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REVIEW

The role of the *MTA* family and their encoded proteins in human cancers: molecular functions and clinical implications

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Abstract *MTA* (metastasis-associated gene) is a newly discovered family of cancer progression-related genes and their encoded products. *MTA1*, the first gene found in this family, has been repeatedly reported to be overexpressed along with its protein product MTA1 in a wide range of human cancers. In addition, the expression of *MTA1*/MTA1 correlates with the clinicopathological properties (malignant properties) of human cancers. MTA proteins are transcriptional co-repressors that function in histone deacetylation and are involved in the NuRD complex, which contains nucleosome remodeling and histone deacetylating molecules. MTA1 expression correlates with tumor formation in the mammary gland. In addition, MTA1 converts breast cancer cells to a more aggressive phenotype by repression of the estrogen receptor (ER) α trans-activation function through deacetylation of the chromatin in the ER-responsive element of ER-responsive genes. Furthermore, MTA1 plays an essential role in c-MYC-mediated cell transformation. Another member of this family, MTA3, is induced by estrogen and represses the expression of the transcriptional repressor Snail, a master regulator of "epithelial to mesenchymal transitions", resulting in the expression of the cell adhesion molecule E-cadherin and maintenance of a differentiated, normal epithelial phenotype in breast cells. In addition, tumor suppressor p53 protein is deacetylated and inactivated by both MTA1 and MTA2, leading to inhibition of growth arrest and apoptosis. Moreover, a hypoxia-inducible factor-1α (HIF-1α) is also deacetylated and stabilized by MTA1, resulting in angiogenesis. Thus, MTA proteins, especially MTA1, represent a possible set of master co-regulatory molecules involved in the carcinogenesis and progression of various malignant tumors. MTA proteins are proposed to be important new tools for clinical application in cancer diagnosis and treatment.

Keywords Metastasis-associated gene 1(MTA1) · Chromatin remodeling · Histone deacetylation · Gene expression · Protein modification · Cancer progression · Metastasis

Abbreviations

MTA	Metastasis-associated gene/protein
HDAC	Histone deacetylase
NuRD	Nucleosome remodeling and histone deacetylation
ER	Estrogen receptor
HIF	Hypoxia-inducible factor

Introduction

Recent advances in molecular biology have resulted in the discovery of a wide variety of new molecules involved in carcinogenesis and cancer progression. Although additional molecules related to cancer will be identified in the future, the existing and new molecules must fulfill two major requirements in order to be clinically useful as molecular targets for the diagnosis and treatment of human cancers. The first is that abnormalities in expression or structure of molecules of interest and their clinical relevance must be definitely demonstrated in human cancers by

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independent studies. The second is the underlying molecular mechanisms necessary for the molecules to exert their functions in carcinogenesis or cancer progression must be determined.

Among a number of cancer-related genes and molecules that have been discovered in the last few years, we identified a candidate metastasis-associated gene by use of a differential cDNA screening method. Thus, we identified a gene that was abundantly overexpressed in highly metastatic rat mammary adenocarcinoma cell lines compared to poorly metastatic cell lines [1, 2]. When this gene was sequenced, it was revealed as a completely novel gene without any homologous or related genes in the database. The rat gene was named *mta1* (metastasis-associated gene 1). A homologous gene was also expressed in human cancer cell lines [1], and its human cDNA counterpart, *MTA1*, was cloned by our group in 2000 [3]. Using surgically resected human tissues, we showed that high levels of *MTA1* mRNA expression were clinicopathologically correlated to the invasive and growth properties of gastrointestinal cancers, including esophageal, gastric and colorectal cancers [4, 5]. Subsequently, several reports from independent research groups followed our observations and showed similar correlations between *MTA1* expression and the malignant potentials of human cancers.

Several genes related to *MTA1* have now been identified, indicating *MTA1* consists of a gene family, which we now call the "*MTA* family". Further studies on molecular biological and biochemical properties of the MTA family have shown that the gene products of the main members of the family (MTA1, MTA2, and MTA3) are tightly associated in a protein complex called NuRD (nucleosome remodeling and histone deacetylation), which has transcriptional regulatory function via histone deacetylation and chromatin remodeling. At the moment, the MTA family has attracted widespread attention as one of the key molecules that plays an indispensable role in the genesis and progression of a wide variety of cancers [6–8]. In this brief review, we will examine the significance of the expression of MTA family members in human cancers and the important molecular mechanisms that are currently known by which MTA proteins exert their functions. Finally, future directions for clinical applications of this protein family for the diagnosis and treatment of human cancers will be discussed.

Members of the MTA family and their protein structures

At present, the MTA proteins represent a family of gene products encoded by three distinct genes (MTA1, MTA2, and MTA3) and six reported isoforms (MTA1, MTA1s, MTA1-ZG29p, MTA2, MTA3, and MTA3L). The molecular masses of the gene products of MTA1, MTA2, and MTA3 are approximately 80, 70, and 65 kDa, respectively. The nucleotide and protein alignment homologies and the phylogenetic comparative analyses are discussed elsewhere [8, 9].

Except for ZG-29p, the MTA family sequences contain several common domain structures [10]. One of these, the BAH (bromo-adjacent homology) domain is involved in protein–protein interactions. Another, the SANT (SWI, ADA2, N-CoR, TFIIIB-B) domain shares a high degree of homology with the DNA-binding domain of the Myb-related proteins, suggesting that this domain may be involved in DNA-binding. The ELM (egl-27 and MTA1 homology) domain has an unknown function [11]. MTA family members also contain a highly conserved GATA-type zinc finger motif, which indicates a direct interaction with DNA [3]. MTA1 has two src-homology (SH)-binding motifs at its C-terminal region, which are known to be important in signal transduction involving many kinase, adaptor and scaffolding proteins [1, 10]. Similar SH2- and SH3-binding domains are also found in MTA2 and MTA3. These common domain structures clearly show that the MTA family is involved in protein–protein and DNA-binding interactions, indicating possible functions in signal transduction and transcriptional regulation.

MTA proteins contain basic nuclear localization signals [1, 10]. They also localize in the nucleus in many cancer cells [4, 8]. However, MTA1 localizes to both the cytoplasm and nucleus in some tumors [12–14]. MTA3 also localizes to the nucleus, but it has no apparent nuclear localization signal [15]. MTA1s, a short splice-variant of MTA1, is predominantly localized in the cytoplasm [16].

The expression of MTA proteins in various cancers and its clinicopathological and biological relevance

Clinicopathological relevance of the increased MTA1 expression in human cancer tissues

Since the first report by us showing that the up-regulation of *MTA1* expression was significantly correlated to the malignant properties of human gastric and colorectal cancers [4], many researchers have been investigating the expression levels of MTA family members, especially MTA1, in various human cancers. This has revealed that the expression levels of MTA family members have clinicopathological significance (The data are summarized in Table 1).

Breast cancer

MTA1 was identified as a candidate progression molecule that was associated with breast cancer metastasis [1, 2] and

growth (the antisense RNA of MTA1 inhibited the growth of highly metastatic breast cancer cell lines [3]). The involvement of MTA1 in the carcinogenesis or progression of human breast cancer was also shown by other data using clinical samples. For example, Martin et al. [17] mapped the chromosomal locus 14q that might be responsible for axillary lymph node metastasis in human breast cancers by comparing the rate of loss of heterozygosity between node-positive and -negative breast cancers. They found that the *MTA1* gene was contained in that gene locus, suggesting that

Table 1 Clinicopathological implications of the increased MTA1 expression in various human cancer tissues

Type of cancer	Method	Clinicopathological implications	Reference
Breast cancer	LOH	Higher LN meta.	[17]
	IHC	Earlier recurrence	[18]
	IHC	Higher tumor grade	[19]
		Higher MVD (angiogenesis)	
Gastrointestinal cancer			
Esophageal	RT-PCR	Deeper adventitial invasion	[5]
		Higher LN meta.	
	IHC	Deeper adventitial invasion	[21]
		Higher LN meta.	
		More advanced stage	
		Poorer prognosis	
Gastric	RT-PCR	Deeper serosal invasion	[4]
		Higher LN meta.	
Colorectal	RT-PCR	Deeper wall invasion	[4]
		Higher LN meta.	
	RT-PCR	Higher expression in cancer tissue	[20]
Carcinoid			
Gastric	RT-PCR	Deeper tumor invasion	[25]
Small intestine	RT-PCR	Malignant carcinoid	[22]
		More liver and LN meta.	
Appendiceal	RT-PCR	Significant increase in malignant tumors	[26]
Pancreatic	IHC	Poorer prognosis (in combination with HDAC1)	[28]
Hepatocellular	RT-PCR	Shorter disease-free survival	[29]
	IHC	Larger tumor size	[12]
		More vascular invasion	
	IHC	More microvascular invasion	[30]
		Higher recurrence rate	
		Poorer survival	
Other cancers			
NSCLC	RT-PCR	Larger tumor size	[31]
		Higher LN meta.	
Thymoma	RT-PCR	More advanced stage	[33]
Ovarian	RT-PCR	Higher LN meta.	[32]
	IHC	More advanced stage	[34]
		Higher FIGO staging	
Prostate	IHC	Metastatic prostate ca.	[35]
Lymphoma	Microarray	Highest expression in diffuse B-cell lymphoma	[36]
HNSCC	Microarray	Higher LN meta.	[37]
	IHC	Higher LN meta.	[38]
		More advanced stage	
		Deeper wall invasion	

NSCLC non-small cell lung cancer, *HNSCC* head and neck squamous cell carcinoma, *IHC* immunohistochemistry, *LOH* loss of heterozygosity, *RT-PCR* reverse transcription-polymerase chain reaction, *MVD* microvessel density, *LN meta.* lymph node metastasis

MTA1 is a strong candidate for a breast cancer metastasis-promoting gene. Furthermore, using immunohistochemistry they examined the MTA1 protein expression in primary human breast cancer samples and demonstrated that node-negative breast cancers with overexpression of MTA1 protein had a higher risk of disease relapse similar to node-positive tumors. Thus, the overexpression of MTA1 may be a useful predictor of early disease relapse [18].

Jang et al. [19] also showed that MTA1 overexpression was closely associated with higher tumor grade and high intratumoral microvessel density in surgically resected human breast cancers, suggesting that MTA1 may be a useful predictor of an aggressive phenotype and a possible angiogenesis-promoting molecule in breast cancer.

Gastrointestinal cancer

By using a reverse-transcription polymerase chain reaction (RT-PCR) method, we demonstrated that the higher expression of *MTA1* mRNA in surgically resected human gastric and colorectal cancer specimens compared to the paired normal counterpart tissues was significantly correlated to the depth of cancer invasion and lymph node metastasis [4]. This study was the first to demonstrate the clinical relevance of MTA1 expression to the malignant potentials of human cancers. Higher expression of *MTA1* mRNA was also shown in colorectal cancers compared to the normal counterpart tissue by another group [20].

Using a RT-PCR method, we found that human esophageal squamous cell cancers overexpressed *MTA1* mRNA. The overexpressing cancer cells showed significantly higher frequencies of adventitial invasion and lymph node metastasis and tended to have a higher rate of lymphatic involvement [5]. Using immunohistochemistry, we further examined the protein expression level of MTA1 in human esophageal squamous cell cancers and reconfirmed the results obtained by RT-PCR [21]. In this study, we also demonstrated that MTA1 was a predictor of poor prognosis after surgery [21].

In another observation, Kidd et al. [22] showed that it was useful to examine the expression of *MTA1* mRNA and MTA1 protein in order to determine the malignant potential and the propensity to metastasize of small intestinal carcinoid (enterochromaffin cell) tumors. When compared to nonmetastatic primary tumors, the expression of MTA1 was increased in malignant small intestinal carcinoids and in metastases to liver and lymph nodes [22–24]. This same group further reported that MTA1 was a good candidate genetic molecular marker to discriminate gastric carcinoids from other gastric neoplasms [25] as well as malignant appendiceal carcinoids from benign tissue [26]. In these studies, MTA1 was thought to be a good marker to define the malignancy of carcinoid tumors.

In addition to cancers of the gastrointestinal tract, the involvement of MTA1 overexpression in carcinogenesis and cancer progression was shown in other gastrointestinal tumors, such as pancreatic cancers and hepatocellular carcinomas. Iguchi et al. [27] examined *MTA1* mRNA expression in pancreatic cancer cell lines and resected pancreatic cancer tissues and found that increased levels of *MTA1* mRNA expression might be involved in the progression of pancreatic cancer. Recently, Miyake et al. [28] showed the expression level of MTA1 protein correlated with poorer prognosis of pancreatic cancer patients.

The possible association of *MTA1* expression with the malignant properties of hepatocellular carcinomas (HCC) was first reported by Hamatsu et al. [29]. In this study, *MTA1* mRNA level was assessed by RT-PCR in resected human HCC tissues, and its high expression predicted a lower disease-free survival rate after curative hepatectomy for HCC. Using immunohistochemistry, Moon et al. [12] examined MTA1 protein expression in resected human HCC specimens. They showed that overexpression of MTA1 was associated with HCC growth and vascular invasion and that nuclear localization of estrogen receptor (ER) α inversely correlated with MTA1 expression, suggesting that MTA1 was involved in negative regulatory mechanisms. Ryu et al. [30] reported that MTA1 was closely associated with microvascular invasion, frequent postoperative recurrence, and poor prognosis in patients with HCC, especially in those with hepatitis B virus (HBV)-associated HCC.

Other cancers

The relationship between MTA1 expression and malignant properties, such as invasion and metastasis, has been investigated in many other carcinomas and sarcomas. High expression of *MTA1* mRNA was correlated clinicopathologically with lymph node metastasis of human non-small cell lung cancers [31] and ovarian cancers [32], and to the advanced stage and invasiveness of thymomas [33]. Dannennmann et al. [34] reported that overexpression of MTA1 protein in ovarian cancer was significantly correlated to more advanced stage and higher FIGO staging. The potential role of MTA1 protein expression has also been suggested in the progression of human endometrial carcinomas [14]. In prostate cancers, Hofer et al. [35] showed that metastatic prostate tumors demonstrated significantly higher intensities of MTA1 protein expression and higher percentages of tissue cores staining positive for MTA1 than in clinically localized prostate cancers or benign prostate tissues. The high expression of MTA1 in diffuse B-cell lymphomas was also reported in human cases [36].

Using DNA microarray analysis, Roepman et al. [37] investigated gene expression patterns in lymph node

metastases of head and neck squamous cell carcinomas. They showed that the *MTA1* gene was the only single gene that showed consistently changed expression between numbers of matched pairs of primary tumor and lymph node metastases. Recently, further evidence was reported showing that overexpression of MTA1 protein in oral squamous cell carcinoma correlated to higher lymph node metastasis, deeper wall invasion and more advanced stage [38].

Biological relevance of MTA proteins to carcinogenesis and cancer progression

In addition to the clinicopathological evidences mentioned above, the biological relevance of MTA proteins to carcinogenesis and cancer progression has been made much clearer by the following important experiments.

The direct evidence to show the association of MTA1 expression with breast cancer malignant properties was first obtained by Mazumdar et al. in 2001 [39]. They demonstrated that forced expression of the MTA1 protein in breast cancer cell line MCF-7 was accompanied by enhancement of the ability of cells to invade an artificial matrix and to grow in an anchorage-independent manner. They also showed that the enhancement was associated with the interaction between MTA1 protein and histone deacetylase, resulting in a repression of ERα-mediated transcription (This will be discussed in more detail later).

The above study was extended by further experiments by the same group where they showed direct in vivo evidence of the involvement of MTA1 in the carcinogenesis of breast cancer in an animal model [10, 40]. This group established transgenic mice that overexpressed MTA1 protein. The MTA1-transgenic mice showed an inappropriate development of mammary glands, and the mice eventually developed hyperplastic nodules and mammary tumors, including adenocarcinomas. Most interestingly, MTA1-transgenic mice were accompanied by high incidence of spontaneous B cell lymphomas, including diffuse large B cell lymphomas [13, 41].

The clinicopathological correlation of MTA1 overexpression with squamous cell carcinomas was reinforced by the experimental results of Mahoney et al. [42]. They transfected *MTA1* cDNA into immortalized human keratinocytes and clearly showed that forced expression of *MTA1* contributed to several aspects of enhanced metastatic behavior, including increased migration, invasion and survival in the anchorage independent state of the immortalized keratinocytes. Furthermore, Qian et al. [43] inhibited *MTA1* expression by RNA interference (RNAi) in a human esophageal squamous cell carcinoma cell line and showed the significant inhibition of in vitro invasion and migration properties of the cancer cells.

Direct evidence showing the role of MTA1 in the progression of pancreatic cancer was provided by Hofer et al. [44]. They transfected *MTA1* cDNA into the pancreatic cell line PANC-1 and demonstrated that enhanced expression of MTA1 promoted the acquisition of an invasive and metastatic phenotype and that it enhanced the malignant potentials of pancreatic adenocarcinomas by modulation of the cytoskeleton via IQGAP1.

Molecular mechanisms of the MTA family, especially MTA1, in carcinogenesis and cancer progression

As mentioned above, it was demonstrated by different approaches and by different laboratories that MTA1 overexpression was closely correlated with carcinogenesis and cancer progression of a wide range of cancers originating in disparate organs and tissues. This strongly indicates that MTA1 may be one of the important key molecules in the cancer progression field. Thus, it will be absolutely necessary to clarify the molecular mechanisms in which MTA family members exert their functions for the clinical utilization of MTA proteins for diagnosis or treatment of human cancers. Here, we introduce the several important functions of MTA proteins that have been clarified, especially those that are concerned with carcinogenesis and cancer progression.

Nucleosome remodeling and histone deacetylation complex and transcriptional regulation

The first notion about the molecular and biochemical functions of MTA1 was accidentally obtained by four independent groups in 1998–1999 [9, 45–48]. In these studies, two disparate chromatin modifying activities, ATP-dependent nucleosome remodeling activity and histone deacetylation, were functionally and physically linked in the same protein complex. This complex has been named the NuRD (Nucleosome remodeling and histone deacetylation), and it contains histone deacetylase (HDAC) 1, HDAC2, the histone binding proteins RbAp46/48 and the dermatomyositis-specific autoantigen Mi-2, which has been shown to have transcription repressing activity. Xue et al. [46] reported that MTA1 protein was found in the NuRD complex, and it had strong transcription repressing activity. Subsequently, Zhang et al. [47] reported that a protein similar to MTA1 (named MTA2) was also a component of the NuRD complex and that MTA2 is highly expressed in rapidly dividing cells. Later, MTA3 was identified as an estrogen-inducible gene product that forms a distinct NuRD complex [15]. We also reported the physical interaction between MTA1 and HDAC1 [49] (Fig. 1).

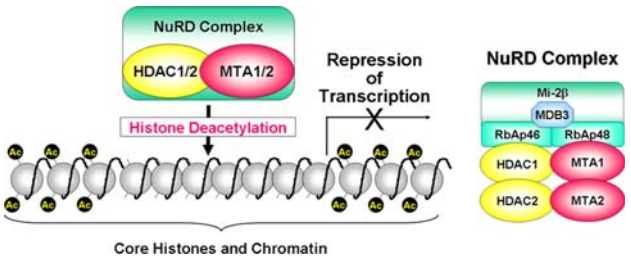

Fig. 1 The fundamental function of MTA proteins. The fundamental function of MTA proteins is chromatin remodeling and histone deacetylation, resulting in repression of transcription. MTA proteins are included in the protein complex named NuRD, which also contains histone deacetylases (HDAC1 and 2), major DNA binding protein 3 (MDB3), histone binding proteins RbAp46/48 and the dermatomyositis-specific autoantigen Mi-2, and has strong transcription repressing activities

Thus, the fundamental functions of the MTA family members appear to be exerted through a NuRD complex that has chromatin remodeling and histone deacetylating properties (There is also deacetylating property of non-histone proteins in the NuRD complex). In addition, the MTA-NuRD complex shows transcriptional repression activities [6–8, 10, 50]. Although all MTA family proteins are found in NuRD complexes, these proteins form distinct NuRD complexes that are thought to target different sets of promoters [9].

Repression of the transactivating function of ERα by MTA proteins

Although the involvement of MTA proteins in NuRD complexes suggested that such complexes might function in chromatin remodeling and histone deacetylation, a direct target of MTA proteins was first identified by Mazumdar et al. in 2000 [39]. MTA1 was identified as a molecule induced by a growth factor, heregulin-beta1 (HRG), which is a natural ligand of the human epidermal growth factor receptors HER3 and HER4 that can also transactivate HER2 (c-erbB-2) in human breast cancer cell lines. They showed that MTA1 directly interacted with the ligand-binding domain of ERα and that HRG stimulated the association of MTA1 and HDAC2 on the chromatin of an ER-responsive element (ERE) in the promoters of the estrogen responsive genes, such as pS2 and c-myc. This explains the phenomenon that activation of HRG/HER2 pathway in ER-positive breast cancers results in the suppression of ERα functions, resulting in more invasive and aggressive phenotypes observed in ER-negative breast cancers [51]. The repressive function of MTA1 on ERα is mediated through histone deacetylation by HDAC1 and HDAC2, suggesting that MTA1 has a potent corepressor function on the transactivation function of ERα through histone deacetylation (Fig. 2a). MTA2 has also been shown to physically interact with ERα and to repress its transactivating function. Furthermore, overexpression of MTA2 rendered cells unresponsive to estrogen and suppressed estrogen-induced colony formation in breast cancer cells [52] (Fig. 2a, b).

Recently, Khaleque et al. [53] showed that MTA1 binds to a heat shock factor 1 (HSF1), the transcriptional activator of the heat shock genes, in vitro and in human breast carcinoma samples. They demonstrated that HSF1-MTA1 complex formation was strongly induced by HRG and that the complex was incorporated into the NuRD complex and participated in repression of estrogen-dependent transcription in breast cancer cell treated with HRG.

Following the report by Mazumdar et al. [39], the same research group reported that several molecules, such as ménages a trios 1 (MAT1), MTA1-interacting coactivator (MICoA) and nuclear receptor interacting factor 3 (NRIF3), all interact with MTA1 and repress the transactivation function of ERα [8]. These three MTA1-binding proteins themselves have coactivator properties upon ERα transactivation. Talukder et al. [54] identified MAT1, an assembly and targeting ring finger factor for cyclin-dependent kinase-activating kinase (CAK), as a MTA1-binding protein. The interactions between CAK and MTA1 apparently regulate the transactivation activity of ERα in a CAK-dependent manner in breast cancer cells. In contrast, MICoA-mediated ERα transactivation functions are opposed by MTA1 through the recruitment of HDACs [55]. Furthermore, the interactions between MTA1 and NRIF3, an estrogen-inducible gene, may be important in modulating the sensitivity of breast cancer cells to estrogen [56]. Singh et al. [57] identified another MTA1-binding partner, Lim-only protein 4 (LMO4). LMO4 was found to be a component of the MTA1 corepressor complex, and its overexpression repressed ERα transactivation functions in a HDAC-dependent manner, proposed to result in the acquisition of the ERα-negative phenotype and increased aggressiveness in breast cancer cells.

A short form of MTA1 protein was subsequently identified and named MTA1s (Fig. 2a) [16]. MTA1s is a splice-variant of MTA1 and contains an ER-binding motif (nuclear binding motif) without any nuclear localization signals at the C-terminus. This protein localizes in the cytoplasm where it sequesters ERα, resulting in the prevention of ligand-induced nuclear translocation of ERα and of stimulation of the malignant phenotype of breast cancer cells. This suggests that the regulation of the cellular localization of ERα by MTA1s may represent a mechanism for redirecting nuclear receptor signaling by nuclear exclusion. MTA1s has also been shown to associate with casein kinase I-gamma2, which is an estrogen-responsive kinase [58].

MTA3 is the latest addition to the MTA family. It was identified as an estrogen-dependent component of the

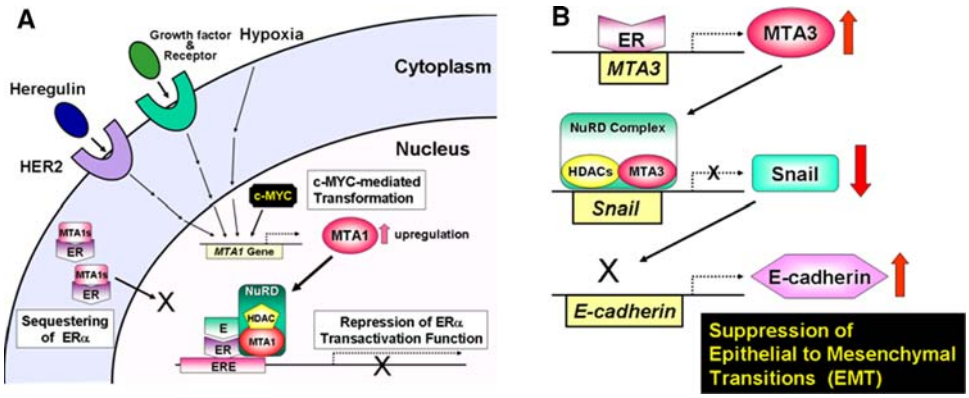

Fig. 2 Roles of MTA family in the carcinogenesis and cancer progression. Schematic presentation of the main functions of MTA family proteins. **a** MTA1 protein is included in NuRD complex that represses the transactivation function of estrogen receptor (*ER*) α, rendering breast cancer cells more phenotypically aggressive. MTA1 protein in NuRD complexes is one of the first downstream targets of c-MYC function, and it is essential for the transformation potential of c-MYC. MTA1s is a splice-variant of MTA1 that localizes in the cytoplasm where it sequesters *ER*α, resulting in the prevention of the ligand-induced nuclear translocation of ERα and stimulation of the development of the malignant phenotype of breast cancer cells. **b** MTA3 protein induced by estrogen represses the expression of the transcriptional repressor Snail, a master regulator of "epithelial to mesenchymal transitions", resulting in the expression of the cell adhesion molecule E-cadherin and maintenance of a differentiated, normal epithelial status in breast cells

Mi-2/NuRD transcriptional corepressor in breast epithelial cells [15]. The absence of MTA3 as well as the absence of ER results in an aberrantly increased expression of the transcriptional repressor Snail, a master regulator of epithelial-to-mesenchymal transitions (EMT). This increased expression of Snail results in reduction of the cell adhesion molecule E-cadherin expression and subsequently changes in epithelial architecture and invasive growth (Fig. 2b). MTA3 is a transcriptional target of ERα, and in the presence of estrogen, ERα directly binds to the MTA3 promoter at the SP1 site in close proximity of the ERE half-site, resulting in stimulation of MTA3 transcription [59, 60]. Thus, MTA3 functions to maintain a differentiated, normal epithelial status in breast cells, which is in stark contrast to MTA1 or MTA1s. Any potential up-regulation of MTA1 may repress MTA3 expression through repression of the ERα function, leading to up-regulation of Snail, down-regulation of E-cadherin, promotion of EMT and consequently an increase in metastatic potential in breast cancer cells. In fact, Mishra et al. [59] reported that MTA3 gene expression was regulated by the endogenous MTA1 and the knockdown of MTA1 resulted in a significant increase in both basal and estrogen-induced promoter activity of the MTA3 gene. Furthermore, Fujita et al. [60] revealed that a transient forced expression of MTA1 lead to loss of MTA3 protein in breast cancer cell lines. Interestingly, the same phenomenon was also observed in ovarian cancer cell line, in which MTA1 overexpression resulted in down-regulation of E-cadherin and MTA3 expression and enhanced expression of the Snail and Slug [34].

The expression of MTA3 inhibits ductal branching in virgin and pregnant mammary glands in MTA3-transgenic mice [61]. This property is in contrast to MTA1-transgenic mice, where the inappropriate development of mammary glands results in the development of hyperplastic nodules and mammary tumors, including adenocarcinomas and lymphomas [8, 40]. MTA3 also represses Wnt4 transcription and Wnt4 secretion, inhibiting Wnt-target genes in mammary epithelial cells. This repression of Wnt4 transcription was found to be mediated through a MTA3-NuRD complex, which interacts with the Wnt4-containing chromatin in an HDAC-dependent manner [61].

Although the fundamental functions of MTA proteins are exerted via transcriptional repression by histone deacetylation, a transcriptional activating function has also been demonstrated. Gururaj et al. [62, 63] showed that Breast Cancer Amplified Sequence (BCAS) 3, a gene amplified and overexpressed in breast cancers, was a chromatin target of MTA1, and the transcription of BCAS3 was stimulated by MTA1. This suggested that MTA1 has a transcriptional coactivator function in addition to a corepressor function. A similar finding has also been suggested for mouse Mta2 protein [64].

Deacetylation of non-histone proteins by the MTA family

The protein targets for deacetylation by HDAC via NuRD complexes containing MTA proteins are not only the chromatin histones but also other non-histone proteins. The tumor suppressor gene p53 product was the first non-histone protein that was reported to be deacetylated by MTA protein-containing NuRD complexes. Luo et al. [65] found that the deacetylation of p53 was mediated by a HDAC1 complex containing MTA2 protein. This MTA2-associated NuRD complex interacted with p53 in vitro and

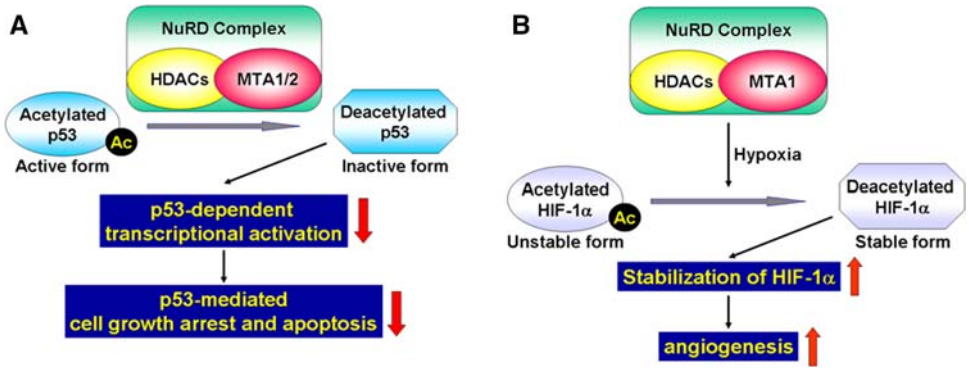

Fig. 3 Roles of MTA family in the carcinogenesis and cancer progression. Deacetylation of non-histone proteins by MTA family proteins. **a** Tumor suppressor p53 protein is deacetylated and inactivated by both MTA1 and MTA2 proteins in NuRD complexes, resulting in inhibition of growth arrest and apoptosis. **b** A hypoxia-inducible factor-1α (HIF-1α) is also deacetylated and stabilized by MTA1 protein, leading to angiogenesis

in vivo and reduced significantly the steady-state levels of acetylated p53. Deacetylation of p53 results in an increase of its own degradation through MDM2 and a reduction in p53-dependent transcriptional activation. This eventually leads to the repression of the normal p53 function that mediates cell growth arrest and apoptosis. The same phenomenon was observed between p53 and MTA1. HDAC1/MTA1 complexes possessed deacetylation activity against p53 protein in human non-small cell carcinoma and human hepatoma cells, and the complexes were found to inhibit p53-induced apoptosis by attenuating the transactivation function of p53 [66] (Fig. 3a, b).

Another important non-histone protein that is deacetylated by HDAC1/MTA1 complexes is hypoxia-inducible factor (HIF)-1α, a key regulator of angiogenic factors [67] (Fig. 3b). The expression of MTA1 is strongly induced under hypoxic conditions in breast cancer cell lines, and MTA1 overexpression enhanced the transcriptional activity and stability of HIF-1α protein. MTA1 physically binds to HIF-1α and deacetylates it by increasing the expression of HDAC1, leading to the stabilization of HIF-1α. These results indicated evidence for positive cross-talk between MTA1 and HIF-1α, which is mediated by HDAC1 recruitment. They also indicated the existence of a close connection between MTA1-associated metastasis and HIF-1α-induced tumor angiogenesis. Furthermore, Moon et al. [68] showed that MTA1 increased the transcriptional activity of HIF-1α and the expression of vascular endothelial growth factor (VEGF), a target molecule of HIF-1α. Conditioned medium collected from MTA1 transfectants increased angiogenesis in vitro and in vivo. This functional link between HIF-1α and MTA1 has been demonstrated in clinical samples of pancreatic carcinoma. Using immunohistochemistry and surgically resected pancreatic carcinomas, Miyake et al. [28] examined the expression of HIF-1α, HDAC1 and MTA1 proteins and suggested that HIF-1α expression, which is associated with a poor prognosis in patients with pancreatic cancers, might be regulated by HDAC1/MTA1 complexes. The contribution of MTA1 protein to tumor angiogenesis was also demonstrated in human breast cancers. Using immunohistochemistry, Jang et al. [19] examined MTA1 protein expression and intra-tumoral microvessel density (MVD) in clinical samples of breast cancer and showed that MTA1 expression was significantly correlated with higher tumor grade and higher tumor MVD. The relationship between MTA1 expression and MVD was also observed in HBV-associated HCC [30]. Recently, Yoo et al. [69] experimentally demonstrated that HBV-X (HBx) protein strongly induced the expressions of MTA1 and HDAC1, resulting in those physical link to HIF-1α. This suggests that positive crosstalk between HBx and MTA1/HDAC1 complex occurs and may be important in stabilizing HIF-1α, which could play a critical role in angiogenesis and metastasis of HBV-associated HCC [69].

The protein members of NuRD complexes, including MTA1 and MTA2 proteins are co-immunoprecipitated with the ataxia teleangiectasia mutated (ATM)- and Rad3-related protein (ATR) [70]. ATR is a phosphatidylinositol—kinase-related kinase that has been implicated in the response of human cells to multiple forms of DNA damage and may play a role in the DNA replication checkpoint. This fact suggests that MTA proteins may contribute to the regulation of DNA checkpoints.

Other possible functions of MTA proteins in cancer

There are other reports suggesting the possible roles of MTA proteins in carcinogenesis and cancer progression. Among them, the most important may be the relationship of MTA1 protein with c-MYC oncoprotein (Fig. 2a). By expression profiling, Zhang et al. [71] identified MTA1 protein as a

target of the c-MYC protein in primary human cancer cells and showed that c-MYC binds to the genomic MTA1 locus and recruits transcriptional coactivators. They also presented data suggesting that MTA1 protein in NuRD complexes was one of the first downstream targets of c-MYC function, essential for the transformation potential of c-MYC, because reduction of MTA1 expression by a short hairpin RNA blocked the ability of c-MYC to transform mammalian cells [71]. There are little data at present concerning the relationship between MTA1 and other important oncogene products such as c-JUN and c-FOS.

As mentioned above, Kumar's group established transgenic mice that overexpressed MTA1 protein and found that the MTA1-transgenic mice showed inappropriate development of mammary glands. These mice also developed hyperplastic nodules and mammary tumors [40]. In this study, the underlying molecular mechanisms were also examined, and the results suggested that MTA1 dysregulation in mammary epithelium and cancer cells triggered down-regulation of the progesterone receptor-B isoform and up-regulation of the progesterone receptor-A isoform, resulting in the up-regulation of the progesterone receptor-A target genes Bcl-XL and cyclin D1 in mammary glands of virgin mice. It would be extremely intriguing and important to examine the HIF-1α/VEGF expressions and angiogenesis in various organs of the MTA1-transgenic mice, although there are no data concerning these questions at present.

Recently, Molli et al. [72] reported that MTA1/NuRD complexes negatively regulated BRCA1 transcription by physically associating with ERE of the BRCA1 promoter in an ERα-dependent manner and that this repressive effect of MTA1 on BRCA1 expression resulted in an abnormal centrosome number and chromosomal instability. The relationship of MTA proteins with tumor suppressor genes other than p53 and BRCA1 remains to be determined.

Our group showed by the yeast two-hybrid system that mouse Mta1 protein physically linked to endophilin 3 and that the binding of those proteins was made between the SH 3-binding domain of Mta1 protein and the SH-3 domain of endophilin 3 [73]. This suggested that MTA1 protein might be involved in the regulation of endocytosis mediated by endophilin 3.

MTA proteins as new molecular targets: clinical implications

On the basis of the available data discussed briefly in this review, it is very likely that MTA proteins have important and critical roles in the genesis and progression of a wide variety of cancers [74]. MTA1 protein can be thought of as a master co-regulatory molecule, strongly and clearly suggesting the possibility that MTA1 protein (or its gene) could be an excellent molecular target for cancer therapy as well as its use in cancer diagnosis/prognosis. Although studies are not yet available which show the "clinical" efficacy of targeting MTA proteins, several experiments have shown that MTA1 protein (or its gene) could be a molecular target for cancer therapy.

The first studies that suggested the possibility of targeting MTA1 were reported by Nawa et al. [3] and Nicolson et al. [74]. They used antisense phosphorothioate oligonucleotides against *MTA1* mRNA and found a growth inhibitory effect on human metastatic breast cancer cell lines. Since these reports, others have shown that inhibition of MTA1 expression can result in the inhibition of the malignant phenotypes of various cancers, as mentioned below.

Various techniques have been used to regulate MTA1 expression. Using RNAi, Qian et al. [43] inhibited MTA1 expression in a human esophageal squamous cell carcinoma cell line and demonstrated significant inhibition of in vitro invasion and migration properties of the cancer cells. The same group further examined the therapeutic value of MTA1 levels in malignant melanoma cells and demonstrated that down-regulation of MTA1 by RNAi successfully suppressed the growth in vitro and experimental metastasis of mouse B16F10 melanoma cells in vivo, suggesting a promising use of the *MTA1* gene as a target for cancer gene therapy [75].

MTA1s may also be a useful target in the treatment of breast cancer. MTA1s functions as a repressor of ERα transcriptional activity by binding and sequestering the ERα in the cytoplasm [16]. MTA1s has a unique C-terminal 33-amino acid region containing a nuclear receptor-box motif that mediates the interaction of MTA1s and ERα. Singh et al. [76] showed that the MTA1s peptide containing this motif could effectively repress the ERα transactivation function, estrogen-induced proliferation and anchorage-independent growth of the human breast cancer cell line MCF-7. Using an animal model, they also showed the effect of MTA1s peptide in blocking the tumor progression of MCF-7 overexpressing ERα.

There is a good possibility that MTA1 will be a target of immunotherapy. In a review on a model for immunotherapy using a vector, disabled infectious single cycle-herpes simplex virus (DISC-HSV), Assudani et al. [77] proposed that MTA1 is a promising antigen for tumor rejection, because it is greatly overexpressed in many different tumors and is only expressed at lower levels in normal tissues. Their initial studies demonstrated the presence of immunogenic MHC class I-restricted peptides of MTA1. Furthermore, MTA1 was identified as a SEREX antigen, and hence it is likely to be capable of inducing a T-cell response in cancer patients [78].

Conclusions and future directions

This review has focused on the clinical and biological significance of the newly emerging gene family named MTA, paying particular attention to its relevance to carcinogenesis and cancer progression, such as invasion and metastasis. The fundamental roles of MTA proteins are thought to be transcriptional corepressors that function through histone deacetylation via NuRD complexes, which contain chromatin remodeling and histone deacetylating molecules. Repression of ERα transactivation function by MTA1 protein through deacetylation of ERE chromatin of the ER-responsive genes has been the most extensively investigated, and the data clearly demonstrate that MTA1 expression results in tumor formation in mammary glands and renders breast cancer cells phenotypically more aggressive. In addition, MTA proteins deacetylate non-histone proteins. For example, the tumor suppressor p53 protein is deacetylated and inactivated by both MTA1 and MTA2 proteins, resulting in inhibition of growth arrest and apoptosis. HIF-1α is also deacetylated and stabilized by MTA1, leading to angiogenesis. Considering the many reports showing the clinical relevance of the expression of *MTA1* mRNA and its encoded protein in a wide variety of human cancers as well as definitive studies showing the molecular and biochemical mechanisms of MTA protein actions, it is likely that MTA proteins, especially MTA1, represent master co-regulatory molecules involved in the carcinogenesis and progression of various malignant tumors. Ultimately this will lead to clinical applications of MTA proteins as a new class of molecular targets for cancer therapy. For example, inhibition of MTA1 expression or function may enhance the chemosensitivity of cancer cells by restoring tumor suppressor function of p53, or it may inhibit tumor angiogenesis by destabilizing the angiogenesis promoting function of HIF-1α. Moreover, inhibitors of MTA proteins may cooperate with HDAC inhibitors, which are now expected to be a new class of anticancer agents. MTA1 will also be clinically useful for the prediction of the malignant potentials of various human cancers, such as esophageal, gastric and colorectal cancers. Thus, evaluating the expression levels of MTA proteins in individual cases of various cancers may provide clinicians with important clues to prognosis and anticancer therapy.

It will be important to understand the physiological functions and underlying mechanisms of MTA proteins in normal cells, because MTA proteins are also expressed in normal cells and tissues, although at lower levels than found in cancer cells. Physiological roles of MTA1 reported are the followings: (1) MTA1 is thought to play a crucial role in postnatal testis development and spermatogenesis [79, 80], (2) The expression level of MTA1 decreases in mouse brain in age-dependent manner, which influences the estrogen-mediated signaling pathway during aging [81], (3) MTA1 protein is a direct stimulator of rhodopsin expression [82], (4) MTA1 stimulates hepatic proliferation in vivo and hepatocyte differentiation in vitro [83]. Furthermore, *Caenorhabditis elegans* has MTA1 homologues, egl-27 and egr-1, which are related to embryonic patterning [11, 84] and NURD complex including egr-1 antagonizes vulval development of *C. elegans*, which is induced by Ras signal transduction pathway [85]. Thus, understanding the physiological functions of MTA proteins will be absolutely necessary to understand the pathological functions of MTA proteins in human cancers. It will be also important to understand MTA1's roles in tissue maintenance via HIF-1α/VEGF expressions against hypoxic condition.

In conclusion, MTA proteins, especially MTA1, are undoubtedly excellent candidates for therapy and diagnosis/prognosis of human cancers and should be intensively studied for their possible clinical applications.

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