MTA family of coregulators in nuclear receptor biology and pathology

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Abbreviations: AP1: activating protein 1; BCAS3: breast carcinoma amplified sequence 3; CAK: cyclin-dependent kinase-activating kinase; EMT: epithelial to mesenchymal transition; ER: Estrogen receptor alpha; ERE: estrogen response element; GPR30: G protein-coupled receptor 30; HCC: human hepatocarcinoma; HDAC: histone deacetylase; HER: human epidermal growth factor receptor; HRG: heregulin; HIF1α: hypoxia inducible factor 1 alpha; LMO4: Lim domain-only protein; MAT1: Ménage à trois 1; MBD: methyl-CpG-binding domain protein; MICA: MTA1-interacting coactivator; MTA: metastases tumor antigen; NR: nuclear receptor; NRIF3: nuclear receptor-interacting factor 3; NuRD: nucleosome remodeling complex; RbAp: retinoblastoma-associated protein; SP1: specificity protein 1; SUMO: small ubiquitin-like modifier.

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Nuclear receptors (NRs) rely on coregulators (coactivators and corepressors) to modulate the transcription of target genes. By interacting with nucleosome remodeling complexes, NR coactivators potentiate transcription, whereas corepressors inhibit transcription of the target genes. Metastasis-associated proteins (MTA) represent an emerging family of novel NR coregulators. In general, MTA family members form independent nucleosome remodeling and deacetylation (NuRD) complexes and repress the transcription of different genes by recruiting histone deacetylases onto their target genes. However, MTA1 also acts as a coactivator in a promoter-context dependent manner. Recent findings that repression of estrogen receptor transactivation functions by MTA1, MTA1s, and MTA2 and regulation of MTA3 by estrogen signaling have indicated the significance of these proteins in NR signaling. Here, we highlight the action of MTA proteins on NR signaling and their roles in pathophysiological conditions.

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Introduction

Metastasis is a complex disease involving a series of events that includes stimulation or repression of numerous gene products in a coordinate manner, resulting in the invasion of neoplastic cells and detachment from the primary tumors, penetration into blood and lymphatics, arrest by adhesion at distant sites, extravasation, induction of angiogenesis, evasion of host antitumor responses, and growth at metastatic sites [Nicolson, 1988]. To identify the genes potentially involved in breast cancer invasion and metastasis, Toh et al. performed differential screening of a cDNA library of metastatic and nonmetastatic adenocarcinoma cell lines from rat mammary glands and cloned a novel gene known as the metastasis-associated tumor gene (mta1) [Toh et al., 1994]. Despite widespread overexpression of the MTA1 protein in human tumors, the molecular functions of the MTA family remained a mystery until the proteomic analysis of the nucleosome remodeling and deacetylation (NuRD) complex identified MTA1 and MTA2 as integral to the histone deacetylase (HDAC) complexes [Xue et al., 1998; Zhang et al., 1998], thus providing clues about the role of the MTA family of proteins in chromatin remodeling. MTA3, the third member of the MTA family, was identified as an estrogen-inducible gene product that forms a distinct Mi-2/NuRD complex [Fujita et al., 2003]. MTA1s, a naturally occurring variant of MTA1, was accidentally discovered by researchers pursuing the role of MTA1 in transcriptional regulation of estrogen receptor-α (ER) [Kumar et al., 2002]. MTA1s is generated by alternative splicing at a cryptic splice-site in exon 14, involving the deletion of 47 base pair nucleotides, and a frame-shift involving the addition of 33 unique amino acids with no homology among sequences from Genbank. The resulting MTA1s gene product lacks the C-terminal region of other MTA family members [Kumar et al., 2002]. Using a polyclonal antibody against purified zymogen granule membrane components from rat pancreas, researchers identified ZG29p, a cDNA coding for the 29-kDa protein, by immunoscreening a hormonally stimulated pancreas cDNA library. ZG29p is an N-terminal truncated form of MTA1, coded by the last seven exons of MTA1 and present in the zymogen granules of the pancreas. ZG29p mediates an interaction with amylase and is involved in condensation-sorting in the exocrine rat pancreas [Kleene et al., 2000].

Domain structure of the MTA family

MTA1 and MTA2 are polypeptides with molecular masses of approximately 80 kDa and 70 kDa, respectively, whereas MTA3 is smaller, approximately 65 kDa. Protein alignment homology of the human MTA proteins revealed 68% and 73% homology of MTA2 and MTA3, respectively, with MTA1. The highest homology was concentrated in the N-terminal half of the proteins, whereas the C-terminal regions of the MTA proteins were divergent (Figure 1A and [Manavathi and Kumar, 2007]). The MTA family, with the exception of the ZG29p variant, contains one BAH domain, one ELM domain, and one SANT domain, which is identical to the DNA binding domain of myb-related proteins. The BAH, ELM, and SANT domains among the MTA family members exhibit
approximately 98% similarity. The functional significance of these domains has not yet been directly tested; it is currently hypothesized from studies of proteins containing such domains. For example, the BAH domain is involved in protein-protein interactions, whereas the SANT domain interacts with histones [Grune et al., 2003; Oliver et al., 2005]. Also, all MTA family proteins contain a GATA-like zinc-finger and a DNA-binding domain found in the GATA transcription factors. Unfortunately, experimental evidence in support of a direct DNA-binding activity of MTA proteins is lacking. The prototypic MTA1 contains two bipartite nuclear localization signals and one basic amino acid-rich nuclear localization signal [Manavathi and Kumar, 2007]. MTA2 contains one basic amino acid-rich nuclear localization signal. MTA3 does not contain a predicted nuclear localization signal, but localizes to both the cytoplasmic and nuclear compartments. Although the N-terminal sequences of MTA1, MTA2, and MTA3 are similar, only MTA1 contains a proline-rich Src-homology 3 domain-interacting region at the C-terminus, providing a biochemical basis for MTA1 interaction with the signaling molecules [Manavathi and Kumar, 2007].

Expression of the MTA genes

MTA genes represent a rapidly growing novel gene family that includes three different genes encoded at three separate loci (MTA1 at 14q; MTA2 at 11q; and MTA3 at 2q) with several alternative splice forms (MTA1s, MTA1-ZG29p, and MTA3L) [Yaguchi et al., 2005]. The existing literature provided the handful of downstream targets of MTA proteins; however, few studies have addressed the regulators of these proteins. Recent findings provide some clues about the regulation of MTA protein expression. Heregulin, a ligand for HER3 and HER4, has been shown to induce the expression of MTA1 in breast cancer cells [Mazumdar et al., 2001]. A recent study that was aimed at isolating novel c-MYC targets by expressing the conditional c-MYC/ER fusion protein in the normal diploid human fibroblasts identified the MTA1 promoter as a c-MYC target [Zhang et al., 2005]. The MTA1 was found to be a mechanistic mediator of c-MYC-regulated transformation. Further, MTA1 is also transcriptionally upregulated under hypoxia through a poorly understood mechanism [Yoo et al., 2006].

It has been demonstrated that estrogen regulates the expression of MTA3 in breast cancer cells [Fujita et al., 2004; Mishra et al., 2004a]. MTA3 contains half estrogen response elements (ERE), along with AP-1 and SP-1 sites, and it has been shown that ER-α, AP-1, and SP-1 transcription factors directly bind onto these half ERE, AP-1, and SP-1 elements on the MTA3 promoter, respectively, and thereby strongly influence MTA3 transcription in breast cancer cells in a hormone-dependent manner [Fujita et al., 2004; Mishra et al., 2004a]. Expression of MTA3 appears to be somewhat tissue-specific, as this protein expresses predominantly in breast cancer cells and B-lymphocytes, whereas MTA1 and MTA2 are ubiquitously expressed. In contrast to MTA3, MTA2 promoter contains ETS-1 binding elements along with SP-1-binding motifs, and MTA2 transcription is regulated by both ETS-1 and SP-1 [Xia and Zhang, 2001]. Information about the regulation of ZP29 gene transcription is not available at this time.

Subcellular localization of the MTA proteins

In general, the MTA proteins contain basic nuclear localization signals and are predominantly localized in the nucleus. Analysis of various mouse tissues suggested that variable, but easily detectable, levels of MTA1 protein are present in multiple organ systems, including lung, liver, kidney, heart, and testes, thus suggesting a physiologic function of MTA1 in normal cellular functions [Mazumdar et al., 2001]. Similarly, MTA1 immunohistochemical assays have documented the predominantly nuclear localization of MTA1 in various cancerous tissues, including ovarian, lung, gastric, and colorectal cancers [Manavathi and Kumar, 2007]. However, in human hepatocarcinoma (HCC) cells and in human B-cell lymphomas, MTA1 localizes to both the nucleus and cytoplasmic compartments [Moon et al., 2004]. In contrast to MTA1, MTA3 localizes to both cytoplasm and nucleus compartments, despite the lack of a putative nuclear localization signal. MTA1s, a smaller protein with 429 amino acids, is predominantly localized in the cytoplasm owing to the loss of the nuclear localization signal due to alternative splicing [Manavathi and Kumar, 2007].

Posttranslational modifications of the MTA proteins

Among MTA family members, MTA1 has been found to be acetylated at lysine 626 by histone acetyltransferase p300 in breast cancer cells [Gururaj et al., 2006]. The lysine 626 site of MTA1 is not conserved among the family members. The acetylated MTA1 and RNA polymerase II (Pol II) complex is recruited onto the ERE site, and it has been shown that ER-α, AP-1, and SP-1 transcription factors directly bind onto these half ERE, AP-1, and SP-1 elements on the MTA3 promoter, respectively, and thereby strongly influence MTA3 transcription in breast cancer cells in a hormone-dependent manner [Fujita et al., 2004; Mishra et al., 2004a]. Expression of MTA3 appears to be somewhat tissue-specific, as this protein expresses predominantly in breast cancer cells and B-lymphocytes, whereas MTA1 and MTA2 are ubiquitously expressed. In contrast to MTA3, MTA2 promoter contains ETS-1 binding elements along with SP-1-binding motifs, and MTA2 transcription is regulated by both ETS-1 and SP-1 [Xia and Zhang, 2001]. Information about the regulation of ZP29 gene transcription is not available at this time.

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expression of the BCAS3 gene. These observations raise an interesting possibility that the corepressor versus coactivator activity of MTA1 may be influenced by MTA1-binding partners, posttranslational modifications of the protein, or both and that this could very well be further affected in a promoter-context manner. MTA1 is also a phosphoprotein (Da-Qiang Li and Kumar, personal communication); however, the responsible kinase(s) are unknown.

Catalytic activity of the MTA proteins

Currently, no evidence supports intrinsic enzymatic activity of the MTA1 proteins. However, the presence of MTA proteins (MTA1, MTA2, and MTA3) in NuRD complexes supports the participation of MTA family members in the deacetylation of histones [Toh et al., 1994; Xue et al., 1998; Zhang et al., 1998] and putative non-histone proteins [Manavathi and Kumar, 2007]. For example, HDAC1-containing complex, known as PID, contains MTA2 and HDAC2 and mediates the deacetylation of p53 [Luo et al., 2000]. PID specifically interacts with p53, and its level reduces the steady-state levels of the acetylated p53. Consequently, deacetylated p53 results in a reduced p53-dependent transcription, as deacetylation of p53 induces its degradation through MDM2, which eventually leads to p53-mediated cell growth arrest and apoptosis [Luo et al., 2000]. Because the levels of PID and MTA2 are especially high in the rapidly dividing cells, there may be a functional interaction between p53, PID, and MTA2, as well as p53 deacetylation, in the cancer cells.

Another notable target of the MTA and HDAC complexes is the hypoxia-inducible factor 1 (HIF-1)α, the master transcriptional regulator that facilitates adaptation to low oxygen availability, commonly known as hypoxia [Yoo et al., 2006]. The expression of MTA1 has been shown to be induced unexpectedly by hypoxia in breast cancer cells, and its level correlates well with HIF-1α [Yoo et al., 2006].

Furthermore, MTA1 enhances the stability and transcriptional activity of HIF-1α under hypoxic stress. The underlying mechanism involves the enhancement of HIF-1α association with HDACs, resulting in the deacetylation of HIF-1α, as deacetylated HIF-1α is resistant to ARD1-mediated ubiquitin-proteosomal degradation [Yoo et al., 2006]. Since MTA1 is a metastasis promoting gene under pathologic conditions [Nicolson, 1988], the mechanistic role of MTA1 in HIF-1α regulation may contribute to the metastatic role of MTA1 in cancer cells.

Because ER-α physically interacts with the MTA1-HDAC complex [Mazumdar et al., 2001], and because ER-α is an acetylated protein [Gururaj et al., 2006], the acetylation status of which directly influences its transactivation functions, it is possible that MTA1 may also regulate ER-α functions by deacetylating ER-α. This possibility has been experimentally tested, and indeed, ER-α is deacetylated by the MTA2/HDAC complex, and the acetylation mutant of ER-α exhibits less transcriptional activity (Figure 1B and [Cui et al., 2006]).

Transcriptional repression of ER-α by the MTA proteins

Estrogen, the ligand for ER, exerts its action by binding to its cognate receptors (ER-α, ER-β and GPR30) [Manavathi and Kumar, 2006]. ER-α and ER-β are transcription and signaling factors. ER regulates cellular functions by binding to the ERE elements on its target genes. The transcriptional activity of ER is influenced by a variety of coactivators and corepressors [McKenna and O’Malley, 2002]. For example, coactivators such as p300 interact with ER-α and support the chromatin remodeling by acetylyzing the histones around the target promoter [McKenna and O’Malley, 2002]. In contrast, corepressor complexes promote the condensation of chromatin, leading to repression of the ability of the nuclear receptor (NR) to promote transcription [McKenna and O’Malley, 2002]. NuRD is one such corepressor complex [Toh et al., 1994; Xue et al., 1998; Zhang et al., 1998]. The NuRD complex contains seven polypeptides: HDAC1, HDAC2, RbAp46, RbAp48, Mi-2α/β, MTA1/2, and p66. Another complex, Six3, has a composition similar to NuRD and also participates in the NR repression of target genes [Ahringer, 2000]. In the NuRD complex, HDAC1 and HDAC2 contribute to deacetylation; Mi-2α and Mi-2β proteins with a chromodomian exhibit a DNA helicase/ATPase activity; and RbAp46 and RbAp48 participate in histone binding [Brackertz et al., 2002]. The p66α and p66b proteins are involved in the interaction with methyl-CpG binding domain proteins (MBD) 2 and 3 and also interact with the tails of all octamer histones to enhance the MBD2-mediated repression [Brackertz et al., 2002]. Further complexity in the role of p66 in the NuRD complex arises from its sumoylation. Small ubiquitin-like modifier (SUMO)-modified forms of p66α efficiently interact with HDAC1, whereas RbAp46 binds to SUMO-p66β [Gong et al., 2006]. Although all MTA family proteins are found in NuRD complexes, these proteins form distinct complexes and are thought to target different sets of promoters [Bowen et al., 2004; Yao and Yang, 2003].

Because the Mi-2/NuRD complex contains both histone deacetylase and chromatin-remodeling ATPase activities, it is thought that such complexes are involved in the hypoacetylation of core histones and that HDAC-containing complexes participate in transcriptional repression. Although the presence of MTA proteins in NuRD complexes has provided clues about the role of MTA family members in chromatin regulation [Toh et al., 1994; Xue et al., 1998; Zhang et al., 1998], direct targets of MTAs were unknown until the discovery that MTA1 directly interacts with ER-α, HDAC1, and HDAC2 [Mazumdar et al., 2001]. The NuRD complex interacts with ER-α and represses its transcriptional activity in an HDAC-sensitive manner [Mazumdar et al., 2001]. Subsequently, MTA1 was found to interact with other proteins such as ménage à trois 1 (MAT1), MTA1-interacting coactivator (MICOA), nuclear
receptor-interacting factor 3 (NRIF3), and Lim-only protein 4 (LMO4) [Mishra et al., 2003; Singh et al., 2005; Talukder et al., 2004; Talukder et al., 2003].

Using a yeast two-hybrid screen with the MTA1 C-terminal domain as bait, Talukder et al identified MAT1 as an MTA1-binding protein. MAT1 is an assembly and targeting factor for cyclin-dependent kinase-activating kinase (CAK), which has been shown to functionally interact with general transcription factor TFIIH, a known inducer of ER transactivation [Talukder et al., 2003]. Estrogen signaling promotes nuclear translocation of MAT1 and interacts with MTA1 in the nucleus. MAT1 binds to the C-terminal GATA domain containing 389-441 amino acids and the N-terminal bromo-domain containing 1-164 amino acids of MTA1, whereas MTA1 binds to the N-terminal ring finger domain of MAT1. MAT1 interacts with the (AF)2 domain of ER-α and co-localizes with ER-α in the nucleus. The interactions between CAK and MTA1 regulate the transactivation activity of ER-α in a CAK-dependent manner in breast cancer cells [Talukder et al., 2003].

MICoA is another well-characterized MTA1 binding partner. MICoA is also an ER coactivator. MICoA cooperates with other ER coactivators, such as p300, and stimulates ER-transactivation functions by associating with endogenous ER-α on its target promoters. MTA1 opposes MICoA-mediated ER-α transactivation functions by recruiting HDACs [Mishra et al., 2003]. NRIF3, another MTA1-binding protein, was also identified using a yeast two-hybrid screen [Talukder et al., 2004]. Interestingly, NRIF3 is an estrogen-inducible gene, and the NRIF3 protein functions as a coactivator for ER-α. NRIF3 interacts with MTA1 through its N-terminal domain, whereas it interacts with ER-α through the NR box. Because NRIF3’s box participates in its interaction with ER-α, estrogen treatment displaces the MTA1/HDAC complex from ER-α and modulates the sensitivitiy of breast cancer cells to estrogen [Talukder et al., 2004]. In addition, a recent study revealed that MTA1 interacts with LMO4, a member of the LIM-only family of transcriptional coregulatory proteins. MTA1 also intensifies the LMO4 protein’s repressor activity on ER-α, implying that LMO4 is a component of the MTA1 corepressor complex [Singh et al., 2005]. It appears that MTA1 represses the ER transactivation functions by recruiting the HDAC complex and other corepressors on ER target genes, which leads to ligand-independent metastasis phenotype (Figure 2, Table 1).

The MTA1s protein, a naturally occurring splice variant of MTA1, was discovered by researchers studying the corepressive activity of MTA1 [Kumar et al., 2002]. MTA1s localizes in the cytoplasm, and its C-terminal stretch of 33-amino acid residues bears no resemblance to any of the previously reported protein sequences. Situated in the early portion of this 33-amino acid region is an NR box motif (LRILL). MTA1s uses this motif to interact and bind with the AF2 domain of ER-α, leading to its cytoplasmic sequestration and subsequent suppression of genomic functions of ER-α (Figure 2). Cytoplasmic sequestration of ER-α augments the rapid cytoplasmic activities of ER, such as MAPK activation. Overexpression of MTA1s in ER-positive breast cancer cells leads to an aggressive phenotype. The nuclear magnetic resonance (NMR)-aided structure of the novel 33-amino acid region of MTA1s revealed its α-helix nature [Singh et al., 2006]. In silico modeling suggested that the NR box motif of the 33-amino acid region of MTA1s binds to the AF2 domain (helix 12) of ER-α and, thus, might impair its interaction with coactivators. Indeed, in tissue culture-based models, the MTA1s 33 amino acid peptide inhibits the recruitment of coactivators onto ER and inhibits its transactivation functions. Further, the MTA1s peptide also exerts an inhibitory effect on estrogen-induced proliferation of breast cancer cells [Singh et al., 2006]. MTA1s has also been shown to interact with casein kinase I-gamma2 (CK1γ2) [Mishra et al., 2004b].

More recently, MTA2 has also been shown to bind to ER-α in breast cancer cells. Like MTA1, MTA2 also represses the transcriptional activity of ER-α by recruiting HDACs onto the ER-α target chromatin (Figure 2 and [Cui et al., 2006]). Moreover, the MTA2/HDAC complex regulates the deacetylation of ER-α and, consequently, its functions. All other MTA family interacting proteins are summarized in Table 1.

**Estrogen-MTA3–mediated regulation of epithelial to mesenchymal transition**

As discussed earlier, MTA3 has also been shown to form a distinct Mi-2/NuRD complex [Fujita et al., 2003]. The MTA3-containing Mi-2/NuRD complex influences E-cadherin expression in breast cancer cells by inhibiting...
expression of the zinc finger (GATA-like zinc finger) transcription repressor Snail [Fujita et al., 2003], which in turn controls the epithelial-to-mesenchymal transition (EMT). This function of MTA3 is linked with the NR-ER-α pathway, as MTA3 is a transcriptional target of ER-α. In the presence of ligand, ER-α directly binds to the MTA3 promoter at the half-ERE/Sp1 binding site and stimulates MTA3 transcription [Fujita et al., 2004; Mishra et al., 2004a]. Because both MTA1 and MTA1s negatively regulate ER-α function and because MTA3 is an estrogen-regulated gene, any potential upregulation of MTA1 or MTA1s may lead to repression of MTA3. As regulated reduction in the level of MTA3 will lead to upregulation of Snail, promotion of EMT, and metastasis of breast cancer cells, these findings suggest a role for MTA1 and MTA1s in the EMT process (Figure 2).

### Table 1. List of currently characterized MTA-interacting proteins.

| MTA family interacting proteins | References |
|---------------------------------|------------|
| MTA1                            | Mishra et al. 2003 |
| MCoA                            | Talukder et al. 2003 |
| MAT1                            | Talukder et al. 2004 |
| NRIF3                           | Singh et al. 2005 |
| LMO4                            | Talukder et al. 2003 |
| MTA1s                           | Singh et al. 2006 |
| ER-α                            | Mishra et al. 2004 |
| CK1γ2                           | Saito et al. 2002 |
| MTA2                            | Luo et al. 2000 |
| MTA3                            | Jaye et al. 2007 |

### MTA1 and hormone action during mammary gland development

Because ER-α plays a pivotal role in mammary gland development and MTA1 is a potent transcriptional corepressor of ER-α, MTA1 is also thought to play an important role in mammary gland development. Consistent with this notion, overexpression of the mammary tumor-like virus (MMTV)-MTA1 transgene in the mouse mammary gland resulted in increased ductal extension, enhanced ductal branching, and proliferation [Bagheri-Yarmand et al., 2004]. In addition, the mammary glands of MMTV-MTA1 mice exhibit an accelerated lobuloalveolar-like precocious differentiation, decreased pregnancy-associated morphogenesis, and delayed involution and tumorigenesis. Furthermore, the noted increase in ductal growth and extensive ductal branching in MTA1-transgenic mice was attributed, at least in part, to the alteration in the ratios of progesterone receptor isoforms [Bagheri-Yarmand et al., 2004]. In contrast to the hyperbranching and tumor formation in MTA1-transgenic mice, MTA3-transgenic mice exhibit hypobranching [Zhang et al., 2006a]. These contrasting physiological differences displayed by MTA family members also support the notion of a lack of functional redundancy among individual MTA proteins.

### Preservation of corepressor activity of MTA1 in Caenorhabditis elegans

Because the NuRD complex plays an important role in nucleosome remodeling and transcriptional regulation, it is anticipated that components of the NuRD complex will play a role in embryonic development. Initially, the biological functions of Mi-2 proteins and components of the NuRD complex forecasted a potential role of the NuRD complex in Caenorhabditis elegans [von Zelewsky et al., 2000]. Indeed, studies with the MTA1 homologues egl-27 and egr-1 in C. elegans identified inactivation of both of these genes in embryos, leading to abnormal patterning of the cells in the embryo. These findings suggested that EGL-27-containing protein complexes regulate the activity of transcription factors involved in embryonic patterning [von Zelewsky et al., 2000]. Furthermore, egr-1 and egl-27 proteins, along with the components of NuRD complex, antagonize the development of vulval tissue, which is induced by the Ras signal transduction pathway. In this pathway, the MTA1-related genes egr-1 and egl-27 act in the synMuvA pathway to repress vulval developmental target genes by local histone deacetylation, which is important for vulval development and embryonic patterning [Chen and Han, 2001]. These studies on developmental aspects identify the importance of MTA coregulators and preservation of their function as transcriptional repressors in chromatin regulation.

### MTA proteins in diseases

Metastasis is the major cause of morbidity and mortality among patients with cancer. Data from cell-based model systems and mouse models have firmly established the significance of MTA1 in cancer metastasis [Manavathi and Kumar, 2007]. For example, MTA1 overexpression in mouse mammary gland epithelium leads to the formation of mammary gland adenocarcinomas [Bagheri-Yarmand et al., 2004]. In addition, MMTV-PyV-mT-transgenic mice, which represent a reliable model for multistage tumorigenesis of human breast cancer, exhibited an altered MTA1 expression in both premalignant lesions and malignant breast carcinoma [Zhang et al., 2006b]. These tumors exhibited an elevated MTA1 nuclear expression and MTA1 in the center of late-stage tumors. In addition to breast cancer,
an analysis of an endometrial tumor microarray containing 70 endometrial adenocarcinomas of various grades showed increased expression of MTA1 in 53 (75.7%) tumors [Balasenthil et al., 2006]. Moreover, MTA1 overexpression was also found in a tissue microarray analysis of 102 gastrointestinal carcinoids [Kidd et al., 2006]. In another study of gastrointestinal carcinoid tumors, 13 (38.2%) of 34 patients with gastric carcinoma showed overexpression of MTA1 mRNA. These findings suggest that higher rates of serosal invasion and lymph node metastasis are linked with MTA1 overexpression and tend to have a higher rate of vascular involvement in these carcinomas [Toh et al., 1997].

Increased expression of MTA1 mRNA was also observed from normal to tumor tissue in human colorectal carcinoma and HCC cells [Giannini and Cavallini, 2005; Hamatsu et al., 2003]. Furthermore, another study showed that MTA1 overexpression correlates well with the acetylated histone 4 in esophageal squamous cell carcinomas [Toh et al., 2004]. This study revealed that 30 (42.9%) of 70 samples (from normal tissue to cancer) displayed overexpression of the MTA1 protein; such tumors invaded deeper into the esophageal wall and these patients showed a significantly higher degree of lymph node metastasis, higher pathological stage, more lymphatic involvement, and poorer prognosis than the remaining cases. A DNA microarray analysis combined with immunohistochemistry analysis showed that MTA1 is also selectively overexpressed in metastatic prostate cancer compared with clinically localized prostate cancer and benign prostate tissue [Hofer et al., 2004]. An analysis of 74 non-small cell lung cancer (NSCLC) samples revealed higher MTA1 mRNA levels in NSCLC samples without lymph node metastasis [Sasaki et al., 2002]. In thymomas, the MTA1 mRNA level was significantly higher in stage IV thymoma than in stage I and II thymomas [Sasaki et al., 2001].

MTA1 also appears to be involved in the development of lymphomas. Data mining analysis of public expression array datasets using Oncomine 2 showed that 75 out of 76 specimens of human lymphomas (98.6%) exhibited MTA1 expression [Balasenthil et al., 2007]. MTA1 expression was observed in the nuclei (32%), as well as in both nucleus and cytoplasm (68%) of diffuse large B-cell lymphoma cases. These data are further supported by the finding that MTA1 transgenic mice, which developed breast adenocarcinoma [Singh et al., 2006], also developed lymphomas during later stages of their life span [Balasenthil et al., 2007]. Further overexpression of MTA1 was observed in various human lymphoma and leukemia cell lines [Balasenthil et al., 2007]. These findings implicate MTA1 in the development of spontaneous B-cell lymphomas. Very little information is available regarding the roles of MTA2 and MTA3 in human cancers.

**Future challenges**

This review highlighted the biological significance of the MTA proteins in the context of nuclear receptor biology. Because the expression of MTA family members is not restricted to cancer cells, one of the major outstanding issues in MTA family research remains the lack of understanding as to the physiological functions and underlying mechanisms in normal cells. On the basis of available data and the fact that MTA1 represents a master coregulatory molecule, it is very likely that MTA1 deregulation may be involved in human diseases other than cancer. Because MTA family members were found in distinct subcellular compartments, it is important to understand the underlying biochemical basis of differential subcellular localization and whether it is further affected by extracellular signals. Furthermore, to fully appreciate the master regulatory function of MTA1 (or other MTA family members), it is of paramount importance to understand the nature of the biochemical switch responsible for corepressor versus coactivator activity of MTA1. In addition to further researching the cellular functions of MTA1, there is a clear need to intensify research connecting the various domains of MTA1 (or other family members) with specific cellular function.

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Review

MTAs in nuclear receptor function

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