Metastasis Tumor Antigen Family Proteins during Breast Cancer Progression and Metastasis in a Reliable Mouse Model for Human Breast Cancer

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Abstract

Purpose: Chromatin remodeling pathways are critical in the regulation of cancer-related genes and are currently being explored as potential targets for therapeutic intervention. The metastasis tumor antigen (MTA) family of proteins, MTA1, MTA2, and MTA3, are components of chromatin remodeling pathways with potential roles in breast cancer. Although all three MTA family proteins have been shown to be associated with metastatic progression of breast cancers, the expression characteristic of MTA1-3 proteins in a multistep breast cancer progression model remains unknown. Structural and functional studies have suggested that they are heterogeneous in the Mi-2/NuRD complex, exhibit tissue-specific patterns of expression, and impart unique properties to estrogen receptor-α (ERα) action. This led us to hypothesize that each member of the MTA family possesses a unique role and interacts with different pathways in the stepwise process of breast cancer development and progression.

Experimental Design: MTA family proteins were examined by immunohistochemistry in breast cancer processes ranging from normal duct, to premalignant lesions, to invasive carcinoma, and to metastasized tumors in PyV-mT transgenic mice, which represents a reliable model for multistage tumorigenesis of human breast cancer. We also determined the association of MTA proteins with the status of cell proliferation, ER, E-cadherin and cytoplasmic β-catenin, and cancer-related coactivators, AIB1 and PELP1.

Results: The expression of all three MTA proteins was altered in primary breast tumors. Each MTA protein had a unique expression pattern during the primary breast tumor progression. Altered expression of MTA1 was observed in both premalignant lesion and malignant carcinoma, but an elevated nuclear expression was observed in ER-negative carcinomas. MTA3 was exclusively expressed in a subset of cells of ER-positive premalignant lesions but not in carcinomas. MTA2 expression seems to be unrelated to ER status. Loss of MTA3 expression and more nuclear localization of MTA1 occurred with loss of E-cadherin and decreased cytoplasmic β-catenin, two molecules essential for epithelial cell adhesion and important tumor cell invasion. At the late stage of tumor formation, MTA1 is usually expressed in the center of tumors. Coincidentally, the distribution of MTA1-positive cells at this stage was complementary to that of AIB1 and PELP1, which were localized to the tumor periphery with relatively active cell proliferation, scattered ER-positive cells and a limited differentiation. In metastasized lung tumors, the expression pattern of MTA-protein expression was distinct from that in primary counterparts.

Conclusions: The findings presented here support the notion that each member of the MTA family might potentially play a stepwise role in a cell type-specific manner during breast cancer progression to metastasis. On the basis of the noted temporal expression patterns of MTA proteins with ER status, cell adhesion—essential regulators (E-cadherin and cytoplasmic β-catenin), and coactivators, we propose that MTA protein—related chromatin remodeling pathways interact with steroid receptors, growth factor receptors, and other transcriptional signaling pathways to orchestrate the governing of events in breast cancer progression and metastasis.

Understanding the molecular processes that control breast cancer provides the foundation for therapeutic intervention. Breast cancer is a complex and multistep disease involving the co-ordinal interaction of multiple genes and the accumulation of multiple molecular and morphologic changes within a cell. One breakthrough in this field was the discovery that chromatin remodeling pathways are critical in the transcriptional regulation of cancer-related target genes (1–4). Histones and chromatin components play key roles in the decision for turning on or turning off genes that govern cell growth, proliferation, and differentiation. Errors in such processes have
severe consequences, such as cell transformation and malignancy. The transcriptional expression of critical genes relies on two opposing enzymes, histone deacetylases and histone acetyltransferases. By dynamic acetylation or deacetylation of histones in the context of ATP-driven chromatin remodelling, the accessibility and transcriptional competence of genes can be determined. The alteration of chromatin structure by histone acetyltransferases and histone deacetylases has been largely connected with carcinogenesis. Intriguingly, the selective inhibition of chromatin remodelling pathways has provided a new therapeutic window in cancer biology (5).

The metastasis tumor antigen (MTA) family of proteins, a group of 70 to 80 kDa polypeptides, have been shown to be functional components of the Mi-2/NuRD complex, a major macromolecular form of histone deacetylases (6, 7), which has been linked to transcriptional repression, cellular proliferation, and cancer (8–20). In vertebrates, the MTA family comprised of three members, MTA1, MTA2, and MTA3. MTA1, the founder of this family, was first identified by differential cDNA library screening of metastatic breast cancer cell lines with increased potential for invasion and metastasis following injection into nude mice (8). Subsequent studies suggested that MTA1 overexpression is also associated with tumor progression and metastasis in multiple cancer types (9–14). The expression level of MTA1s, a natural variant of MTA1, is increased in estrogen receptor (ER)-negative human breast cancers (15). MTA2, encoded by the MTA2 gene and evolved earlier than other MTA members, is highly expressed in cervical cancer tissue and its expression correlates well with rapid cell division (16). It has been proposed that MTA2 interacts with p53 and such interaction plays an important role in tumor progression (17). The expression of MTA3 is closely tied to ER function in human breast cancer and regulates invasive growth of breast cancers (18).

Although three MTA proteins are components of the NuRD and share homologous domains, several lines of evidence suggest that they demarcate functionally specialized forms of the NuRD complex (19–22). Subunit heterogeneity in the Mi-2/NuRD complex might result in functionally specialized activity. Based on the functional data and the fact that these proteins exhibit tissue-specific patterns of expression, it has been proposed that MTA proteins may exhibit specific transcriptional functions in a cell type-specific manner. For example, several recent studies suggest that the MTA family imparts unique properties to ERα action by directly inhibiting ERs (MTA1 and MTA1s) and by indirectly regulating ERα target genes (MTA3; refs. 19, 20). Heregulin signaling pathways result in increased biosynthesis of MTA1 (12) and estrogen signaling is necessary for high levels of MTA3 expression (18). In breast cancer, these two pathways represent critical determinants of cell growth. There is no direct evidence yet to show the function of MTA2 in breast cancer, although data suggests that MTA2 may have the potential to repress, as well as activate, gene transcription (21, 22). These observations suggest that although all three genes are cancer-related, their regulation may depend on functional specificity and interaction with specific pathways.

To provide further information on the functional specificity and coordinating interactions of the MTA family of proteins in breast cancer, we characterized the expression profile of these proteins in different stages of breast cancer using such in situ techniques as immunohistochemistry. To determine the functional roles of the MTA family and their interaction with other factors, we correlated MTA protein expression with the status of cell proliferation, steroid receptor (ER), growth factor receptor (Neu), epithelial cell adhesion regulators (E-cadherin and β-catenin), and other breast cancer–related coregulators (AIB1 and PELP1). To date, no studies have addressed the question of how chromatin remodelling pathways regulate secondary tumor growth after remote metastasis. We have also determined the association of MTA family members and associated biomarkers in lung metastasis. Because this investigation requires distinct stages and detailed information of breast tumor progression, for our study, we took advantage of PyV-mT transgenic mice, a reliable mouse model for human breast cancer. The PyV-mT transgenic mouse model represents distinct pathologic stages resembling human breast cancer and shares many signaling pathways that are important in human breast diseases (23–25). The recently published data firmly establishes the usefulness of the PyV-mT system for the study of the multistep progression of mammary lesions from normal duct through hyperplasia to malignancy and metastasis. In addition, the PyV-mT model, compared with other genetically engineered mice, has the advantage of reduced tumor latency and increased tumor metastasis (23–25). The similarities between PyV-mT premalignant lesions and many types of human atypical hyperplasias emphasize the value of this model system. Previous comprehensive characterization of premalignant PyV-mT mammary lesions, including mammary transplantation experiments and immunohistochemical stains commonly used in both mouse and human histopathology provided a basis for direct comparisons of the PyV-mT model with human breast disease (23–25).

Materials and Methods

Mice and tissue collection. The right fourth inguinal mammary glands were removed from FVB mice, expressing PyV-mT transgene under the control of mouse mammary tumor virus promoter (The Jackson Laboratory, Bar Harbor, ME) and fixed in 4% paraformaldehyde. All procedures involving mice were conducted in accordance with NIH regulations concerning the use and care of experimental animals.

Immunohistochemistry. Paraffin-embedded glands were sectioned to 4 μm. Immunohistochemistry was done with the indirect enzyme-labeling procedure as previously described (26). The primary antibodies used were rabbit polyclonal anti-MTA1 (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit polyclonal anti-MTA2 (eBioscience, San Diego, CA), rabbit polyclonal anti-MTA3 (Bethyl Laboratories, Montgomery, TX), rabbit polyclonal anti-PELP1 (27), rabbit polyclonal anti-AIB1 (28), rabbit polyclonal anti-Neu (Santa Cruz Biotechnology), rabbit polyclonal anti-ERα (Santa Cruz Biotechnology), rabbit polyclonal anti-E-cadherin (Santa Cruz Biotechnology), mouse monoclonal anti-β-catenin (BD Transduction Laboratories, San Jose, CA), rabbit polyclonal anti-caspase-3 (R&D Systems, Minneapolis, MN) and mouse monoclonal anti-CD34 (Neomarkers, Fremont, CA). Horseradish peroxidase–conjugated secondary antibodies were purchased from Amersham Biosciences (Piscataway, NJ). Controls included antigen preabsorption of the primary antibodies or using the same dilution of preimmune serum from same species. All three MTA family protein antibodies were detected by Western blotting and these antibodies indeed selectively recognize a specific type of MTA member (see refs. 29, 30; vendors information).

Bromodeoxyuridine labeling. Mice were given i.p. injection of 50 mg/kg body weight bromodeoxyuridine (BrdUrd; Sigma Chemical Co., St. Louis, MO) 2 hours before the termination of the
MTA family proteins exhibit differential expression patterns in primary tumors. Mammary tumors in PyV-mT transgenic mice consist of pathologically distinct stages, ranging from MIN (intraluminal epithelial proliferation, a term describing mouse mammary premalignant lesions including hyperplasia and ductal carcinoma in situ; refs. 32–34), early stage of tumor, and advanced carcinoma (23–25). To assess the spatial and temporal distribution of MTA family proteins during breast cancer progression, we assayed the expression of MTAs during breast tumor progress in stages including hyperplasia, ductal carcinoma in situ, tumor as well as advanced carcinoma by immunohistochemical assays.

In normal duct, MTA1 was expressed in 10% of the epithelial cells with a weak immunoreactivity in the nuclear compartment (Fig. 1A). When primary lesions progress to the MIN, the expression of MTA1 significantly increases and MTA1 was localized in both nuclear and cytoplasmic compartments (Fig. 1B). A marked increase of nuclear MTA1 signal was observed in late carcinoma stage (Fig. 1C). The increased MTA1 expression was reflected both in the percentage and intensity of positive epithelial cells.

In normal ducts, MTA2 expression in epithelial cells showed a strong nuclear pattern in 50% to 80% of cells, depending on the status of cell proliferation and differentiation (Fig. 1D). In both noninvasion focal lesion and in carcinoma, there was a clear nuclear staining of MTA2 with a mosaic pattern among positive and negative cells (Fig. 1E and F). It seems that MTA2 expression decreases in carcinomas as compared with elevated levels in premalignant lesions (Fig. 1F versus E).

In normal ducts, MTA3 expression was detected in both the nucleus and cytoplasm in 30% of epithelial cells (Fig. 1G). There was a moderate enhancement in nuclear MTA3 expression in the cells at MIN stages (Fig. 1H). A dramatic decrease in the ratio of MTA3-positive cells was observed during the transition from MIN to tumors (Fig. 1H and I). In late stage carcinomas, MTA3-positive cells were barely detected (Fig. 1). The data clearly show that each MTA protein has a unique expression profile in the development and progression of primary breast tumor.

Association of MTA protein expression with the status of ERα and Neu. Clinical ER expression is used to determine the prognosis of human breast cancers (35). Consistent with the established notion that loss of ER in mouse models also correlates with tumor progression to malignancy (34, 36), we found that ER expression dramatically decreases upon progression of hyperplasia to ductal carcinoma in situ and carcinoma (Fig. 2A). Recent data suggests that MTA1 acts as an ER corepressor (12), whereas MTA3 is an ER-regulated gene (18). In the present study, we found that MTA3-positive cells are present during hyperplasia and noninvasive focal lesions that usually contain high ER-expressing cells (Fig. 1H). In contrast, the population of MTA3-negative cells remarkably expanded with the decrease of ER-positive cells (Fig. 1H and I, and Fig. 2A). MTA3-positive cells were barely detectable during progression of the primary tumors to the ER-negative carcinoma stage (Figs. 1I and 2A). MTA1 expression increases in both premalignant and malignant stages of tumors compared with normal ducts (Fig. 1B and C). Intense MTA1 nuclear expression was observed in ER-negative carcinomas (Fig. 1C and F, and Fig. 2A). Coincident with the MTA1 alteration, Neu expression becomes progressively more intense during tumor development.

Fig. 1. Immunohistochemical detection of MTA family proteins in normal duct, MIN, and carcinoma of mammary glands of PyV-mT mice. Compared with weak nuclear expression of MTA1 in normal duct cells (A), MIN cells present enhanced MTA1 immunoreactivity in both nuclear and cytoplasmic compartments (B), whereas carcinoma cells have a remarkable increase of nuclear MTA1 staining (C). Typical nuclear MTA2 staining is in normal duct (D) and lesions (E, F). Normal duct (G) and MIN have MTA3-positive cells (H), whereas invasive carcinoma cells barely show MTA3 expression (I). All micrographs are at the same magnification. Magnification, ×400. Bars, 20 μm.
and peaks in late stage carcinomas (data not shown; ref. 25). MTA2 was expressed in both ER-positive and negative cells (Figs. 1E and F, and Fig. 2A). Together, the above observations show that a coincidental loss of MTA3 and ER was accompanied by increased expression of MTA1 and Neu during transition from ER-positive premalignant lesions to ER-negative carcinoma.

**MTA proteins and differential expression of E-cadherin/cytoplasmic β-catenin in primary tumors.** E-cadherin and its partner, cytoplasmic β-catenin, are essential for maintaining the cell-cell junction and are important for tumor cell invasion and metastasis (18, 19, 37–39). Disruption of the cadherin-catenin complex correlates with tumor dedifferentiation, infiltrative growth, lymph node metastasis, and worse patient prognosis (18, 37–39). Data from in vitro cell culture–based studies suggest that MTA3 up-regulates E-cadherin by inhibiting the expression of Snail, leading to an event associated with changes in epithelial architecture and invasive growth (18). In contrast, ample evidence has implicated overexpression of MTA1 in invasive and metastatic growth (9–12). In this study, we observed that E-cadherin is highly expressed at the cell membrane and in the cytoplasm of MIN (Fig. 2B). Interestingly, a clear transition from high expression to down-regulation of E-cadherin in MIN was observed, which coincides with the progression of hyperplasia to ductal carcinoma in situ (Fig. 2B and C). This decrease becomes more drastic upon the onset of carcinomas (Fig. 2D). Eventually, the membranous E-cadherin was lost with only a faint cytoplasmic signal (Fig. 2D). The expression level of cytoplasmic β-catenin shows a similar tendency to that of E-cadherin during tumor progression (Fig. 2E and F). MTA3 was mainly expressed in the margin area of premalignant lesions consisting of strong E-cadherin-positive cells (Figs. 1H and 2B). Expression of MTA3 begins to decrease towards the center of premalignant lesion and dramatically decreases and is even lost in carcinoma (Fig. 1H). MTA1, on the other hand, is highly expressed in the center of late stage tumor cells with E-cadherin negativity (Figs. 1C and 2D). MTA2 seems to be expressed independently on E-cadherin (Figs. 1E and F, and Fig. 2B, C, and D). Together, these findings suggest that MTA3 has an important role in controlling the cadherin-catenin complex associated with epithelial architecture, whereas MTA1 may be more involved in the regulation of invasive cells.

**Complementary expression pattern of corepressors and coactivators in primary carcinomas.** Because different areas of breast tumors may have different coregulator needs, we next examined the status of MTA1 with the coactivators, PELP1 (27) and AIB1 (28). We consistently noticed that MTA1 expression in carcinoma was restricted to the tumor centers (Fig. 3A and D). Because this area contains ER-negative cells, we also found an intense staining of Neu in the center of the tumor (data not shown; ref. 25). In contrast to the MTA1-positive cell population, tumor cells expressing coactivators, AIB1 and PELP1, were limited to the tumor margins (Fig. 3B, C, and E) and thus, were complementary to the MTA1-positive cell population. Proliferating BrdUrd-positive cells were predominantly found in the peripheral area containing AIB1- and PELP1-positive cells (Fig. 3F). Given the evidence that tumor centers are characterized by the presence of ER-negative cells with high levels of Neu (data not shown; ref. 25) and apoptosis (Fig. 3G), the finding of the overexpression of MTA1 in the tumor center suggests that this protein may be involved in the regulation of cell survival. The noted localization of AIB1 and PELP1 in the periphery (also populated by ER-positive cells), strengthens the ER coactivator functions of AIB1 and PELP1. In addition, it is also possible that the corepressor MTA1 acts in concert with coactivators for regulating cell survival and growth via different pathways.

**Differential expression of MTA proteins in lung metastasis.** There are few studies that specifically investigate the transcriptional regulation in primary breast tumors in relationship to their distant metastasis. In this context, the expression of chromatin remodeling pathways in distant metastasis is unknown. Therefore, we next examined the status of MTA family members in distant lung metastasis sites. We found that MTA1 was expressed in most of the tumor cells, primarily in the nuclei (Fig. 4A). Almost 80% of the cells in distant metastases were MTA2-positive (Fig. 4B). In contrast to the primary tumor, there were high levels of MTA3 expression in distant tumor cells with an intense staining towards the periphery as compared with the expression in the tumor centers (Fig. 4C). As expected, there was no expression of ERs in the lung tumors (data not shown). As shown in Fig. 4, we noticed clear evidence of secondary tumors in the lung accompanied by an increased cell proliferation as evident by the presence of BrdUrd-labeled cells in the margin area (Fig. 4D) and apoptosis as judged by caspase-3 immunostaining (Fig. 4E). Interestingly, we also found evidence of significant E-cadherin expression in these...
tumors from the periphery to the center (Fig. 4F). A closer examination revealed two layers of endothelium surrounding some lung lesions (Fig. 4A, inset) indicating the presence of endothelial-covered tumor emboli, raising the possibility that such metastases might have arisen as “invasion-independent intravascular” metastases (40, 41). In addition, we detected an endothelial marker, CD34, in some secondary nodules in lungs (Fig. 4G) and we could not detect CD34-positive endothelial cells covering the metastatic lesion (Fig. 4G). Therefore, this result also suggests the presence of endothelial-covered and non–endothelial-covered metastatic lesions, and further opens a situation of coexistence of invasion-independent (40, 41) and invasion-dependent mechanisms (37–39).

Discussion

The growing body of evidence in favor of the widespread, deregulated expression of MTA family members strengthened the notion of their potential role in human breast tumors (13, 19, 20). The differential structural and functional nature of the MTA family members raises the possibility that MTA proteins might regulate gene expression in a spatiotemporal manner (21, 22). Therefore, we set out to examine the functional specificity and cell stage–specific expression of MTA proteins in breast cancer. Here, we document a comprehensive expression analysis of MTA proteins and their relationship to known cancer-associated pathways during multi-stage breast cancer progression.

In general, human breast cancer represents a heterogeneous disease involving the accumulation of multiple molecular and morphologic changes within a cell. Human investigations remain limited to retrospective studies that provide relative risk statistics. In addition, other limitations of human studies include the diversity of human genetic background and the quality of tumor and tissue procurements. Most importantly, many studies using human breast cancer tissues are often confounded by the known or unknown contributions of therapy, especially adjuvant endocrine therapy. As an alternative, in recent years, genetically engineered mice have emerged as powerful tools for understanding the principles of tumorigenesis and evaluating response to therapy (42). Among various available mouse models, the PyV-mT transgenic mouse model has proven to be a valuable model as it reflects the complexity of human breast cancer, especially in terms of pure genetic background and multistep progression from normal through hyperplasia to malignancy and metastasis (23–25, 34). Thus, this model could be followed to study the development and progression of breast cancer, which is difficult to achieve in human samples. In addition to the reproducing nature of the morphologic characteristics of human counterparts, PyV-mT mouse model also exhibits several molecular pathways that are common in human breast cancer (23–25, 34, 42). We therefore took advantage of the PyV-mT transgenic model to perform a comprehensive expression-based analysis of MTA proteins during the multistage process of breast cancer progression.

In this study, we showed that PyV-mT breast tumors have distinct characteristics of multistage tumorigenesis similar to their human counterparts, including the transition to dedifferentiation by the loss of ER, a clear malignant switch characterized by increased cell proliferation, loss of E-cadherin, and decreased cytoplasmic β-catenin. Our approach included the use of in situ localization, rather than global gene analysis,
of the molecules of interests. Because the expression of transcriptional regulators is heterogeneous in the mammary glands, global analyses such as RT-PCR and Western blot may not provide the desired analysis due to the dilution of positive cells by negative cells in addition to the lack of spatiotemporal considerations.

Although previous reports have shown that altered individual expression of MTA1 and MTA3 is associated with certain steps in breast cancer progression (10, 13, 18), our present comprehensive investigation represents, for the first time, that each MTA protein has a unique expression pattern in a step and cell type–specific manner. Previous studies in human breast cancer tissue have found increased MTA1 expression in more advanced and metastatic carcinomas, while MTA3 expression is intimately linked with ER-positive carcinoma. This may be due to the fact that most of the coregulators have been shown to possess functions that are not necessarily linked with the ER pathway. Tumor cells exhibited a similar pattern of AIB1 and PELP1 expression. We also found that MTA1-positive cells were primarily localized in the center of the carcinoma, whereas AIB1- and PELP1-expressing cells were limited to the periphery of tumor, an area with significant proliferative activity.

Another notable finding of this study is the relationship of the MTA proteins with the expression of E-cadherin and cytoplasmic β-catenin. Cadherin-catenin complex is required for the production of adhesion junctures and for the regulation of epithelial architecture (18). MTA3-E-cadherin connection has been linked to epithelial mesenchymal transition (EMT), an event associated with tumor cell invasion and metastasis (18). Previous studies have implicated the essential role of EMT in the progression of noninvasive tumor cells into malignant, metastatic carcinomas, during which epithelial cells lose their cell-cell junctions and acquire mesenchymal characteristics, leading to migratory abilities (37, 38). Recently, a p53-associated, calcium-binding protein known as fibroblast-specific protein-1 has been shown to regulate EMT, and subsequently, control long distant metastasis in the PyV-mT mouse model (39). Here, we show that loss of MTA3 expression was observed along with the loss of E-cadherin and decreased cytoplasmic β-catenin expression. This finding is in accordance with the notion that MTA3 plays an important role in maintaining epithelial adhesion junctures and implicates MTA3 as a regulator of the onset of EMT (18). On the other hand, the increased nuclear expression of MTA1 in E-cadherin-negative

### Fig. 4
Immunolocalization of MTA family proteins in lung metastasis of PyV-mT breast cancer. Secondary tumors in the lung show high levels of immunoreactivity to MTA1 (A), MTA2 (B) and MTA3 (C). Distribution of proliferating cells labeled by BrdUrd (D) and apoptotic cells stained for caspase-3 (E) and E-cadherin-positive cells (F) in lung lesions. G, CD34 immunostaining in a secondary lung lesion. Inset in (A), a high magnification of two layers of endothelium surrounding a lung lesion. Photomicrographs are at same magnification. Magnification, ×400. Bars, 20 μm.
cells suggests that MTA1 may be involved in the regulation of invasive tumor cells. Further documentation of in vivo evidence regarding the MTA-cadherin connection contributing to the regulation of EMT is ongoing.

Most breast cancer-related mortalities are caused not by the primary tumor itself but by subsequent metastases. However, studies involving the status of MTA family members in secondary tumors after distant metastasis remained undocumented. We found that metastatic lung tumors reestablish their growth through cell proliferation in the peripheral tumor area in conjunction with the restoration of E-cadherin expression. Interestingly, we also discovered that MTA proteins were expressed differently in lung metastases as compared with their expression in the primary breast tumors. Thus, we envisioned that the establishment of secondary distant metastasis is regulated differently from primary tumors. Likewise, MTA4 was highly expressed in metastasized tumors in the absence of ER expression, raising the possibility that in addition to ER (18), MTA4 might regulate alternative cellular pathways in metastasized tumors. Because tumor metastasis is a complicated biological process, uncertainties remain regarding the relative contribution of tumor cell invasion versus angiogenic activity. A generally described paradigm of invasion–essential metastasis process involves EMT (which changes their epithelial characteristics into mesenchymal type cells) of tumor cell to invade into surrounding stroma, invasation into the circulation, extravasation, and secondary growth in distal organs (37, 38). However, the changing view suggests an alternative metastatic pathway that is dependent not on invasiveness, but on tumor angiogenesis in spontaneous and transgenic mouse mammary tumors (40, 41). In the PyV-mT model, we observed two categories of metastatic lung lesions: endothelial-covered emboli (Fig. 4A, inset), probably representing the invasion-independent process (40, 41), and non-endothelial-covered emboli (Fig. 4C), indicating the invasion-dependent process (37, 38). Therefore, it implicates the coexistence of both invasion-dependent (37, 38) and invasion-independent (40, 41) metastatic processes in the PyV-mT model, and MTA proteins may involve both pathways. Indeed, further elucidation of the contribution by MTA proteins to these two metastatic processes will lead to new knowledge of molecular mechanisms of metastasis.

In this report, we have described the altered expression of MTA family proteins during breast tumorigenesis and metastasis in the PyV-mT model. The regulation of chromatin remodeling signaling proteins, such as MTA family members, plays a crucial role in tumor progression/metastasis. A hierarchy of different mechanisms at multiple levels, including genetic, epigenetic, and transcriptional regulations, may finally define the MTA proteins’ activity in a dynamic, as well as a cell-specific and tissue-specific manner. The regulation of MTA protein expression might be more complicated than one expects. We cannot exclude the possibility that the different expressions of MTA family proteins might be the result of tumorigenesis. Because most of our current understanding of MTA functions in cancer cells is derived from tissue culture model systems, it will be important to combine these approaches with whole animal models (such as genetically engineered mouse models) as well as with human tumor specimens, to gain a comprehensive view of the regulation and functions of MTA protein expression that may be important in tumor progression and metastasis.

In summary, our present study supports the idea that each MTA protein may play a unique stepwise role in a cell type–specific manner during the progression of breast cancer to more invasive phenotypes. On the basis of the relationship of MTA proteins with the status of ER, Neu, E-cadherin/cytoplasmic β-catenin, and cancer-related coactivators, we propose that the MTA family of chromatin remodeling components interact with steroid receptors and growth factor receptors; to orchestrate the governing of the events occurring in breast cancer progression.

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References


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