CUL4A Induces Epithelial–Mesenchymal Transition and Promotes Cancer Metastasis by Regulating ZEB1 Expression

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Abstract

The ubiquitin ligase CUL4A has been implicated in tumorigenesis, but its contributions to progression and metastasis have not been evaluated. Here, we show that CUL4A is elevated in breast cancer as well as in ovarian, gastric, and colorectal tumors in which its expression level correlates positively with distant metastasis. CUL4A overexpression in normal or malignant human mammary epithelial cells increased their neoplastic properties in vitro and in vivo, markedly increasing epithelial–mesenchymal transition (EMT) and the metastatic capacity of malignant cells. In contrast, silencing CUL4A in aggressive breast cancer cells inhibited these processes. Mechanistically, we found that CUL4A modulated histone H3K4me3 at the promoter of the EMT regulatory gene ZEB1 in a manner associated with its transcription. ZEB1 silencing blocked CUL4A-driven proliferation, EMT, tumorigenesis, and metastasis. Furthermore, in human breast cancers, ZEB1 expression correlated positively with CUL4A expression and distant metastasis. Taken together, our findings reveal a pivotal role of CUL4A in regulating the metastatic behavior of breast cancer cells. Cancer Res; 74(2); 520–31. ©2013 AACR.

Introduction

Breast cancer, the most frequent malignancy in women worldwide (1, 2), is a complex and intrinsically heterogeneous disease. It is believed that breast cancer develops through the accumulation of a wide spectrum of genomic aberrations (3). Amplification of 13q34 is found in 5% of all human breast cancers and as high as 20% of basal-type breast cancers (4), the subtype of breast cancers most often associated with aggressive growth and poor prognosis (5), and in other malignant tumors (6, 7). Several candidate genes, including CUL4A, have been proposed for this region (4, 8, 9). CUL4A mRNA and protein levels are highly correlated with 13q34 amplification and have been hypothesized to be the candidate driver of this amplicon based on its amplification and significant elevation in breast cancers (4, 10), as well as in other cancers (9, 11–15). The fact that overexpression of CUL4A is associated with poor prognosis in node-negative breast cancers and other malignancies, such as ovarian carcinoma (16, 17), further supports its possible role in the aggressive behavior of certain cancers.

CUL4A, a member of the cullin family of proteins that composes the multifunctional ubiquitin ligase E3 complex, is essential for the ubiquitination of several well-defined tumor suppressor genes such as p21 (18), p27 (19), and p53 (20). Changes in CUL4A potentially exert pleiotropic effects that alter cellular functions, including proliferation, differentiation, and apoptosis. Thus, CUL4A may act as an oncogene, but whether CUL4A plays a role in breast cancer metastasis remains unknown.

The vast majority of patients with breast cancer succumb to their disease as a result of metastasis (21, 22). Currently, treatment options for metastatic breast cancers are limited and ineffective. Therefore, a tremendous effort has been focused on the understanding of the mechanisms by which metastasis occurs to provide a more rational approach in the development of future metastatic breast cancer treatments. However, how metastases are formed remains less understood. Mounting evidence shows that in epithelial cancers, including breast cancers, induction of epithelial–mesenchymal transition (EMT) is a major event that provides mobility to cancer cells to generate metastases (23). EMT is characterized by the loss of epithelial characteristics and acquisition of a mesenchymal phenotype, which confers the ability for cancer cells to invade adjacent tissue and migrate to distant sites (24), where these cancer cells proliferate to generate new tumors. Hence,
Figure 1. CUL4A expression is correlated with metastasis of human cancers. A and B, CUL4A expression was analyzed by quantitative RT-PCR (A) and Western blotting (B) in human breast cancer cell lines. C–J, immunohistochemical analysis of CUL4A expression in human breast (C and D), gastric (E and F), ovarian (G and H), and colorectal (I and J) cancers using tissue microarrays. C, E, G, and I, from left to right are representative images showing CUL4A expression, and semiquantification (D, F, H, and J) of CUL4A expression in normal tissues, primary cancer tissues without or with distant metastasis. Normal, normal tissues; no distant met, primary cancers without distant metastasis (in situ and lymph node metastasis); distant met, primary cancers with distant metastasis. Scale bars, 50 μm (C, E, G, and I) and 20 μm in the insets (C, E, G, and I); **, P < 0.01 is based on the Student t test (D, F, H, and J). Error bars, SD.
clarifying the regulation of proliferation and EMT will greatly benefit our understanding of tumor metastasis.

In this study, we found that CUL4A overexpression induced proliferation and EMT in normal and malignant human mammary epithelial cells, resulting in enhancement of growth, migration, and invasion in vitro and metastasis in vivo. Depletion of CUL4A inhibited proliferation, led to mesenchymal-to-epithelial transition (MET) and blocked distant metastasis. These functional effects of CUL4A were exerted through control of ZEB1 transcriptional expression via trimethylation of H3K4 (H3K4me3). Our findings provide a novel mechanistic role of CUL4A in breast cancer metastasis and a reasonable explanation of our clinical observation that CUL4A expression is correlated with breast cancer metastasis, suggesting that CUL4A may serve as a potential therapeutic target for advanced breast cancers.

Materials and Methods

Chemicals and antibodies

Lipofectamine 2000 transfection and TRizol LS Reagents were purchased from Invitrogen. Antibodies against CUL4A, fibronectin, Histone H3, H3K4me1, H3K4me2, H3K4me3, H3K9me3, and H3K27me3 were purchased from Abcam. E-cadherin, N-cadherin, vimentin, ZEB1, p21, p27, Ki67, and β-actin antibodies were from Cell Signaling Technology. α-Catenin antibody was from BD Biosciences. Unless otherwise noted, all other chemicals were from Sigma.

Histologic and immunohistochemical analyses

The tumors, lungs, and livers dissected from mice were fixed in 4% paraformaldehyde in PBS overnight and subsequently embedded in paraffin wax. Sections cut at a thickness of 4 μm were stained with hematoxylin and eosin (H&E) for histologic
Figure 3. CUL4A regulates the transition between epithelial and mesenchymal phenotypes in human breast cancer cells. A, representative phase-contrast images of MDA-MB-468 and MDA-MB-231 cells showed CUL4A-modulated morphologic changes. B–D, expression of epithelial and mesenchymal marker was analyzed by immunofluorescence stains (B), Western blotting (C), and qRT-PCR (D). E, sections of xenograft tumors were stained with H&E. F and G, expression of CUL4A, epithelial and mesenchymal marker in xenograft tumors was analyzed by immunohistochemical staining (F) and Western blotting (G). Scale bars, 20 μm (A), 20 μm (B), 50 μm (E and F), and 20 μm in the inset in E and F. Each experiment in A–D is repeated at least three times. n = 5 for subcutaneous transplantation. *, P < 0.05; **, P < 0.01 based on the Student t test. Error bars, SD.
analysis. Tissue microarrays for breast (BR954), colon (C0812), and ovary (OV806 and OV8010) cancers were from Alenabio, and for gastric cancer (HStm-Ade080CD-01) were from Shanghai Outdo Biotech. Clinical and pathologic information was provided by the manufacturers. Immunohistochemical analysis was performed for different markers in these arrays as described previously (25). The proportion of stained cells (lower, <30% staining; higher, ≥30% staining) was semiquantitatively determined following published protocols (26).

**Cell culture**

The human breast cancer cell lines, MDA-MB-468, MDA-MB-231, BT549, MCF7, HCC1569, normal human breast epithelial cell line, MCF10A, and HEK 293 Phoenix amphotere packing cells were purchased from the American Type Culture Collection, where they were characterized by DNA fingerprinting and isozyme detection. Cell culture was according to the manufacturer’s protocol. All the cell lines were grown at 37°C in a 5% CO2/95% air atmosphere and were revived every 3 to 4 months.

**Chromatin immunoprecipitation–quantitative PCR**

The chromatin immunoprecipitation (ChIP) Kit was purchased from Millipore and ChIP experiments were carried out essentially as described previously (27). Immunoprecipitated DNA was analyzed on the ABI PRISM 7900HT sequence detection system. The primers used for detection of promoters after ChIP are available upon request.

**In vivo tumor growth and metastasis**

Nude mice were purchased from Shanghai Slac Laboratory Animal Co. Ltd. and maintained in microisolator cages. All animals were used in accordance with institutional guidelines and the current experiments were approved by the Use Committee for Animal Care. For subcutaneous inoculation, different numbers of tumor cells were resuspended in PBS medium with 50% Matrigel and inoculated subcutaneously into 8-week-old nude mice. The tumors were measured every 3 days after appearance of tumors and the tumor volume was calculated by the formula: length × width²/2. The mice were killed 40 days after the inoculation. For metastasis assays, cells were resuspended in PBS at a concentration of 1 × 10⁷ cells/mL. Cell suspension (0.1 mL) was injected into tail veins of nude mice. All of the mice were killed by CO₂ 60 days after inoculation.

**Statistical analysis**

Data were described as the mean ± SD. Association between ZEB1 and CUL4A expression in breast tissue microarray was assessed using the Spearman rank correlation test. Comparisons between different groups were undertaken using the Student two-tailed t test. The criterion of statistical significance was P < 0.05. Statistical analysis was done with SPSS/Win11.0 software (SPSS Inc.).

**Results**

**CUL4A is highly expressed in malignant cancers**

CUL4A was highly expressed in breast cancer cells, especially in invasive cancer cells compared with normal mammary epithelial cells (Fig. 1A and B). We then analyzed CUL4A expression in normal breast tissues and breast cancers without or with distant metastasis using breast cancer tissue microarray. Consistent with a previous report (10), CUL4A was highly expressed in breast cancer tissues compared with adjacent normal breast tissues (Fig. 1C and D). Most importantly, we found that CUL4A overexpression was significantly correlated with distant metastasis (Fig. 1D). In
our samples, all patients with breast cancer with distant metastasis, but only 8 of 21 (38.1%) patients with breast cancer without distant metastasis, had high CUL4A expression (Fig. 1D).

Because CUL4A overexpression or amplification has been reported in other cancers, we examined CUL4A expression in other types of carcinomas. CUL4A exhibited high expression in ovarian (Fig. 1E and F), colorectal (Fig. 1G and H), and gastric (Fig. 1I and J) cancer tissues compared with adjacent normal tissues and CUL4A overexpression was consistently significantly correlated with distant metastasis in those types of carcinomas. These results collectively indicate a functional role of CUL4A in aggressive behaviors of cancers. We then used breast cancer as a model to verify the function and underlying mechanisms of CUL4A in promoting cancer metastasis.
Figure 6. CUL4A regulates ZEB1 transcriptional expression through H3K4 trimethylation. A, supervised hierarchical clustering of the genes differentially expressed after CUL4A overexpression. B, gene set enrichment analysis was carried out using ConceptGen. Edge indicates significant overlap between two gene sets. The P values for enrichment between CUL4A signature and others determined with ConceptGen are shown. (Continued on the following page.)
CUL4A promotes proliferative capacity of breast cancer cells

To test the oncogenic activity of CUL4A in breast cancers, we retrovirally established stable overexpression of CUL4A in MCF10A and MDA-MB–468 cells (designated as MCF10A–CUL4A and MDA-MB–468–CUL4A), and silencing CUL4A in MDA-MB–231 and BT549 cells (designated as MDA-MB–231–shCUL4A and BT549–shCUL4A). The levels of CUL4A in these resultant cell lines were verified by Western blotting (Fig. 2A). Compared with vector-only controls, both MCF10A–CUL4A and MDA-MB–468–CUL4A cells had significant increases in cell proliferation by MTT assay (Supplementary Figs. S1A and S2B) and generated more numbers and larger colonies (Supplementary Figs. S1B and S2C). In contrast, silencing CUL4A in MDA-MB–231 and BT549 cells significantly reduced cell proliferation (Fig. 2D; Supplementary Fig. S1C) and clonogenicity (Fig. 2E; Supplementary Fig. S1D). Consistent with these observations, the expression of two major proliferation related protein, p21 and p27, was modulated upon CUL4A expression. CUL4A overexpression significantly decreased the expression levels of both p21 and p27, whereas silencing CUL4A dramatically increased their expression levels (Fig. 2A). Taking together, these results suggest that CUL4A is an important regulator of proliferation in breast cancer cells.

CUL4A regulates the transition between epithelial and mesenchymal phenotypes in breast cancer cells

To investigate whether CUL4A positively regulates cell migration and invasion, we first observed the morphologic changes and found that both MCF10A–CUL4A and MDA-MB–468–CUL4A cells exhibited fibroblastic morphology (Supplementary Figs. S2A and S3A). This observation was further confirmed by expression analysis of epithelial and mesenchymal markers. We showed that CUL4A overexpression decreased the levels of epithelial markers (E-cadherin and α-catenin) and increased the levels of mesenchymal markers (N-cadherin, fibronectin, and vimentin) in both cell lines (Supplementary Figs. S2B, S3B and S3C). Moreover, mRNA levels correlated with the corresponding protein levels (Supplementary Figs. S2C and S3D), suggesting that CUL4A affected the expression of epithelial and mesenchymal markers at the transcript level.

Conversely, both MDA-MB–231–shCUL4A and BT549–shCUL4A cells reverted to an epithelial phenotype as compared with their respective control cells (Fig. 3A; Supplementary Fig. S2A). Consistent with this, silencing CUL4A increased levels of epithelial markers, and decreased levels of mesenchymal markers (Fig. 3B–D; Supplementary Fig. S2B and S2C).

MDA-MB–468–CUL4A, MDA-MB–231–shCUL4A, and their corresponding control cells were subcutaneously injected into nude mice. Xenograft tumors from MDA-MB–468–CUL4A cells also showed a mesenchymal morphology, indicating that CUL4A overexpression maintained EMT in vivo (Fig. 3E). In contrast, changes consistent with MET were observed in xenograft tumors from MDA-MB–231–shCUL4A cells (Fig. 3E). These observations were further confirmed by expression analysis of epithelial and mesenchymal markers using both immunochemical staining (Fig. 3F) and Western blotting (Fig. 3G) in these tumors. Taken together, these findings suggest that CUL4A plays an important role in regulating EMT–MET plasticity of breast cancer cells.

CUL4A promotes migratory and invasive capacities of breast cancer cells in vitro

The effect of CUL4A on cell migration was first assessed by wound healing assay. Both MCF10A–CUL4A and MDA-MB–468–CUL4A cells had significantly faster closure of the wound area compared with their control cells (Supplementary Figs. S3A and S4A). This result was confirmed by Boyden chamber assay (Supplementary Figs. S3C and S4B). Moreover, MCF10A–CUL4A and MDA-MB–468–CUL4A cells showed a greater degree of invasion through Matrigel (Supplementary Figs. S3C and S4B). In contrast, silencing CUL4A dramatically reduced the migratory and invasive capacity of MDA-MB–231 and BT549 cells (Fig. 4C and D; Supplementary Fig. S3B and S3D), suggesting that restoration of an epithelial phenotype through MET may dampen or inhibit their mobility potential. These results indicate that CUL4A promotes migratory and invasive behaviors in breast cancer cells.

CUL4A promotes tumorigenesis and metastasis in vivo

To extend our in vitro observations, we investigated whether CUL4A could regulate tumorigenic and metastatic capacity of breast cancer cells in vivo. MDA-MB–468–CUL4A, MDA-MB–231–shCUL4A, and their corresponding control cells were subcutaneously injected into nude mice. As expected, the tumors from MDA-MB–468–CUL4A cells grew more rapidly at the implantation site than their cells (Fig. 5A and B). Increased cell proliferation in MDA-MB–468–CUL4A–derived tumors was further confirmed by ki67 level (Supplementary Fig. S4A). In contrast, silencing CUL4A in the typically aggressive MDA-MB–231 cells led to a dramatic decrease in cell proliferation (Supplementary Fig. S4B), tumor volume, and weight (Fig. 5C and D).

We then investigated the functional relevance of CUL4A for metastasis in vivo. MDA-MB–468–CUL4A, MDA-MB–231–shCUL4A, and their corresponding control cells were injected into nude mice through the tail vein. CUL4A overexpression not only significantly increased the number of mice with distant metastasis (Fig. 5E), but also dramatically increased the number of metastatic tumors in both lung and liver of each mouse.

(Continued.) C and D, protein and mRNA levels of ZEB1 were measured in breast cancer cells with CUL4A overexpression or silencing by Western blotting (C) and qRT-PCR (D) assay. E, the abundance of H3 lysine methylation was assessed by Western blotting using whole-cell lysate; total H3 was used as a loading control. F, schematic presentation of three regions relative to the ZEB1 transcriptional start site used as primers to test histone occupied abundance. G and H, ChIP was performed to assess H3K4me3 occupancy in MDA-MB–468–CUL4A (G) and MDA-MB–231–shCUL4A (H) cells. IgG was used as negative control (G and H, left). “Percentage of input” indicates the ratio of DNA fragment of each promoter region bound by H3K4me3 to the total amount of input DNA fragment without H3K4me3 antibody pull-down. *, P < 0.01 in D, G, and H is based on the Student’s test. All experiments except expression array are repeated three or four times. Error bars, SD.

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Figure 7. ZEB1 mediates CUL4A-induced EMT and metastasis. A, silencing ZEB1 restored the epithelial marker expression and decreased mesenchymal markers in MDA-MB-468–CUL4A cells. B, silencing ZEB1 inhibited CUL4A-driven Transwell migration and Matrigel invasion in MDA-MB-468–CUL4A cells. C, the total numbers of mice with distant metastasis at 60 days after injection of MDA-MB-468–CUL4A cells with or without silencing ZEB1 into tail vein. Of note, 468 in (C) indicates MDA-MB-468 cell line. (Continued on the following page.)
(Fig. 5F and G; Supplementary Fig. S4C). Silencing CUL4A in MDA-MB-231 cells inhibited metastatic behavior, both in terms of the number of mice with distant metastasis (Fig. 5E) and the number of metastatic tumors in the lung and liver of each mouse (Fig. 5H and I; Supplementary Fig. S4D). Therefore, the in vivo results further demonstrate the critical role of CUL4A in breast cancer metastasis.

**CUL4A regulates ZEB1 expression through H3K4 trimethylation**

To better understand the mechanisms by which CUL4A engaged in breast cancer development and progression, we performed gene expression profiling on MDA-MB-468–CUL4A and its control cells. Microarray analysis identified a list of genes significantly differentially expressed after CUL4A overexpression, including upregulation of ZEB1 (Fig. 6A; Supplementary Table S1). Furthermore, gene set enrichment analysis indicated that proliferation, neoplastic metastasis and invasion, cell movement and motility, and ZEB1-related gene signatures (28) were significantly enriched in CUL4A overexpression cells (Fig. 6B), supporting the idea that CUL4A regulates proliferation, EMT and cancer invasion, and metastasis. These data also led us to hypothesize that CUL4A exerts these functions possibly via ZEB1. To test this, we first determined whether ZEB1 is a downstream target of CUL4A in breast cancer cells. Expression of ZEB1 in the cells with altered CUL4A expression was further evaluated by Western blotting and quantitative real-time (qRT)-PCR. MCF10A–CUL4A and MDA-MB-468–CUL4A cells exhibited greatly increased both ZEB1 protein and mRNA levels, whereas silencing CUL4A in MDA-MB-231 and BT549 cells dramatically decreased its protein and mRNA levels (Fig. 6C and D). Similar observations were made in xenograft tumors from MDA-MB-468–CUL4A and MDA-MB-231–shCUL4A cells (Supplementary Fig. S5B and S5C), suggesting the regulation of ZEB1 expression by CUL4A is at transcriptional level. This was confirmed by ZEB1 gene promoter luciferase assay (Supplementary Fig. S5A).

We then explored how CUL4A regulates ZEB1 expression at the transcriptional level. Cullin-RING ligase complexes are frequently involved in chromatin regulation (29, 30). To determine whether CUL4A regulates specific histone modifications in breast cancer cells, histone modification patterns were measured after modulation of CUL4A expression. Among histone H3K4, H3K9, and H3K27, we found that only H3K4me3 was affected by CUL4A expression (Fig. 6E). Ectopic expression of CUL4A increased H3K4me3, whereas silencing CUL4A decreased this modification.

Because H3K4me3 is associated with active transcription, we tested whether CUL4A expression was correlated with the H3K4me3 modification at the ZEB1 gene promoter in breast cancer cells. Quantitative ChIP (qChIP) assay was performed in MDA-MB-468–CUL4A and MDA-MB-231–shCUL4A cells. We found that CUL4A expression was associated with increased H3K4me3 levels at region −616 to −371bp and +239 to +464 bp of the ZEB1 promoter in MDA-MB-468–CUL4A cells (Fig. 6F and G). Less occupancy of those ZEB1 gene promoter regions by H3K4me3 was detected in MDA-MB-231–shCUL4A cells (Fig. 6H). The occupancy of chromatin repressors such as methylated H3K9 and H3K27 at the ZEB1 gene promoter was not changed by altered CUL4A expression (Supplementary Fig. S5D). These results clearly indicate that CUL4A induces transcriptional activation of ZEB1 through regulating H3K4me3 and enriching H3K4me3 to the ZEB1 gene promoter.

**ZEB1 is a mediator for CUL4A-induced EMT, migration, invasion, and tumor metastasis**

To test whether CUL4A-induced metastatic capacity was mediated by ZEB1, short hairpin RNAs (shRNA) were used to silence ZEB1 gene expression by virally transfecting MDA-MB-468–CUL4A cells with two distinct ZEB1 shRNAs (Fig. 7A). Knockdown of ZEB1 in MDA-MB-468–CUL4A cells resulted in increase in epithelial marker expression and decrease in mesenchymal marker expression at protein (Fig. 7A) and transcriptional levels (Supplementary Fig. S6A), and was accompanied with the reduction of migratory and invasive capacities (Fig. 7B; Supplementary Fig. S6B). Taken together, these results show that ZEB1 mediates CUL4A-induced EMT, migration, and invasion in breast cancer cells.

To verify whether ZEB1 eventually mediates CUL4A-induced metastasis in vivo, MDA-MB-468–CUL4A cells with or without silencing ZEB1 were injected into nude mice through the tail vein. Silencing ZEB1 not only significantly decreased the number of mice with distant metastasis (Fig. 7C), but also dramatically decreased the number of metastatic tumors in both lung and liver of each mouse (Fig. 7D and E; Supplementary Fig. S6C). Therefore, the in vivo results further demonstrated the critical role of ZEB1 in mediating CUL4A-promoted metastatic behavior in breast cancer cells.

To recognize any clinical correlation of CUL4A and ZEB1, we analyzed ZEB1 expression in the same human breast cancer tissue microarray. Highly positive correlation between CUL4A and ZEB1 expression was drawn (Fig. 7F). Similar to CUL4A, ZEB1 was highly expressed in breast cancer tissues compared with their adjacent normal breast tissues, and high level of ZEB1 was significantly correlated with distant metastasis (Fig. 7G). This result is consistent with and further supports our above in vitro and in vivo analyses.

**Discussion**

To our knowledge, this is the first study to show that CUL4A plays a functional role in metastasis. CUL4A overexpression in breast cancer cells induces proliferation, EMT, migration, and
invasion in vitro, and enhanced tumorigenic and metastatic capacities in vivo. In contrast, silencing CUL4A reversed these events in otherwise aggressive and invasive breast cancer cells. We also showed a mechanistic link between CUL4A and ZEB1 through CUL4A-mediated regulation of H3K4me3, which subsequently leads to transcriptional upregulation of ZEB1 expression. Knockdown of ZEB1 attenuated CUL4A function and had effects similar to those elicited by direct silencing CUL4A.

The putative role of CUL4A as an oncogene in cancer development is supported by the observations that CUL4A is highly expressed in breast cancers and other malignant tumors relative to normal tissues (4, 9, 10). The fact that CUL4A ubiquitinates and degrades several well-known tumor suppressor genes (18–20, 31) lends further support to, and potential mechanistic insight into, the possibility of CUL4A as an oncogene. Perhaps the most convincing evidence is the genetic study showing that deletion of Cul4a in mouse resulted in dramatically increased resistance to UV-induced skin carcinogenesis (18). Consistent with these reports, we showed that CUL4A overexpression promoted breast cancer cell proliferation and enhanced tumor formation in vivo. Interestingly, our study points to a novel function of CUL4A in breast cancer metastasis through regulating EMT.

First, breast cancer cells with ectopic expression of CUL4A displayed an EMT phenotype, including the associated stimulatory effects on migration and invasion in vitro. Interestingly, our results indicate that CUL4A not only promotes EMT, but silencing CUL4A also leads to MET. This observation suggests that EMT–MET is a fluid process. Consistent with the notion that EMT is essential for tumor cells to disseminate from adjacent tissues and seed new tumors in distant sites, all of these characteristics induced by CUL4A in vitro culminated to increased numbers of distant metastases in vivo. These experimental findings provide a mechanistic framework to explain the clinical observations that patients with breast cancer with high levels of CUL4A in tissues have higher probabilities of distant metastasis and significant shorter overall and disease-free survivals (16), and that amplification of the CUL4A-containing 13q34 region is associated with aggressive basal subtype of breast cancers (4).

The roles of several transcription factors as EMT regulators have been extensively reported. In our effort to elucidate the mechanism how CUL4A modulates EMT in breast cancer cells, we identified ZEB1 as an effective mediator of these CUL4A-induced phenomena. The mechanistic connection between CUL4A and ZEB1 was previously unknown, indeed, regulation of ZEB1 is not well understood. In this study, we showed that modulation of CUL4A expression altered the methylation status of H3K4 at the ZEB1 gene promoter, which in turn transcriptionally controlled ZEB1 expression. However, we did not detect any influence of CUL4A expression on the methylation status of H3K9 and H3K27, nor did we find recruitment of H3K9me3 and H3K27me3 on ZEB1 gene promoter. Thus, we conclude that CUL4A transcriptionally activates ZEB1 expression through regulation of H3K4 trimethylation and recruitment of H3K4me3 to ZEB1 gene promoter, and consequently promotes EMT in vitro and metastasis in vivo. How CUL4A modulates H3K4me3 requires further clarification; a few reports suggest that CUL4A regulates H3K4me3 possibly through a substrate-specific adaptor WDR5 (30), which is an essential component of the mixed-lineage leukemia histone methylation complexes that catalyze the critical trimethylation at H3K4 (32, 33).

Our observations that silencing CUL4A in aggressive breast cancer cells with high level of CUL4A dramatically blocked tumor growth and metastasis in vivo provide us a therapeutic option by targeting CUL4A in clinical practice. Considering that CUL4A is highly expressed in all breast, ovarian, colorectal, and gastric cancers with distant metastasis we analyzed, and that the vast majority of patients with breast cancer succumb to their disease as a result of distant metastasis (21, 22), together with our previous report that tumor cells with high CUL4A levels are sensitive to thalidomide treatment (12), our studies suggest a promising therapeutic target in certain subtypes of breast cancers and probably in other types of metastatic malignant tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: J.-H. Mao, G. Wei
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Oncogenic Role of CUL4A in Cancer Metastasis

CUL4A Induces Epithelial–Mesenchymal Transition and Promotes Cancer Metastasis by Regulating ZEB1 Expression

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