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Knock Down of LINC00504 Represses Proliferation and Invasion via Regulation of miR-140-5p in Breast Cancer

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1 # Knock down of LINC00504 represses proliferation and invasion via
2 regulation of miR-140-5p in Breast cancer

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15

16 **Abstract**

17 **Background:** Breast cancer is one of the most common cancer in the world. Emerging evidence
18 has demonstrated the critical role of long noncoding RNAs (lncRNAs) in the development of breast
19 cancer. In this study, we aimed to investigate the role of LINC00504 in breast cancer progression.
20 **Methods:** Quantification real-time PCR was used to analyzed the expression levels of LINC00504
21 and miR-140-5p in breast cancer tissues and cell lines. Cell proliferation, migration and invasion
22 were assessed by Cell Counting Kit-8, transwell assay and Immunofluorescence. Dual-luciferase

reporter assay and RNA Immunoprecipitation assay were performed to verify the interaction between LINC00504 and miR-140-5p. The expression levels of VEGFA, CDH1 and VIM were demonstrated by western blot assays.

Result: Here, we found that LINC00504 is up regulated in breast cancer tissues and cell lines. Down regulation of LINC00504 mediated by shRNA suppressed the proliferation, migration, and invasion of breast cancer cells in vitro and in vivo. Furthermore, LINC00504 was found to competitively regulate miR-140-5p via targeting VEGFA. Inhibition of miR-140-5p attenuated the knockdown-LINC00504 induced inhibition of breast cancer cell proliferation and invasion.

Conclusion: Taken together, our results demonstrated the mechanism of the LINC00504–miR-140-5p–VEGFA axis in breast cancer cell proliferation and invasion and may lead to new lncRNA-based diagnostics or therapeutics for breast cancer.

Keywords: breast cancer, LINC00504, proliferation, invasion, miR-140-5p, VEGFA

1 Background

Breast cancer is the most common type of gynecological tumor in China [1]. Despite efforts by diagnostic techniques and patient management, there has been little progress in improving the overall survival of breast carcinoma patients. In addition, it has been reported that there is an apparent trend in incidence and mortality rates of breast cancer [2, 3]. Development of suitable therapy to increase patient survival rate has been limited because the pathophysiological mechanisms contributing to breast carcinoma are largely unknown [4]. Therefore, uncovering the molecular mechanisms for development and progression of breast carcinoma is necessary for developing effective therapies.

The long noncoding RNAs (lncRNAs) are a new category of noncoding RNAs with over 200

45 nucleotides and are deficient in protein coding ability [5-7]. More and more evidence has shown
46 that LncRNAs play important role in various human cancers, including breast cancer [8], liver
47 cancer [9], gastric cancer [10] and so on. LncRNAs have been linked to every stage of cell life,
48 including cell proliferation, differentiation, apoptosis, and motility. For instance, Knockdown of
49 Long Noncoding RNA GHET1 Inhibits Cell Proliferation and Invasion of Colorectal Cancer [11].
50 Therefore, identifying the mechanism of regulation of LncRNA is essential for tumor diagnosis and
51 therapy. A long noncoding RNA， called LINC00504， is a newly identified lncRNA. Feng J et al
52 firstly reported that A noncoding RNA LINC00504 interacts with c-Myc to regulate tumor
53 metabolism in colon cancer [12]. However, the expression of LINC00504 in breast cancer and its
54 biological effects has not been reported.
55 In this study, we explored the expression of LINC00504 in breast cancer tissues and cells by using
56 qRT-PCR, CCK-8 and other molecular biology experiments at the breast cancer tissue and cell level,
57 we demonstrated that LINC00504 is upregulated in human breast cancer tissues and cell lines.
58 Besides, we uncovered the oncogenic function of LINC00504 via regulating the miR-140-5p–
59 VEGFR pathway during breast cancer development. These results might provide a new insight for
60 the treatment of breast cancer.
61
62 **2 Materials and methods**
63
64 **2.1 Tissue Specimen and Cell Culture**
65 28 BC specimens and their matched adjacent normal tissues were collected after surgical
66 resection at Shanghai Tenth People’s Hospital. All the patients underwent surgical resection and

67 were diagnosed with breast cancer by rapid pathology. And the tissues were immediately snap-
68 frozen in liquid nitrogen after resection and stored at −80°C. The study protocol was approved by
69 Shanghai Tenth People's Hospital Institutional Review Board (The Certificate Number: SHSY-IEC-
70 KY-4.0/17-23/01), and all patients signed informed consent. Human breast cancer cell lines BT549,
71 T47D, MCF-7, SKBR3 and MDA-MB-231 were purchased from the Institute of Biochemistry and
72 Cell Biology of the Chinese Academy of Sciences (Shanghai, China). Human normal breast cell line
73 MCF-10A was purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA).
74 Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, Grand Island, NY)
75 supplemented with 10% of fetal bovine serum (FBS), 100U/ml penicillin and 100mg/ml
76 streptomycin (Invitrogen, Carlsbad, CA, USA). The cells were maintained in a humidified incubator
77 at 37°C and 5% CO_2.
78
79 **2.2 Transfection and lentivirus transduction**
80 Oligonucleotide transfection was performed with Lipofectamine 2000 reagent (Invitrogen, Carlsbad,
81 CA, USA). The modified miR-140-5p mimics, miRNA inhibitors (miR-140-5p in), shRNA targeting
82 LINC00504 (sh-LINC00504), and corresponding negative controls were chemically enhanced
83 oligonucleotides which designed and synthetized by Applied Biosystems (Foster city, CA, USA).
84 LINC00504 overexpression plasmids also was chemically synthetized by Applied Biosystems. The
85 empty lentiviral vector sh-control was used as a control. Recombinant lentivirus plasmids were used
86 to infect cells with 5 mg/mL Polybrene (Sigma, St. Louis, MO, USA).
87
88 **2.3 Cell cycle analysis**

89 After transfection, cells were harvested and adjusted to the concentration of 1×106 cell/mL. Then

90 pre-cold ethanol (75%) was used to dissolve the cells, followed by incubation at 4°C for 4h. After

91 that, cells were washed with cold PBS again. Next, cells were stained with BD Pharmingen™

92 PI/RNase for 30 min at room temperature, followed by flow cytometer at different cell cycle phase

93 (G1, S, and G2). 10000 cells were measured for each sample.

94

95 **2.4 Real-Time Quantitative PCR (qPCR) Analysis**

96 TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from the tissues and

97 cell lines according to the manufacturer's instructions. RNA was reverse-transcribed to cDNA by

98 means of the PrimeScript RT Reagent Kit (TaKaRa, Dalian, China). SYBR Premix Ex Taq (TaKaRa)

99 was used to detect LINC00504 and miR-140-5p expression. PCR was carried out at least in triplicate,

100 and the results were analyzed on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems,

101 Foster City, CA). LINC00504 and miR-140-5p expression levels were quantified by the expression

102 of GAPDH and U6, respectively. The relative expression was calculated using the $2^{-\Delta\Delta CT}$ method.

103

104 **2.5 Cell proliferation，Cell migration and invasion assays**

105 Cell proliferation was detected by Cell Counting Kit-8 (CCK-8; Dojindo, Tokyo, Japan). Cells were

106 seeded in 96-well plates with density 5×103 cells/well and incubated in 37°C with 5% CO2. Next,

107 the CCK-8 assay solution (10μl) was added to each well at indicated time. The absorbance at 450nm

108 was measured with an enzyme immunoassay analyzer (Thermo Fisher Scientific, Shanghai, China).

109 A transwell chamber (8-μm pore size, Corning, Cambridge, MA, USA) was used to perform cell

110 migration and invasion assays. Transfected cells (2×10^5 cells/mL) were resuspended in 200 μL of

111 the serum-free medium and seeded in the upper chamber. Next, the cells were placed on the top side

112 of the membrane (without Matrigel for the migration assay) or placed on the top side of the

113 membrane precoated with Matrigel (BD Biosciences) (for the invasion assay). After incubation at

114 37°C for 48 hours, the cells migrated or invaded to the lower side of the membrane were fixed in

115 20% methanol and stained with 0.1% crystal violet for 15min. The cells was counted in five

116 randomly selected visual fields under an inverted Phase-contrast Microscope (Olympus).

117

118 **2.6 Mice experiments**

119 For xenograft mouse experiments, SKBR3 or MDA-MB-231 (5×10^6 cells) under different

120 treatments were subcutaneously injected into 8-week-old athymic nude mice (Bikai). Tumor size

121 and weight was measured every 6 days, and the tumor volume was calculated as $0.5 \times L \times W^2$, with

122 L representing length and w representing width.

123

124 **2.7 Immunofluorescence**

125 SKBR3 and MDA-MB-231 were cultured on glass slides and fixed with 4% paraformaldehyde for

126 15 min. Then, the cells were washed three times with phosphate-buffered saline (PBS) for 5 min

127 and incubated with blocking buffer (PBS solution containing 3% fetal bovine serum (FBS), 1% goat

128 serum, and 0.1% Triton X-100) for 2h at room temperature. Next, cells were incubated with the

129 primary antibody (anti-VIM, anti-CDH1) diluted in PBS at 4°C overnight. The slides were then

130 washed with PBS three times prior to being incubated with Alexa Fluor®-488/555 fluorescent

131 conjugated secondary antibody for 1 h in the dark. Afterward, the slides were washed three times in

132 PBS prior to being mounted with Pro-Long® Gold Antifade Reagent with 4′,6-diamidino-2-

133 phenylindole (DAPI: Molecular Probes, Eugene, OR, USA). The slides were observed using an
134 LSM 800 Confocal Microscope (Carl Zeiss AG, Oberkochen, Germany).
135
136 **2.8 The luciferase reporter assay**
137 MiR-140-5p was found to be directly regulated target by LINC00504 using miRcode bioinformatics
138 tools (http://www.mircode.org/). The theoretical binding sequence for miR-140-5p in the
139 LINC00504 gene and its mutant sequence were cloned into the psiCHECK-2 vector (Promega,
140 Madison, WI, USA) to construct a dual luciferase reporter plasmid. The wild-type (wt) 3′-UTR
141 fragment of LINC00504 and its mutant (mut) of the miR-140-5p binding site were cloned into a the
142 psiCHECK-2 vector to form the reporter vector， which named as Wt-LINC00504 and Mut-
143 LINC00504, respectively. SKBR3 and MDA-MB-231 cells were transfected with Wt (or Mut)
144 reporter plasmid and an NC mimic or miR-140-5p mimic for 48 hours. The luciferase activity was
145 detected using a Dual Luciferase Reporter Gene Assay Kit (Beyotime Institute of Biotechnology,
146 Shanghai, China) according to the manufacturer's protocol. The relative luciferase activity was
147 normalized to Renilla luciferase activity.
148
149 **2.9 RNA immunoprecipitation (RIP) assay**
150 RIP was performed using a Magna RIP RNA-Binding Protein Immunoprecipitation kit (Millipore,
151 Bedford, MA) according to the manufacturer's instructions. Briefly, cells were transfected with
152 miR-140-5p mimics, miR-140-5p NC mimics, and LINC00504, then were lysed in lysis buffer.
153 Subsequently, cell lysates were incubated with anti-Ago2 (Abcam) or anti-IgG (Abcam) and protein
154 A/G magnetic beads. The magnetic bead-bound complexes were purified by Dnase and Proteinase

155 K (Applied Biosystems). Lastly, qRT-PCR assays were used to determine the relative enrichment of
156 LINC00504 mRNA, with a LightCycler 480 Probes Master Kit on a LightCycler 480 instrument.
157
158 **3.0 Statistical analysis**
159 Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Data are
160 presented as mean ± standard deviation (SD) of at least three independent experiments. Differences
161 between two groups or more than two groups were evaluated, respectively, by Student's t test or
162 one-way analysis of variance (ANOVA). Spearman rank-correlation was performed to calculate the
163 correlation coefficient between LINC00504 and miR-140-5p expression levels.
164
165 **3 Results**
166
167 **3.1 LINC00504 is up regulated in breast cancer tissues and cells**
168 Bioinformatics analysis was used to identify the expression of LINC00504 in breast cancer patients
169 and healthy people in The Cancer Genome Atlas (TCGA) database. We found a significant increase
170 of the level of LINC00504 in the breast cancer patients compared to healthy control (Figure.1A).
171 Furthermore, Real-time qpcr was used to investigate the expression of LINC00504 in breast cancer
172 tissues and adjacent normal tissues. The results showed that LINC00504 was expressed at higher
173 levels in tumor tissues compared with adjacent normal tissues, which also confirmed the results of
174 bioinformatics (Figure.1B). We also measured the LINC00504 expression in five human breast
175 cancer cell lines (BT549, T47D, MCF-7, SKBR3 and MDA-MB-231) and in MCF-10A, the normal
176 breast cell line. Same as before results, LINC00504 expression was obviously higher in five breast

177 cancer cell lines compared with the normal breast cell (Figure.1C). These results demonstrated that

178 LINC00504 was up regulated in breast cancer.

179 **3.2 knock down of LINC00504 suppressed breast cancer cell proliferation, migration and**

180 **invasion in vitro and in vivo**

181 The physiological role of LINC00504 was explored using SKBR3 and MDA-MB-231 cells, which

182 were transfected with sh-control and sh-LINC00504. The significantly decrease of LINC00504

183 expression in these sh-LINC00504 transfected cells was confirmed by qRT-PCR (Figure 2A). Then

184 we performed CCK-8 assays to analyze the effect of LINC00504 knockdown on proliferation. We

185 found the inhibition of the two cell lines proliferation when compared to their corresponding

186 controls (Figure 2B,2C). Similarly, LINC00504 knockdown dramatically decreased the colony

187 formation by SKBR3 and MDA-MB-231 cells (Figure.2D). Cell cycle analysis was used to explore

188 the cell distribution at different cell phases. The results showed that knockdown of LINC00504

189 increased the numbers of SKBR3 and MDA-MB-231 cells in G1 phase and decreased the numbers

190 of MDA-MB-231 cells in both S and G2 phase, as for SKBR3, the numbers were reduced in G2

191 phase (Figure.2E). In xenograft mice models, we found that LINC00504 knockdown significantly

192 inhibited tumor growth (Figure.3), which further supports our previous tests that LINC00504 boosts

193 breast cancer cell proliferation.

194 Next, transwell assays was used to study whether LINC00504 can Influence breast cancer cell

195 migration and invasion. The results showed that LINC00504 knockdown suppressed breast cancer

196 cell migration and invasion (Figure 4A, B). Immunofluorescence was used to detect the variety of

197 VIM and CDH1, which were the characteristic proteins of the epithelial–mesenchymal transition

198 (EMT) [13, 14]. The results demonstrated that knockdown of LINC00504 inhibited the expression

199 of VIM and enhanced the expression of CDH1 (Figure 4C, D), which also proved that LINC00504
200 boosts breast cancer cell migration and invasion. Collectively, LINC00504 knockdown impaired
201 further progression of breast cancer cells.
202
203 **3.3 MIR-140-5p was a target of LINC00504 in breast cancer**
204 There has been reported that lncRNA could act as a ceRNA to exert its regulatory functions. To
205 further reveal the underlying mechanism responsible for LINC00504 functions in breast cancer, we
206 examined a set of miRNAs that were predicted to interact with LINC00504 using predication
207 software miRcode and RNA22. MIR-140-5p was found to be a promising target of LINC00504 and
208 the predicted bonding site of miR-140-5p in the LINC00504 sequence is showed in (Figure 5A).
209 After transfection with sh-LINC00504, qRT-PCR detection revealed that miR-140-5p expression
210 was significantly increased after knocking down of LINC00504(Figure 5B). To further verify the
211 interaction between miR-140-5p and LINC00504, luciferase reporter vectors were constructed
212 which contained a wild-type (wt) or mutated (mut) miR-140-5p-binding site in LINC00504. The
213 results of dual-luciferase reporter assays showed that miR-140-5p suppressed the luciferase activity
214 of the LINC00504-wt reporter vector, whereas barely influenced that of the LINC00504-mut
215 reporter vector, (Figure 5C). Furthermore, RIP assay was used to examine the potentially
216 endogenous interaction between LINC00504 and miR-140-5p. The data presented that LINC00504
217 was substantially enriched by miR-140-5p overexpression with anti-Ago2 in MDA-MB-231 and
218 SKBR3 cells (Figure 5D and 5E). using Spearman's correlation analysis, we found that the levels of
219 LINC00504 were statistically correlated with that of miR-140-5p among breast cancer tissue
220 samples (Figure 5F). These data indicated that miR-140-5p is a direct target of LINC00504 in breast

221 cancer.

222

223 **3.4 LINC00504 promoted the progression of breast cancer by the miR-140-5p-VEGFA axis**

224 Next, we explored the effect of miR-140-5p in LINC00504-driven promotion of breast cancer
225 progression. We knocked down LINC00504 and inhibited miR-140-5p in the same time in SKBR3
226 and MDA-MB-231 cells. CCK8, transwell migration and invasion assays showed that LINC00504
227 knockdown significantly inhibited cell proliferation, migration and invasion while miR-140-5p
228 inhibition in the meantime abrogated these effects (Figure 6A,B,C), which demonstrated that miR-
229 140-5p plays a key role in LINC00504-related oncogenic effects on breast cancer cells. It has been
230 reported that MicroRNA-140-5p inhibits invasion and angiogenesis through targeting VEGFA in
231 breast cancer [15]. Thus, we supposed that VEGFA is involved in the LINC00504/miR-140-5p-
232 dependent malignant progression of breast cancer cell. We knocked down LINC00504 and inhibited
233 miR-140-5p in the same time in SKBR3 and MDA-MB-231 cells. The expression of VEGFA,
234 CDH1 and VIM was detected by Western blotting. The results showed that VEGFA and VIM was
235 downregulated while the expression of cdh1 was increased when knockdown of LINC00504.
236 However, the converse expression of the proteins was observed when knockdown of LINC00504
237 and inhibition of miR-140-5p existed simultaneously (Figure 6D). These results suggested that
238 LINC00504 induced tumor development via inhibition of miR-140-5p and by targeting VEGFA.

239

240 **4 Discussion**

241 Breast cancer is the most common and mortality-related malignant tumor in female around the world
242 [16]. The incidence of breast cancer is increasing rapidly during recent years [17]. Early breast

243 cancer often does not have typical symptoms and signs, and it is not easy to attract attention. It is
244 often in the middle and late stages when found [18]. However, the underlying mechanism that
245 regulates breast cancer development remains largely unknown. It is crucial to develop novel
246 molecular biomarkers for the diagnosis and prognosis of breast cancer. Here, we found that
247 LINC00504 was significantly upregulated in breast cancer. And LINC00504 was critical for the
248 proliferation, migration and invasion of breast cancer cells, which indicated that LINC00504 may
249 be a new biomarker for breast cancer.
250 Emerging evidence showed that dysregulation of lncRNAs has been demonstrated to be involved in
251 tumorigenesis and progression of breast cancer [19-21], suggesting the possibility of lncRNAs to
252 serve as novel target for breast cancer diagnosis and therapy. LINC00504 is a newly found lncRNA,
253 which was highly expressed in colon cancer. Feng J et al firstly reported that LINC00504 interacts
254 with c-Myc to regulate tumor metabolism in colon cancer. However, the detail function and
255 underlying mechanism of LINC00504 on breast cancer still remain unclear. In this study, we showed
256 that LINC00504 was highly expressed in breast cancer tissues and cell lines. By in vitro and in vivo
257 assays, we showed that knockdown of LINC00504 remarkably inhibited cell proliferation. Similarly,
258 a clear weakening trend of cell migration and invasion was observed with LINC00504 depletion in
259 breast cancer. Moreover, we firstly manifested that LINC00504 knockdown resulted in decreased
260 EMT in breast cancer cells. All these results hinted that LINC00504 might contribute to the
261 metastasis of breast cancer.
262 Previous evidence showed that lncRNAs can serve as competitive endogenous RNA (ceRNA) to
263 sponge miRNAs [22-24]. To further explore the underlying molecular mechanism by which
264 LINC00504 regulates breast cancer, we made a predication and found that miR-140-5p was a

265 promising candidate. The gene encoding miRNA-140-5p is located in chromosome 16, which has
266 been proven to function in several cancer cells [25-27]. For instance, Yunfeng et al. demonstrated
267 that miR-140-5p could suppress tumor growth and metastasis of non-small cell lung cancer by
268 targeting IGF1R [28]. In hypopharyngeal squamous cell carcinoma, miRNA-140-5p suppresses
269 tumor cell migration and invasion by targeting ADAM10-mediated Notch1 signaling pathway [29].
270 And miRNA-140-5p inhibits invasion and angiogenesis through targeting VEGF-A in breast cancer
271 [30]. Above studies indicate that miRNA-140-5p may be a tumor suppressor. To further investigate
272 the correlation between LINC00504 and miRNA-140-5p in breast cancer tumorigenesis, we
273 performed luciferase reporter assays. Results showed that LINC00504 direc tly combined to
274 miRNA-140-5p in breast cancer cells. We also found that LINC00504 significantly inhibited
275 miRNA-140-5p expression, enhanced VEGFA and VIM expression and reduced CDH1 level. Taken
276 together, our results indicated that the oncogene LINC00504 promoted breast cancer progression by
277 negatively regulating miRNA-140-5p, a tumor suppressor via targeting VEGFA. The
278 LINC00504/miRNA-140-5p may act as a novel therapeutic target for the treatment of breast cancer.

279 **5 Conclusion**

280 Taken together, our results demonstrated the mechanism of the LINC00504–miR-140-5p–VEGFA
281 axis in breast cancer cell proliferation and invasion and may lead to new lncRNA-based diagnostics
282 or therapeutics for breast cancer.

283 **Abbreviations**

284 **lncRNAs**: long noncoding RNAs

285 **ATCC**: American Type Culture Collection

286 **DMEM**: Dulbecco's modified Eagle's medium

287 **RT-qPCR**: Real-Time Quantitative PCR

288 **RIP**: RNA immunoprecipitation

289 **IF**: Immunofluorescence

290 **TCGA**: The Cancer Genome Atlas

291 **EMT**: epithelial–mesenchymal transition

292 **ceRNA**: competitive endogenous RNA

293

294 **Declarations**

295 ***Ethics approval and consent to participate***

296 The study protocol was approved by Shanghai Tenth People's Hospital Institutional Review Board
297 (The Certificate Number: SHSY-IEC-KY-4.0/17-23/01), and all patients signed informed consent.
298 The animal study protocol was approved by Guangdong Provincial People's Hospital Research
299 Ethics Community (The Certificate Number: No. GDREC2018218A).

300 ***Consent for publication***

301 Not applicable

302 ***Availability of data and materials***

303 The datasets used and/or analysed during the current study are available from the corresponding
304 author on reasonable request.

305 ***Competing interests***

306 The authors declare that they have no competing interests

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310 ***Authors' contributions***

311 TYH contributed to the acquisition, analysis and interpretation of the data. TYH, SLW and QSQ

312 designed and drafted the manuscript. All authors read and approved the final manuscript.

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395 **Figure legends**

396 Figure1. LINC00504 was up regulated in breast cancer tissues and cells. The expression of
397 LINC00504 in breast cancer patients and healthy people was assessed in The Cancer Genome Atlas

(TCGA) database(A). qRT-PCR assay was used to assess the expression of LINC00504 in 28 pairs breast cancer tissues and adjacent noncancerous tissues(B), breast cancer cell lines (BT549, T47D, MCF-7, SKBR3 and MDA-MB-231) and normal breast cell line MCF-10A(C). **P<0.01 vs normal tissues or MCF-10A.

Figure2. The effect of LINC00504 knockdown on the proliferation, cell cycle distribution of breast cancer cells in vitro. SKBR3 and MDA-MB-231 cells were transfected with sh-control or sh-LINC00504. LINC00504 expression was detected by qRT-PCR when transfected in the two cells(A). transfected cells proliferation was detected by CCK-8 and colony formation assays(B-D). Flow cytometer was used to analyze the transfected cells at different cell cycle phase (G1, S, and G2)(E). **P<0.01 vs sh-control.

Figure3. The effect of LINC00504 knockdown on the proliferation in vivo. Transfected cells were planted in mice, and tumor growth was measured. **P<0.01 vs sh-control.

Figure4. The influence of LINC00504 knockdown on invasion, and EMT of breast cancer cells. Transfected cells migration and invasion capacities were assessed by transwell assays(A-B). Immunofluorescence was used to detect the variety of VIM and CDH1 in Transfected cells. **P<0.01 vs sh-control.

Figure5. LINC00504 directly binded to miR-140-5p in SKBR3 and MDA-MB-231 cells. The potential binding sites between LINC00504 and miR-140-5p, and the mutant in the seed region(A). The expression of miR-140-5p was detected in sh-control or sh-LINC00504 transfected cells(B). Dual-luciferase reporter assays were performed by transfecting with LINC00504-WT or LINC00504-Mut constructs into SKBR3 and MDA-MB-231 cells with miR-NC mimics or miR-140-5p(C). SKBR3 and MDA-MB-231 cells were transfected with miR-NC mimics or miR-140-5p mimics, followed by the measurement of LINC00504 mRNA enrichment with anti-Ago2 by qRT-PCR, and anti IgG served as control(D,E). Relationship between levels of LINC00504 and miR-140-5p in breast cancer tissues(F). **P<0.01 vs sh-control or miR-NC.

Figure6. LINC00504 promoted the migration, invasion, and EMT of breast cancer cells, and it was regulated by miR-140-5p-VEGFA axis. SKBR3 and MDA-MB-231 cells were transfected with sh-control, sh-LINC00504 or sh-LINC00504+miR-140-5p inhibitor, followed by the trial of colony formation for cell proliferation(A), transwell assays for cell migration(B) and cell invasion(C), western blot analysis for VEGFA, CDH1, VIM levels(D). **$P<0.01$ vs corresponding control.

Figures

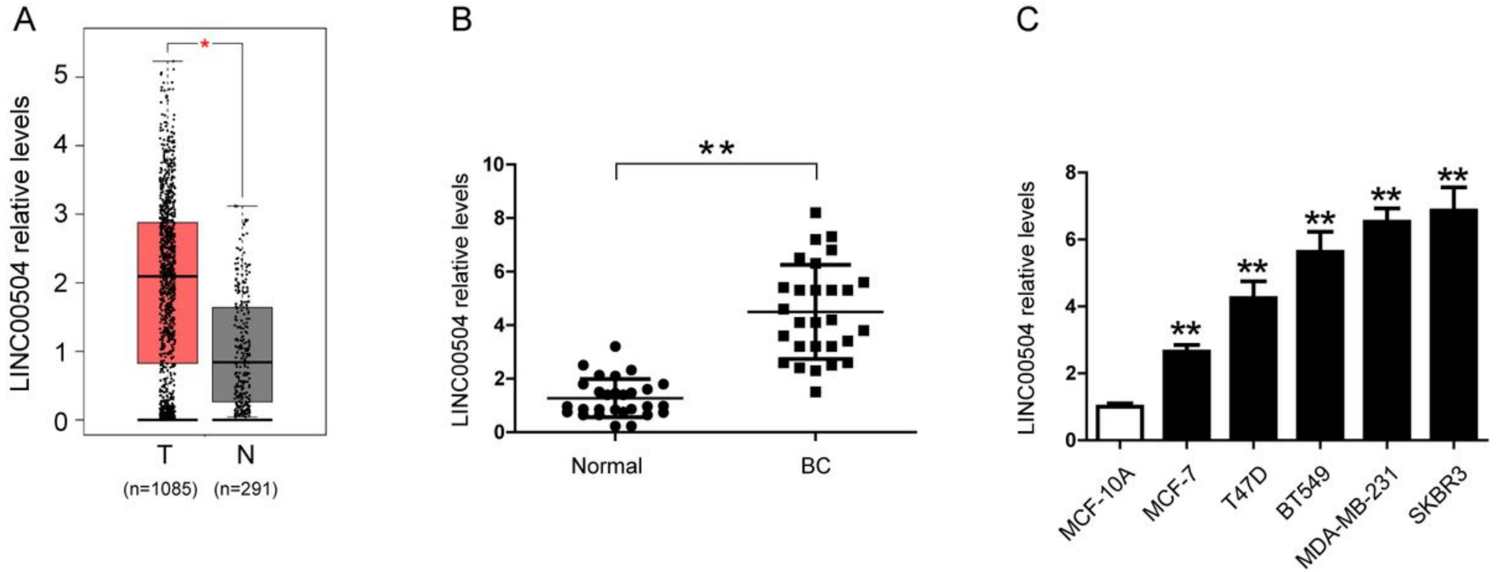


Figure 1

LINC00504 was up regulated in breast cancer tissues and cells. The expression of LINC00504 in breast cancer patients and healthy people was assessed in The Cancer Genome Atlas (TCGA) database(A). qRT-PCR assay was used to assess the expression of LINC00504 in 28 pairs breast cancer tissues and adjacent noncancerous tissues(B), breast cancer cell lines (BT549, T47D, MCF-7, SKBR3 and MDA-MB-231) and normal breast cell line MCF-10A(C). **P<0.01 vs normal tissues or MCF-10A.

Figure 2

The effect of LINC00504 knockdown on the proliferation, cell cycle distribution of breast cancer cells in vitro. SKBR3 and MDA-MB-231 cells were transfected with sh-control or sh- LINC00504. LINC00504 expression was detected by qRT-PCR when transfected in the two cells(A). transfected cells proliferation was detected by CCK-8 and colony formation assays(B-D). Flow cytometer was used to analyze the transfected cells at different cell cycle phase (G1, S, and G2)(E). **P<0.01 vs sh-control.

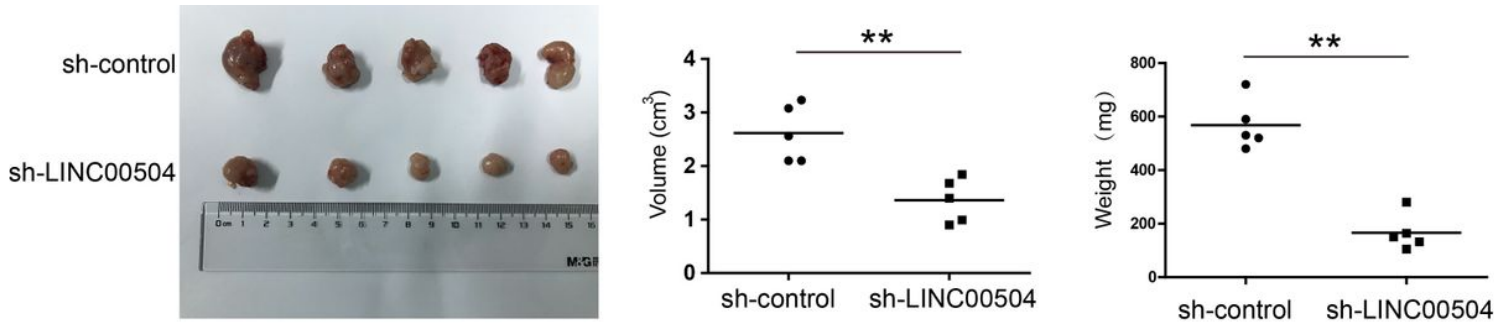


Figure 3

The effect of LINC00504 knockdown on the proliferation in vivo. Transfected cells were planted in mice, and tumor growth was measured. **P<0.01 vs sh-control.

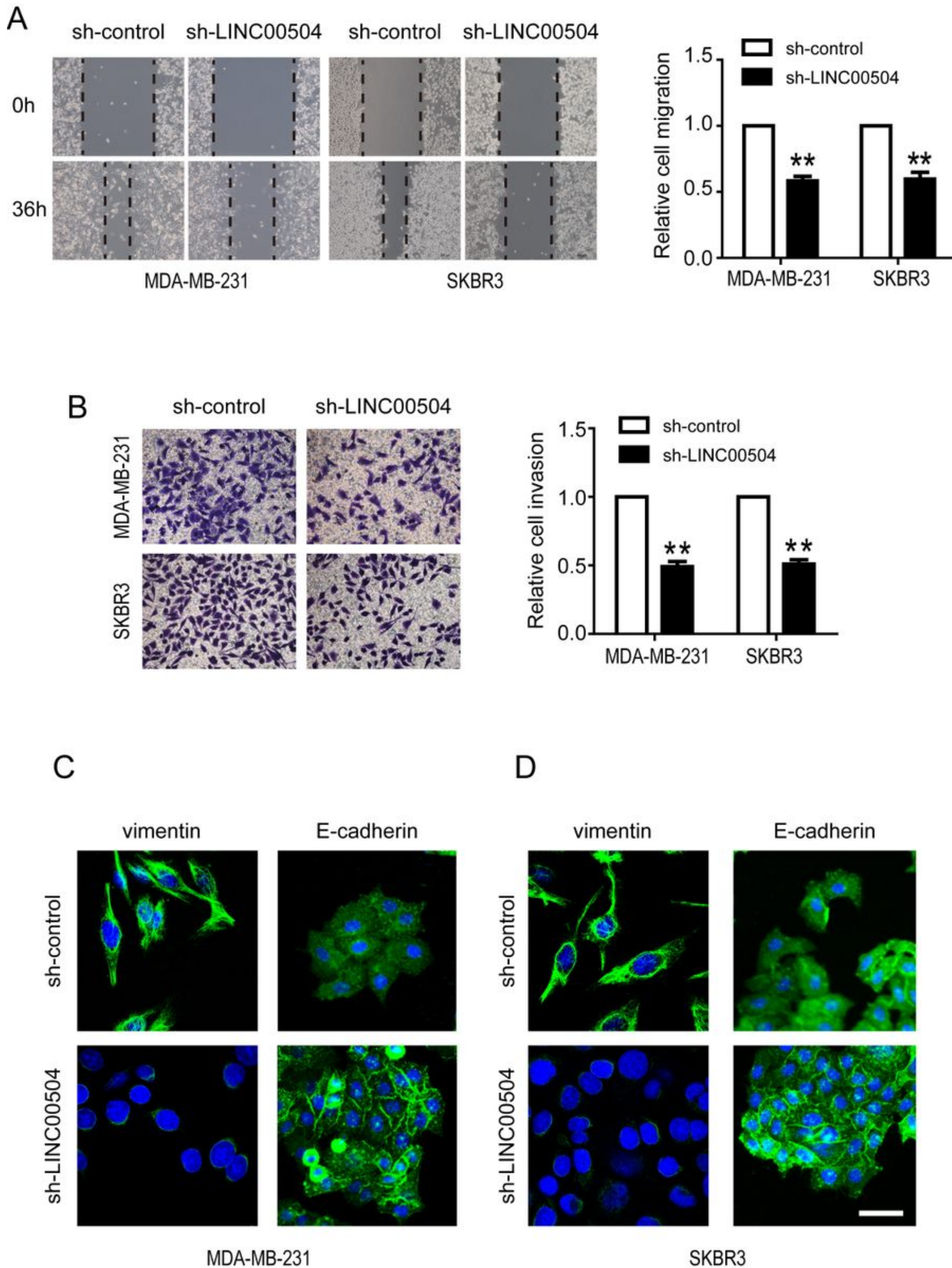
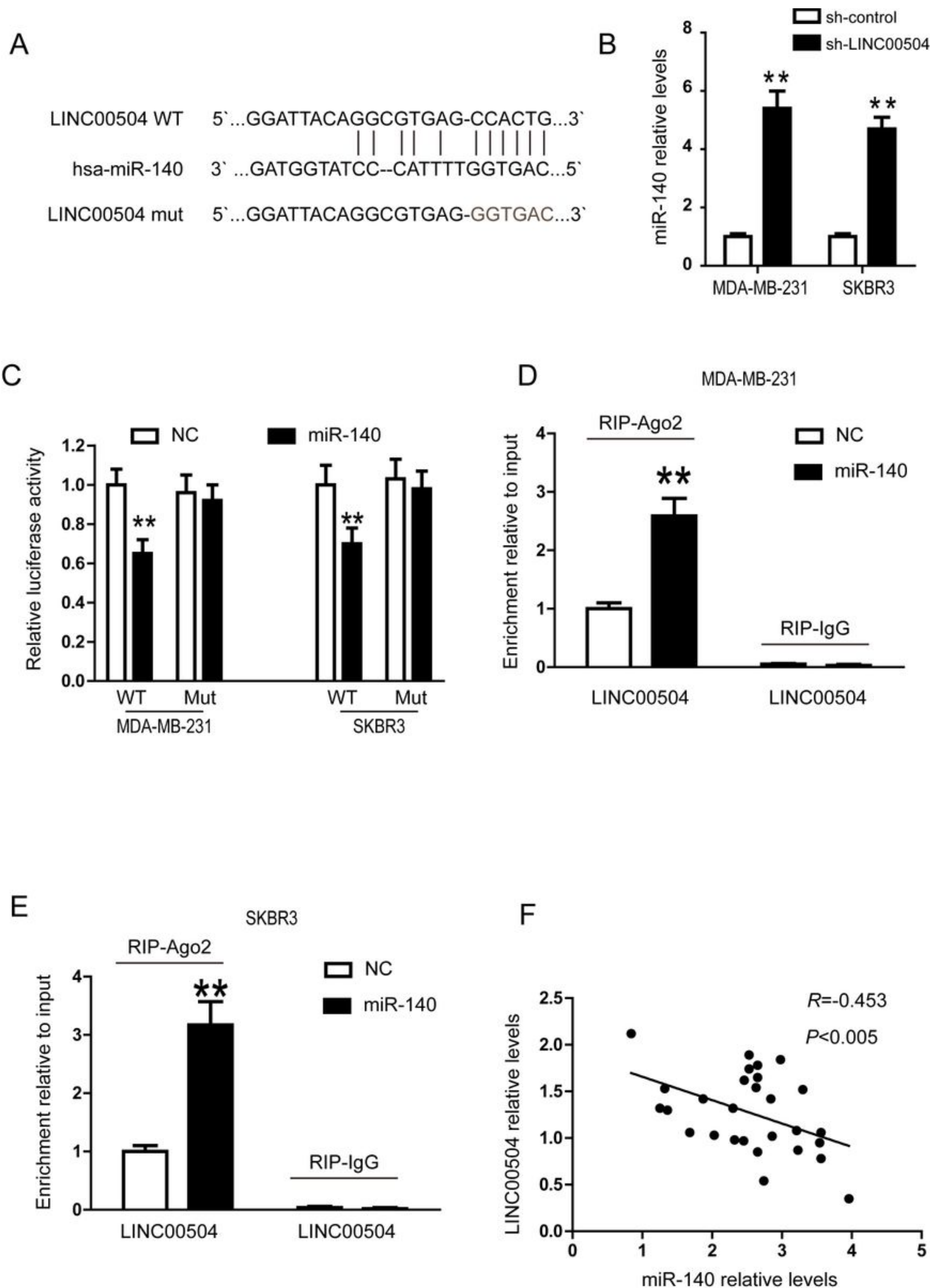


Figure 4

The influence of LINC00504 knockdown on invasion, and EMT of breast cancer cells. Transfected cells migration and invasion capacities were assessed by transwell assays(A-B). Immunofluorescence was used to detect the variety of VIM and CDH1 in Transfected cells. **P<0.01 vs sh-control.



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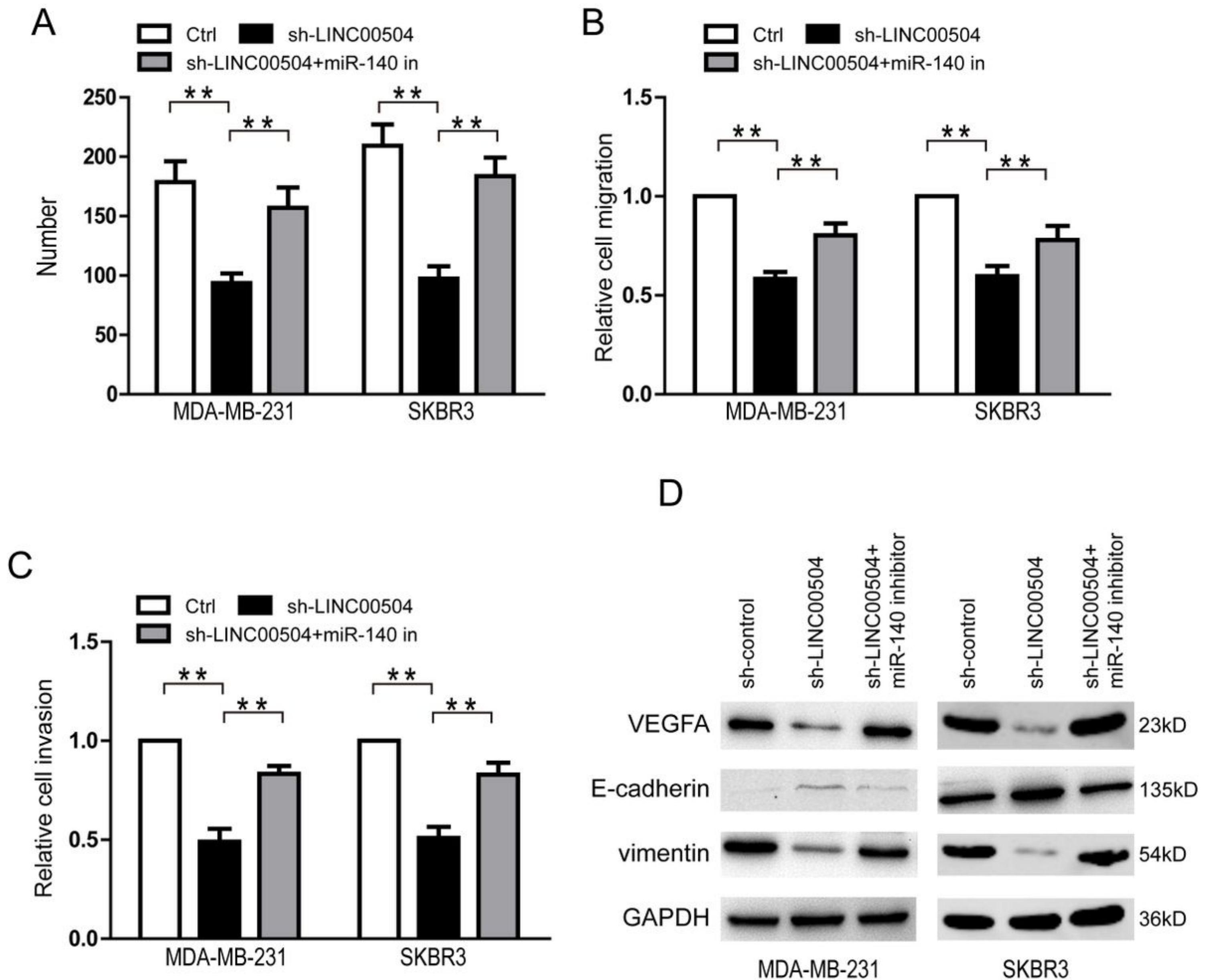

Figure 6

LINC00504 promoted the migration, invasion, and EMT of 428 breast cancer cells, and it was regulated by miR-140-5p-VEGFA axis. SKBR3 and MDA-MB-231 cells were transfected with sh-control, sh-LINC00504 or sh-LINC00504+miR-140-5p inhibitor, followed by the trial of colony formation for cell proliferation(A), transwell assays for cell migration(B) and cell invasion(C), western blot analysis for VEGFA, CDH1, VIM levels(D). **P<0.01 vs corresponding control.