The Novel Metastasis Promoter Merm1/Wbscr22 Enhances Tumor Cell Survival in the Vasculature by Suppressing Zac1/p53-Dependent Apoptosis

Youya Nakazawa1,2, Hiroyuki Arai2, and Naoya Fujita1

Abstract

Understanding metastasis is integral to curative cancer treatments. Using a mouse genetic screening model, we identified Merm1/Wbscr22 as a novel metastasis promoter that includes a methyltransferase fold in its structure. Merm1 showed high levels of expression in invasive breast cancer. Ectopic expression of Merm1 in nonmetastatic cells enhanced metastasis formation without affecting cell growth and motility. The intact methyltransferase fold of Merm1 was required for metastasis formation. Interestingly, Merm1 expression promoted cell survival after entrapment in the lung microvasculature. Consistent with these results, knockdown of endogenous Merm1 in tumor cells reduced lung retention and metastasis formation. On the basis of comparative transcriptome analysis, Merm1 expression was negatively correlated with the expression of tumor suppressor Zac1. We confirmed that Merm1 suppressed Zac1 expression with histone H3 methylation at Lys9 in the Zac1 promoter region. Zac1 can induce apoptosis through its ability to transcriptionally coactivate p53, which regulates apoptosis in the vasculature and is often downregulated in metastasis. We found that Zac1 knockdown reduced the p53-dependent apoptosis that was enhanced by Merm1 knockdown, thereby increasing lung retention of metastatic cells. Our findings show that Merm1 enhances cancer cell survival in the vasculature by suppressing Zac1/p53-dependent apoptosis, thereby enhancing metastasis.

Introduction

Metastasis and relapse are the leading causes of cancer mortality, resulting in more than 5 million deaths annually worldwide. Primary cancer is becoming increasingly treatable with the development of new cancer treatments, whereas metastasis remains difficult to control and treat. The difficulty in treating metastasis arises from the fact that metastasis is a dynamic and multistep pathology, and each step requires the cooperative action of a wide variety of genes (1, 2). The complicated steps can result in the selection of untreatable metastatic tumor cells. Particularly in the vasculature, almost all cancer cells are destroyed because of strong selection pressures such as mechanical stress and immunologic assault, and less than 0.01% of tumor cells survive (3). Mimicking the dynamic state in the vasculature would be an excellent tool to screen factors that are essential for the development of untreatable metastatic cancer cells.

In this study, we screened metastasis-promoting genes through an experimental metastasis model by intravenous injection. This metastasis model provides a good simulation of the cell dynamics of tumor metastasis in the vasculature. In fact, Minn and colleagues identified a set of genes that mediates breast cancer metastasis to the lungs through this metastasis model (4). Brown and colleagues also identified the gene that is essential for lung-homing of breast cancer through the similar model (5). We successfully isolated Williams–Beuren syndrome chromosomal region 22 (Wbscr22) cDNA from surviving cells in the lungs. Wbscr22 is one of 26 genes in the chromosomal region that is deleted in Williams–Beuren syndrome, characterized by distinctive facial features, mental retardation, supravalvular aortic stenosis, hypercalcemia, and hypertension (6). There is no report suggesting a relationship between Williams–Beuren syndrome and tumors or metastasis. Wbscr22 contains a nuclear localization signal and an S-adenosyl-l-methionine–dependent methyltransferase fold (SAM-dependent MTase fold), but its function is completely unknown (7). Our study showed that the intact SAM-dependent MTase fold of Wbscr22 was important in promoting metastasis formation. Furthermore, knockdown of endogenous Wbscr22 in tumor cells reduced metastasis formation. For the first time, we clarified the function of Wbscr22 and named it metastasis-related methyltransferase 1 (Merm1). Further analysis demonstrated that Merm1 repressed the expression of the tumor suppressor gene Zac1/Lot1/Plagl1 with Lys9 methylation.
methylation of the core histone H3 in the promoter region. Methylation of Lys9 and Lys27 of the core histone H3 (H3-Lys9 and H3-Lys27) has been previously reported to lead to transcriptional repression by regulating the recruitment of chromatin remodeling enzymes (8–10). In tumors, H3-Lys9 and -Lys27 methylation were recently shown to be profoundly involved in the epigenetic gene expression control of both tumor promoter and suppressor genes (10–12). Zac1 is a C2H2 zinc finger transcription factor that has been shown to exhibit antiproliferative effects through G1 arrest and apoptosis induction (13–15). Huang and colleagues showed that Zac1 directly bound to the p53 N-terminal activation region and enhanced the transcriptional activity of p53 (13). Some reports have indicated that the apoptosis of tumor cells usually occurs shortly after entrapment in the vasculature (16–19). p53 was partly involved in the apoptosis induction in the vasculature (18), and many mechanisms have been reported the p53 downregulation in the course of metastasis (20–23).

Characteristic of Merm1-mediated metastasis promotion was to be accompanied by enhanced cell survival after entrapment in the vasculature. Acquisition of resistance to cell death in the vasculature is an indispensable step to hematogenous tumor spread. According to the classic view, resistance to cell death is important for the development of a primary cancer and much less important for subsequent cancer progression. However, many recent reports have indicated that the acquisition of resistance to cell death is strongly associated with metastasis (16–18). This link has significant implications for cancer therapy. Our results suggest that Merm1 is involved in tumor cell survival in the vasculature by suppressing p53 function. These findings suggest that Merm1 has a therapeutic potential in controlling metastasis.

Materials and Methods

Animals

Female C57BL/6N mice, C3H/HeN mice, BALB/c-nu/nu mice, and C.B-17/ker-scid mice were purchased from Charles River Japan. All animal procedures were performed using protocols approved by the Japanese Foundation for Cancer Research Animal Care and Use Committee.

Cell lines

CHO and MDA-MB-231 cells were obtained from the American Type Culture Collection. Dunn, L8, and Saos-2 cells were obtained from the RIKEN Cell Bank. C26, NL14, and NL17 cells were previously established in our laboratory (24). B16, B16-F10, and B16-BL6 cells were provided by Dr. Isaiah J. Fidler (MD Anderson Cancer Center, Houston, TX), A375M, SBC3, and KM12 cells were provided by Dr. Takao Yamori (Japanese Foundation for Cancer Research, Tokyo, Japan).

cDNA library and plasmids

A cDNA library of B16-BL6 cells was constructed in a pQCXIN vector (Clontech). Cloned human and mouse Merm1 were subcloned into a pRetroQ vector (Clontech). A V5 tag was added to the carboxy terminus of Merm1. shRNA was constructed in a pSIREN-RetroQ vector (Clontech). The targeting sequences are shown in Supplementary Materials and Methods. Substitution of the glycine codons with arginine codons in mouse and human Merm1 to generate the methyltransferase-dead mutant was accomplished using the Quick-Change site-directed mutagenesis kit (Stratagene).

PCR and chromatin immunoprecipitation

The pQCXIN multiple-cloning sites containing cDNA inserts were amplified using PCR from genomic DNA of CHO-M1 and CHO-M2 cells. qRT-PCR (quantitative RT-PCR) to amplify cDNA was performed with the primers shown in Supplementary Materials and Methods. Chromatin immunoprecipitation (ChIP) was performed using the SimpleChIP Enzymatic Chromatin IP kit (Cell Signalling Technology) and antibodies to pan-methyl histone H3 (Lys9) and tri-methyl histone H3 (Lys27; Cell Signaling Technology). The promoter region of human Zac1 was amplified from immunoprecipitated genomic DNA with the following primers: forward, 5′-CAGTGTGTGCTGTGAGGATTACACGT-3′; and reverse, 5′-AACCTAACCTTCTGTGCGGAGCA-3′. The sequence was confirmed using the ABI 3130 Genetic Analyzer (Applied Biosystems).

In vivo screening

The cDNA library-infected CHO cells (CHO/library; 1 × 10⁶ cells) were intravenously inoculated into the lateral tail vein of a 7-week-old female C57BL/6N mouse. After 28 days, the mouse lung was enzymatically digested. The digested lung was cultivated in medium containing geneticin (Invitrogen). Surviving CHO cells (CHO-M1) were intravenously inoculated, and CHO-M2 cells were separated using the same in vivo screening.

Western blotting

Cells were solubilized in a lysis buffer containing 2% NP-40, 0.2% sodium dodecyl sulfate, and 0.5% sodium deoxycholate. We used antibodies to human Merm1 (GeneTex), mouse Merm1 (rabbit polyclonal antibody raised against amino acids 1–16), human Zac1 (Aviva), V5 tag (Invitrogen), p53AIP1 or β-actin (Sigma). The LAS3000 system (FUJIFILM) was used for visualization and quantification of signals. In some experiments, cells were incubated with 10 or 30 μmol/L Nutlin-3 (Sigma) for 48 hours and then solubilized.

Immunostaining and immunohistochemistry

Cells were fixed and permeabilized with 4% paraformaldehyde and 0.2% Triton X-100 in PBS. Labeling was carried out by incubation with a human Merm1 antibody (GeneTex)
followed by incubation with Alexa Fluor 488-conjugated goat anti-rabbit IgG antibody, Hoechst 33342 and Texas Red-phalloidin (Invitrogen). The specimens were visualized using a fluorescence confocal microscope (FV1000; Olympus). For immunohistochemistry, AccuMax tissue arrays (ISU ABXIS) were deparaffinized, activated by heat, and incubated with human Merm1 antibody (GeneTex). Color was developed with the Vectastain ABC Kit (Vector Laboratories). All the specimens were obtained after informed consent.

**Metastasis and lung retention assay**

About 5 x 10^5 CHO cells and 1 x 10^6 A375M cells were intravenously inoculated into the lateral tail vein (CHO: 7-week-old female BALB/c-nu/nu mice, A375M: 7-week-old female C.B-17/1cr-1scid mice). About 2 x 10^6 LM8 cells were subcutaneously injected into the backs of 7-week-old female C3H/HeN mice. After 20 (CHO), 35 (A375M), or 28 (LM8) days, the mice were euthanized, and surface lung and liver metastatic foci were counted. To image tumor cells in lung microvasculature, 5 x 10^6 calcein AM (Invitrogen)-labeled cells were intravenously injected into the lateral tail vein. The mice were euthanized after 12 hours, and the lungs were extirpated. Cryosections of the lungs were visualized with the IX71 fluorescent microscope (Olympus), and the tumor foci in the field of view were counted using a modified hot-spot method (25). Briefly, the area with the highest density of tumor foci was identified at low power. The density of tumor foci in this area was then measured at high magnification.

**Transcriptome analysis**

A microarray analysis was performed according to standard Affymetrix protocols using GeneChip Human Genome U133 Plus 2.0 and GeneChip Mouse Genome 430 2.0 arrays. The microarray data sets were deposited in the National Center for Biotechnology Information Gene Expression Omnibus under the accession no. GSE22065. To select significant genes based on differential expression, probes were selected with a fold change cutoff of more than 2-fold increase or decrease.

**Statistical analysis**

Data are shown as means ± SD. Mann–Whitney U test was performed to determine statistical significance in metastasis assays. Other assays were evaluated by Student’s t test. P values less than 0.05 were considered statistically significant. All statistical tests were 2-sided.

**Results**

**Identification of Merm1/Wbscr22 as a novel metastasis promoter**

To identify novel metastasis-promoting factors, we performed in vivo genetic screening using a mouse experimental metastasis model. The retroviral cDNA library generated from the highly metastatic mouse melanoma B16-BL6 cell line was introduced into noncancerous CHO cells (CHO/library). Mouse received an intravenous injection of the CHO/library, and cells surviving 28 days after the injection were collected from the lung and named CHO-M1 cells. CHO-M1 cells were intravenously inoculated, and then CHO-M2 cells were separated using the same in vivo selection. Two cDNA inserts were amplified from the genomic DNA of those cells (Fig. 1A). We read the sequence of the lower band and identified it as Merm1/Wbscr22. Merm1/Wbscr22 is one of the genes in the chromosomal region that is deleted in Williams–Beuren syndrome (6). Although Merm1 is known to have a typical domain of methyltransferase (SAM-dependent MTase fold) and a nuclear localization signal (7), no studies have discussed the functions of Merm1. Human melanoma A375M cells, which are known as highly-metastatic tumor cells (26), showed strong nuclear Merm1 expression (Fig. 1B). To verify the specificity of the antibody, we confirmed Merm1 expression by Western blotting (Fig. 1C). Immunohistochemistry of tissue array on which various cancer tissues were spotted showed strong Merm1 expression in invasive ductal carcinomas (IDC) compared with corresponding normal tissues (Fig. 1D). An additional analysis using tissue array of IDC suggested that Merm1 expression were frequently upregulated in IDCs (25/50; 50%). IDC is a very common type of breast cancer that is likely to invade lymph and blood systems. About 10% of all cases are diagnosed as metastatic IDC in the first medical examination. IDC prognosis is evaluated by histopathological grades (27). As shown in Figure 1D, poorly differentiated IDCs (Black’s nuclear grade 1) expressed Merm1 more frequently (Fig. 1D, left, 18/32; 56%) than moderately differentiated IDCs (Fig. 1D, middle, Black’s nuclear grade 2, 7/18; 39%). Although this difference is quite small, there may be some kind of relationship between Merm1 expression and poorer prognosis of IDC.

**Requirement of an intact methyltransferase fold in Merm1-induced metastasis formation and cell survival after entrapment in the vasculature**

Merm1 contains a highly conserved DXGXGXGXXG motif (in which X may be any amino acid) in the SAM-dependent MTase fold. This motif is essential for SAM binding and contains noncharged small amino acids such as glycine (28, 29). In fact, substitution of these glycines with other amino acids has been used to destroy methyltransferase activity (30). We generated methyltransferase-dead Merm1 (Merm1-MD) in which the Gly residues in the DXGXGXGXXGX motif were substituted with Arg (DXRXRXRXXR). CHO cells were infected with retroviral vectors encoding nothing (CHO/mock), mouse Merm1-WT (CHO/Merm1-WT), or mouse Merm1-MD (CHO/Merm1-MD; Fig. 2A). CHO/Merm1-WT, but not CHO/mock and CHO/Merm1-MD, formed many metastatic foci in the lungs and livers (Fig. 2B). This suggested that the intact SAM-dependent MTase fold of Merm1 was essential for promoting metastasis. No significant difference was observed in in vitro and in vivo proliferation (Supplementary Fig. S1) and migration ability (data not shown) among the different cell types. Given these facts, we hypothesized that Merm1 would affect the state of tumor cells in the bloodstream, but not the state of tumor growth at metastatic sites. Calcein AM is a cell-permeable dye that is hydrolyzed by intracellular esterases into the green fluorescent calcein, which is retained well in the cytoplasm in living cells but rapidly disappears from the cytoplasm.

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when cells die. Twelve hours after injection of calcein AM-labeled CHO cells, we extirpated lungs and prepared cryosections for microscopy. As shown in Figure 2C, only Merm1-WT expression significantly enhanced cell survival after tumor cell entrapment in the lung microvasculature.

**Merm1 mediates tumor metastasis by regulating tumor cell survival in the vasculature**

To observe the function of endogenous Merm1 in tumor cells, we examined Merm1 expression in mouse tumor cells with different metastatic potentials (Fig. 3A, left) and in human metastatic tumor cells (Fig. 3A, right). Metastatic potentials of mouse tumor cells were previously examined by intravenous injection (24, 31, 32). Interestingly, Merm1 expression was correlated with their metastatic ability in mouse tumor cells of the same origin. We generated short hairpin RNAs (shRNA) targeting mouse and human Merm1 (Fig. 3B and C). Consistent with the results of ectopic expression in CHO cells (Fig. 2B), Merm1 knockdown inhibited experimental metastasis of A375M cells to the lungs and spontaneous metastasis of LM8 cells to the lungs (Fig. 3D). We then ascertained that Merm1 knockdown inhibited tumor survival in the lung microvasculature (Fig. 3E) and did not affect the *in vitro* or *in vivo* proliferation (Supplementary Fig. S2A and S2B). Merm1 was suggested to enhance tumor cell survival in the vasculature, resulting in increased metastasis formation.

**Merm1 inhibits expression of the tumor suppressor Zac1 with histone H3-Lys9 methylation of the Zac1 promoter**

We performed comparative transcriptome analysis using cDNA microarray. LM8/shRNA2 cells were compared with LM8/control cells, and A375M/shRNA2 cells were compared with A375M/control cells. Merm1 knockdown was confirmed in both LM8 and A375M cells (Fig. 4A). There was no significant difference in the expression levels of cell adhesion-relating molecules (selectins, cadherins, and CAM). An adhe-
sion assay of A375M cells to human umbilical vein endothelial cells (HUVEC) confirmed that Merm1 was not likely to affect tumor cell adhesion to vascular endothelial cells (Supplementary Fig. S2C). We used the threshold of 2-fold increase or decrease to select significant genes (Supplementary Tables S1 and S2). Only 1 gene, Zac1/Lot1/Plagl1, satisfied the criteria in both LM8 and A375M cells. The transcription of Zac1 was upregulated by Merm1 knockdown in both cell lines (Fig. 4A). We confirmed the negative correlation between Merm1 and Zac1 in LM8 and A375M cells at both the mRNA and the protein levels (Fig. 4B). We also found a negative correlation between Merm1 and Zac1 expression in 14 other human tumor cell lines (Fig. 4C). These results suggest that Merm1 has a negative effect on Zac1 transcription.

Methyltransferases containing a SAM-dependent MTase fold regulate gene transcription via DNA or histone methylation activity (25, 26). However, Merm1 does not possess the characteristic catalytic center (Pro-Cys motif) and DNA-binding motif of DNA methyltransferases (33). Therefore, we assumed that Merm1 mediated histone methylation, but not DNA methylation. We, thus, examined H3-Lys9 and H3-Lys27 methylation states in the Zac1 promoter region. H3-Lys9 methylation in the Zac1 promoter region (−424 to −308) was observed in A375M/control cells, but not in A375M/shRNA2 cells (Fig. 4D), and H3-Lys27 methylation was not observed in either cell line. These findings suggested that Merm1 repressed Zac1 expression by direct or indirect H3-Lys9 methylation in the Zac1 promoter region. To specify the direct link between Merm1 and H3-Lys9 methylation of the Zac1 promoter, we performed an in vitro methylation assay using recombinant histone H3 and Merm1. However, purified Merm1 did not methylate histone H3 in this assay. Merm1 might require native conditions or functionally associated proteins to methylate histone H3.
Figure 3. Knockdown of endogenous Merm1 in tumor cells reduced metastasis formation and tumor survival in the vasculature. A, endogenous expression of Merm1 in various tumor cell lines was assayed. In the left panels, the metastatic abilities of mouse tumor cells of the same origin were compared. B, the effects of control shRNA (control) or Merm1 shRNAs (shRNA1 and shRNA2) on Merm1 expression were confirmed. C, human melanoma A375M cells were treated with control shRNA or Merm1 shRNA2 Merm1 expression was confirmed by immunostaining with a human Merm1 antibody. Merm1 and actin filaments were visualized with Alexa Fluor 488-conjugated secondary antibody and Texas Red-phalloidin, respectively. Scale bar, 20 μm. D, LM8/control or Merm1 knockdowned LM8/shRNA1 and LM8/shRNA2 cells were subcutaneously injected into the backs of C3H/HeN mice (n = 18). A375M/control or Merm1 knocked down A375M/shRNA1 and A375M/shRNA2 cells were intravenously inoculated into C3H/HeN mice (n = 12). After 28 (LM8) or 35 (A375M) days, surface lung metastatic foci were counted. Data are obtained from 2 independent experiments. Bars, average. **, P < 0.01; *, P < 0.05 by Mann–Whitney U test. E, calcein AM–labeled LM8 or A375M cells were intravenously injected into C3H/HeN or C.B-17/Scid mice (n = 3). After 12 hours, cryosections of the lungs were visualized with a fluorescent microscope (bottom), and tumor foci were counted in 3 independent fields of view for each mouse (top). Data are representative of 2 independent experiments. Scale bar, 500 μm. Column, means ± SD. **, P < 0.01; *, P < 0.05 by Student’s t test.
Merm1 suppresses Zac1/p53-dependent apoptosis, resulting in enhanced cell survival in the vasculature and metastasis formation.

We then generated Zac1 shRNAs and found that shRNA3 and shRNA4 effectively attenuated Zac1 expression (Fig. 5A). Nutlin-3 inhibits p53 degradation by interfering with the p53-MDM2 interaction (34). The amount of the p53-regulated proapoptotic factor p53AIP1 (35) increased in proportion to the p53 accumulation due to Nutlin-3 (Fig. 5B). In the presence of 30 μmol/L Nutlin-3, Merm1 knockdown enhanced the expression of p53AIP1, and this effect was eliminated by simultaneous Zac1 knockdown. An annexin V-PI staining assay confirmed that actual level of apoptosis (Fig. 5C). Merm1 knockdown increased the population of cells in late apoptosis (annexin V positive, PI positive), but Zac1 knockdown reduced the same population. Thus, Merm1 inhibited p53 transcriptional activity through Zac1 repression, resulting in apoptosis inhibition. Consistent with these results, Zac1 knockdown rescued Merm1 knockdown-mediated cell death in the vasculature (Fig. 5D). We also confirmed that Zac1 knockdown...
eliminated the inhibition of metastasis by Merm1 knockdown (Fig. 5E). Our results indicate that Merm1 promoted metastasis formation by enhancing cell survival in the vasculature via suppressing Zac1/p53-dependent apoptosis.

Merm1-mediated metastasis promotion can be realized on the premise that tumor cells have a normal p53 function. Sequence analysis of cDNA isolated from LM8, SBC3, KM12, and A375M cells showed that they did not have a mutation in
p53 protein, including its DNA-binding domain (Supplementary Fig. S3A and S3B). On the other hand, MDA-MB-231 cells, which had mutant p53 (Supplementary Fig. S3A), and Saos-2, which are known to lose the coding region of p53 (36), expressed very low levels of Merm1 (Fig. 3A). CHO cells also had a mutation in the DNA-binding domain of p53 (Supplementary Fig. S3C). However, this mutation of p53 (T211K) was reported to abrogate its ability to induce cell-cycle arrest but have no effect on its ability to downregulate DNA replication (37). Mutant p53 of CHO cells probably retain partial p53 function involving apoptosis initiation, and this could explain why CHO cells could acquire metastatic ability by ectopic expression of Merm1 (Fig. 2B). These suggest that Merm1 suppresses persisting p53 function through repression of Zac1, leading to acquisition of metastatic ability.

Discussion

Recently, the presence of chemotherapy-resistant cancer cells, for example, dormant cancer cells or cancer stem cells, has been shown to be involved in metastasis formation (38, 39). These cells are growing very slowly or not at all, rendering proliferation-targeted therapies ineffective. In other words, these cells are just surviving. To establish effective treatments for metastasis, it is necessary to identify and understand the specific mechanisms that control tumor cell survival. In this study, we discovered a novel mechanism that promotes cell survival and metastasis by inhibiting p53-dependent apoptosis. Although deletion and mutation of p53 are not the first-hit mutations in tumor initiation, p53 inactivation is important in subsequent tumor progression (40). In the vasculature, tumor cells must overcome immunologic assault or mechanical shear forces and almost all tumor cells die (3, 19). Such cell death partly depends on p53. Zhang and colleagues showed that hypoxia in the primary tumor upregulated MDM2 expression and decreased p53 protein levels, which rendered tumor cells less sensitive to apoptosis and more prone to metastasis (18). In another study, Lewis and colleagues showed that the absence of functional p53 induced metastasis without affecting primary tumor growth in a mouse model (23). Here, we clarified a novel critical mechanism of p53 inactivation during the process of metastasis formation. Merm1 inhibited p53-dependent apoptosis by repressing the p53 coactivator Zac1 and promoted tumor cell survival in the vasculature. Because Merm1 had no effect on tumor proliferation (Supplementary Fig. S1, S2A, and S2B), the enhanced cell survival in the vasculature appears to be directly reflected in the metastatic capacity of tumor cells. Furthermore, Merm1 expression enhanced metastasis to both the lungs and livers (Fig. 2B), indicating that Merm1 does not affect tumor proliferation in an organ-specific manner.

Our results indicate that Merm1 is a potential target for controlling cancer metastasis. Because Merm1 was originally isolated from mouse melanoma cell lines, we tried to examine the expression of Merm1 in melanoma clinical samples. Although we found significant upregulation of Merm1 expression at the mRNA level in melanoma, there was no relationship between Merm1 upregulation and poor prognosis of melanoma. We are now collecting tissue sections of melanoma for further analysis with immunohistochemistry. In breast cancer, we found strong expression of Merm1 in tumor tissues (Fig. 1D), whereas another group reported Zac1 downregulation, which we believe could have resulted from Merm1 expression (15). Because Merm1-targeted therapy would be effective only when tumor cells had normal p53 function, we need to pay attention to the p53 status. Interestingly, CHO cells had mutant p53 (Supplementary Fig. S3C) but acquired metastatic ability by ectopic expression of Merm1 (Fig. 2B). Mutant p53 expressed in CHO cells was reported to retain partial p53 function(s) (37). Merm1 might enhance metastatic ability of CHO cells by suppressing the retained p53 function(s). These results suggest that Merm1 promoted metastasis by attenuating unidentified metastasis suppressor function(s) of p53. In addition, the completely unknown physiological function of Merm1 is a great hindrance to the clinical application of Merm1. The unclear relationship between Merm1 and histone H3-Lys9 methylation should also be studied. Further studies are necessary to elucidate the feasibility of clinical application of Merm1.

As shown in Supplementary Tables S1 and S2, we found gene expression changes associated with Merm1 knockdown. Although many genes were selected using the threshold of 2-fold increase or decrease, only 1 gene, Zac-1, was commonly upregulated in both LM8 and A375M cells. There might be other Merm1-regulated genes that were listed in the Tables. Because other genes might be specific for each cell line and might not be common Merm1-regulated genes, we did not examine other genes in this study. However, further studies of Lys9 methylation on their promoter would aid to identify biomarkers for Merm1-targeting therapy.

Disclosure of Potential Conflicts of Interests

No potential conflicts of interest were disclosed.

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