

UXT, a novel DNMT3b-binding protein, promotes breast cancer progression via negatively modulating lncRNA MEG3/p53 axis

Zhong-Feng Huang

Xiangya Hospital Central South University

Yu-Ling Tang

Central South University Third Xiangya Hospital

Zhao-Long Shen

Central South University Third Xiangya Hospital

Kai-Yan Yang

Central South University Third Xiangya Hospital

Kai Gao (✉ gaokai1501@163.com)

The Third Xiangya Hospital of Central South University <https://orcid.org/0000-0002-8725-3101>

Primary research

Keywords: UXT, lncRNA MEG3, DNMT3b, p53, breast cancer

Posted Date: September 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-63588/v1>

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

[Read Full License](#)

Version of Record: A version of this preprint was published at Molecular Therapy - Oncolytics on December 1st, 2021. See the published version at <https://doi.org/10.1016/j.omto.2021.12.008>.

Abstract

Background

UXT is highly expressed in tumor tissues, which function as a vital role in tumor progression. However, the exact role and potential mechanism of UXT in the tumorigenesis of breast cancer remains largely unknown.

Methods

Expression patterns of UXT and MEG3 were examined using qRT-PCR and western blot, respectively. The capacity of cell proliferation, apoptosis, migration and invasion was assessed using CCK-8, flow cytometry and transwell assays. The methylation of MEG3 promoter was determined using methylation specific PCR. Co-immunoprecipitation was performed to measure the UXT/DNMT3b interaction. RNA-immunoprecipitation was subjected to evaluate the regulation of MEG3 on p53 activity. The xenograft tumor experiment was further conducted to certify the molecular mechanism.

Results

UXT was up-regulated while MEG3 was down-regulated in breast cancer tissues and cell lines. UXT knockdown or MEG3 overexpression significantly inhibited the proliferation, promoted the apoptosis, and weakened the migration and invasion of MCF7 and ZR75 cells. The hypermethylation of MEG3 promoter was found in breast cancer cells, which was modulated by highly expressed DNMT3b. UXT inhibited the level of MEG3 via recruiting DNMT3b to its promoter. Mechanistically, MEG3 positively regulated the transcriptional activity of p53 via binding with it, thus regulating cell apoptosis, migration and invasion. The xenograft tumor experiment indicated that UXT negatively regulated the MEG3/p53 axis in a DNMT3b-dependent manner to promote the tumor growth.

Conclusions

UXT, a novel DNMT3b-binding protein, aggravates the progression of breast cancer through MEG3/p53 axis, which may provide a new insight involving in the treatment of breast cancer.

Introduction

In recent decades, cancers have been regarded as the leading cause of human death, which were traditionally recognized as genetic disorder. Although the increasingly apparent that epigenetic alterations, such as microRNA dysregulation, DNA methylation and histone modifications, have been found to play crucial roles in the development and progression of cancers, there is still unable to exactly explain the pathogenesis of cancers. Emerging evidence showed that several epigenetic modifier genes

exert crucial functions participating in malignant tumor transformation and progression [1]. Therefore, it is urgent to get a better understanding of genetic and epigenetic alterations in the development of cancers for searching new therapeutic targets in cancer treatment.

Ubiquitously expressed transcript (UXT), a putative member of an α -class prefoldin protein family, is first discovered by Andreas Schroer in 1998 [2, 3]. Located in Xp11.23-p11.22, *UXT* gene is composed of seven exons and encodes a protein of 157 amino acids [3]. UXT is ubiquitously expressed and predominantly localizes in the nucleus [4]. It has been reported that UXT is markedly elevated in some human tumor tissues including bladder, breast, ovary, and thyroid, but not in the matching normal [3]. Abrogation of UXT protein expression by small interfering RNA leads to human osteoblast sarcoma U2 (U2OS) cell death [5]. Besides, UXT also suppresses cell transformation via interacting with survival stimulatory factors like Evi1 [6], and acts as an oncogene of sarcoma promoting cell proliferation *in vitro* and tumor progression *in vivo* [5]. All these findings indicated that UXT might be a novel target against tumor. Nevertheless, further research remains to be done to explore the underlying mechanisms and regulatory networks of UXT.

Long non-coding RNAs (lncRNAs) are a heterogeneous group of non-coding transcripts longer than 200 nucleotides [7]. Abnormal expression of lncRNAs has been demonstrated to be involved in cancer tumorigenesis, development, and progression [8–10]. Maternally expressed gene 3 (MEG3), located on human chromosome 14q32, constitutes the imprinted domain reciprocally imprinted with the paternally expressed gene DLK1 [11]. It is a lncRNA with a length of ~ 1.6- kb nucleotides, and expresses in many human normal tissues [12]. However, the expression of MEG3 is lost or decreased in various tumor such as meningioma, colon cancer, nasopharyngeal carcinoma, and leukemia [13, 14], implying its anti-tumor functions.

p53, a star of tumor suppress gene, responses to diverse stress stimulation to prevent tumor development and avoid unnecessary pathological consequences [15, 16]. In tumor, p53 is frequently mutated or lost [17]. Our previous study demonstrated that UXT binds to MDMX and suppresses the basal activity of p53, thereby inducing glycolysis by activated NF- κ B in sarcoma [5]. Zhu et al. also found that ectopic expression of MEG3 inhibits hepatoma cells proliferation and induces apoptosis by interacting with p53 protein [18]. Therefore, both UXT and MEG3 participated in the regulation of p53 activity. However, whether there is the interaction between UXT and MEG3 remains unknown.

In the present study, we investigated the role of UXT and its potential mechanisms in tumorigenesis of breast cancer. We found that UXT was up-regulated whereas MEG3 was down-regulated in both breast cancer tissues and cell lines. Further mechanistic experiments revealed that UXT modulated the expression of MEG3 through methylation of MEG3 promoter via directly binding to DNMT3b, in turn inhibiting apoptosis, enhancing migration and invasion, and accelerating tumor growth, which provide the theory basis for strategies development against cancer based on UXT, MEG3 and p53.

Materials And Methods

Patient and tumor sample preparation

13 pairs of breast cancer and adjacent non-tumor tissue specimens were obtained from surgical specimens at The Third Xiangya Hospital of Central South University after informed consent. Breast cancer was diagnosed by pathologist. Adjacent non-tumor tissue specimens were taken from a standard distance (3 cm) from the margin of resected neoplastic tissues of patients with tumors who ensured surgical breast ablation. All these specimens were snap-frozen in liquid nitrogen after excision. This study was approved by the Medical Ethics Committee of The Third Xiangya Hospital of Central South University. Informed consent was obtained from each participant and the procedures were carried out in accordance with the approved guidelines.

Xenograft assays

All animal experiments were performed in accordance with relevant institutional and national guide lines and regulations. Total of twenty six-week-old immune-deficient mice (BALBC/C-nu/nu, Vital River Co.) were randomly divided into 4 group (siNC, siUXT, siUXT + siDNMT3b, siUXT + DNMT3b) and injected subcutaneously with indicated cells (1×10^6) suspended in 100 μ l 0.9% sodium chloride (NaCl) solution. The volume of the xenograft tumor was calculated as $0.5 \times \text{length} \times \text{width}^2$. Tumor growth was observed every three days, and until 30 days, the mice were sacrificed and the tumors were harvested, weighed, and photographed.

Immunohistochemistry

Xenograft tumors were fixed in 4% paraformaldehyde solution for 48 h, then embedded in paraffin and processed into 5 μ m sections. The sections were stained with indicated antibodies (UXT, 1:50, Cell Signaling Technology, USA; DNMT3b, 1:100, Cell Signaling Technology, USA; p53, 1:100, Cell Signaling Technology, USA) and visualized by diaminobenzidine (DAB).

Cell culture, plasmid construction and transfection

The human breast cells MCF10A and breast cancer MCF7 and ZR75 cells were obtained from ATCC. These cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA) and 1% antibiotics at 37 °C with 5% CO₂. All cell transfection experiments were carried out by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's instructions.

For gain-of-function of MEG3, full-length Homo sapiens MEG3 sequences (NR_002766) were cloned into the pcDNA3.1 vector. pcDNA3.1 vector was available commercially from OriGene. And then MCF7 and ZR75 cells were transfected with the vector pcDNA3.1-MEG3 to stably expressing MEG3 in two cell lines. qRT-PCR assay was performed to examine the high expression of MEG3. The cell line with stably expressing empty vector pcDNA3.0 served as control.

For loss-of-function of UXT, target sequences for UXT siRNA (⁴³⁴UAC AAG GCC UGC AGA AUU U⁴⁵² and ³⁶²GCA ACA GCC UCA CCA AGG A⁵⁸⁰) were design and chemically synthesized by GeneParma. MCF7 and

ZR75 cells were transfected with 200 pmol siRNA. After incubated for 48 h, corresponding assay were performed. Western blot was used to detect the low expression of UXT. The non-specific control siRNA duplexes were used as the control.

Total RNA extraction and quantitative RT-PCR

Total RNA was isolated using Trizol Reagent (Invitrogen) according to the manufacturer's instruction. Subsequently, 1 µg of total RNA was used to make cDNA (Takara), which served as the template of quantitative RT-PCR that was performed by Premix Ex TaqTM (Takara). The n-fold change in gene relative expression was determined on the basis of $\Delta\Delta C_t$ value. The following primer pairs were used:

MEG3: forward 5'-AGACCCGCCCTCTGACTGAT - 3',

reverse 5'-AGGAGCCCACTTCCCACA - 3';

UXT: forward 5'-GACAAGCCGATTCCCAGCGTT-3'

reverse 5'-TAGACCCTGGTGACACAGTTGCTT-3';

β -actin: forward 5'-CTGTCCACCTTCCAGCAGATGT-3',

reverse 5'-CGCAACTAAGTCATAGTCCGCC-3'.

DNA extraction, bisulfite DNA modification and methylation specific PCR

Genomic DNA from MCF7 and ZR75 cells was extracted using DNeasy Blood and Tissue Kit (Qiagen, Gaithersburg, MD, USA) according to the manufacturer's guidelines. Sodium bisulfite modification of DNA and subsequent purification was performed according to the manufacturer's guidelines for sodium bisulfite conversion of unmethylated cytosine in DNA using EpiTect Bisulfite kit (Qiagen). Bisulfite-treated genomic DNA was subjected to an optimized methylation-specific PCR, and was performed as Zhou et al. described [19].

Total protein extraction and western blot

Cells were lysed in RIPA buffer supplemented with protease inhibitor cocktail (Roche) for 30 min on ice to extract total protein. Protein concentration was determined by BCA protein assay (ThermoScientific). The protein samples were boiled in 1 × sodium dodecyl sulfate buffer for 5 minutes. Protein in the same amount was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto poly-vinylidene fluoride membranes. The membranes were blocked with TBST (100 mmol/l Tris-HCl pH7.4, 150 mmol/l NaCl and 0.05% Tween20) containing 5% non-fat milk for 1 h, following incubated with indicated antibody at 4 °C overnight. After washed for three times, the membranes were probed by another matching second antibody. Eventually, it was visualized by enhanced chemiluminescence reagents super signal (Thermoscientific). Mouse monoclonal anti-p53 antibody (1:1000), mouse monoclonal anti-DNMT3b antibody (1:1500), mouse monoclonal anti-UXT antibody (1:1000) and mouse

monoclonal anti- β -actin antibody (1:2000) were purchased from Cell Signaling Technology. Image J were used for quantitative analysis of gray ribbon.

Co-immunoprecipitation

After quantitative analysis of extracted total protein by BCA, 200 μ g of total cell lysate were incubated with a Flag antibody overnight at 4 °C in a rocker platform. Protein A/G plus agarose beads (#sc-2003; Santa Cruz Biotechnology) were then added, in accompany with incubated on a rocker platform for an additional 2 h at 4 °C. After collected by centrifugation and washed three times in PBS, the beads were resuspended in SDS loading dye and subjected to SDS-PAGE. The following experimental procedure is the same that in western blot described above.

Luciferase Reporter Assay

p53-TA-luc and internal control plasmid pRL-SV40 were co-transfected into HEK293T cells. The luciferase assay was performed by using a dual-luciferase reporter assay system (Promega). Luciferase activity was normalized against an internal control to correct for the variations in transfection efficiency.

Flow cytometry analysis of apoptosis

Cells transfected with pcDNA3.0 or pcDNA3.0-MEG3 were harvested by trypsinization. After double staining with FITC-Annexin \square and Propidium isodide (PI), the cells were analyzed using flow cytometry (FACScan, BD, Biosciences). Cells were distributed into four parts including viable cells, dead cells, early apoptotic cells, and apoptotic cells. This experiment was independently performed at least three times.

Migration and invasion

Migration assays were performed 24-well BD BiocoatTM MatrigelTM Invasion Chambers (8 μ m pore size; BD Biosciences). After indicated treatment, 5×10^4 cells suspended in 300 μ l medium with 2% FBS were seeded on the top chamber of the transwell and medium containing 10% FBS were added to bottom chamber. After 19 h of incubation, the cells that migrated through the membrane were fixed using 70% methanol and stained with crystal violet. The average number of cells was calculated from sixteen fields of each insert. For invasion assay, the insert were covered by Matrigel diluted by serum-free medium with ratio 1: 7.

RNA immunoprecipitation

After indicated treatment, cells were washed twice in PBS with PMSF, lysed in RIPA buffer (Thermoscientific), and incubated at 4 °C for 30 minutes with rotation. After centrifugated at 13,000 RPM for 15 minutes at 4 °C, the supernatant were divided into two parts, which respectively used to test the expression of RNA-binding protein of interest by western blotting and retrieve RNA for comparison in qRT-PCR. Samples were successively washed two times with low salt wash buffer (RIPA buffer) and high salt wash buffer (50 mM Tris, pH 7.4, 1 M NaCl, 1 mM EDTA, 0.1% SDS, 1% NP-40, and 0.5% sodium deoxycholate). 100 μ l each out of 1 ml of the beads suspension during the last wash was removed to test the efficiency of immunoprecipitation by western blotting. Remained beads and 1% input sample were

treated with proteinase K buffer containing 117 μ l of RIP wash buffer, 15 μ l of 10% SDS, 18 μ l of 10 mg/mL proteinase K at 55 °C for 30 minutes with shaking to digest the protein. The RNAs in the buffer were extracted by TriZol reagent. Quantitative PCR was performed to detect the MEG3 presented in the immune complex.

Statistical analysis

All experiments were independently performed at least three times. The values are presented as mean \pm standard deviation (SD). Differences were assessed by two-tailed Student's *t*-test or multi-groups one-way (ANOVA). Analysis was performed using GraphPad prism (prism 5 for windows). $P < 0.05$ was considered as significantly statistical difference.

Results

Up-regulation of UXT and down-regulation of MEG3 were observed in breast cancer.

To investigate the expression of UXT and MEG3, we collected breast tumor tissues and their adjacent tissues from 13 cases of patients and conducted the following experiments such as immunohistochemistry, qRT-PCR and western blot analysis. As shown in Fig. 1A, the positive expression of UXT in tumor tissues was significantly stronger than the adjacent tissues. The data of qRT-PCR and western blot analysis also displayed that UXT was highly expressed while MEG3 was low expressed in breast tumor tissues compared with adjacent normal tissues (Fig. 1B-1D). Moreover, we further examined the levels of UXT and MEG3 in various breast cancer cell lines including ZR75 and MCF7, as well as normal breast cell MCF10A. As expected, higher level of UXT and lower level of MEG3 were also observed in breast cancer cell lines, compared with that of control (Fig. 1E-1G).

Knockdown of UXT or overexpression of MEG3 inhibited proliferation, promoted apoptosis and delayed migration and invasion of MCF7 and ZR75 cells.

To explore the biological effects of UXT and MEG3 in the tumorigenesis of breast cancer, we down-regulated UXT expression by using siRNA duplexes and up-regulated MEG3 expression through introducing pcDNA 3.1-MEG3 into ZR75 and MCF7 cells, following by testing the alteration of proliferation, apoptosis, migration and invasion. As shown in Fig. 2A, the level of MEG3 both in ZR75 and MCF7 cells was remarkably increased after transfection of pcDNA 3.1-MEG3. As expected, the mRNA and protein levels of UXT were also knocked down after transfection of its specific siRNA (Fig. 2B and 2C). Along with the increasing level of MEG3 or decreasing level of UXT, CCK-8 assay presented the inhibited cell viability of ZR75 and MCF7 cells (Fig. 2D), and the increased apoptotic cells (Fig. 2E). Furthermore, both migratory and invasive cells were remarkably reduced in plasmid pcDNA3.1 MEG3 group (Fig. 2G). Likewise, UXT knockdown by siRNA also inhibited the capacity of migration and invasion of MCF7 and ZR75 cells (Fig. 2H). Therefore, these data indicated that there is a negative correlation between UXT and MEG3 in regulating the progression of breast cancer.

UXT bound with DNMT3b to promote MEG3 methylation, in turn down-regulating MEG3 expression.

To assess the methylation of MEG3 promoter, methylation-specific PCR was conducted, and we found that the bright band of 160 kb was only present in the methylated group of positive, MCF7 and ZR75 cells, whereas there was no same band existing in corresponding un-methylated group and both group of negative cells (Fig. 3A), suggesting that the hypermethylation of MEG3 promoter in breast cancer cells. It has been demonstrated that the methylation of MEG3 is regulated by DNA (cytosine-5)-methyltransferase 3b (DNMT3b) [19]. Thus, DNMT3b level in breast cancer was detected in clinical specimens (13 cases) and cell lines. The results showed that DNMT3b was highly expressed in breast tumor tissues and cell lines (Fig. 3B-3D). Knockdown of DNMT3b increased the level of MEG3 (Fig. 3E-3G). Moreover, the data of co-immunoprecipitation identified the interaction between UXT and DNMT3b (Fig. 3H). Since silencing of UXT obviously reduced the level of DNMT3b (Fig. 3H), while knockdown of DNMT3b did not alter the expression of UXT (Fig. 3I), indicating that DNMT3b might be a downstream effector in UXT-mediated breast cancer tumorigenesis. Furthermore, the elevated level of MEG3 induced by UXT knockdown was dramatically reversed by DNMT3b overexpression (Fig. 3J). In sum up, these data implied that the negative regulation of UXT on MEG3 expression was depended on DNMT3b-mediated methylation modification.

MEG3 bound with p53 to regulate its transcriptional activity.

To explore the downstream mechanism of MEG3, we detected the expression of p53 in normal and breast cancer cells and found that p53 was down-regulated in breast cancer cells (Fig. 4A). Moreover, overexpression of MEG3 also induced the increased level of p53 (Fig. 4B). Thus, we hypothesized whether MEG3 could bind to p53 and regulate its transcription. As MEG3 contains three conserved motifs, named as M1, M2 and M3, several relevant MEG3 deletion mutants was generated for further confirmation. Luciferase reporter assay was performed to test the mutant for the ability to activate p53-mediated transcription activity and the results showed that each deletion mutants failed to stimulate the transcriptional activity of p53 (Fig. 4C), indicating that full length of MEG3 is crucial for p53 transcription. Furthermore, RNA immunoprecipitation (RIP) assay suggested that MEG3 could directly bind to p53 (Fig. 4D). Knockdown of p53 diminished the effects of MEG3 overexpression on apoptosis, migration, invasion, which was further verified by the detection of apoptosis-related proteins including Bax, Bcl-2, p21 and p53 (Fig. 4E-4G). All above, it is suggested that p53 was involved MEG3-mediated apoptosis, migration and invasion of MCF7 and ZR75 cells.

UXT negatively regulated the MEG3/p53 axis in a DNMT3b-dependent manner to promote cancer growth in vivo.

To identify whether the regulation of UXT on MEG3/p53 axis in a DNMT3b-dependent manner is fit *in vivo*, the stable cells of UXT knockdown combined with DNMT3b knockdown or overexpression were constructed and then subcutaneously injected into the right flank of nude mice to confirm the influence of UXT and DNMT3b on tumor growth. As shown in Fig. 5A and 5B, the inhibition of UXT knockdown on tumor growth was further strengthened by DNMT3b knockdown but weakened by DNMT3b overexpression. Similarly, increasing levels of MEG3 and p53 were presented by the knockdown of both

UXT and DNMT3b while the up-regulation of MEG3 and p53 induced by UXT knockdown was almost abolished by DNMT3b overexpression (Fig. 5C and 5D). Whereas, the data of DNMT3b using qRT-PCR exerted the opposite trend (Fig. 5E). As expected, immunohistochemical staining of p53 and DNMT3b further validated the conclusion (Fig. 5F). Thus, UXT negatively regulated the MEG3/p53 axis in a DNMT3b-dependent manner to promote the growth of nude mice xenograft.

Discussion

Cancer is still the main threat for human health. The discoveries of new approaches and/or targets against cancer are always hotspot in this field. It has been reported that UXT, a putative member of an α -class prefoldin protein family, is overexpressed in a number of human tumor tissues but not in the matching normal tissues [3]. Herein, we identified that UXT is also more abundant in breast cancer tissues or cells than normal tissues or cells through analyzing its expression in specimens from breast cancer patients and breast cancer cells, respectively. The reasons for UXT involving in tumor progress were often attributed to enhancing NF- κ B activities through forming an integral component of the NF- κ B enhanceosome [20], corrupting centrosome activity by forming a novel component of centrosomal processes associated with γ -tubulin [5], or interacting with estrogen receptor or androgen receptor [21, 22], and so on. In the present study, we found that UXT negatively regulates the MEG3/p53 axis in a DNMT3b-dependent manner to promote the apoptosis and inhibit migration and invasion of breast cancer cells, as well as the growth of nude mice xenograft.

Due to the important role in cell physiological activities and development of tumors, lncRNA has been emphasized in therapy of tumor. MEG3, an imprinted gene, is highly expressed in normal human tissue but lost or down-regulated in major human tumors [13]. Researches on MEG3 for a long period of time suggest a fact that MEG3 is a tumor suppressor [12]. In breast cancer, down-regulated expression of MEG3 is associated with poor prognosis and serves as an unfavorable risk factor for survival of patients [23]. Overexpression of MEG3 suppresses breast cancer cell proliferation, invasion, and angiogenesis [24]. Here, our results showed that MEG3 was significantly down-regulated in breast cancer cells and tissues versus normal cells and tissues. Overexpression of MEG3 slowed down the proliferation, accelerated the apoptosis, delayed the migration and invasion of MCF7 and ZR75 cells.

Promoter hypermethylation is considered as the major reason for low expression of MEG3 [25, 26]. Epigenetic changes are the vital factor for tumor occurrence and development and also utilized as biomarkers for cancer early detection [27]. DNA methylation modifies occur the most frequently in epigenetic changes and function through silence gene expression via blocking the interaction between DNA and protein. DNA methylation is catalysed by a family of DNA methyltransferase (DNMT) enzymes, namely, DNMT1, DNMT3a, and DNMT3b. DNMT1 is responsible for maintaining the methylation pattern of the genome in daughter cells during cell division, whereas DNMT3a and DNMT3b are essential for *de novo* methylation [28]. Zhou et al. suggested that elevating DNMT3b expression activated the methylation of MEG3 [19], which is consistent with our results on MEG3 regulation. Intriguingly, we further identified that it is UXT which directly bound to DNMT3b to modulate its expression.

p53, a tumor suppressor gene, plays a critical role in tumor initiation, development and progression [29]. Zhu et al. indicates that MEG3 functions in hepatoma cells through activation of p53 via interacting with p53 DNA binding domain [18]. Sun et al. suggests that overexpressed MEG3 in breast cancer inhibited the proliferation, colony formation, migration and invasion capacities by enhancing p53's transcriptional activity on its target genes, including p21, Maspin and KAI1 [30]. Through investigating the interaction between MEG3 and p53 in breast cancer cells, we found that MEG3 did not only regulate the transcriptional activities of p53 but also bound to p53 protein. Furthermore, when siRNA p53 were transfected into cells pre-transfected with pcDNA3.1 MEG3, inhibition of p53 could abolish MEG3-mediated cell apoptosis, migration and invasion.

In conclusion, UXT participated in tumor progress through down-regulating the expression of MEG3 via methylation by binding to DNMT3b, in turn inhibiting the expression of p53 to influence the apoptosis, migration, invasion and tumor growth of breast cancer cells. Those provide a theory basis on drug development of UXT against cancer.

Declarations

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of The Third Xiangya Hospital of Central South University. Informed consent was obtained from each participant and the procedures were carried out in accordance with the approved guidelines.

Consent for publication

The informed consent obtained from study participants.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

None.

Author's Contribution

guarantor of integrity of the entire study: Zhongfeng Huang

study concepts: KaiGao

study design: KaiGao

definition of intellectual content: KaiGao

literature research: Zhongfeng Huang

clinical studies: Zhongfeng Huang, Yuling Tang, Zhaolong Shen, Kaiyan Yang

experimental studies: Zhongfeng Huang, Yuling Tang, Zhaolong Shen, Kaiyan Yang

data acquisition: Zhongfeng Huang

data analysis: Zhongfeng Huang

statistical analysis: Zhongfeng Huang

manuscript preparation: KaiGao

Acknowledgements

We would like to give our sincere gratitude to the reviewers for their constructive comments.

References

1. Whayne TF. Epigenetics in the development, modification, and prevention of cardiovascular disease. *Mol Biol Rep.* 2015;42(4):765–76.
2. Schroer A, Schneider S, Ropers H, Nothwang H. Cloning and characterization of UXT, a novel gene in human Xp11, which is widely and abundantly expressed in tumor tissue. *Genomics.* 1999;56(3):340–3.
3. Zhao H, Wang Q, Zhang H, Liu Q, Du X, Richter M, Greene MI. UXT is a novel centrosomal protein essential for cell viability. *Mol Biol Cell.* 2005;16(12):5857–65.
4. Markus SM, Taneja SS, Logan SK, Li W, Ha S, Hittelman AB, Rogatsky I, Garabedian MJ. Identification and characterization of ART-27, a novel coactivator for the androgen receptor N terminus. *Mol Biol Cell.* 2002;13(2):670–82.
5. Qi M, Ganapathy S, Zeng W, Zhang J, Little JB, Yuan ZM. UXT, a novel MDMX-binding protein, promotes glycolysis by mitigating p53-mediated restriction of NF-kappaB activity. *Oncotarget.* 2015;6(19):17584–93.
6. McGilvray R, Walker M, Bartholomew C. UXT interacts with the transcriptional repressor protein EVI1 and suppresses cell transformation. *FEBS J.* 2007;274(15):3960–71.
7. Spizzo R, Almeida MI, Colombatti A, Calin GA. Long non-coding RNAs and cancer: a new frontier of translational research? *Oncogene.* 2012;31(43):4577–87.

8. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature*. 2010;464(7291):1071–6.
9. Huarte M. The emerging role of lncRNAs in cancer. *Nat Med*. 2015;21(11):1253–61.
10. Liz J, Esteller M. lncRNAs and microRNAs with a role in cancer development. *Biochim Biophys Acta*. 2016;1859(1):169–76.
11. Miyoshi N, Wagatsuma H, Wakana S, Shiroishi T, Nomura M, Aisaka K, Kohda T, Surani MA, Kaneko-Ishino T, Ishino F: **Identification of an imprinted gene, Meg3/Gtl2 and its human homologue MEG3, first mapped on mouse distal chromosome 12 and human chromosome 14q.** *Genes Cells* 2000, **5**(3):211–220.
12. Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: a tumor suppressor. *J Mol Endocrinol*. 2012;48(3):R45–53.
13. Sun M, Xia R, Jin F, Xu T, Liu Z, De W, Liu X. Downregulated long noncoding RNA MEG3 is associated with poor prognosis and promotes cell proliferation in gastric cancer. *Tumour Biol*. 2014;35(2):1065–73.
14. Zhang X, Zhou Y, Mehta KR, Danila DC, Scolavino S, Johnson SR, Klibanski A. A pituitary-derived MEG3 isoform functions as a growth suppressor in tumor cells. *J Clin Endocrinol Metab*. 2003;88(11):5119–26.
15. Golomb L, Volarevic S, Oren M. p53 and ribosome biogenesis stress: the essentials. *FEBS Lett*. 2014;588(16):2571–9.
16. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell*. 1997;88(3):323–31.
17. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science*. 1991;253(5015):49–53.
18. Zhu J, Liu S, Ye F, Shen Y, Tie Y, Zhu J, Wei L, Jin Y, Fu H, Wu Y, et al. Long Noncoding RNA MEG3 Interacts with p53 Protein and Regulates Partial p53 Target Genes in Hepatoma Cells. *PLoS One*. 2015;10(10):e0139790.
19. Zhou C, Huang C, Wang J, Huang H, Li J, Xie Q, Liu Y, Zhu J, Li Y, Zhang D, et al. lncRNA MEG3 downregulation mediated by DNMT3b contributes to nickel malignant transformation of human bronchial epithelial cells via modulating PHLPP1 transcription and HIF-1 α translation. *Oncogene*. 2017;36(27):3878–89.
20. Sun S, Tang Y, Lou X, Zhu L, Yang K, Zhang B, Shi H, Wang C. UXT is a novel and essential cofactor in the NF-kappaB transcriptional enhanceosome. *J Cell Biol*. 2007;178(2):231–44.
21. Nwachukwu JC, Li W, Pineda-Torra I, Huang HY, Ruoff R, Shapiro E, Taneja SS, Logan SK, Garabedian MJ. Transcriptional regulation of the androgen receptor cofactor androgen receptor trapped clone-27. *Mol Endocrinol*. 2007;21(12):2864–76.
22. Sanchez-Morgan N, Kirsch KH, Trackman PC, Sonenshein GE. UXT Is a LOX-PP Interacting Protein That Modulates Estrogen Receptor Alpha Activity in Breast Cancer Cells. *J Cell Biochem*. 2017;118(8):2347–56.

23. Zhang JJ, Guo SH, Jia BQ. Down-regulation of long non-coding RNA MEG3 serves as an unfavorable risk factor for survival of patients with breast cancer. *Eur Rev Med Pharmacol Sci*. 2016;20(24):5143–7.
24. Zhang CY, Yu MS, Li X, Zhang Z, Han CR, Yan B. Overexpression of long non-coding RNA MEG3 suppresses breast cancer cell proliferation, invasion, and angiogenesis through AKT pathway. *Tumour Biol*. 2017;39(6):1010428317701311.
25. Benetatos L, Dasoula A, Hatzimichael E, Georgiou I, Syrrou M, Bourantas KL. Promoter hypermethylation of the MEG3 (DLK1/MEG3) imprinted gene in multiple myeloma. *Clin Lymphoma Myeloma*. 2008;8(3):171–5.
26. Sheng X, Li J, Yang L, Chen Z, Zhao Q, Tan L, Zhou Y, Li J. Promoter hypermethylation influences the suppressive role of maternally expressed 3, a long non-coding RNA, in the development of epithelial ovarian cancer. *Oncol Rep*. 2014;32(1):277–85.
27. Tarasenko N, Nudelman A, Rozic G, Cutts SM, Rephaeli A. Effects of histone deacetylase inhibitory prodrugs on epigenetic changes and DNA damage response in tumor and heart of glioblastoma xenograft. *Invest New Drugs*. 2017;35(4):412–26.
28. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004;429(6990):457–63.
29. Wormann SM, Song L, Ai J, Diakopoulos KN, Kurkowski MU, Gorgulu K, Ruess D, Campbell A, Doglioni C, Jodrell D, et al. Loss of P53 Function Activates JAK2-STAT3 Signaling to Promote Pancreatic Tumor Growth, Stroma Modification, and Gemcitabine Resistance in Mice and Is Associated With Patient Survival. *Gastroenterology*. 2016;151(1):180–93 e112.
30. Sun L, Li Y, Yang B. Downregulated long non-coding RNA MEG3 in breast cancer regulates proliferation, migration and invasion by depending on p53's transcriptional activity. *Biochem Biophys Res Commun*. 2016;478(1):323–9.

Figures

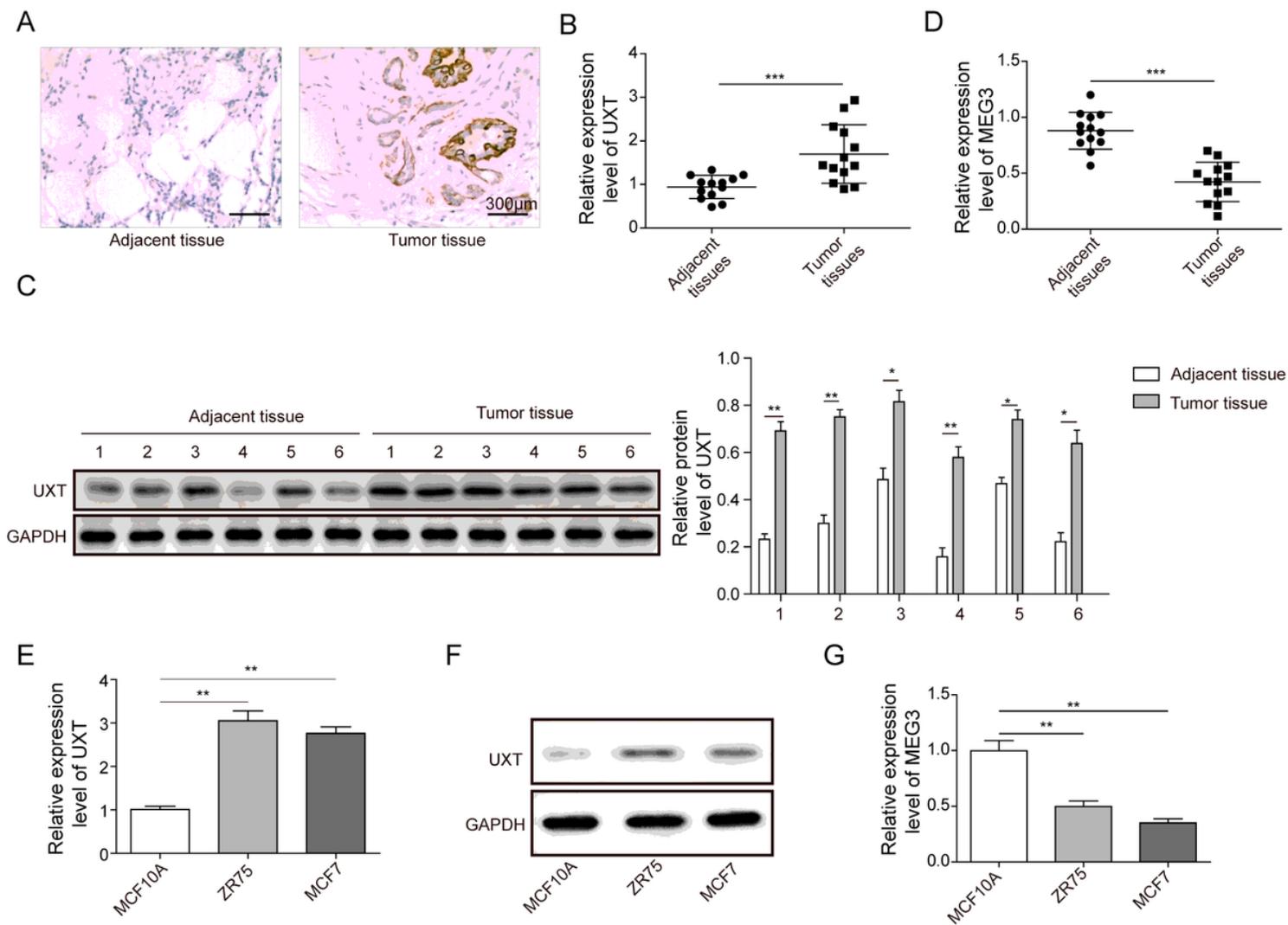


Figure 1

The expression patterns of UXT and MEG3 in breast cancer. A. Immunohistochemistry analysis on UXT expression of breast cancer and adjacent non-tumor tissue specimens from 13 cases of patients. Scale bar, 300 µm. B. qRT-PCR analysis on UXT level in breast cancer and adjacent non-tumor tissue specimens from 13 cases of patients using qRT-PCR assay. C. Western blot analysis of UXT in breast cancer and adjacent non-tumor tissue specimens from 13 cases of patients. D. qRT-PCR analysis of MEG3 expression in breast cancer and adjacent non-tumor tissue specimens from 13 cases of patients. E. qRT-PCR analysis of UXT expression in breast cancer cells and normal breast cells. F. Western blot analysis of UXT expression in breast cancer cells and normal breast cells. G. qRT-PCR analysis of MEG3 expression in breast cancer cells and normal breast cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

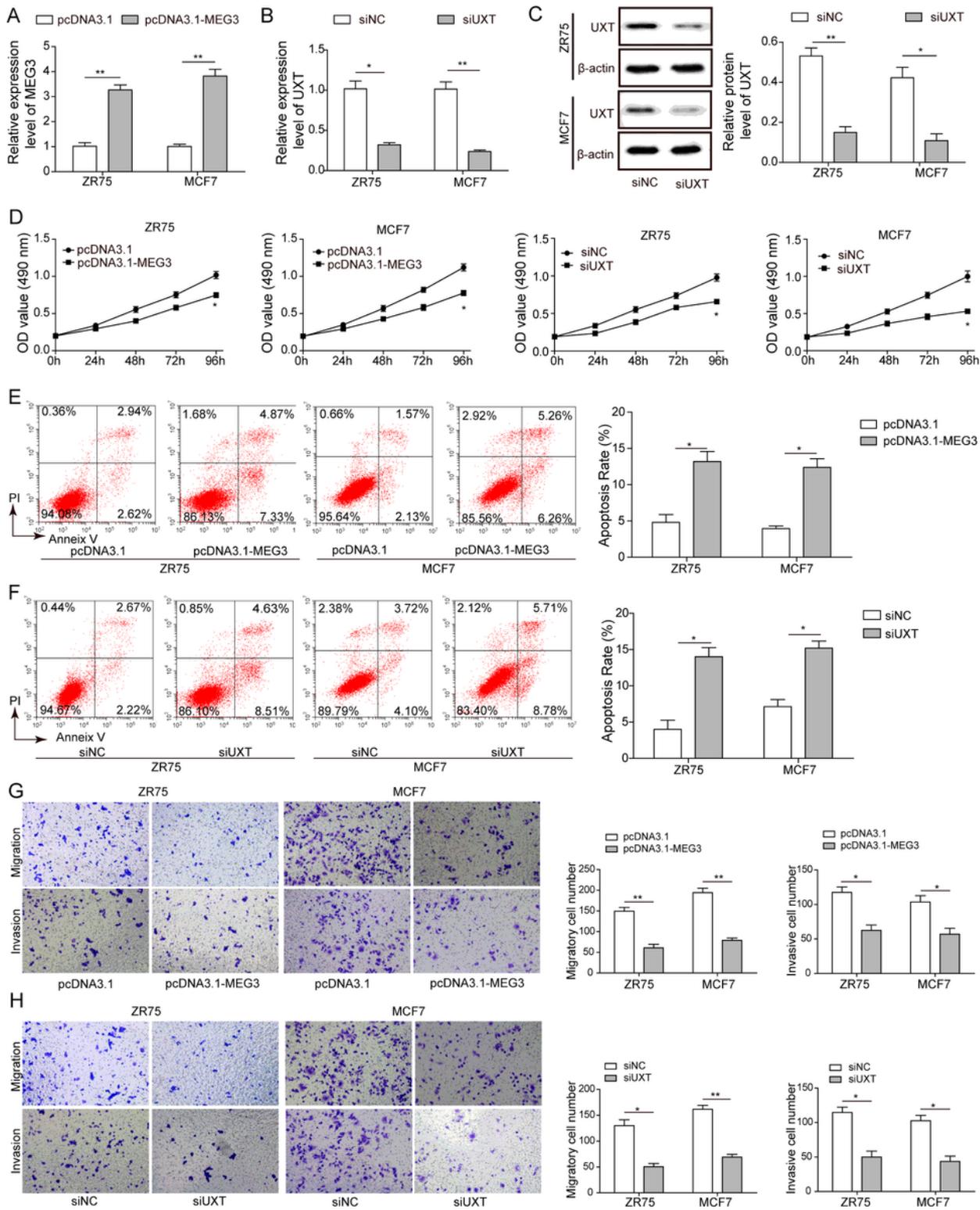


Figure 2

The effects of UXT and MEG3 on cell apoptosis, migration and invasion in MCF7 and ZR75 cells. MCF7 and ZR75 cells were transfected by plasmid pcDNA3.0 MEG3 or siRNA UXT, and the proliferation, apoptosis, migration and invasion were detected. Plasmid pcDNA3.0 and nonspecific control siRNA duplexes were respectively used as the matching control. A. MEG3 expression level was determined using qRT-PCR assay in ZR75 and MCF7 cells. B, C. The mRNA and protein levels of UXT were examined using

qRT-PCR and western blot analysis in ZR75 and MCF7 cells. D. CCK-8 assay was performed to assess the cell viability within MEG3 overexpression or UXT knockdown. E. The effects of MEG3 overexpression or UXT knockdown on cell apoptosis were detected using flow cytometry. F. Transwell assay was employed to assess the cell migration and invasion in ZR75 and MCF7 cells. * $P < 0.05$, ** $P < 0.01$.

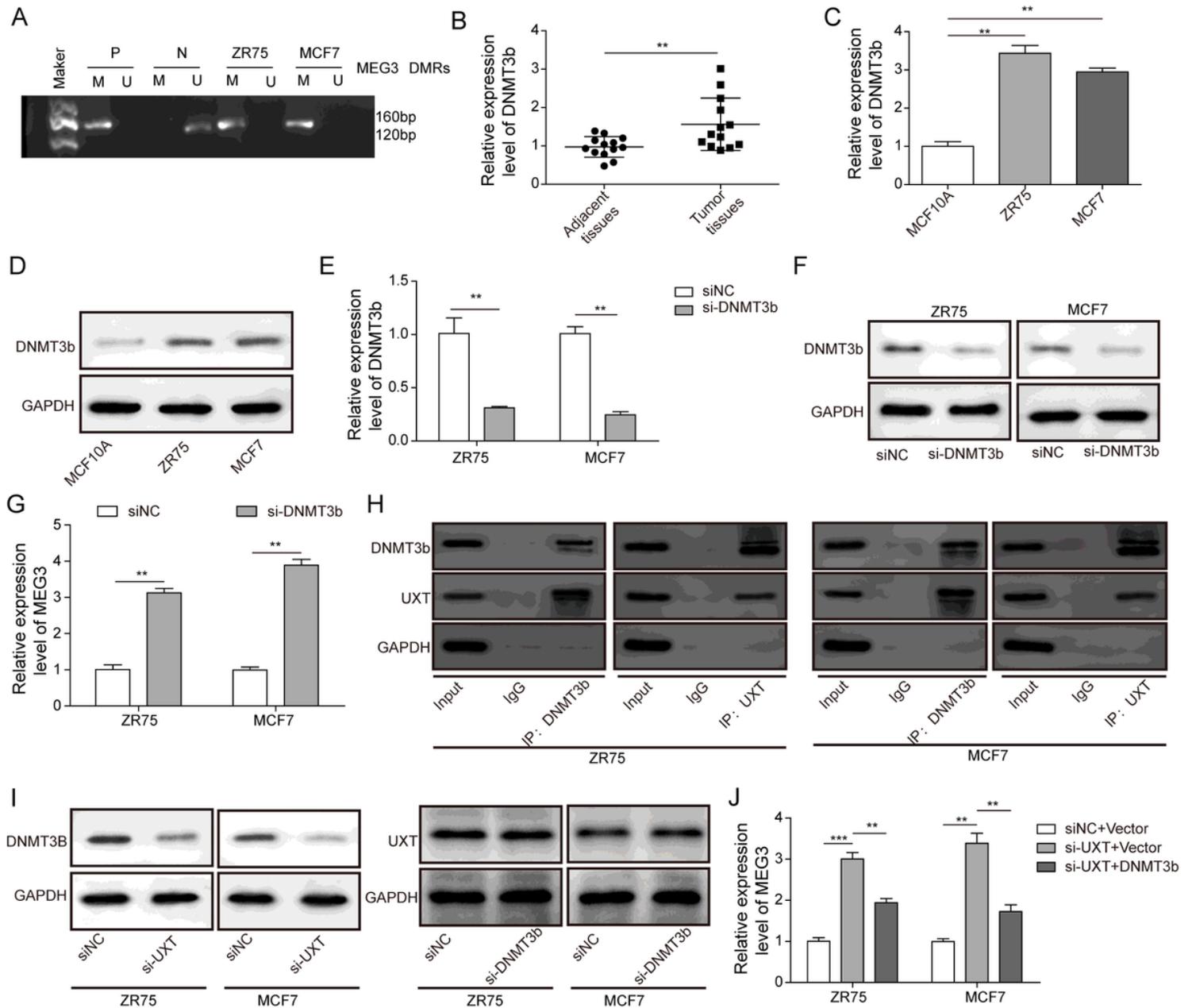


Figure 3

UXT regulated the methylation modification of MEG3 through binding to DNMT3b. A. MS-PCR assay was used to test the methylation of promoter DMR in MEG3. B. qRT-PCR analysis of DNMT3b expression of breast cancer and adjacent non-tumor tissue specimens from 13 cases of patients. C. qRT-PCR analysis of DNMT3b expression in breast cancer cells and normal breast cells. D. Western blot analysis of DNMT3b expression in breast cancer cells and normal breast cells. E, F. DNMT3b mRNA and protein levels were determined using qRT-PCR and western blot analysis. G. The effects of DNMT3b knockdown on MEG3 expression. H. Co-immunoprecipitation analysis on the interaction between DNMT3b and UXT. I.

Knockdown analysis on the interaction between DNMT3b and UXT. J. The effects of UXT combined with DNMT3b on MEG3 levels in ZR75 and MCF7 cells. **P<0.01, ***P<0.001.

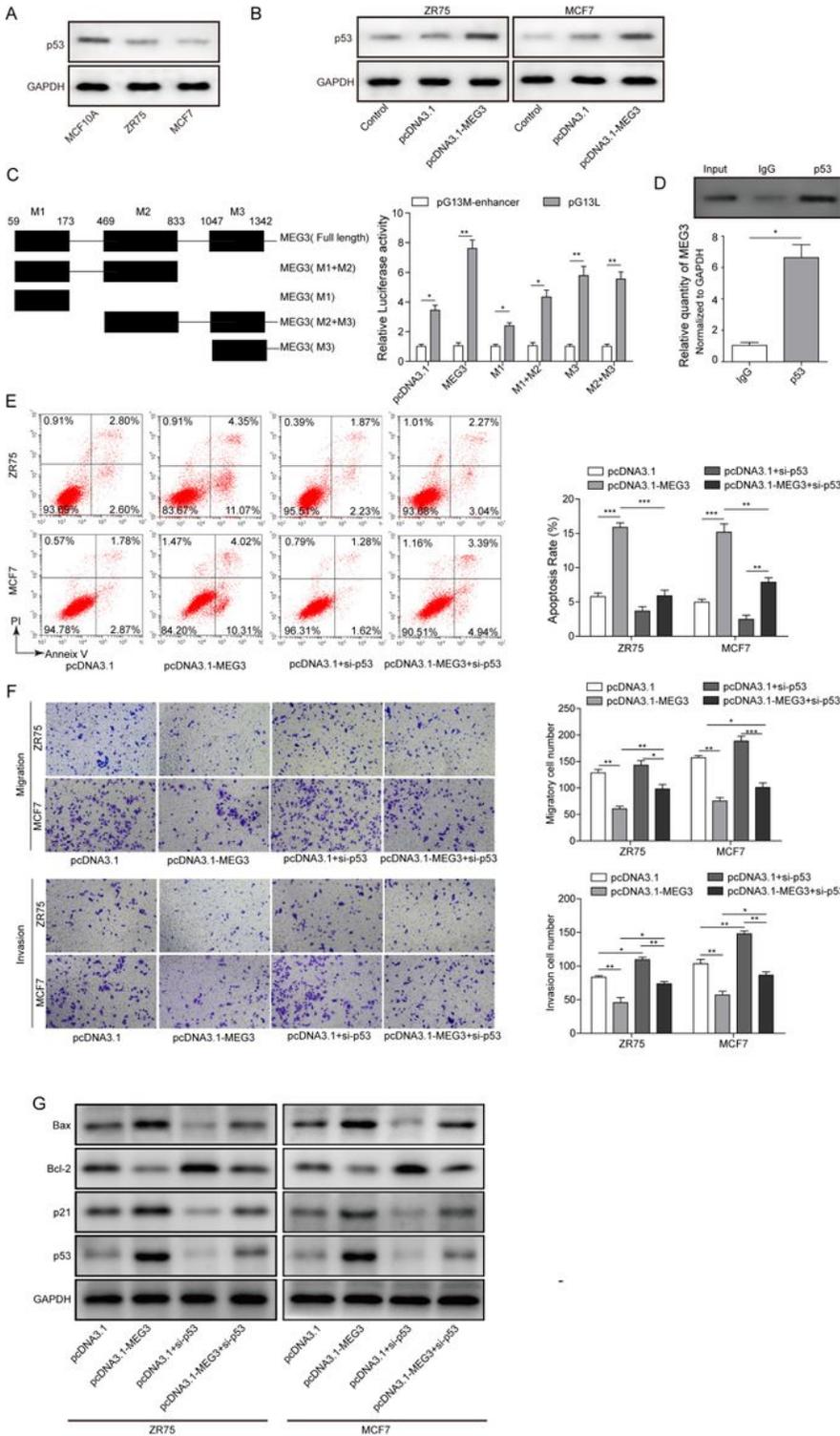


Figure 4

The regulation of MEG3 on p53. A. The expression of p53 in normal and breast cells were detected by western blot. B. The alteration of p53 after overexpression of MEG3 was tested by western blot. C. Luciferase fluorescence intensity changed by transfected with various component of MEG3 was

measured via luciferase reporter assay. D. RNA immunoprecipitation was used to test the interaction between p53 and MEG3. E. Flow cytometry was performed to detect the apoptotic cell. F. Transwell assay was subjected to assess cell migration and invasion of ZR75 and MCF7 cells. G Western bolt analysis on the alterations of p53, p21, Bax and Bcl-2. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

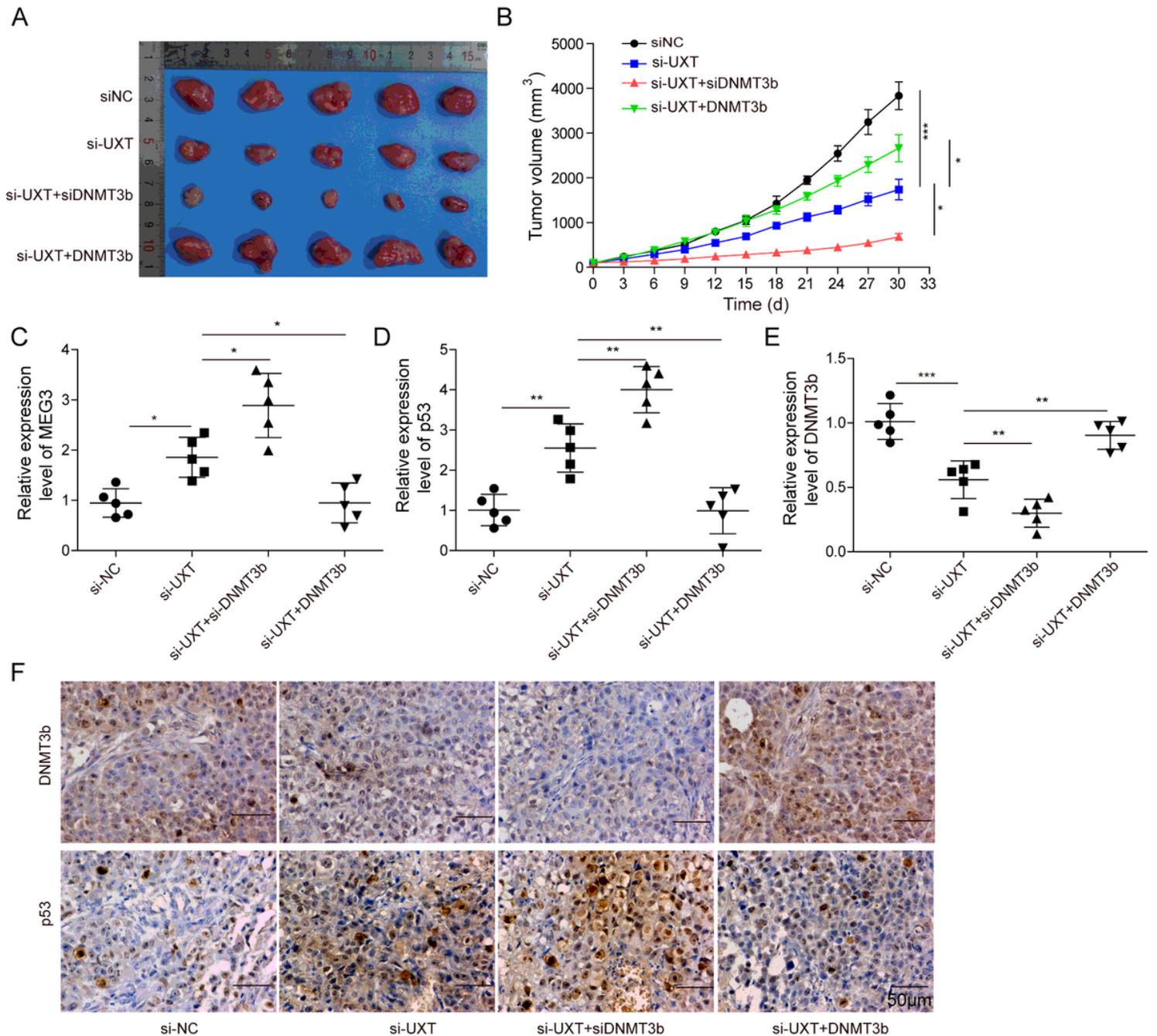


Figure 5

The effects of UXT and MEG3 on nude mice xenograft. After constructed the stable cells of UXT knockout combined with DNMT3b knockdown or overexpression by using matched plasmid, these cells were subcutaneously injected into the right flank of nude mice. A, B. The image and curve of tumor volume varied with time. C-E. qRT-PCR analysis of MEG3, p53 and DNMT3b expression levels was performed. F. Immunohistochemistry analysis of DNMT3b and p53. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.