor-driving oncogenic in several cancers, including hepatocellular carcinoma (Chang *et al.* 2017), osteosarcoma (Lv *et al.* 2018), pancreatic cancer (Wei *et al.* 2017) and lung cancer (Tantai *et al.* 2015). However, several studies also confirmed that XIST inhibited tumor process in prostate cancer, gastric cancer and breast cancer (DL *et al.* 2016; Liu *et al.* 2015; Du *et al.* 2017). In the present study, we detected that XIST was significantly down-regulated in TNBC tissues and cells. Functional assay indicated overexpressed XIST inhibited TNBC cell proliferation, EMT and induced apoptosis *in vitro* as well as suppressed TNBC tumorigenesis *in vivo*. We

firstly verified that XIST functioned as a tumor suppressor in TNBC.

Recently, studies have highlighted an lncRNA-mRNA interaction which is very similar to the miRNA regulation of mRNA and lncRNAs are reported to bind to miRNA and work as a sponge of miRNA (Xiong *et al.* 2017; Zhang *et al.* 2017b). XIST has been reported could bind various miRNAs in some common cancers. Wei *et al.* found XIST promoted pancreatic cancer proliferation by sponging miR-133a to regulate EGFR (Wei *et al.* 2017). XIST promoted gastric cancer progression through TGF- $\beta$ 1 via directly interacting with miR-185 (Zhang *et al.* 2018). XIST interacted with miR-124 to modulate bladder cancer growth, invasion and migration (Xiong *et al.* 2017). Furthermore, in lung cancer, XIST regulated tumor growth by miR-140/iASPP axis (Tang *et al.* 2017). MiR-454 is a





**Figure 6.** XIST exerted anti-tumor effects via regulating miR-454. MDA-MB-468 and MDA-MB-231 cells were transfected with XIST, vector, XIST + NC, or XIST + miR-454 for 24 h. (A) The expression of miR-454 was examined by qRT-PCR after transfection. (B) Cells proliferation was detected using CCK-8 assay. (C) Flow cytometry was used to analysis cell apoptosis. (D, E) Western blot was utilized to determine the change of EMT related protein (including E-cadherin, N-cadherin, Snail and Vimentin). \* $P < 0.05$ .

recently identified cancer-related miRNA. MiR-454 was found to be increased in colorectal cancer, lung cancer and hepatocellular carcinoma, and functioned as a potential oncogene to regulate cancer development (Liang *et al.* 2015; Yu *et al.* 2015; Zhu *et al.* 2016). Besides that, miR-454 was observed to be down-regulated in gastric cancer, glioblastoma, and osteosarcoma, which played anti-tumor effects (Fang *et al.* 2015; Niu *et al.* 2015; Song *et al.* 2017). In this study, the expression of miR-454 was significantly increased in TNBC tissues and cells, knockdown of miR-454 inhibited TNBC cells progression which was consistent with previous study (Lu *et al.* 2017). After that, miR-454 was predicted and confirmed to be a target of

XIST. Thus, we further explored whether XIST/miR-454 responsible for the progression of TNBC, and results indicated that overexpressed XIST exerted anti-tumor effects via direct interaction with miR-454 *in vitro*.

In conclusion, we confirm that XIST was decreased in TNBC tissues and cell lines, XIST restoration inhibited TNBC cell proliferation and EMT, and induced apoptosis *in vitro* and suppressed TNBC tumor growth *in vivo*. Additionally, we first identified the regulatory network of XIST/miR-454 axis in TNBC progression, which may shed new light on metastasis and therapies of TNBC. In the future, we will explore the downstream signaling pathway targeted by XIST/

miR-454 axis to provide more valuable information on the molecular regulation mechanisms of TNBC.

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