OCT4 Suppresses Metastasis in Breast Cancer Cells Through Activation of STAT3 Signaling

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Research article

Keywords: OCT4, STAT3, EMT, Breast cancer, metastasis

Posted Date: September 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-66884/v1

Abstract

Background: Metastatic breast cancer is the major cause of death in breast cancer patients. Activation of epithelial-mesenchymal transition (EMT) induces migration and invasion of breast cancer cells (BCCs). OCT4 (*POU5F1*) is a key transcription factor for reprograming and plays an important role in self-renewal. Recent studies recovered OCT4 may correlate with cancer progression. However, it is no sufficient proofs to verify how OCT4 plays in metastasis of breast cancer. In this present study, we show the role of OCT4 in the migration and invasion of BCCs *in vitro* and metastasis *in vivo*.

Methods: PCR, Western Blot and Immunofluorescence staining were performed to determine to OCT4 expression in BCCs. Wound-healing assay and invasion assay were utilized to analyze the mobility of BCCs. Tumor metastasis was assessed with nude mice by subcutaneously injection. IHC assay was used to evaluate phosphorylated signal transducer and activator of transcription 3 (p-STAT3) expression in breast cancer tissues and normal breast tissues. To study whether OCT4 regulate EMT through STAT3 signal, we used shRNA to knockdown *STAT3* gene expression in BCCs.

Results: OCT4 changed cell morphology of BCCs, decreased cell adhesion, and inhibited migration, invasion and metastatic ability of BCCs. In the meantime, overexpression of OCT4 activated STAT3 signaling and changed EMT-related protein expressions in BCCs. However, knockdown of STAT3 in BCCs with overexpression of OCT4 could facilitate EMT.

Conclusion: Our data demonstrate that OCT4 suppresses EMT in BCCs through activation of STAT3 signaling, which is a key mechanism in impeding BCCs migration and invasion. Collectively, these data suggest that elevating OCT4 expression may be an effective method for reducing the metastatic potential of BCCs, which could also contribute to developing new methods for diagnosis and new molecular target therapies in breast cancer metastasis.

Background

Breast cancer (BC) is the main malignant tumor in women worldwide (Polyak et al., 2012). In advanced cancer patients, distant metastasis from breast cancer is a major cause of cancer-related deaths. However, the mechanism of this clinical problem remains unclear (Gao et al., 2012). Polarized epithelial cells transdifferentiate into mesenchymal cells during tumor metastasis, with several molecular pathways that mediate the epithelial-mesenchymal transition (EMT) in cancer cells having been identified (Kalluri et al., 2009; Polyak et al., 2009; Thiery et al., 2009; Dave et al., 2012). EMT is induced by several molecular pathways, such as Wnt, TGFβ-Smad, PI3K-AKT, STAT3 etc. (Lamouille et al., 2014).

The transcription factor signal transducer and activator of transcription 3 (*STAT3*) is regulated during cancer progression and metastasis. STAT3 regulates the initial events of tumor development also in addition to tumor progression and invasion (Huang 2007). Nevertheless, several recent reports have revealed that a loss of STAT3 expression in patient tumors increases risk of prostate cancer recurrence and metastasis (Pencik et al., 2015). IL-6/STAT3 signaling suppresses metastatic prostate cancer upon

ARF expression (Culig et al., 2016). STAT3 degrades SNAI by promoting activation of GSK3β and thus, suppresses adenoma-to-adenocarcinoma transition in Apc^{min} mice (Lee et al., 2012). Therefore, it is necessary to further study the exact role of STAT3 in breast cancer metastasis. In addition, stem cell pluripotent markers, including OCT4, Nanog, SOX2 and Kalf4, act as transcription factors and regulates STAT3 expression in human embryonic stem cells (Boyer et al., 2005), which may correlate with cancer progression. Therefore, it is necessary to analyze the role of stem cell pluripotent markers in EMT process in breast cancer metastasis.

OCT4, encoded by *POU5F1*, plays a key role in embryonic development and stem cell pluripotency (Nichols et al., 1998). The aberrant expression of OCT4 blocks progenitor-cell differentiation and triggers dysplasia in epithelial tissues (Hochedlinger et al., 2005). Nevertheless, the aberrant expression of OCT4 is linked to a variety of human cancers (Zhu et al., 2015; Lemos et al., 2016; Villodre et al., 2016). Previous studies have demonstrated that OCT4 expression is absent in HeLa and MCF-7 cells, whereas OCT4 is highly expressed in nTera cells (Cantz et al., 2008). In accordance with our previous study, OCT4 is low expressed in breast cancer tissues and cells compared to the normal breast tissues and cells (Jin et al., 2019). However, the underlying molecular mechanisms of OCT4 expression in breast cancer cells (BCCs) metastasis are not yet clear.

In this present study, we show the role of OCT4 in EMT and metastasis in BCCs. The data demonstrate that OCT4 expression is associated with suppression of migration and invasion as well as regulation of EMT process in BCCs. Concurrently, OCT4 was shown to activate STAT3 to regulate EMT-related proteins in BCCs. Finally, we provide evidence that OCT4 suppresses EMT and inhibits metastasis phenotype in BCCs via activation of STAT3 signaling.

Methods

Patients’ samples and Cell culture

Paraffin-embedded tissues including normal breast tissues and breast cancer tissues were collected from patients at the Second Hospital of Jilin University. The study was approved by the Ethics Committee of Jilin University (Changchun, Jilin, PR China). The patients with medical records did not receive any neo-adjuvant therapy. All the cancer cases were classified and graded according to the International Union Against Cancer (UICC) staging system for breast cancer.

Human breast cancer cell line MDA-MB-231 (triple-negative type) and human breast cancer cell line MCF-7 (luminal type) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (GIBCO, USA), which was supplemented with 10% fetal bovine serum (FBS) (BI, Israel) at 37 °C in a humidified 5% CO_2 atmosphere.

Lentivirus Production and Lentivirus Transduction

Lentivirus vector pLV-EF1α-OCT4-IRES-EGFP and packaging plasmids expressing gag-pol, pVSVG, and rev genes were obtained from the Institute of Biochemistry and Cell Biology of Shanghai Life Science

Research Institute, Chinese Academy of Science. Lentivirus production and transduction were performed according to our previous protocol (Liu et al., 2015). MDA-MB-231 cells and MCF7 cells were seeded on a 6-well plate and were infected with lentivirus expressing OCT4 in the presence of 5 mg/ml of polybrene for 24 h. After this, the cells were validated using PCR and Western Blot.

Western Blot

Western Blot analysis was conducted according to our previous protocol (Liu et al., 2016). The following antibodies were used: OCT4 (1:1000, Abcam, ab19857). β-actin (1:2000, CST, #3700). STAT3 (1:1000, CST, #12640S). p-STAT3 (Tyr705) (1:1000, CST, #9145). E-cadherin (1:1000, CST, #3195). Vimentin (1:1000, CST, #5741). Snail (1:1000, Abcam, ab17732-100). GAPDH (1:2000, Transgene, HC301).

Reverse-transcription PCR (RT-PCR)

Total RNA was isolated using the TRIzol reagent (Invitrogen, USA). A total of 0.5 μg of total RNA underwent reverse transcription by using the M-MLV reverse transcriptase (TaKaRa, Japan) and random primer (TaKaRa, Japan) at 42 °C for 60 min. The PCR primers are shown in Table S1. Amplification was conducted under the following conditions: PCR reactions were denatured at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. This was followed by a final extension of 5 min at 72 °C. *GAPDH* was used as an endogenous control. The results were analyzed by Quantity One 4.4.1 software (Bio-Rad Laboratories Inc, Hercules, CA, USA).

Immunofluorescence Assay

Cells were seeded onto small coverslips. After being washed three times with PBS, the cells were fixed with 4% paraformaldehyde for 10 min at room temperature. The cells were incubated with 0.1% Triton X-100 and BSA for 1 h, before being incubated with OCT4 antibody (1:200, Abcam, ab19857) at 4 °C overnight. On the next day, after being washed three times with PBS, cells were incubated with a secondary antibody (1:1000, CST, #8889) for 1 h. Immunofluorescence staining for F-actin was performed using red phalloidin staining for F-actin (AAT-23102, AAT Bioquest) and DAPI staining for nuclei (D8417, SIGMA).

Cell adhesion and spreading assay

We utilized the impedance-based iCELLigence Real-Time Cell Analysis system (ACEA Biosciences) for the detection of MDA-MB-231 and MCF-7 cell adhesion and spreading on fibronectin (354008, CORNING). Briefly, fibronectin was diluted to 10 μg/ml in DMEM media and added on to wells on E-plate (ACEA Biosciences) and incubated for 30 min at 37 °C. The media were removed and 15000 cells were added. Cell adhesion and spreading was measured with the RT-CES system every 30 min for 24 h.

Wound-healing assay

Cancer cells were cultured on 6 well plates, before being wounded by scratching with a 200-μl pipette tip, and washed 3 times with PBS. The cells were cultured in DMEM, which was supplemented with FBS free

medium at 37 °C in a humidified 5% CO_2 atmosphere for 48 h. The wounds were photographed at the beginning (0 h) and 48 h.

Migration assay

The Boyden chamber was used for this assay and cells in serum-free medium were placed into the upper chamber (8-μm pore size; Millipore, Billerica, MA, USA). Medium containing 10% FBS was added to the lower chamber. After 24 h of incubation, we removed the upper membrane cells with cotton wool. In the end, the migratory cells were stained by DAPI and counted using the Image J software.

Invasion assay

The invasive potential of cancer cells was determined using the Boyden chamber. Matrigel (356234, CORNING) was coated on the upper chamber (8-μm pore size; Millipore, Billerica, MA, USA) of a 24-well plate and incubated at 37 °C for 30 min. After this, cells outside of a serum medium were placed into the upper chamber that coated with Matrigel. Medium containing 10% FBS was added to the lower chamber. After incubation for 24 h, the cells and Matrigel remaining on the upper membrane were removed with cotton wool. In the end, the invasive cells were stained by crystal violet staining solution and counted using the Image J software.

Animal experiments

Mice were housed under hygienic conditions according to the Chinese guidelines governing animal experimentation, and their care was in accordance with institution guidelines. Animal experiments were approved by the Chinese veterinary authorities. All the mice used in the study were females that were 3–5 weeks old. Each group showed 5 mice. For subcutaneous injections, 5×10^6 MDA-MB-231 cells (MDA-MB-231-pEF and MDA-MB-231-OCT4 cells) and 5×10^6 MCF-7 cells (MCF7-pEF and MCF7-OCT4 cells) in 100 μl of PBS were injected into nude mice. After 3–4 weeks, the primary tumor reached 1000 mm^3 in volume. At the end of the experiment, lungs, livers, spleen and kidney were collected to quantify metastatic tumor cells. Tissues were placed into formalin solution and H&E staining was performed on paraffin-embedded lung, liver, spleen and kidney sections, before the metastatic area was quantified. The metastatic index was calculated as the number of metastatic foci.

Short hairpin RNA (shRNA) transfection

Four shRNAs were designed, based on the STAT3 mRNA sequence NM_003150 and were constructed into the vector U6-MCS-Ubiquitin-Cherry-IRES-puromycin (GeneChem, Shanghai, China). Cells were transfected using EndoFectinTM Max Transfection Reagent (GeneCopoeia). A negative control cell line was generated by transfecting cells with non-targeting shRNA. ShRNA sequences were listed in Table S2. The shRNA-mediated suppression of STAT3 and p-STAT3 expressions was confirmed using the Western Blot as described above.

Immunohistochemistry (IHC)

All samples were fixed in 4% paraformaldehyde overnight at 4 °C, before being dehydrated in different concentrations of ethanol. Immunohistochemistry staining and analytical methods were performed according to the protocol of UltraSensitiveTM SP(Mouse/Rabbit)IHC Kit (Maxim). P-STAT3 (Tyr705) (1:1000, CST, #9145) was used in the experiments.

Statistical analysis

Differences between means of independent groups were tested using the unpaired Student's *t*-test. Analyses were carried out using GraphPad Prism 7 (GraphPad software). Categorical variables were compared using the Chi-square test among different groups. *P* values less than 0.05 were considered to be statistically significant.

Results

Overexpression of OCT4 alters morphology of BCCs

Our previous study showed that OCT4 was low expressed in breast cancer tissues and breast cancer cells compared with normal breast tissues and cells. To assess the role of OCT4 in metastasis of BCCs, we overexpressed OCT4 in MDA-MB-231 (high metastatic potential) and MCF-7 cells (low metastatic potential), before identifying its efficiency using RT-PCR and Western Blot, respectively (Fig. 1a). OCT4 expression in the nucleus of MDA-MB-231-OCT4 and MCF-7-OCT4 cells was detected by Immunofluorescence assay (Fig. 1b). In particular, we observed that adhesive abilities of MDA-MB-231-OCT4 and MCF-7-OCT4 were significantly reduced compared with the control group (Fig. 1c). The cell-spread area and cell-adhesion in OCT4 overexpression monolayers decreased significantly in comparison to control group, indicating that OCT4 may be correlated with cancer cell adhesion and metastasis.

Overexpression of OCT4 reduces adhesion and degrades of fibronectin, and associates with remodeling of actin networks in BCCs

After this, we examined the effects of OCT4 on cell attachment substrates. We pre-coated cell culture plates with fibronectin matrix, before allowing MDA-MB-231 cells and MCF-7 cells to adhere to this fibronectin matrix. We observed that the overexpression of OCT4 in MDA-MB-231 cells and MCF-7 cells exposed to fibronectin significantly decreased the abilities of cell adhesion and spreading (Fig. 2a). Additionally, overexpression of OCT4 significantly reduced filamentous actin (F-actin) networks, which was shown using phalloidin staining. Conversely, overexpression of OCT4 led to moderating cytoplasmic F-actin staining and pronounced clusters of actin (Fig. 2b). The results indicate that OCT4 alters F-actin remodeling, which is indispensable for cell migration.

Overexpression of OCT4 inhibits migration, invasion and metastasis in vitro and in vivo

To evaluate whether OCT4 played a role in breast cancer progression, we then examined the effects of OCT4 on cancer cell metastasis. We analyzed the migratory and invasive abilities of MDA-MB-231 and MCF-7 cells *in vitro*. Consistent with our hypothesis, OCT4 overexpression inhibited migration both in MDA-MB-231 and MCF-7 cells compared to the control group, which was proven by the results of the wound-healing assay and migration assay (Fig. 3a and b). In addition, we found OCT4 also inhibited invasion in MDA-MB-231 and MCF-7 cells, which was determined using the invasion assay (Fig. 3c).

We used a xenograft model created by subcutaneous injection to validate the effects of OCT4 on BCCs metastasis *in vivo*. Cells were inoculated into nude mice. Three to four weeks after the injection, the tumors formed in each mouse. After this, we collected lungs, livers, spleens and kidneys from the nude mice. We found that only lung had metastatic loci, with the group with OCT4 overexpression showing a reduced number of lung metastatic loci compared with the control group. This was determined through hematoxylin and eosin (H&E) staining of lung sections (Fig. 4a and b). Therefore, our data *in vivo* complemented the results of functional *in vitro* studies involving OCT4.

OCT4 activates STAT3 signaling and reverses EMT

Previous studies have shown that the STAT3 signaling pathway is involved in cancer progression (Groner et al., 2017), while *STAT3* gene is bound by OCT4 in human embryonic stem cells (Boyer et al., 2005; Lister et al., 2009; Jung et al., 2010). To understand the mechanism related to the effects of OCT4 on migration and invasion in BCCs, we analyzed STAT3 and phosphorylation of STAT3 (Tyr705) in MDA-MB-231 and MCF-7 cells, which were transduced by OCT4 overexpression. We observed that OCT4 significantly increased phosphorylation of STAT3 at Tyr705 in MDA-MB-231-OCT4 and MCF-7-OCT4 cells compared with control groups (Fig. 5a).

EMT is an essential process for tumor metastasis. Epithelial protein E-cadherin is downregulated and mesenchymal proteins, such as Vimentin and Snail, are upregulated during EMT process (Lamouille et al., 2014). Therefore, we investigated the effects of OCT4 on EMT-related proteins. Overexpression of OCT4 decreased the expression of Vimentin and Snail but increased E-cadherin expression in BCCs (Fig. 5b). These combined changes suggest that OCT4 affects STAT3 activation and inhibits EMT process.

Since OCT4 could activate STAT3 signaling in BCCs, we evaluated p-STAT3 expression in normal breast tissues and different subtypes of breast cancer tissues in humans. Analysis of IHC showed p-STAT3 expression in myoepithelial cells, although there was no expression in glandular epithelium cells of breast normal tissues. In addition, p-STAT3 had lower levels of expression in luminal A and luminal B subtypes, while p-STAT3 was not expressed in human epidermal receptor 2 (Her2) and triple negative breast cancer (TNBC) subtypes (vascular endothelial cells used as positive control, Fig. 5c). We further investigated the relationship between p-STAT3 expression and clinical parameters. We found that p-STAT3 expression was correlated to molecular subtype, estrogen receptor (ER), progestogen receptor (PR) and Ki67 status (Table 1). After this, we confirmed the expression of p-STAT3 and STAT3 using Western Blot and found

that STAT3 was partially inactivated in human breast cancer tissues (Fig. 5d), indicating that STAT3 might be a negative regulator in breast cancer progression.

Table 1
Association of p-STAT3 expression with clinicopathological parameters of breast cancer patients.

Characteristics	No.	p-STAT expression		*P* value
		positive	negative	
Age (years)				
< 50	14	4 (28.57%)	10 (71.43%)	*P* = 0.694
≥ 50	26	5 (19.23%)	21 (80.77%)	
Size (cm)				
≤ 2	24	6 (25%)	18 (75%)	*P* = 0.781
2–5	14	2 (14.29%)	12 (85.71%)	
> 5	2	1 (50%)	1 (50%)	
Lymph node status				
Negative	22	5 (22.73%)	17 (77.27%)	*P* = 0.262
1–3	9	2 (22.22%)	7 (77.78%)	
4–9	4	2 (50%)	2 (50%)	
> 9	5	0 (0%)	5 (100%)	
Molecular subtype				
Luminal A	10	5 (50%)	5 (50%)	*P* = 0.002**
Luminal B	10	4 (40%)	6 (60%)	
HER2-enriched	10	0 (0%)	10 (100%)	
Triple-negative	10	0 (0%)	10 (100%)	
ER status				
Negative	20	0 (0%)	20 (100%)	*P* = 0.001***
Positive	20	9 (45%)	11 (55%)	
PR status				
Negative	23	0 (0%)	23 (100%)	*P* = 0.000***
Positive	17	9 (52.94%)	8 (47.06%)	
HER2 status				

Significance of association was determined using χ^2 test.

Characteristics	No.	p-STAT expression		*P* value
		positive	negative	
Negative	25	2 (8%)	23 (92%)	*P* = 0.622
Positive	15	2 (13.33%)	13 (86.67%)	
Ki67 status				
≤ 20%	20	8 (40%)	12 (60%)	*P* = 0.020*
> 20%	20	1 (5%)	19 (95%)	
Significance of association was determined using χ^2 test.				

Knockdown of STAT3 causes the recurrence of the EMT process

Having observed the elevated expression of p-STAT3 in BCCs with overexpression of OCT4, we sought to investigate its significance in EMT. For this, we executed the knockdown of *STAT3* expression in BCCs with overexpression of OCT4, before examining the expressions of EMT-related proteins. We found that knockdown of *STAT3* caused the recurrence of the EMT process in BCCs with overexpression of OCT4, although OCT4 expression was not altered significantly (Fig. 6a). Moreover, the migratory and invasive abilities of cells with overexpression of OCT4 were also restored after knockdown of *STAT3* (Fig. 6b and c). Our combined data demonstrate that OCT4 engages the EMT process in BCCs through activation of STAT3 signaling, which is a key mechanism for impeding BCCs metastasis (Fig. 6d).

Discussion

In this present study, we provided evidence from clinical, BCCs and animal studies that the OCT4-driven transcriptional program suppresses breast cancer metastasis. Clinically, OCT4 expression is higher in normal breast tissues compared to breast cancer tissues, which does not correlate with clinicopathological variables (Jin et al., 2019). Moreover, we identified that OCT4 regulates STAT3 signaling, which is involved in migration and invasion of BCCs *in vitro*. We further provided insight into the molecular mechanism of OCT4 in regulating downstream target genes. EMT involves loss of the epithelial phenotype and transition into a mesenchymal phenotype. At the same time, there are molecular alterations to regulate cell-cell adhesions and mobility of epithelial cells. These alterations may induce cancer cell migration and invasion into the surrounding tissues (Foroni et al., 2012). In this study, OCT4 suppresses Snail and Vimentin expression, while it activates E-cadherin expression via STAT3 signaling activation, resulting in debilitating breast cancer metastasis.

OCT4 has been previously reported to have either tumor-suppressing (Cantz et al., 2008; Ge et al., 2010; Hu et al., 2011; Matsuoka et al., 2012; Shen et al., 2014; Fu et al., 2016) or tumor-promoting actions (Gao et al., 2014; Tang et al., 2015; Lemos et al., 2016; Villodre et al., 2016). Consistent with tumor-suppressive

function, OCT4 has been shown to suppress the metastasis potential of BCCs via Rnd1 downregulation, while OCT4 overexpression leads to the upregulation of E-cadherin expression, even in BCCs with high E-cadherin level (Shen et al., 2014). Conversely, OCT4 has also been considered to facilitate cancer progression by increasing BIRC5 and CCND1 expression in hepatocellular carcinoma (Cao et al., 2013). OCT4 promotes drug-resistance and metastasis in lung cancer by regulating downstream PTEN and TNC genes (Tang et al., 2015). In embryonic stem cells, OCT4 shows antiapoptotic effects in response to stress through the activation of STAT3 signaling pathway (Guo et al., 2008). Consistent with our demonstration OCT4 abrogates EMT via activating STAT3 signaling. Nevertheless, *STAT3* is considered to be an oncogene, and activated STAT3 is present in many tumors of mice and human. Moreover, a recent study has stated that co-expression of *OCT4* and *Nanog* results in cells obtaining stem cell characteristics and promotes EMT through activation of the STAT3/Snail signaling pathway in hepatocellular carcinoma (Yin et al., 2015). Although *STAT3* initially has been considered as an oncogene, several recent studies have shown that STAT3 can also act as a tumor suppressor in the very same cancers (de la Iglesia et al., 2008; de la Iglesia et al., 2008; Musteanu et al., 2010; Lee et al., 2012; Sonnenblick et al., 2012; Gordziel et al., 2013; Grabner et al., 2015; Pencik et al., 2015). In this present study, we demonstrated that STAT3 plays an unexpected tumor-suppressive role in breast cancer. Indeed, STAT3 is not activated in breast cancer tissues. Meanwhile, other recent researches showed that disruption of STAT3 signaling promoted KRAS-induced lung tumorigenesis (Grabner et al., 2015). Loss of STAT3 or IL-6 accelerates the progression of metastatic prostate metastasis (Pencik et al., 2015). In addition, loss of STAT3 in intestinal epithelial cells interferes with the formation of early adenomas in tumor-prone Apc^{Min} mice However, at later stages of tumorigenesis, there is a loss of STAT3-enhanced progression of benign adenomas to invasive carcinomas (Musteanu et al., 2010). Therefore, STAT3 signaling is mainly regulated by upstream genes and STAT3 function is possibly influenced by context in terms of the oncogenic driver mutation, cancer type or the specific tumor microenvironment.

Meanwhile, several recent studies demonstrated that OCT4 downregulates the EMT regulator Snail via *Tgfβ3* and *TgfβR3*. When combined with SOX2, this activates a specific cluster of the miR-200 miRNA family, which subsequently represses the EMT regulator Zeb2 that facilitates the EMT (Li et al., 2010; Wang et al., 2013). Additionally, it has been reported that E-cadherin was an important downstream OCT4 effector for establishing cell adhesion properties required to maintain a pluripotent state (Livigni et al., 2013). Increasing evidence has shown that *Snail* acts as a *E-cadherin* gene repressor for triggering EMT, which binds to the E-box site in the promoter of *E-cadherin* and suppresses *E-cadherin* transcription (Jiao et al., 2002). Moreover, STAT3 suppresses adenoma-to-adenocarcinoma transition through activation of GSK3β and degradation of Snail in Apc^{min} mice (Lee et al., 2012). *Snail* is also known to upregulate *N-cadherin* and *Vimentin* expressions (Nieto 2002). In present study, we found that knockdown of *STAT3* expression triggers Snail and Vimentin upregulation and E-cadherin downregulation. Thus, OCT4 regulates these EMT markers via STAT3 signaling.

Conclusion

In summary, we demonstrate, for the first time, a novel molecular mechanism for OCT4 in breast cancer, in which OCT4 inhibits EMT and breast cancer metastasis by activating STAT3 signaling. Furthermore, *STAT3* is not only an oncogene, but also a tumor suppressor gene in breast cancer. From a clinical point of view, the expression level of OCT4 in breast tumor specimens could be an effective diagnosis and prognosis biomarker. This may provide new insights for predicting the progression of breast cancer. Hence more intensive studies are required in this field before this vision can become a clinical reality. Therefore, further investigations are necessary to provide the explicit mechanism by which OCT4 activates STAT3 signaling pathway in BCCs.

Abbreviations

BC
Breast cancer; EMT:Epithelial-mesenchymal transition; STAT3:Signal transducer and activator of transcription 3; BCCs:Breast cancer cells; Her2:Human epidermal receptor 2; TNBC:Triple negative breast cancer; ER:Estrogen receptor; PR:Progestogen receptor.

Declarations

Acknowledgements

We thank to Professor Hongwen Gao, Department of Pathology, the Second Hospital of Jilin University, Changchun, China, for providing patients' samples.

Authors' contributions

Chengshi Quan, Xiangshu Jin and Yanru Li were involved in the study conception and design. Huinan Qu, Da Qi, Xinqi Wang and Yan Lu were participated in data acquisition, analysis. Yuan Dong, Yingying Liang and Qiu Jin did the experiments. Xiangshu Jin wrote the manuscript. All authors have read and approved the manuscript for publication.

Funding

This work was supported by the Opening Project of Animal Experiments from the Science and Technology Development Projects of Jilin Province [grant number 20170623093-TC].

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jilin University (Changchun, Jilin, PR China). Prior donor consent and the approval of the Institutional Research Ethics Committee from all patients were

obtained for use of the data. Mice were housed under hygienic conditions according to the Chinese guidelines governing animal experimentation, and their care was in accordance with institution guidelines. Animal experiments were approved by the Chinese veterinary authorities.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Figures

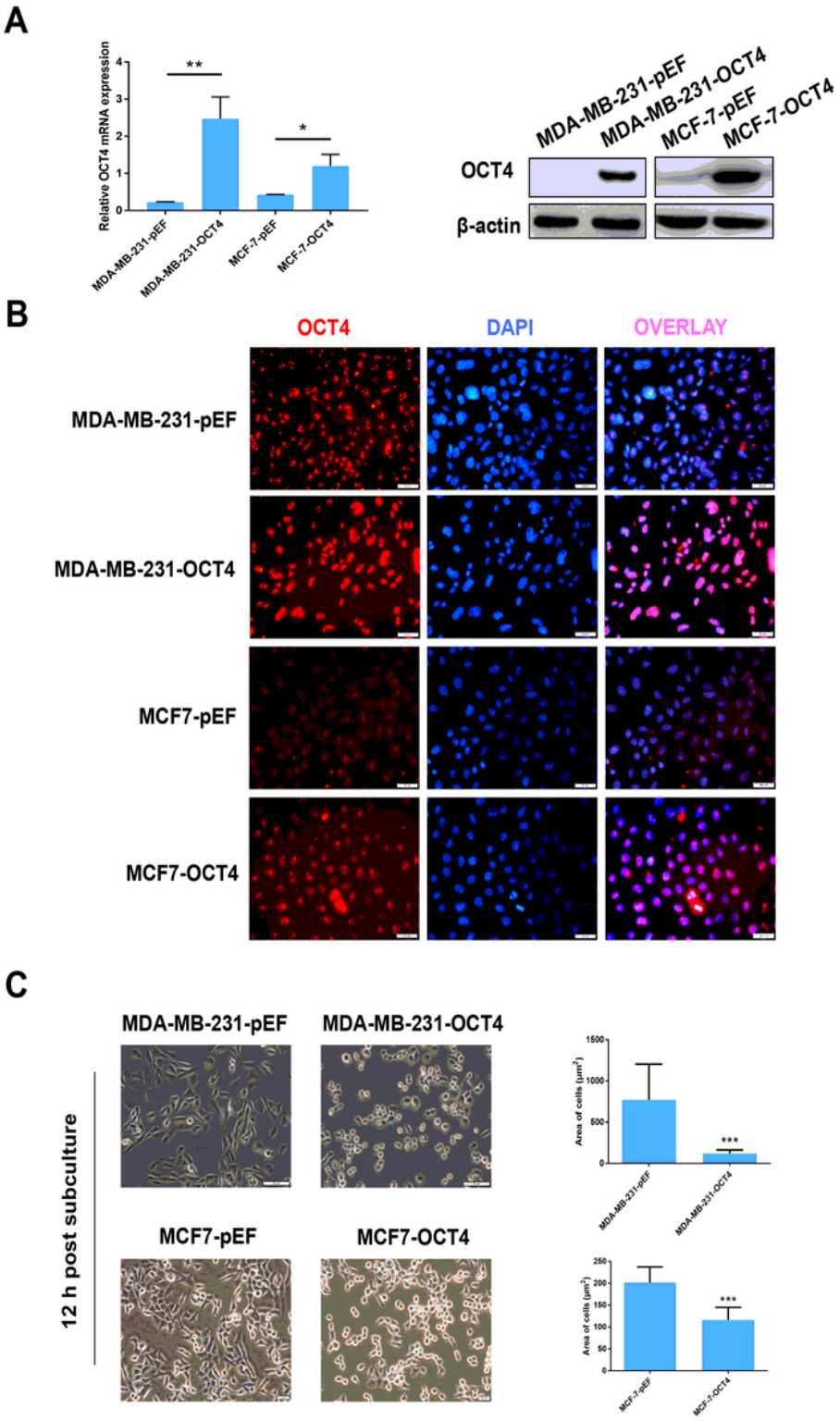

Figure 1

Overexpression of OCT4 alters morphology of BCCs. a Using lentivirus transduction, OCT4 was overexpressed in MDA-MB-231 and MCF-7 cells. MDA-MB-231 and MCF-7 cells transduced with empty vector controls (MDA-MB-231-pEF, MCF-7-pEF) and OCT4 overexpression (MDA-MB-231-OCT4, MCF-7-OCT4) were detected by RT-PCR and Western Blot. b Immunofluorescence assay showed OCT4 (red) expression in the nucleus of MDA-MB-231-OCT4 and MCF-7-OCT4 cells. DAPI (blue) represented cell

nucleus. Images were taken under fluorescent microscope with 200× magnification. c Cell-spread areas in BCCs with overexpression of OCT4 were observed to decrease significantly in comparison to the control group at 12 h post subculture. Images were taken under light microscope at 100× magnification. The experiments were repeated three times. ***, P<0.001.

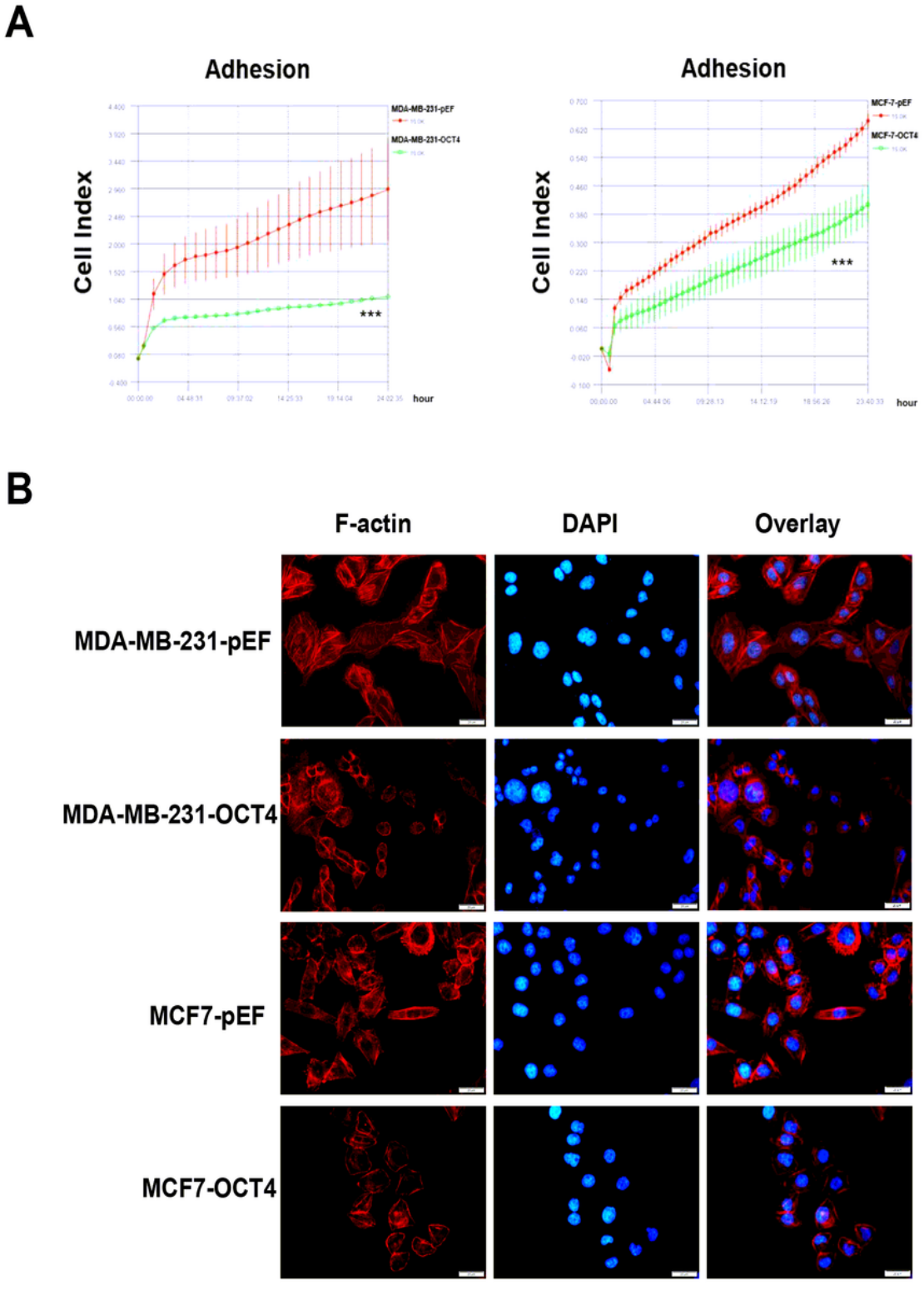

Figure 2

Overexpression of OCT4 reduces adhesion and degrades of fibronectin, which is associated with remodeling of actin networks in BCCs. a MDA-MB-231 and MCF-7 cells with OCT4 overexpression were plated in suspension and allowed to adhere to fibronectin. Fibronectin was plated at 10 μg/ml and cell impedance was monitored for 24 h after plating by using iCELLigence Real-Time Cell Analysis system. b Immunofluorescence images of MDA-MB-231 and MCF-7 cells with OCT4 overexpression showed F-actin (red), which represented filamentous actin as determined by phalloidin staining. DAPI (blue) represented cell nucleus. Images were taken under fluorescent microscope at 200× magnification. Each experiment was repeated three times. ***, $P<0.001$.

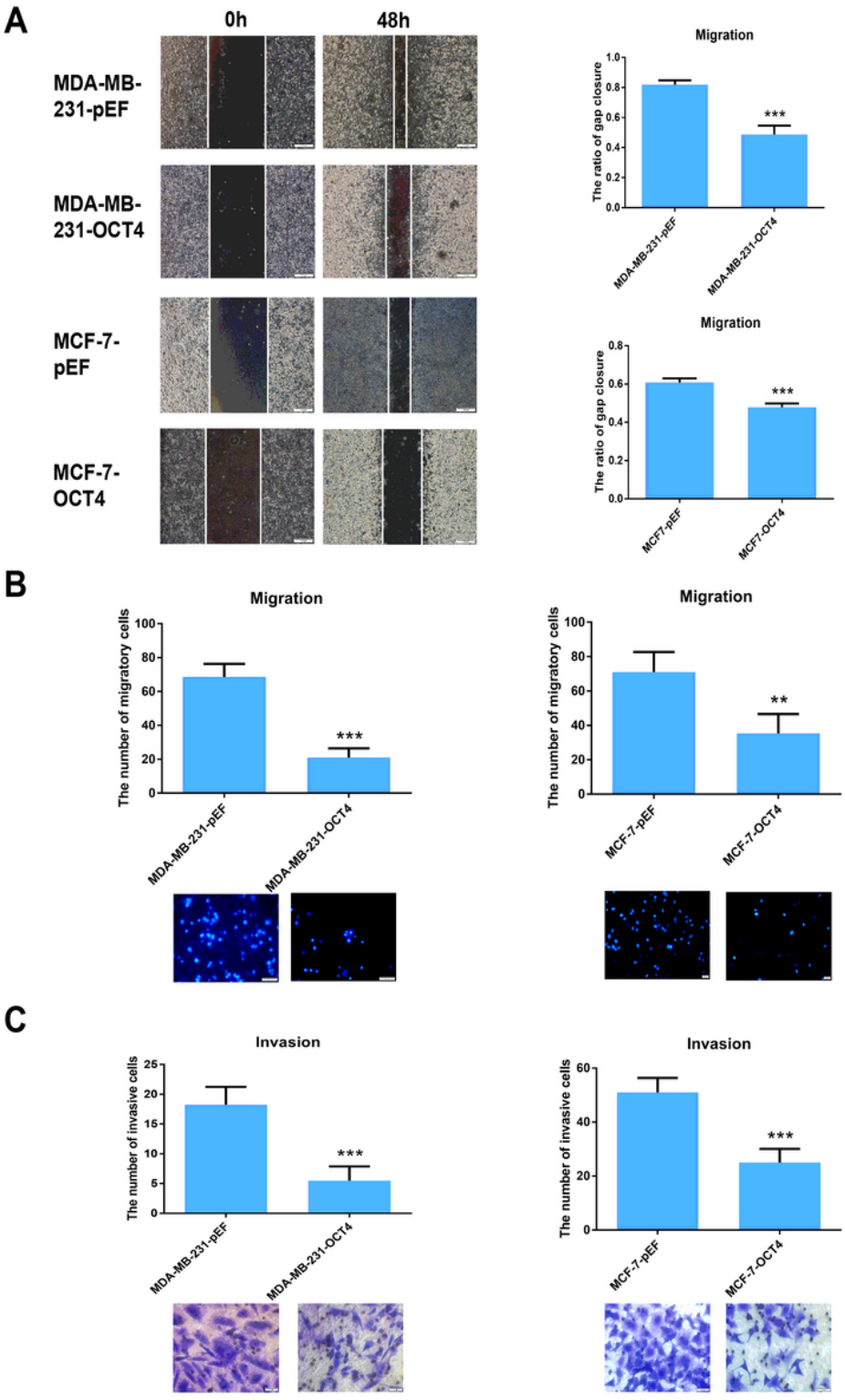

Figure 3

Overexpression of OCT4 inhibits migration and invasion in BCCs in vitro. a The migration of MDA-MB-231 and MCF-7 cells with overexpression of OCT4 were compared with the control group by the wound-healing assay. Images were taken under light microscope with 40× magnification. b The migration of MDA-MB-231 and MCF-7 cells with overexpression of OCT4 were compared with the control group by the trans-well assay. Images were taken under fluorescent microscope with 100× magnification. c The

invasion of MDA-MB-231 and MCF-7 cells with overexpression of OCT4 were compared with the control group by the trans-well (coated with Matrigel) assay. Images were taken under light microscope with 200× magnification. **, P<0.01. ***, P<0.001.

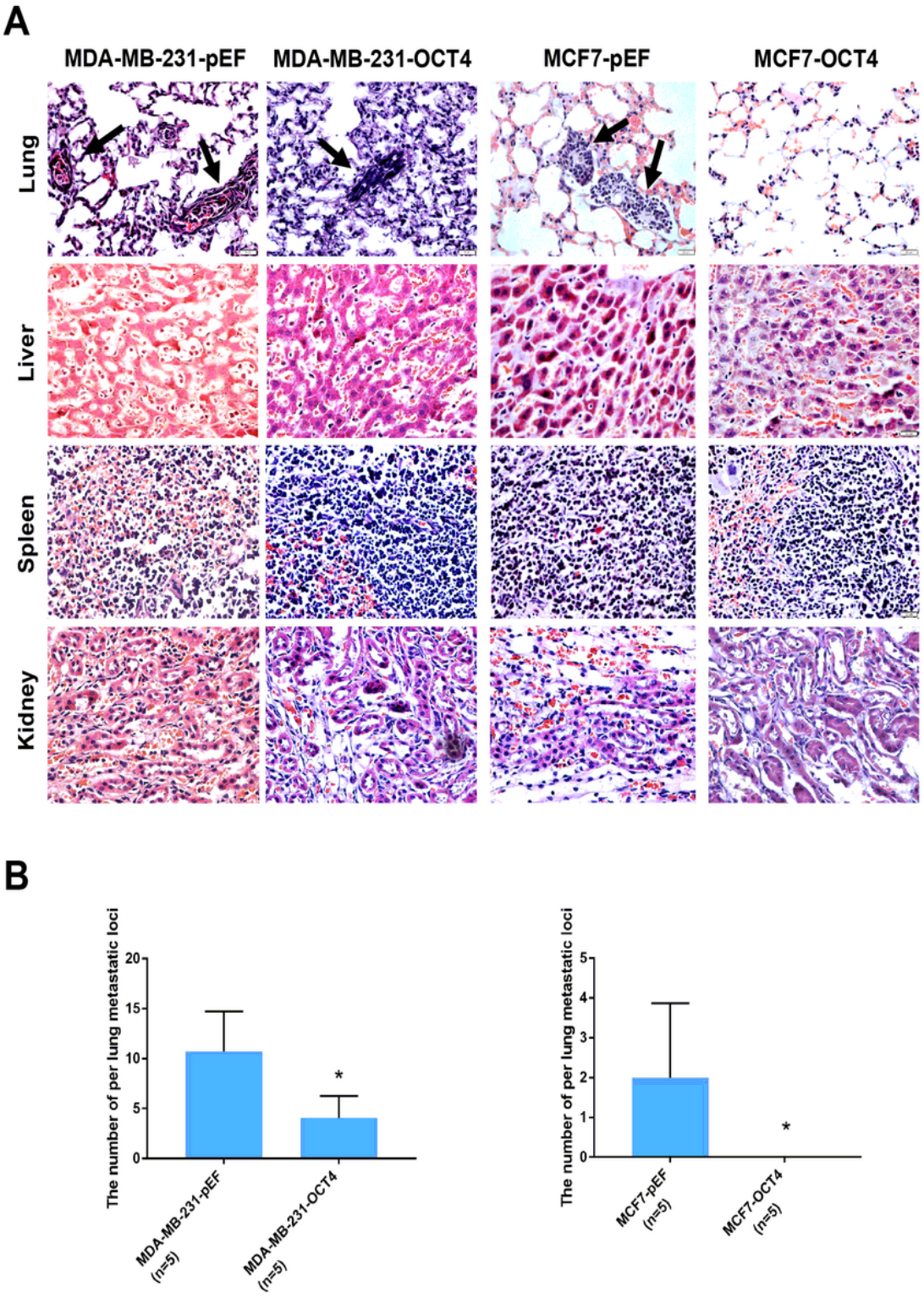

Figure 4

OCT4 suppresses metastasis in BCCs in vivo. a Balb/c nude mice subcutaneous transplant tumor models were established using MDA-MB-231 and MCF-7 cells transduced by OCT4 overexpression and empty

vectors, respectively. Mice from MDA-MB-231 groups were sacrificed after 22 days, while mice from MCF-7 groups were sacrificed after 28 days. H&E staining was applied to sections of lungs, liver, spleen and kidney, which were dissected from the mice. The arrows were metastatic tumor cells. Images were taken under light microscope with 400× magnification. b Quantity of metastatic loci to per lung was counted. *, P<0.05.

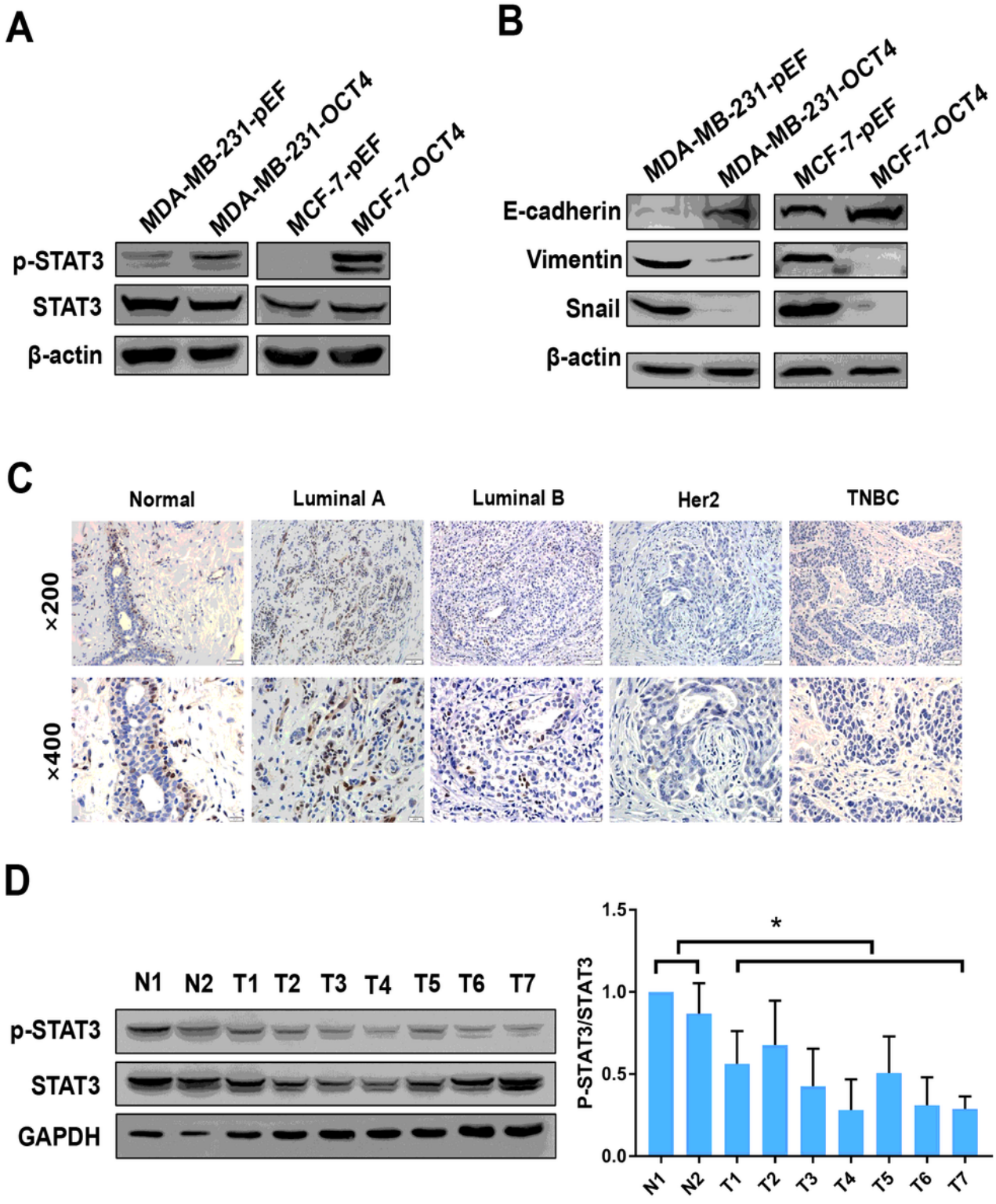

Figure 5

OCT4 activates the transcription factor signal transducer and activator of transcription 3 (STAT3) signaling and suppresses epithelial-mesenchymal transition (EMT) in BCCs. a Western Blot analysis of changes in activation of STAT3 protein as assessed by phosphorylation of STAT3 at Tyr705 in MDA-MB-231 and MCF-7 cells transduced by OCT4 overexpression and empty vectors, respectively. b Using Western Blot, the expressions of EMT markers, including E-cadherin, Vimentin and Snail, were analyzed in MDA-MB-231 and MCF-7 cells transduced by OCT4 overexpression and empty vectors, respectively. c IHC staining for p-STAT3 expression was performed in human normal breast tissues and human different breast cancer subtypes tissues. Images were taken under light microscope with 200× and 400× magnification. d The protein expressions of p-STAT3 and STAT3 in breast normal tissues (N) and breast cancer tissues (T) were further confirmed using Western Blot. *, $P<0.05$. ***, $P<0.001$.

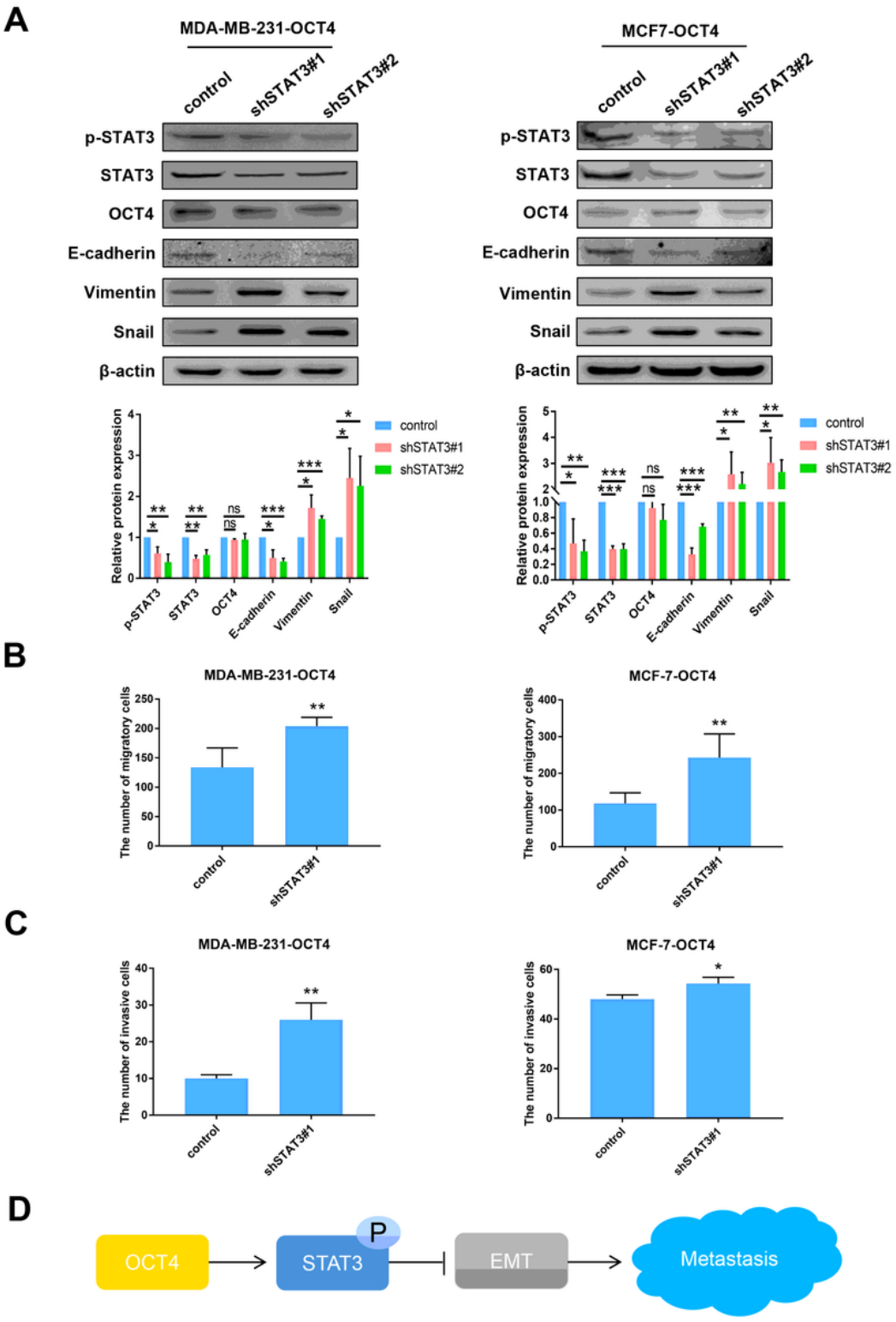

Figure 6

Knockdown of STAT3 causes the recurrence of the EMT process and promotes migration and invasion in breast cancer cells with overexpression of OCT4. a MDA-MB-231-OCT4 and MCF-7-OCT4 cells were transfected with shRNA for STAT3. Non-targeting shRNA was used as negative controls. STAT3, p-STAT3, OCT4, E-cadherin, Vimentin and Snail expressions were detected by Western Blot. b Knockdown of STAT3 in MDA-MB-231-OCT4 and MCF-7-OCT4, which showed that the migratory abilities were significantly

enhanced compared to the control group. c Comparison with the control group, which showed that the invasive abilities were significantly enhanced in MDA-MB-231-OCT4 and MCF-7-OCT4 cells. d Proposal model of OCT4 function in metastasis of BCCs. *, P<0.05. **, P<0.01. ***, P<0.001.

Supplementary Files

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- TableS2.docx
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