PRMT7 Induces Epithelial-to-Mesenchymal Transition and Promotes Metastasis in Breast Cancer

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Abstract

Epithelial-to-mesenchymal transition (EMT) enables metastasis. E-cadherin loss is a hallmark of EMT, but there remains an incomplete understanding of the epigenetics of this process. The protein arginine methyltransferase PRMT7 functions in various physiologic processes, including mRNA splicing, DNA repair, and neural differentiation, but its possible roles in cancer and metastasis have not been explored. In this report, we show that PRMT7 is expressed at higher levels in breast carcinoma cells and that elevated PRMT7 mediates EMT and metastasis. PRMT7 could inhibit the expression of E-cadherin by binding to its proximal promoter in a manner associated with altered histone methylation, specifically with elevated H4R3me2s and reduced H3K4me3, H3Ac, and H4Ac, which occurred at the E-cadherin promoter upon EMT induction. Moreover, PRMT7 interacted with YY1 and HDAC3 and was essential to link these proteins to the E-cadherin promoter. Silencing PRMT7 restored E-cadherin expression by repressing H4R3me2s and by increasing H3K4me3 and H4Ac, attenuating cell migration and invasion in MDA-MB-231 breast cancer cells. Overall, our results define PRMT7 as an inducer of breast cancer metastasis and present the opportunity for applying PRMT7-targeted therapeutics to treat highly invasive breast cancers. Cancer Res; 74(19); 5656–67. ©2014 AACR.

Introduction

Cancer metastasis leads to 90% of the mortality from solid tumors (1). Metastasis is a complex cascade involving infiltration of primary cancer cells into surrounding tissues followed by intravasation and extravasation into distant organs, and survival of invaded cells in foreign microenvironment to form metastatic colonization (2). Breast cancer is a heterogeneous disease (3), and can be classified into four subtypes, i.e., luminal, HER2+, basal-like, and normal breast-like (4). This heterogeneity ultimately brings about the risk in evaluating breast cancer metastasis and the difficulty in therapy. The epithelial-to-mesenchymal transition (EMT) is referred to changes in cell phenotypes from epithelial to mesenchymal states; and EMT process has been implicated in various biologic processes, including gastrulation, neural crest cell emigration, and cancer progression (5). In addition, EMT is frequently considered as the initial step of tumor cell metastasis.

At the molecular level, loss of E-cadherin expression is the most predominant hallmark of EMT (6). Besides, many transcription factors, such as Snail, Twist, ZEB1, ZEB2, and FOXC2, have been shown to directly or indirectly repress the E-cadherin promoter activity and subsequently to induce EMT (7–10). Over the past years, there have been some hints that epigenetic modifications are involved in regulation of E-cadherin expression, e.g., Snail recruits histone deacetylase1/2 (HDAC1/2), histone demethylase LSD1, histone methyltransferases Suv39H1 and G9a, and DNA methyltransferases (DNMT) to E-cadherin promoter to repress its expression (11–13). Moreover, protein arginine methyltransferase5 (PRMT5) was found to be recruited to the Snail complex via interaction with the scaffold protein AJUBA to participate in the transcriptional silencing of E-cadherin (14). Histones are often subject to multiple posttranslational modifications, particularly methylation and acetylation (15); for instance, the well-characterized modifications H3K4me3, H3Ac, and H4Ac are closely correlated with transcriptional activation (16), whereas the symmetrically dimethylated H4R3 (H4R3me2s) mediated by PRMT5 and PRMT7 is associated with gene repression (17). Conceivably, epigenetic dysregulation of histone modifications is relevant to oncogenesis; studies in this field will hopefully provide a greater mechanistic insight into human tumorigenesis (18).
PRMT7 belongs to the type II methyltransferase capable of generating symmetric dimethyl-arginine (SDMA) modifications of proteins (19). It has been indicated that PRMT7 may participate in a variety of physiologic processes, e.g., PRMT7 plays a role in male imprinted gene methylation by catalyzing H4R3me2s (20), and the SnRNP assembly and biogenesis are thought to be mediated by PRMT7 via post-translational modification (21). Also, PRMT7 is involved in regulation of the DNA damage repair genes by mediating H2AR3me2s and H4R3me2s (22). Recently, PRMT7 has been shown to repress the MLL4 target genes by increasing H4R3me2s levels and antagonize MLL4-mediated H3K4me3 in cellular differentiation (17). However, whether and how PRMT7 is involved in tumorigenesis and metastasis is currently unclear.

YY1 is a transcriptional factor with two opposite capabilities of either repressing or activating gene transcription (23). YY1 was first identified as a repressor of adeno-associated virus P5 promoter (24). YY1 can increase Snail expression by binding to its 3’ enhancer in melanoma cells (25). It has been indicated that silencing of YY1 disrupts the hypoxia-induced HIF1α stability and significantly suppresses growth of metastatic cancer cells (26). Significantly, previous studies have established that YY1 can interplay with P300 and CBP, as well as HDAC1, 2, 3 to recruit HDACs to repress gene transcription (27, 28). Moreover, YY1 is also able to recruit PRMT1 to activate transcription of target genes (29). These data imply that YY1 may play a critical role in gene transcription regulation by recruiting epigenetic modification enzymes.

In this study, we identified PRMT7 as a key mediator of EMT in breast cancer, both in vitro and in vivo. We presented evidence that PRMT7 induced an EMT program by inhibiting the E-cadherin expression through a cascade of epigenetic mechanisms involving the interplays among various histone modifications at the gene’s promoter. These data provide an insight into the mechanisms of the epigenetic control of EMT in cancer, as well as a base for the exploration of PRMT7 as a potential target for the therapeutic intervention for highly invasive breast cancers.

**Materials and Methods**

**Cell culture**

MCF10A, MCF7, MDA-MB-231, MDA-MB-435, 4T1, BT549, MDCK, and HEK293T cell lines were purchased from ATCC, where all the cell lines were characterized by DNA fingerprinting and isozyme detection. Cells were immediately expanded and frozen such that they could be revived every 3 to 4 months. The MDA-MB-231HM cell line was obtained from The Oncology Hospital, Fudan University, Shanghai, China. MCF10A cells were cultured as previously described (30). MCF7, 4T1, and BT549 cells were cultured in RPMI-1640 medium supplemented with 10% FBS (ExCell Bio) and 0.023 IU/mL insulin. MDA-MB-231, MDA-MB-231HM, and MDA-MB-435 cells were cultured in L15 medium with 10% FBS at 37°C without CO2. MDCK and HEK293T cells were cultured in DMEM containing 10% FBS.

**Antibodies and plasmids**

The following antibodies were used: antibodies against E-cadherin, N-cadherin, vimentin, fibronectin, β-catenin (BD Biosciences), β-actin (Sigma-Aldrich), MMP2, MMP9 (Genetex), PRMT7, H3K4me3, H3Ac, H4Ac (Upstate Biotechnology), YY1, HDAC3 (Santa Cruz Biotechnology), H4R3me2s (Abcam), His, GFP (Sungene Biotech), and Flag (Abmart).

A description of plasmids is detailed in Supplementary Materials and Methods.

**Human breast cancer specimens and immunohistochemistry**

A total of 114 paraffin-embedded human breast cancer specimens used in this study were obtained from China-Japan Union Hospital of Jilin University, China. The tissue sections were deparaffinized and rehydrated. Antigen retrieval was done by heating samples in 10 mmol/L citrate buffer (pH 6.0) at 95°C for 15 minutes. Endogenous peroxidase activity was blocked with peroxidase (Dako), and sections were incubated with anti-PRMT7 antibody for 1 hour, followed by incubation with secondary antibody (Dako) for 30 minutes. The immunostaining was developed with 3,3-diaminobenzidine. Finally, sections were counterstained with hematoxylin.

**Immunofluorescence, immunoprecipitation, and Western blotting**

Immunofluorescence was performed as described previously (30). Immunoprecipitation was carried out as described in Supplementary Materials and Methods.

**RNA extraction, reverse transcription, and real-time RT-PCR**

Experiments were carried out as described in Supplementary Materials and Methods.

**Wound healing, cell invasion, migration, and proliferation assays**

Experiments were performed as described previously (30). For wound healing assay, indicated 5 × 10^5 cells were plated in 6-well plates. The scratches were created using a 10-μL pipette tip. Then, the progression of migration was examined and photographed after 24 hours.

**Glutathione S-transferase pull-down assay**

The procedure was performed basically as described previously (31). Briefly, GST, GST-PRMT7, and GST-HDAC3 protein expressions were induced by adding 0.1 mmol/L IPTG at 25°C for 8 hours with shaking, and then purified on glutathione-Sepharose4B according to the manufacturer’s instructions (GE Healthcare).

**Luciferase reporter assay**

The experiments were performed as described previously (30). The pGL4.15-E-cadherin (−420/+32) and pGL4.20-N-cadherin reporter gene vectors were constructed by inserting the PCR amplification products from the human genome. Vimentin promoter construct was a gift from Dr. Karen M. Ridge (Northwestern University, Chicago, IL).
Chromatin immunoprecipitation and re-chromatin immunoprecipitation

The chromatin immunoprecipitation (ChIP) protocol was described elsewhere (32), and the re-ChIP was done using essentially the same protocol as for the primary ChIP. The bead elutes from the first IP were incubated with 75 μL of 10 mmol/L DTT at 37°C for 30 minutes and diluted 1:20 with ChIP dilution buffer followed by re-ChIP with the secondary antibodies. The primers for the E-cadherin promoter were 5’-TGGTTGTGGCAGCTGACT-3’ and 5’-GACCTGACCGTGTCTGATT-3’.

Animal studies

Briefly, the MCF7-Vector, MCF7-PRMT7 (2 × 10⁶) cells were injected into the tail veins of 5-week old female BALB/c nude mice. Two months later, the mice were sacrificed and lungs were fixed in formalin before embedded in paraffin using the routine procedure. Hematoxylin and eosin (H&E) staining was performed on sections from paraffin-embedded tissues. For bioluminescence imaging, mice were injected i.p. with 150 mg/kg D-luciferin (GoldBio) 10 minutes before imaging.

Statistical analysis

Data are presented as mean ± SD as indicated. The Student t test (two-tailed) was used to determine statistical significance of differences between groups. P < 0.05 was considered statistically significant. All statistical analyses were carried out using the SPSS 17.0 statistical software.

Results

PRMT7 expression was associated with malignant breast carcinomas

We first compared the expression level of PRMT7 in non-cancerous mammary epithelial MCF10A cell and in multiple breast cancer cell lines. Western blotting showed that PRMT7 expression was remarkably higher in cells known to be highly metastatic (MDA-MB-231, MDA-MB-231HM, and MDA-MB-435; Fig. 1A). To further clarify the clinical relevance of PRMT7 expression to breast carcinomas, we examined the PRMT7 expression level in the Oncomine database (33). Data from nine (total, 12) breast cancer cohorts showed that increased expression of PRMT7 mRNA was found in mammary carcinomas in contrast with normal breast tissues (Supplementary Table S1). Further analysis indicated that the PRMT7 expression was closely associated with higher tumor grades and stages, and the enhanced expression of PRMT7 predicted a very poor clinical outcome (Supplementary Table S2). Thus, results from both in vitro and clinical specimens have proved that higher PRMT7 expression is critical for malignant breast cancer progression.

Ectopic expression of PRMT7 induced an EMT program

We next intended to elucidate the biologic effects of PRMT7 deregulation on breast cancer progression. We first tested whether overexpression of PRMT7 could induce EMT in human normal mammary epithelial MCF10A cells, which express very little endogenous PRMT7. MCF10A cells were used in this study to establish two stable cell lines (PRMT7-1 and PRMT7-2) via virus infection that constitutively overexpress the PRMT7 protein, in which PRMT7 overexpression was comparable with the endogenous PRMT7 expression in MDA-MB-231 and MDA-MB-435 cells (Fig. 2A). We found that whereas MCF10A cells expressing a control vector retained the typical polarized monolayer epithelial morphology with tight cell-to-cell adhesion, PRMT7-1 and PRMT7-2 cells displayed a spindle-like, fibroblastic phenotype (Fig. 2B). Apart from morphologic changes, a switch of expression from the epithelial to mesenchymal marker genes was prominent. Specifically, the PRMT7-1 and PRMT7-2 cells were characterized by a significant reduction of E-cadherin expression and a concurrent upregulation of N-cadherin, vimentin, and fibronectin at both mRNA and protein levels (Fig. 2C and Supplementary Fig. S1A). This change was further verified by inspecting the subcellular presence of proteins using immunofluorescence staining. As expected, immunofluorescent microscopy showed loss of E-cadherin staining from cell membrane in the PRMT7-1 and PRMT7-2 cells compared with its strong membrane staining in control cells, whereas mesenchymal markers N-cadherin, vimentin, and fibronectin exhibited a reverse trend (Fig. 2D).

Furthermore, we detected the increase in the endogenous mRNA levels of some other classical EMT inducers, including Twist, Snail, ZEB1, and ZEB2, along with the enhanced PRMT7 expression (Supplementary Fig. S1B).
We then performed a loss-of-function study in a triple-negative breast cancer cell line MDA-MB-231 that expresses PRMT7 at an elevated level. We first established stable MDA-MB-231 cell lines by lentiviral infection of PRMT7 shRNAs, and the two independent shRNAs targeting PRMT7 were used (Fig. 2E). We found that although PRMT7 depletion did not convert the spindle-like morphology of MDA-MB-231 cell to epithelial morphology, it did augment the expression of epithelial marker E-cadherin with a concomitant decrease of mesenchymal markers vimentin and fibronectin, as demonstrated at both mRNA and protein levels (Fig. 2F and Supplementary Fig. S1C). These changes were also confirmed by indirect immunofluorescence staining (Fig. 2G). Our data further demonstrated that PRMT7 silencing could not reverse the mesenchymal phenotype of MDA-MB-231HM and MDA-MB-435 cell lines, despite the obvious change in the EMT-associated markers (Supplementary Fig. S2A and S2B). We next wanted to determine whether PRMT7 could also induce EMT in MCF7 human breast cancer cells and in MDCK canine renal epithelial cells. The in vitro gain-of-function analyses using a lentiviral PRMT7 overexpression strategy unveiled that PRMT7 overexpression in MCF7 cells was comparable with endogenous PRMT7 in MDA-MB-231 and MDA-MB-435 cells, and that both MCF7-PRMT7 cells and MDCK-PRMT7 cells exhibited morphologic transformation and EMT-related markers changes (Supplementary Fig. S2C–S2G). Collectively, our data demonstrated that ectopic expression of PRMT7 was sufficient to induce an EMT program.

Overexpression of PRMT7 facilitated migratory and invasive abilities

Epithelial cells that have undergone EMT are characterized by their accelerated migratory and invasive abilities, and MMP2 and MMP9 are closely relevant to tumor invasion
Therefore, we asked whether overexpression of PRMT7 can lead to the increased MMP2 and MMP9 activities. Our real-time PCR analysis revealed that MMP2 and MMP9 mRNA levels were 110.2- and 16.9-fold higher, respectively, in MCF10A-PRMT7 cells (Fig. 3A). Nevertheless, only MMP2 protein expression was elevated (Supplementary Fig. S4A). MMP2 and MMP9 are synthesized as proenzymes in cells before being cleaved to become catalytically activated when secreted (35). As shown in Fig. 3B, PRMT7 significantly augmented the mature MMP2 and MMP9 activities, as measured by gelatin zymography. Subsequently, we observed that elevated PRMT7 expression significantly increased the wound closure in cultured MCF10A cells (Fig. 3C). In addition, the wound healing assay also showed that knockdown of PRMT7 expression remarkably decreased the migratory speed of MDA-MB-231 cells compared with that of nonspecific shCtrl control (Fig. 3D). Next, we used the Transwell migration and Matrigel invasion assays to assess the effects of PRMT7 on the migration and invasion both in normal breast epithelial cells and in aggressive breast cancer cells, in both PRMT7 gain-of-function and loss-of-function cell lines. The results demonstrated that overexpression of PRMT7 prominently enhanced the migratory and invasive capabilities of MCF10A cells (Fig. 3E and G). In contrast, PRMT7 depletion in the invasive MDA-MB-231 cells reduced the migratory and invasive behaviors (Fig. 3F and H). Likewise, we also showed that PRMT7 could increase the motility of MCF7 and MDCK cells as judged by the Transwell assay (Supplementary Fig. S3A–S3C).

To rule out the possible interference of abnormal PRMT7 expression to migration and invasion, we evaluated the role of PRMT7 in mediating cell proliferation. We found that PRMT7 caused a significant reduction in MCF10A cell proliferation as compared with MCF10A-Vector cells (Supplementary Fig. S4B). We then confirmed that knockdown of PRMT7 expression enhanced the proliferation ability of MDA-MB-231 cells (Supplementary Fig. S4C). In addition, although PRMT7 inhibited cell proliferation, the motility of PRMT7-mediated cells was remarkably increased.

### Simultaneous occurrence of the elevated H4R3me2s and the reduced H3K4me3/H4Ac at E-cadherin promoter during EMT

Although PRMT7 was found both in the nucleus and in cytoplasm, it was mainly localized in the nucleus (Supplementary Fig. S5A). We then tested whether PRMT7 could repress the promoter activity of EMT-related genes. As a result, we found that PRMT7 alone was able to inhibit the transcription of E-cadherin promoter-driven luciferase reporter in a dose-dependent manner (Fig. 4A). However, it did not affect the activity of other mesenchymal gene promoters, including N-cadherin and vimentin (Supplementary Fig. S5B and C). Then we detected the enrichment of PRMT7 at the E-cadherin promoter, but not at the N-cadherin and vimentin promoters in MCF10A-PRMT7 cells as revealed by ChIP assays (Fig. 4B). It was previously reported that the PRMT7-induced H4R3me2s (a gene repressive marker) antagonized the MLL4-mediated H3K4me3 (a hallmark of gene activation; ref. 17). We, therefore, examined these histone modifications at the EMT-related marker gene promoters by ChIP analysis, and we detected a dramatic elevation of H4R3me2s together with a marked reduction of H3K4me3 at the epithelial marker E-cadherin promoter, whereas the H4R3me2s modification was not found at the promoters of mesenchymal markers N-cadherin and vimentin (Fig. 4C). Because the knockdown of endogenous PRMT7 caused a partial loss of EMT and inhibition of migratory and invasive abilities in MDA-MB-231 cells (see Figs. 2 and 3), we performed ChIP assays to assess the presence of PRMT7 and different histone hallmarks at the promoter of

<table>
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Table 1. Association between PRMT7 expression and clinicopathologic tumor types in breast carcinomas

**NOTE:** P values are based on the χ² test. Significant P values are marked in bold.

*Few samples were unable to be analyzed.
E-cadherin in MDA-MB-231-shPRMT7#1 cells. As expected, the results showed that depletion of PRMT7 led to a reduction of binding of PRMT7 to E-cadherin promoter, with a decreased H4R3me2s and an elevated H3K4me3 enrichment at E-cadherin promoter (Fig. 4D).

We next intended to determine if the PRMT7-generated H4R3me2s affected histone acetylation in an EMT model, as inspired by the recent discovery that a decreased H3K4Ac level at the E-cadherin promoter occurred in hypoxia-induced EMT (31). Our ChIP assays detected a significant decrease of H3Ac and H4Ac at the E-cadherin promoter in MCF10A cells stably expressing PRMT7. Scale bars, 50 μm. E, Western blot analyses of PRMT7 expression in MDA-MB-231-shCtrl, shPRMT7#1, and shPRMT7#2 cells. F, immunoblots of epithelial marker E-cadherin and mesenchymal markers vimentin and fibronectin after PRMT7 silencing in MDA-MB-231 cells. G, immunofluorescence for EMT markers in PRMT7 silencing MDA-MB-231 cells. Scale bars, 50 μm.

YY1 recruited PRMT7–HDAC3 complex to repress E-cadherin expression

As an epigenetic modifier, PRMT7’s function to regulate gene transcription may rely on the participation of a transcription factor. As reported, HDAC3 was activated and bound to E-cadherin promoter in hypoxia/HIF1α-induced EMT (31). In this study, we verified that PRMT7 upregulated the HDAC3
protein level in MCF10A cells (Supplementary Fig. S6A). As shown in Supplementary Fig. S6B, overexpression of PRMT7 led to an increase of HDAC3 binding to E-cadherin promoter. Inversely, silencing of PRMT7 decreased HDAC3 binding on the E-cadherin promoter (Supplementary Fig. S6C). On the basis of the fact that the transcription factor YY1 can interact with HDAC3 (28), we were curious to find out whether YY1 was involved in PRMT7-mediated E-cadherin regulation. First, we found that YY1 expression was not obviously changed in the MCF7-His-PRMT7 cell line (Supplementary Fig. S7A). Then, coimmunoprecipitation assays with His-tag antibody against target proteins followed by Western blotting with the anti-YY1 and anti-HDAC3 indicated that PRMT7 was coprecipitated with YY1 and HDAC3 (Fig. 5A). This interaction was confirmed with endogenously expressed proteins in MDA-MB-231 cells (Fig. 5B). To determine whether PRMT7 could interact with YY1 and HDAC3 in vitro, GST pull-down assays were carried out using GST-fused PRMT7 protein and affinity-purified Flag-YY1 and Flag-HDAC3 by using anti-FLAG M2-agarose. The results demonstrated that PRMT7 was able to interact with YY1 and HDAC3 in vitro, respectively (Fig. 5C). Moreover, Co-IP and GST pull-down experiments also verified that YY1 interacted with HDAC3 in vitro (Supplementary Fig. S7B and S7C). Thus, our results have proved that YY1 can interact with PRMT7 and HDAC3 in vitro to form a ternary YY1–PRMT7–HDAC3 complex.

Furthermore, ChIP-re-ChIP with anti-YY1 followed by anti-PRMT7 and anti-HDAC3 antibodies in MCF10A-PRMT7 clones validated the cooccupancy of YY1, PRMT7, and HDAC3 at the E-cadherin promoter (Fig. 5D). Also, we infected MDA-MB-231 cells with lentiviral particles that expressed YY1-specific shRNA#1, #2, and #3, and MDA-MB-231-shYY1#1, shYY1#3 cell lines were selected for further characterization (Supplementary Fig. S7B and S7C). Thus, our results have proved that YY1 can interact with PRMT7 and HDAC3 in vitro to form a ternary YY1–PRMT7–HDAC3 complex.

To determine whether PRMT7 could promote breast cancer cell metastasis in vivo, the MCF7-PRMT7 cells were injected into the tail veins of nude mice. Strikingly, we discovered that MCF7-PRMT7 cells effectively metastasized to the lung region of nude mice in 2 months, in contrast with the MCF7-Vector cells, as illustrated by the bioluminescence imaging (Fig. 6A). Noticeably, histologic observation showed that 7 out of 9 mice bearing MCF7-PRMT7 tumors had a large number of macroscopic lung metastases, whereas no visible metastasis was found in mice transplanted with MCF7-Vector cells (Fig. 6B). We further explored whether PRMT7 had an impact on tumorigenesis in vivo. Surprisingly, ectopic expression of PRMT7 apparently reduced the weights and volumes of the
MCF7 primary tumors when the nude mice were subcutaneously injected with MCF7-PRMT7 cells for 5 weeks (Fig. 6C and D; Supplementary Fig. S8A). However, our MTT assay revealed that PRMT7 did not repress the proliferation of MCF7 cells in vitro (Supplementary Fig. S8B). Together, these data clearly demonstrate that PRMT7 plays a pivotal role in promoting breast cancer cell metastasis in vivo.

Discussion

EMT is implicated in the initial step of cancer metastasis and is indispensable for cancer cells to break through the barriers formed by normal tissues to realize a metastasis. The heterogeneity in breast cancer also aggravates the risk of breast carcinomas' progression and metastasis, and exacerbates the therapeutic resistance (3). Despite the intensive studies on the trigger of EMT and its causal relationship with cancer progression, however, the epigenetic regulatory mechanisms that control EMT and cancer metastasis have not been clearly defined. In this study, we focused on an important epigenetic modifier, PRMT7, whose physiologic roles have been established in male imprinted gene methylation, mRNA splicing, DNA repair, and neural cell differentiation (17, 20–22). To date, PRMT7 has not been studied in association with cancer progression, although other members of the PRMT family, such as PRMT1, PRMT4/CARM1, PRMT5 and PRMT6, have been found to be either upregulated or downregulated in prostate cancer, breast cancer, colon carcinoma, and melanoma (36–39). Data arising from this study unraveled, for the first time, the functional role of PRMT7 in regulating the EMT in breast cancer. We found that PRMT7 was expressed at a high level in aggressive and metastasized breast cancer cells and breast carcinomas tissues (Fig. 1), and this finding was supported by the Oncomine database analysis (Supplementary Tables S1 and S2). More significantly, we showed that PRMT7 could induce the EMT (Fig. 2), and promote mammary epithelial cell MCF10A migration and invasion (Fig. 3). However, we did not detect primary tumor growth in nude mice subcutaneously injected with MCF10A-PRMT7 cells (data not shown). We also demonstrated that PRMT7 caused a dramatic enhancement of the migratory, invasive, and metastatic abilities of the triple-negative breast cancer MDA-MB-231 cells evidently reduced the migratory and invasive behaviors (Fig. 3). Hence, our study provides significant insights into the novel roles of PRMT7 as a promoting factor of breast cancer metastasis through epigenetic control of EMT.

Apart from the classic EMT inducers (Snail, Twist, ZEB1, and ZEB2) that have been identified to promote tumor metastasis, aberrant expression of EMT marker genes is also associated with cancer metastasis. For instance, loss of E-cadherin promoted tumor metastatic dissemination, and both N-cadherin and vimentin overexpression enhanced metastasis of breast tumors and hepatocellular carcinoma (7, 40, 41). Apparently, understanding how the EMT markers contribute to breast cancer metastasis process may provide useful insights into the molecular mechanisms underlying cancer cell invasion and metastasis. In this study, we intended to investigate the mechanistic roles of PRMT7 in modulating the EMT process and the invasiveness and metastatic behavior in human breast cancer. We showed that PRMT7 could effectively inhibit the

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Specific histone hallmarks were associated with E-cadherin promoter in PRMT7-mediated EMT. A, luciferase reporter assay of E-cadherin promoter cotransfected with increasing amounts of PRMT7 expression plasmid in HEK293T cells. B, qChIP results using anti-PRMT7 to measure levels of PRMT7 presence at the promoters of EMT marker genes in MCF10A-PRMT7 cells. C, qChIP results were used to measure the levels of histone marks at the promoters of EMT marker genes in MCF10A-PRMT7 cells. D, qChIP results showing decreased PRMT7 and H4R3me2s, and increased H3K4me3 levels at the E-cadherin promoter after PRMT7 silencing. E and F, changes of histone acetylation level at E-cadherin promoter after PRMT7 overexpression or knockdown. G, qChIP was used to measure the levels of histone marks and PRMT7 presence at E-cadherin promoter in MCF10A-PRMT7 cells after TSA treatment. All are representative data from three independent experiments.
activity of E-cadherin promoter in a dosage-dependent manner, but it did not affect N-cadherin and vimentin. We further demonstrated that PRMT7 bound to the E-cadherin promoter in the EMT model used in this study. It has been established that PRMT7 is the major enzyme that catalyzes the symmetrical dimethylation on arginine-3 of histone H4 (H4R3me2s), which occurs at several repressed genes (17, 42). In line with this, we detected an increased H4R3me2s level at the E-cadherin promoter, which was accompanied by a decreased H3K4me3 level (Fig. 4C). Moreover, increasing evidence suggests that histone acetylation/deacetylation is an important regulatory mechanism of E-cadherin during EMT in several cancers (43). Thus, the reduced H3Ac and H4Ac levels at the E-cadherin promoter by PRMT7 overexpression observed in this study (Fig. 4E) also supported this notion. Unexpectedly, however, depletion of PRMT7 only increased H4Ac level at E-cadherin promoter but did not change the H3Ac level (Fig. 4F). Presumably, this was, in part, caused by the decreased H4R3me2s modification upon the PRMT7 silencing. Our preliminary analysis suggests that the PRMT7-generated H4R3me2s modification may probably prevent histone H4 from being acetylated, more effectively than it does to H3 acetylation. In addition, we observed that treatment of the HDAC inhibitor TSA led to the increased H3Ac and H4Ac levels at the E-cadherin promoter by YY1 silencing. All data are representatives of three independent experiments. **, P < 0.01; *, P < 0.05.

Figure 5. PRMT7 formed a ternary complex with YY1 and HDAC3, and was recruited to E-cadherin promoter by YY1 to repress the E-cadherin transcriptional activity. A, coimmunoprecipitation assays showing that PRMT7 pulled down YY1 and HDAC3 in MCF7-PRMT7 cells. B, endogenous PRMT7, YY1, and HDAC3 were coimmunoprecipitated from MDA-MB-231 cells, and the bound endogenous PRMT7, YY1, and HDAC3 were examined by immunoblotting. C, PRMT7 directly interacted with YY1 and HDAC3 in vitro. GST pull-down assays were performed with indicated GST-fused PRMT7 protein and purified Flag-YY1 and Flag-HDAC3. Coomassie Brilliant Blue staining of GST and GST-PRMT7 proteins was shown. D, recruitment of YY1 and PRMT7, YY1, and HDAC3 onto E-cadherin promoter. Schematic representation of the E-cadherin promoter region, including the primer location with TSS region. ChiP-re-ChIP was performed in MCF10A-PRMT7 cells. E, Western blot analyses of YY1 expression in MDA-MB-231-shCtrl, shYY1#1, shYY1#2, and shYY1#3 cells. F, qChIP results were used to measure the change of YY1, PRMT7, and HDAC3 at E-cadherin promoter in MDA-MB-231-shYY1#1, shYY1#3 cells. G, qChIP results showing the decreased H4R3me2s and increased H3Ac and H4Ac levels at the E-cadherin promoter after YY1 silencing. All data are representatives of three independent experiments. **, P < 0.01; *, P < 0.05.
at E-cadherin promoter, along with reduced presences of PRMT7 and H4R3me2s (Fig. 4G). This phenomenon implies that histone acetylation may negatively regulate H4R3me2s modification at E-cadherin promoter. In addition, it has been known that a common mechanism of E-cadherin epigenetic silencing is via the DNMT-mediated DNA hypermethylation, as elucidated in hepatocellular, breast, and prostate cancers (44, 45). Previous studies have demonstrated that the PRMT5-mediated H4R3me2s recruits DNMT3a to repress gene expression (46). Therefore, it would be intriguing to investigate in the future work whether the PRMT7-mediated E-cadherin loss is also regulated by the recruitments of DNMTs.

Inspired by the recent discovery that HDAC3 serves as an essential corepressor to inhibit epithelial gene expression in hypoxia-induced EMT (31), we investigated the possible involvement of HDAC3 in PRMT7-mediated E-cadherin silencing in EMT. Indeed, we found that HDAC3 was able to bind to the E-cadherin promoter, which led to the reduced histone acetylation at E-cadherin promoter (Fig. 4E and Supplementary Fig. S6B). Our results also revealed that PRMT7 interacted directly with both YY1 and HDAC3 to repress E-cadherin expression in vitro (Fig. 5C), and this finding further confirmed the earlier report of the interplay between YY1 and HDAC3 (28). Undoubtedly, the interplay between proteins in vivo is more complicated than we can detect in vitro; nevertheless, understanding of the spatial and transient regulation of these protein complexes should shed light on the roles of clinically significant epigenetic modifying enzymes.

Figure 6. PRMT7 promoted the in vivo distant metastasis of breast cancer cells. A, representative bioluminescence images of lung metastases in mice via tail vein injection of indicated cells, and the metastases were quantified by measuring the photo flux (mean of 9 mice). Results of three representative mice from each group are shown. B, after 2 months, mice were killed and lung metastatic nodules were examined macroscopically or detected by H&E staining. White arrows, metastatic nodules; scale bars, 100 μm. C and D, primary tumor weights and volumes in BALB/c nude mice that received transplants of indicated cells. Error bars in C and D, mean ± SD (n = 9). ***P < 0.001.
Furthermore, our re-ChIP study showed that YY1/PRMT7 and YY1/HDAC3 bound to the E-cadherin promoter and that YY1 silencing resulted in decreased PRMT7 and HDAC3 binding (Fig. 5D–G). Noticeably, the present literatures have pointed to the opposite comprehensions toward the function of YY1 in tumor progression; i.e., YY1 is thought to inhibit cell invasion in breast and lung cancer cells (47), whereas it is found to be overexpressed in breast cancer and can enhance the migration and invasion in nontumorigenic MCF10A mammary cells (48). Apparently, results from this study support the latter viewpoint, as we showed that PRMT7 contributed to the acquisition of migratory and invasive capabilities of breast cancer cells and that PRMT7 directly interacted with YY1 in vitro (Figs. 3 and Fig. 5C).

Recently, the EMT program has been found to generate breast cancer stem cell (CSC)–like cells (49), and PRMT7 is associated with the pluripotency of mouse embryonic stem cells (50). Although CSCs and embryonic stem cells share some similarities, we did not identify CD44+CD24− cell populations in PRMT7-expressing MCF10A cells by FACS (data not shown), suggesting that the PRMT7-mediated EMT is irrelevant to CSCs in nontumorigenic breast epithelial cells. In addition, we observed that the PRMT7 mRNA level decreased during TGFβ-induced EMT in the MCF10A cell line (data not shown), implying that PRMT7 may play little roles in a TGFβ-induced EMT event.

Although our study has provided evidence that the wild-type PRMT7 can inhibit E-cadherin activity by mediating the repressive H4R3me2s modification and to induce EMT program (see Figs. 2 and Fig. 4), whether catalytically inactive PRMT7 mutant mediates EMT program via stimulating other signaling pathways or via interacting with transcriptional coactivators is still unknown. Moreover, despite the fact of high expression of PRMT7 in breast cancer specimens (see Fig. 1, Table 1), the prognostic significance of the YY1–PRMT7–HDAC3 coexpression needs further investigation. In this regard, experiments with tissue microarrays (TMA) and immunohistochemistry of YY1, PRMT7, and HDAC3 expression in large scales of breast carcinoma samples will be helpful. Perspectives, these prognostic analyses will contribute to the clarification of the correlation between coexpression of YY1–PRMT7–HDAC3 and poor outcomes of breast cancer.

In conclusion, we have defined in this study a novel function of PRMT7 as an inducer of EMT and a promoting factor of breast cancer metastasis. We present evidence that the ectopically expressed PRMT7 mediates the EMT program and promotes breast cancer metastasis by suppressing E-cadherin transcriptional activity. This finding leaves an open opportunity to develop a new therapeutic strategy for invasive and metastatic breast carcinomas by blocking the YY1–PRMT7–HDAC3 ternary complex.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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