MTA1 Promotes STAT3 Transcription and Pulmonary Metastasis in Breast Cancer

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Abstract

Overexpression of the prometastatic chromatin modifier protein metastasis tumor antigen 1 (MTA1) in human cancer contributes to tumor aggressiveness, but the role of endogenous MTA1 in cancer has not been explored. Here, we report the effects of selective genetic depletion of MTA1 in a physiologically relevant spontaneous mouse model of breast cancer pulmonary metastasis. We found that MTA1 acts as a mandatory modifier of breast-to-lung metastasis without effects on primary tumor formation. The underlying mechanism involved MTA1-dependent stimulation of STAT3 transcription through action on the MTA1/STAT3/Pol II coactivator complex, and, in turn, on the expression and functions of STAT3 target genes including Twist1. Accordingly, we documented a positive correlation between levels of MTA1 and STAT3 in publicly available breast cancer data sets. Together, our findings reveal an essential modifying role of the physiologic level of MTA1 in supporting pulmonary metastasis of breast cancer. Cancer Res; 73(12); 3761–70. ©2013 AACR.

Introduction

Breast cancer is the most common cancer diagnosed among women and is the second leading cause (next to lung cancer) of cancer-related death among women. Breast cancer induction and progression are associated with oncogenic activation, loss of checkpoint control tumor suppressor function, and growth sustained by growth factors and steroids. The breast cancer metastasis to the distant sites, including lungs, is the primary cause of mortality in patients with breast cancer. Among other mechanisms, cancer metastasis depends on the deregulation of signaling components as well as aberrant transcription of genes coding critical regulatory proteins. For example, STAT3 is one of the widely upregulated and hyperactivated signaling molecules in human cancer, and deregulated expression of STAT3 target genes have been intimately linked with metastatic potential of breast cancer cells and tumors (1, 2). STAT3 expression was found to be constitutively active in many cancers including head and neck, mammary, multiple myelomas, and other hematologic malignancies (2, 3). STAT3 besides regulating its own transcription also regulates the expression of genes involved in antiapoptosis, prosurvival, tumor angiogenesis, invasion, and metastasis (1–5). STAT3 transcriptional activity is mainly due to its phosphorylation of tyrosine residue present in transcription activation domain. STAT3 interacts and recruits with other transcriptional factors or coactivators to its chromatin targets to activate their transcription (1, 6). In spite of a widely documented overexpression of STAT3 in human cancer including breast, the molecular basis of increased STAT3 transcription by a cancer-relevant physiologic factor remains poorly understood.

Regulation of fundamental cellular processes requires alterations in the transcription of regulatory genes by dynamic participation of chromatin modifiers at the target gene chromatin (7). One family of ubiquitously expressed chromatin modifiers is the metastasis tumor antigen 1 (MTA1), an integral component of the nucleosome remodelers and histone deacetylation (NuRD) complexes (7). MTA1 modifies DNA accessibility of transcriptional factors at the target gene chromatin. In addition to its role as a corepressor, MTA1 is also a bona fide coactivator as it stimulates the expression of BCAS3, PAX5, and p19ARF independent of the NuRD complex (7–10). In recent years, MTA1 has emerged as one of the most widely upregulated genes in human cancer, including in breast cancer (7, 11). MTA1 is also thought to

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play an important role in mammary gland development as genetic depletion impairs mammary gland morphogenesis and branching of mammary duct (12). Consistent with this notion, overexpression of the MTA1 in the mouse mammary gland resulted in increased ductal extension, enhanced ductal branching, and proliferation, a delayed involution, and tumorigenesis (13). In addition, MTA1 overexpression in the Rat1 cells is transforming in nature (14) and promotes epithelial-to-mesenchymal transition in the HC11 and NMuMG model systems (15).

Despite a large body of work in support of MTA1 overexpression in human cancer and tumor aggressiveness, the contribution of the physiologic level of MTA1 in breast-to-lung metastasis continues to be unknown. Here, we attempt to investigate this outstanding question in the field by investigating the influence of selective genetic depletion of MTA1 on breast-to-lung metastasis. Here, we found that MTA1 acts as a mandatory modifier of breast-to-lung metastasis. The underlying mechanism involves MTA1 stimulation of STAT3 transcription and in turn, the expression and functions of STAT3 target genes.

Materials and Methods

Cell line authorization statement and cell culture
All the cell lines used in this study are either from Dr. Rakesh Kumar’s or Dr. Yi Zheng’s laboratory and have been tested, authenticated, and previously used in the peer-reviewed articles from the laboratory (12, 14, 16). Stat3 wild-type (WT) and knockout mouse embryonic fibroblasts (MEF) were kindly provided by Dr. Yi Zheng (17).

Generation of PyVMT-tg/MTA1<sup>−/−</sup> mice strain
The MMTV-PyVMT-tg mice were described previously (18, 19). For generation of PyVMT-tg/MTA1<sup>−/−</sup> mice, MTA1<sup>+/+</sup> female mice (12) were first bred with PyVMT-tg/wt to produce double heterozygous mice. Then the double heterozygous males were bred with MTA1<sup>+/−</sup> female to produce PyVMT-tg/MTA1<sup>−/−</sup> mice. The genetic background of these mice are 25% FVB, 37.5% B6, and 37.5% 129Sv. The PyVMT-tg/MTA1<sup>−/−</sup> female mice and PyVMT-tg/wt mice with both MTA1 copies were monitored for the palpable breast tumors and potential lung metastasis at the time of sacrifice.

Western blot analysis, RT-PCR, Northern blotting, reporter assays, cell migration, and invasion assays and immunohistochemistry
These methods were carried out as previously described (12–16). Primers used for reverse transcription (RT)-PCR and quantitative polymerase chain reaction (qPCR) analysis were provided in Supplementary Table S1A.

Chromatin immunoprecipitation assay and electrophoretic mobility shift assay
Chromatin immunoprecipitation (ChIP) assay and electrophoretic mobility shift assay (EMSA) were carried out according to the protocol described previously (20, 21) and the PCR was carried out using the primers mentioned in Supplementary Table S1B and S1C.

Microarray data analysis
Publicly available microarray datasets containing tumor samples from various types of cancer were curated from Gene Expression Omnibus (GEO) at http://www.ncbi.nlm.nih.gov/geo/ to analyze the correlation of transcript levels of MTA1 with STAT3. The datasets used for the analyses were GSE2034 (breast cancer), GSE20565 (ovarian cancer), and GSE1431 (prostate cancer). The probes used to detect the mRNA levels of MTA1 in the first 3 datasets are 202247_s_at and 211783_s_at, and those for STAT3 levels were 208991_at and 208992_s_at. Likewise, for the prostate cancer dataset, the probes used for detection of MTA1 were 38633_at, 1642_at, and 1643_g_at and those for STAT3 are 239_at and 39708_at. In general, after summarization and normalization [using robust multiaarray average (RMA) algorithm] and subsequent log transformation of data in each dataset with GeneSpring GX 11.0, the resulting values were used for correlating MTA1 levels with STAT3.

Results

MTA1 is required for breast-to-lung metastasis
Despite an overwhelming evidence of wide-spread MTA1 overexpression in breast cancer metastasis (7, 11, 22), its causative role in breast-to-lung metastasis remains unknown. We determined the influence of selective genetic depletion of MTA1 in a spontaneous breast-to-lung tumor model, the mouse mammary tumor virus (MMTV)-driven polyoma virus-middle T antigen-transgenic mice (PyVMT-tg; ref. 18). To this end, we first generated the MMTV-PyVMT-tg/MTA1<sup>−/−</sup> mice by breeding the MTA1<sup>−/−</sup> female mice with the PyVMT-tg/wt mice to produce double heterozygous mice. Then the double heterozygous males were bred with MTA1<sup>+/−</sup> female to produce PyVMT-tg/MTA1<sup>−/−</sup> mice, which were deprived of MTA1. There was no compensatory effect of the depletion of MTA1 on the levels of MTA2 or MTA3 (Fig. 1A). The relevance of this whole animal model to human breast cancer is supported by the fact that the breast tumors do metastasize to lung (23). We unexpectedly found that there was no significant phenotypic effect of the depletion of one or both copies of MTA1 on the volume of palpable primary mammary tumors in PyVMT-tg/MTA1<sup>−/−</sup> mice as compared with mammary tumors in the control PyVMT-tg/MTA1 mice (Fig. 1B). However, we were surprised to notice a significant reduction in the number and size of lung metastases in the PyVMT-tg/MTA1<sup>−/−</sup> mice as well as in PyVMT-tg/MTA1<sup>−/−</sup> mice compared with control PyVMT-tg/MTA1 mice with MTA1 gene (Fig. 1C), suggesting that MTA1 may be preferentially required for breast-to-lung metastasis. Because depletion of either one or both copies of MTA1 substantially compromised the ability of breast tumors to metastasize to lung, we decided to compare the WT animal with those with depletion of both copies of MTA1 in the subsequent studies. Deletion of MTA1 also compromised the number and size of micro-lung metastases in the PyVMT-tg/MTA1<sup>−/−</sup> mice compared with control PyVMT-tg/MTA1 mice (Fig. 1D). Because PyVMT-tg represents the most potent and rapid oncogene (18, 19), and tumorigenesis in murine models is profoundly influenced by genetic strains (24), it is possible that the endogenous MTA1 may not be an essential component for the formation of...
MTA1-STAT3 Signaling in Breast Cancer Metastasis

MTA1 targets STAT3 to promote breast cancer cell invasion

While these studies were in progress, results from another project in the laboratory involving microarray analysis of the Rat1 fibroblasts overexpressing MTA1 (14) identified STAT3 as one of the targets of MTA1 (unpublished data). Considering the previously established role of STAT3 in cancer metastasis (1, 2), we hypothesized that MTA1 may use STAT3 to influence its metastatic effects in the model system used. In this context, we found evidence of increased STAT3 mRNA and protein expression by MTA1 overexpression in the HC11 murine mammary epithelial cells, Rat1 and MCF-7 human breast cancer cells as compared with control cells (Fig. 2A and B). We also noticed a reduced level of total STAT3 protein in the primary tumors from the PyVMT-tg/MTA1 mice as compared with PyVMT-tg/MTA1 mice (Fig. 2C), suggesting a potential role of STAT3 in breast-to-lung metastasis but not in the primary tumors generated by a potent oncopogene PyVMT transgenic based PyVMT-tg/MTA1−/− model used here.

Mechanistically, we found that the expression of MTA1 stimulates Stat3-promoter luciferase activity in the MCF-7 cells (Fig. 2D), suggesting that MTA1 may act as an upstream activator of Stat3-transcription. Accordingly, Stat3 promoter exhibits a reduced transcription activity in the MTA1−/− MEFs as compared with the MEFs with the WT MTA1 (Fig. 2E). Furthermore, selective knockdown of STAT3 or inclusion of a pharmacologic inhibitor of STAT3, AG490 (26), and Piceatanol (27) in an invasive tumor cell line previously established from the PyVMT-tg mice (25) inhibits cell migration (Fig. 2F). In addition, STAT3 pharmacologic inhibitor piceatannol also inhibited the migration of the MCF-7 cells stably expressing MTA1 (Fig. 2G). Furthermore, the meta-analysis of the available microarray data sets revealed a strong positive correlation between the levels of MTA1 and STAT3 in patients with breast cancer (Fig. 2H) as well as in multiple human cancer types including, lung, prostate, and ovarian cancers (Fig. 3A–C). Together these results suggest that STAT3 may be a mechanistic mediator of MTA1-driven invasive/metastasis potential of mammary epithelial cells.

MTA1, an upstream transactivator of Stat3 transcription

It is generally accepted that the STAT3 regulates its own transcription via binding to STAT3-binding elements (SBE), which are largely conserved in the murine or human Stat3 promoter (4, 5). To understand the basis of MTA1 regulation of Stat3 transcription, we next carried out MTA1-ChIP using the primers encompassing the SBE region (−140 to −470 bp in the murine Stat3 promoter) in the HC11 cells. We found that MTA1 and STAT3 are corecruited to the same −140 to −470 bp region in the Stat3 promoter (Fig. 4A) and such recruitment was increased in cells with MTA1 overexpression as compared with the control cells (Fig. 4B). Because MTA1 enhances Stat3 transcription, we analyzed the corecruitment of RNA polymerase II (Pol II), an indicator of active transcription, and MTA1 on the Stat3 promoter. Sequential double ChIP analysis revealed the recruitment of MTA1/PoI I coactivator complex but not the components of MTA1/NuRD complex, such as HDAC2, MBD3, CHD4, or RbBP4 onto the SBE region of the
Stat3 promoter (Fig. 4C), suggesting that MTA1 is a positive regulator of the Stat3 transcription. Indeed, coexpression of MTA1 and STAT3 stimulates Stat3-promoter-driven transcription in an additive manner as compared with the stimulation of the Stat3 promoter activity by STAT3 or MTA1 alone (Fig. 4D). In addition, MTA1 and STAT3 did not show any stimulatory effect on the mutant Stat3 promoter-luc activity (Fig. 4D).

Because the net levels of STAT3 were dependent on the status of MTA1 (Fig. 2A and B), we next examined the possibility of a mutual essentiality of MTA1 and STAT3 coassociation in the Stat3 transcription using the WT, MTA1−/− and STAT3−/− MEFs. We found the recruitment of MTA1 onto Stat3 chromatin only in WT MEFs but not in the STAT3−/− MEFs (Fig. 4E), suggesting a facilitating role of STAT3 for the noted recruitment of MTA1 onto Stat3 chromatin. In contrast, STAT3 recruitment onto Stat3 chromatin was not completely compromised in the MTA1−/− MEFs as compared with the WT MEFs (Fig. 4E). These findings suggest that MTA1 uses STAT3 to get recruited onto Stat3 chromatin to manifest its coactivator activity upon Stat3 transcription.

Because MTA1 stimulates Stat3 transcription through a MTA1/STAT3 coactivator complex (this study), we next tested whether these 2 proteins could interact. We found that indeed, endogenous MTA1 interacts with STAT3 in the ZR-75 cells (Fig. 5A). Furthermore, 35S-labeled recombinant MTA1 directly binds to the C-terminal transcriptional activation domain.
We also observed reciprocal interaction between the 35S-labeled recombinant STAT3 and MTA1 (Fig. 5C). Because STAT3 is known to be activated by growth factors, we next showed that serum stimulation of the ZR-75 cells promotes the interaction between the endogenous STAT3 and MTA1 (Fig. 5D), presumably due to translocation of the activated STAT3 to the nuclear compartment. Because STAT3 transactivation domain is also known to interact with p300 (28), we also found evidence of serum-dependent association of MTA1 with STAT3 in the ZR-75 breast cancer cells (Fig. 5D). We next verified the coexistence of MTA1/STAT3/PoII in the same fractions using sucrose gradient sedimentation experiment in the ZR-75 nuclear extract (Fig. 5E). These findings raised the possibility of a signal-dependent recruitment of MTA1/STAT3 coactivator complex onto the Stat3 target gene chromatin, and consequently, in the expression and functions of STAT3 target genes. In support to these findings, we noticed the recruitment of MTA1 and STAT3 onto Stat3 promoter only in presence of serum stimulation (Fig. 5F). To show a direct binding of the MTA1/STAT3/PoII complex onto the Stat3 promoter DNA, we carried out an EMSA analysis using the PCR product encompassing the region -140 to -470 of the Stat3 promoter using the nuclear extracts prepared from the HC11 cells (Fig. 5G). As expected, we observed the formation of specific STAT3 protein–DNA complexes only under conditions of serum stimulation. The identity of protein–DNA complexes was established by supershifting the protein–DNA complexes by inclusion of antibodies against MTA1 or STAT3 or PoII at the expense of

Figure 3. MTA1 expression positively correlates with the levels of STAT3 in patients with cancer. A, meta-analysis showing the correlation between MTA1 and STAT3 in lung cancer data set GSE4573. B, correlation of MTA1 with STAT3 transcripts in prostate cancer dataset GSE1431 with primary tumor samples. (n = 88). C, correlation between MTA1 and STAT3 transcript levels in ovarian cancer dataset GSE20565. Secondary ovarian tumor samples (n = 44).

Figure 4. Recruitment of MTA1/STAT3/PoII onto Stat3 chromatin. A, ChIP analysis for the recruitment of MTA1 and STAT3 onto Stat3 chromatin (−140 to −470 bp) in the HC11 cells. B, ChIP analysis for the recruitment of Stat3 promoter onto Hc11 cells stably expressing pcDNA and MTA1. C, double ChIP analysis for the recruitment of RNA Pol II > T7-MTA1 into Stat3 chromatin in HC11 cells stably expressing pcDNA and MTA1. D, double ChIP analysis for the recruitment of MTA1 and STAT3 onto Stat3 chromatin in HC11 cells stably expressing pcDNA and MTA1. E, double ChIP analysis for the recruitment of RNA Pol II > T7-MTA1 into Stat3 chromatin in HC11 cells stably expressing pcDNA and MTA1.
the primary protein–DNA complex (Fig. 5G). Together, these findings reveal the participation of the MTA1/STAT3/Pol II coactivator complex in the noticed MTA1 regulation of Stat3 transcription (Fig. 5H).

**MTA1 stimulates Twist1 transcription in STAT3-dependent and -independent manner**

Results in the preceding sections suggest that STAT3 may be a downstream effector of MTA1 overexpression-associated increased invasiveness of mammary epithelial cells. Consistent with this notion, we found that MTA1 overexpression in the MCF-7 cells leads to the increased expression of STAT3 target genes VEGF, MMP-2, MMP-9, and Twist (Fig. 6A–D), all with roles in promoting cancer cell invasion and metastasis. Earlier studies have shown that STAT3 transcriptionally regulates Twist1 expression and thereby STAT3-Twist1 pathway plays an important role in the invasion of cancer cells (29). Because MTA1 now regulates the expression of STAT3 and consequently its function (this study), we considered the possibility of MTA1 regulation of Twist1 expression via STAT3. We found

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**Figure 5.** MTA1/STAT3/Pol II coactivator complex regulates Stat3 transcription. A, Western blot analysis for STAT3, MTA1, and RNA Pol II after immunoprecipitating the nuclear extracts from the ZR-75 cells with antibodies against STAT3 or MTA1 or RNA Pol II. B, in vitro interaction of MTA1 and STAT3. The GST-STAT3 fusion protein and its 2 deleted versions were used in the glutathione S-transferase (GST) pull-down assays with in vitro-translated 35S-labeled full-length MTA1. C, in vitro interaction of STAT3 and MTA1. The GST-MTA1 fusion protein was used in the GST pull-down assay with in vitro-translated 35S-labeled full-length STAT3. D, Western blot analysis of interactions among the MTA1, Pol II, and STAT3 in the ZR-75 cells stimulated in the presence or absence of serum before immunoprecipitating the nuclear lysates with an anti-MTA1 antibody. E, ZR-75 cell nuclear extract was fractionated onto a 3% to 30% sucrose gradients by sedimentation. The fractions were subjected to Western blot analysis with the indicated antibodies. F, recruitment of MTA1 and STAT3 onto Stat3 promoter in the HC11 cells in presence and absence of serum. G, EMSA analysis showing the binding of MTA1, STAT3, or Pol II to the −140 to −470 region of the Stat3 promoter in the nuclear extracts of the HC11 cells. H, proposed model to represent the recruitment of MTA1/STAT3/Pol II coactivator complex onto the Stat3 promoter leading to its stimulation.
that MTA1 overexpression promotes the levels of Twist1 protein in the MCF-7, Rat1 cells, and MEFs (Fig. 6C). We also observed an increased Twist1 mRNA expression by STAT3 or MTA1 or both proteins together in the MCF-7 cells (Fig. 6D). Accordingly, MTA1 overexpression in the MCF-7 cells was accompanied by an elevated Twist-promoter activity as compared with vector transfected cells (Fig. 6E), whereas Twist-promoter activity was compromised in the MTA1−/− MEFs as compared with control MEFs (Fig. 6F). In addition, genetic depletion of MTA1 in MEFs from the WT, MTA1−/− heterozygous, or MTA1−/− homozygous mice (Fig. 6G) as well as selective knockdown of MTA1 in the MCF-7 cells reduces the levels of Twist1 (Fig. 6H). To further establish the hierarchy of molecules, we next depleted STAT3 either by siRNA in the MCF-7 cells or genetic depletion in the STAT3−/− MEFs inhibited the expression of Twist1 and not MTA1, which is upstream of STAT3 (Fig. 6I). Similar to the findings in the cell lines, we also observed reduced level of Twist1 in the primary mammary gland tumors from the PyVMT-tg/MTA1−/− mice as compared with tumors from the PyVMT-tg/MTA1 mice (Fig. 6J). These findings suggest that MTA1 regulates STAT3-Twist1 pathway.

To gain a mechanistic understanding of the observed MTA1 regulation of Twist1 expression, we next carried out a detailed...
Figure 7. MTA1/STAT3 coactivator complex regulates Twist1 transcription. A, ChIP analysis of MTA1 recruitment onto the Twist1 chromatin in the HC11 cells. B, ChIP analysis of MTA1 recruitment onto the Twist1 chromatin in the STAT3+/+ and STAT3−/− MEFs. C, Twist1 promoter luciferase activity in the MCF-7 cells after cotransferring with pcDNAs for STAT3, STAT1, MTA1, MTA1 + STAT3, or MTA1 + STAT1. D, recruitment of the MTA1–Pol II, MTA1–STAT3, MTA1–STAT1, and MTA1–HDAC2 complexes onto the Twist1 promoter in the HC11 cells. E, ChIP analysis of MTA1 recruitment onto the Twist1 promoter in the STAT3−/− MEFs after STAT1 knockdown. F, Western blot analyses of STAT1 in the STAT3−/− MEFs after treating with STAT1-siRNA. G, Western blot analysis of STAT1 interaction with MTA1 in MCF-7 nuclear extracts after immunoprecipitating with MTA1. H, Twist1 promoter activity in the STAT3−/− MEFs after cotransferring alone with pcDNA, MTA1, STAT3, or both MTA1 + STAT3 and MTA1 + STAT1. I, EMSA analyses of STAT3, STAT1, and MTA1 binding to the mouse Twist1 promoter in the nuclear extracts prepared from STAT3+/+, STAT3−/−, and STAT3+/− MEFs after treating with STAT1-siRNA. Lack of free probe in lanes 2 to 11 is suggestive of a saturated binding of DNA–protein complex with the probe. J, effect of overexpression of STAT3 or Twist1 on the migration and invasion of MTA1−/− MEFs as compared with the WT MEFs. Error bars in panels C, H, and J represent the SD. K, proposed working model of the noted MTA1 regulation of STAT3-TWIST1 axis in supporting the invasiveness of breast cancer cells.
MTA1-mediated breast cancer invasiveness (Fig. 7K). We hypothesized that MTA1 recruitment onto the putative Twist1 promoter in STAT3−/− MEFS may involve binding sites for STAT3 homologs such as STAT1, which was also known for its role in cancer invasion and metastasis (30). Hence, we measured the effect of STAT3 or STAT1 in the presence or absence of MTA1 on the Twist1 promoter activity in the MCF-7 cells. We found that MTA1 cooperates with both STAT3 and STAT1 to stimulate Twist1 transcription (Fig. 7C). In addition, we also noticed the recruitment of MTA1/Pol II, MTA1/STAT3, and MTA1/STAT1 complexes onto only one region (−456 to −292) of the Twist1 promoter in the HC11 cells (Fig. 7D), suggesting the involvement of MTA1/Pol II/STAT3 or STAT1 coactivator complexes in the transcriptional regulation of Twist1 chromatin by MTA1. Furthermore, MTA1 failed to get recruited onto the region −456 to −292 of the Twist1 promoter in STAT3−/− MEFS after selective knockdown of STAT1 (Fig. 7E and F), suggesting STAT1 may substitute STAT3 in the noted regulation of Twist1 transcription by MTA1 in STAT3−/− MEFS as illustrated in Fig. 7H. As expected, STAT1 does interact with MTA1 in vivo (Fig. 7G).

To directly show the binding of the endogenous STAT3 or STAT1-containing MTA1 coactivator complexes onto the Twist1 promoter, we next conducted EMSA analyses using the PCR product encompassing the region −456 to −292 of the Twist1 promoter using the nuclear extracts prepared from serum-stimulated MEFS. We found the formation of specific MTA1/STAT/DNA complexes and that such complexes were effectively supershifted by STAT3 or STAT1 or MTA1 antibodies in the WT MEFS (Fig. 7I, lanes 1–6) and STAT1 or MTA1 antibodies in the nuclear extracts prepared from the STAT3−/− MEFS (Fig. 7I, lanes 7–11). Similar to the ChIP studies, depletion of STAT1 in the STAT3−/− MEFS completely abrogated the noted MTA1-containing protein–DNA complexes (Fig. 7I, lanes 12–15). In addition, we found a decreased migration and invasion of MTA1−/− MEFS as compared with the corresponding WT MEFS, whereas MTA1−/− MEFS overexpressing either STAT3 or Twist1 showed increased migration and invasion (Fig. 7J). These results suggest that MTA1 regulates Twist1 transcription via directly interacting with the Twist1 promoter via STAT3 or STAT1. These findings revealed an inherent role of MTA1 in the regulation of Twist1 expression in both STAT3-dependent or -independent manner and, in turn, supporting MTA1-mediated breast cancer invasiveness (Fig. 7K).

Discussion

The morbidity and mortality of patients with breast cancer predominantly results from invasion and metastasis of the primary tumor cells to secondary sites. In this context, MTA1 is one of the most widely upregulated gene products in human cancers, and its overexpression promotes oncogenesis and metastasis (7, 11). However, physiologic significance of the normal level of MTA1 in this process remains unknown. Here, we made an attempt to delineate the physiologic contribution of MTA1 in supporting the formation of spontaneous breast tumors driven by the powerful PyVMT oncogene. Our finding that genetic depletion of MTA1 in the PyVMT-tg/MTA1−/− mice was sufficient to selectively impair breast-to-lung metastasis is important as it reveals an essential modifying role of the physiologic level of MTA1 in metastasis. As the model system used here does contain MTA2, MTA3, and the fact that there was no significant effect of MTA1-depletion on the primary mammary tumors, these studies allowed us to discover a selective modifying action of the normal levels of MTA1 in metastasis for the first time. This is an interesting revelation of the significance of the normal level of MTA1 whereas its overexpression acts as an oncogene.

The results from the mechanistic studies underlying the noticed selective blockage of breast-to-lung metastasis by MTA1-depletion also identified an unusual genomic target of MTA1 in the physiologic setting, the STAT3, which is constitutively upregulated and activated signaling molecule in cancer that plays an important role in human cancer invasion (1). This is exciting, as these findings now place MTA1 as the first upstream coactivator of STAT3 and raise the possibility that commonly observed upregulation of STAT3 in human cancer might be mechanistically tied with the upregulation of MTA1 oncogene as suggested by the meta-analysis of publically available data sets. Because the level of MTA2 and MTA3 remains intact in the PyVMT-tg/MTA1−/− mice, it is possible that the MTA1-STAT3 axis represents a specific pathway with implication in breast-to-lung metastasis. In brief, our findings presented here reveal an important role of the physiologic level of MTA1 in promoting/supporting breast-to-lung metastasis. It will be important to understand whether the noted function of MTA1 may not be limited to breast-to-lung metastasis or MTA1 acts as a modifier of secondary metastasis in general. We hope that the findings and model systems presented here may help in addressing these hypotheses and contribute to the development of antimetastatic strategies to target the component(s) of the MTA1-STAT3 axis.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: R.-A. Wang, M.R. Pillai, R. Kumar
Development of methodology: S.B. Pakala, R.-A. Wang, S.D.N. Reddy
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.B. Pakala, R.-A. Wang, K. Ohshiro, R. Pires, L. Costa
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References


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