The Metastasis Tumor Antigens- an Emerging Family of Multifaceted Master Coregulators*

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Key words: MTA, Chromatin modifiers, NuRD Complex, Transcriptional regulation, Development

Summary
Regulation of fundamental genetic processes demands dynamic participation of transcription factors, their coregulators, and multiprotein chromatin remodeling activities at target genes. One family of chromatin modifiers that is ubiquitously expressed is the metastasis tumor antigens (MTA), which are integral parts of nucleosome remodeling and histone deacetylation (NuRD) complexes. MTA family members exist in distinct NuRD complexes, and functional redundancy is lacking among MTA family members. MTA proteins regulate divergent cellular pathways, including hormonal action, epithelial-to-mesenchymal transitions, differentiation, protein stability and development, and cell fate programs by modifying the acetylation status of crucial target genes. Intriguingly, at least one member of this family, MTA1, itself undergoes acetylation and acts as a coactivator in certain contexts. We discuss the roles of the MTA family of chromatin modifiers, with an emphasis on their physiologic functions.

Text
Dynamic alterations in chromatin structure facilitate or repress access of transcription complexes to target DNAs, leading to transcriptional changes and the regulation of functions associated with the gene products (1). These complexes modify DNA accessibility for cofactors by affecting DNA-histone interactions, nucleosome sliding, or relocation. In addition, the transcriptional state is influenced by covalent modification of the core histones (2). Among histone modifications, acetylation plays a pivotal role in chromatin remodeling (3). Core histones are subject to reversible acetylation at select lysine residues in their N-terminal domains through the coordinate activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs) (3). Histone acetylation correlates with transcriptional activity, whereas deacetylation favors transcriptional repression. The mechanism of action of HATs is extensively reviewed elsewhere in the literature (4). Here we discuss the current understanding of the MTA family of coregulators, which are essential components of HDAC-containing NuRD transcriptional complexes.

The NuRD Complex, a Dynamic Switch for Histone Deacetylation and Chromatin Remodeling
Deacetylation of histones via HDACs is carried out by two major complexes, Sin3 and NuRD (5,6). The Sin3 complex contains seven polypeptides: HDAC1, HDAC2, RbAp46, RbAp48, Sin3, SAP18, and SAP30. This complex participates in nuclear hormone receptor repression of target genes (5,7). NuRD complexes share four core proteins (HDAC1, HDAC2, RbAp46 and RbAp48) with the Sin3 complex and contain Mi-2α/β, MTA1/2, and p66 (5). In the NuRD complex, HDAC1/2 participate in the deacetylation process; Mi-2α/β proteins with a chromodomain exhibit a DNA helicase/ATPase activity; and RbAp46/48 participate in histone binding. The roles of p66α/β in NuRD complex are complex, as p66 proteins are sumoylated and SUMO-modified p66α efficiently interacts with HDAC1, whereas RbAp46 binds to SUMO-modified p66β (8,9). All three MTA family proteins are found in NuRD complexes; however, recent data suggest that they occur in distinct complexes (10), and thus in principle, may target different sets of promoters (10,11). The precise role of the MTA proteins in the NuRD complexes is not fully appreciated. Due to the abundant presence of the MTA family members in normal tissues, their NuRD corepressor complexes are presumed to target diverse genes affecting a variety of...
cellular pathways with roles in both normal and pathologic states.

MTA Proteins, Family with Multiple Functionality
MTA proteins represent a small family of gene products encoded by three distinct genes with different chromosomal loci in humans, i.e., MTA1 at 14q32, MTA2 at 11q12-q13.1, and MTA3 at 2p21 and six reported isoforms (MTA1, MTA1s, MTA1-ZG29p, MTA2, MTA3 and MTA3L) (12). Two homologues of the human MTA1 gene also exist in *C. elegans*, egl-27 and egr-1 (**lin-40**) (13,14). Among MTA family members, the expression pattern of MTA3 is restricted, whereas MTA1 and MTA2 are ubiquitously expressed.

MTA1, the founding member of the MTA family of genes, was originally identified through differential screening of a cDNA library from rat metastatic breast tumors (15). Subsequent studies found a widespread upregulation of MTA1 in human tumors (12,16). In spite of a strong correlation between MTA1 upregulation and cancer, the molecular function of the MTA family remained a mystery until proteomic analyses of the NuRD complex identified MTA1 and MTA2 as integral subunits, thus providing essential clues to the chromatin modifying roles of MTA proteins (17,18).

Analysis of the primary structure of MTA proteins shows that human MTA2 and MTA3 are 68% and 73% identical to human MTA1, respectively, with the highest homology concentrated in the N-terminal half of the proteins (Fig. 1). In contrast, the C-terminal halves of MTA proteins are divergent. The MTA proteins (with the exception of the ZG29p variant) contain one BAH domain, one ELM domain, and one SANT domain that is similar to the DNA binding domain of myb-related proteins. Our understanding of the functional significance of these domains in MTA family is derived from studies of other proteins containing such domains. For example, the BAH domain is involved in protein-protein interactions (19), whereas the SANT domain binds to histone tails (20). Also, all MTA family members contain a GATA zinc-finger DNA binding domain (Fig.1); MTA1 contains two bipartite nuclear localization signals and one basic amino acid–rich nuclear localization signal. MTA2 contains one basic amino acid–rich nuclear localization signal (using the PSORTII program) and thus, predominantly localizes to the nucleus (Fig.1). In human hepatocarcinoma (HCC) cells, MTA1 localizes to both the nucleus and cytoplasmic compartments (21), which raises the possibility of an NuRD complex-independent function of MTA1. Although the physical organization of MTA1, -2 and -3 are similar at the N-terminus, the C-terminus of MTA1 contains a Src-homology 3 domain (11), which makes it the only MTA that could interact with signaling molecules.

Naturally Occurring MTA Variants
MTA1s is a C-terminal truncated variant of MTA1 that predominantly localizes to the cytoplasm of estrogen receptor (ER)-positive breast cancer cells (22). MTA1s is generated by alternative splicing at a cryptic splice site located in (or near) exon 14, resulting in the deletion of 47 nucleotides and the entire C-terminal portion of MTA1, and a translational frameshift leading to the addition of 33 novel amino acids that include a nuclear receptor (NR) interacting motif (NR box) (22). Among other MTA family members, ZG29p is an N-terminal truncated form of MTA1, encoded by the last seven exons of MTA1 (23). The ZG29p protein contains two nuclear localizing signals, but in contrast to MTA1, is excluded from the nucleus by a mechanism that is yet to be identified. ZG29p occurs primarily in zymogen granules, with a small amount appearing in the Golgi complex and rough endoplasmic reticulum (23). Recent data suggest the existence of multiple splice variants of MTA1 (24). MTA3L is a longer form of MTA3 that contains 594 aa, versus the 515 amino acids of MTA3 (25). The mRNAs encoding MTA3 and MTA3L differ in their 3′ terminal exons and produce two highly similar proteins with unique carboxyl termini.

Regulation of ER pathway by MTA family members
Although the presence of MTA1/MTA2 in NuRD complexes provided clues about the role of MTA proteins in chromatin remodeling (17,18), direct targets of MTAs remained
unknown until the discovery that MTA1 directly interacts with ERα and represses its transactivation activity in an HDAC-sensitive manner (26). Subsequently, the MTA1s variant was also found to inhibit ERα transactivation by sequestering ERα in the cytoplasm via a direct interaction between the ERα and the MTA1s NR box (22). More recently, MTA2 was also found to suppress ERα transactivation activity (27). A deeper understanding of the corepressor activity of MTA1 upon the ERα pathway was revealed by the identification of three novel MTA1 binding partners that by themselves exerted coactivator function upon ERα transactivation. These MTA1 binding proteins are MAT1, a component of cyclin-dependent kinase activated kinase (CAK) complex (28), MICoA (29), and NRIF3 (30). In addition, MTA1 interacts with LMO4 and facilitates the ability of LMO4 to transcriptionally repress ERα (31). LMO4, a member of the LIM-only family of transcriptional coregulatory proteins, consists of two LIM protein-protein interaction domains that enable it to interact with both ERα and its corepressor MTA1. These observations suggest that the functional status of ERα is regulated by association with different MTAs and their assorted binding proteins (Fig. 2).

The C-terminal 33 amino acids of MTA1s bear no obvious resemblance to known protein motifs, with the exception that they contain a NR-box motif (-LRILL-). Recent studies using nuclear magnetic resonance spectroscopy suggested an α-helical structure for this 33 amino acid sequence region (32). MTA1s uses this motif to interact with the AF2 domain of ERα, leading to cytoplasmic sequestration of the receptor and, consequently, to inhibition of the genomic functions of ERα. Intriguingly, MTA1s is phosphorylated by CKI-gamma2 in an antiestrogen-dependent manner. Phosphorylation by CKI-gamma2 potentiates the corepressor activity of MTA1s on ERα (33) (Fig 2). Recent findings suggest that MTA3 regulates epithelial–mesenchymal transitions by inhibiting the zinc finger transcriptional repressor, Snail (25). Further MTA3 is downstream of the ERα pathway, as ERα directly binds to the MTA3 promoter at the half-ERE/Sp1 binding site and regulates MTA3 transcription (34-36).

**MTA1/2-dependent Deacetylation of Non-histone Proteins**

In addition to deacetylation of histones, MTA1/2/HDAC complex interacts with and deacetylates non-histone proteins. MTA proteins deacetylate p53, HIF1α, and ERα (27,37,38) (Fig. 3). Deacetylated p53 results in decreased p53-dependent transcription, as deacetylation of p53 marks it for degradation through MDM2, leading to cell growth arrest and apoptosis. Another non-histone target of MTA1/HDAC complex for deacetylation is HIF-1α, a transcriptional regulator of hypoxia. MTA1 enhances the stability and transcriptional activity of HIF-1α under hypoxic conditions. The underlying mechanism involves MTA1 association and deacetylation of HIF-1α, leading to increased steady-state levels of HIF-1α due to resistance of deacetylated HIF-1α to ubiquitin-proteasome degradation (Fig. 3). It is important to note that deacetylation of HIF-1α protects from degradation while deacetylation of p53 promotes degradation. Because the acetylation status of ERα is closely linked to transactivation, a recent study found that MTA2/HDAC complex involves in deacetylation of ERα and modulates its transcriptional activity (27).

**MTA Family in Development, Cell Fate Determination, and Beyond**

**MTA1 homologues in embryonic development.** Coordinate regulation of transcription factors and coregulators is particularly important during embryonic development. Components of the NuRD complex appear to play a regulatory role during development in *C. elegans* (39). For example, inactivation of the MTA1 homologues egl-27 and egr-1 (also known as *lin-40*) in *C. elegans* leads to abnormal patterning of cells in the embryo, suggesting that EGL-27-containing complexes regulate the activity of transcription factors involved in embryonic patterning (14). Further, mutations in egl-27 lead to defects in cell polarity and migration in the worm (13). EGR-1 and EGL-27 proteins in conjunction with NuRD components antagonize vulval development (39). EGR-1 (LIN-40) inhibits vulval fate specification by downregulating *lin-
Hox expression, leading to enhanced cell fusion between vulval precursor cells and the hypodermal syncytium at an early larval stage (40). These developmental studies highlight the importance of MTA coregulators in processes affecting developmental decisions such as cell migration, polarity, and pattern formation.

**MTA1 in mouse mammary gland development.** A recent study showed that forced overexpression of MTA1 in the mouse mammary gland epithelium was accompanied by extensive ductal branching and proliferation in virgin glands, which was mechanistically linked to an altered ratio of progesterone receptor A and B isoforms (41). These data suggest a role for MTA1 in mammary gland development. In older animals, MTA1 leads to the formation of mammary gland adenocarcinomas. Virgin mammary glands from MTA1 transgenic mice also exhibit upregulation of β-catenin and cyclin D1, which may account for late-stage tumor formation in these animals. Since β-catenin and cyclin D1 are indicators of an activated Wnt pathway, these findings raise an interesting possibility of cross-talk between MTA1 and the Wnt pathways.

**MTA3 regulation of the Wnt pathway.** In spite of a role for MTA3 in epithelial to mesenchymal transitions (EMT) in cancer cell lines (25), more recent data suggest that forced overexpression of MTA3 in the mammary epithelium leads to inhibition of ductal side branchin in virgin glands due to reduced cell proliferation and differentiation (42). Furthermore, MTA3-induced hypobranching in the mammary gland is mechanistically linked with the direct corepressor activity of the MTA3/HDAC complex on Wnt4 chromatin. These effects of MTA3 are opposite those of MTA1 in virgin mammary gland (41). These findings support the notion of physiological differences among the MTA family members and underscore the lack of functional redundancy among individual MTA proteins. Since the Wnt pathway controls the growth and differentiation of many organ systems, it will be important to know the potential contribution of MTA proteins in such tissues.

**MTA family members in lymphocyte fate determination.** In addition to EMT, MTA3 participates in B lymphocyte development (43), which is regulated in part by a negative feedback mechanism involving the transcriptional repression of BCL6 and Blimp-1 (44). Inhibition of BCL6 in germinal center B cells leads to derepression of Blimp-1, resulting in differentiation of these B cells into plasma cells. Such a “biological double circuit,” where BCL6 represses Blimp-1 expression and Blimp-1 represses BCL6 expression, controls B-cell differentiation (43,44). The MTA-dependent repression mechanism also participates in T-lymphocyte functions as MTA1 overexpression in T lymphocytes potentiates transcriptional repression mediated by BCL11B, whereas MTA1 depletion inhibits BCL11B transcriptional repression activity (45).

**Coactivation by MTA1, A New Avenue of Investigation**

In addition to the corepressor activities of MTA family members, a recent report suggests that MTA1 may also act as a coactivator of the breast cancer amplified sequence 3 (BCAS3) protein (46). In this context, MTA1 is acetylated at lysine 626 by histone acetyltransferase p300. Interestingly, the lysine 626 site of MTA1 is not conserved in MTA2 or MTA3. The acetylated MTA1 and RNA polymerase II (Pol II) complex are recruited onto the BCAS3 promoter in the vicinity of half-ERE elements. It appears that MTA1 upregulates BCAS3 gene expression by acting as a coactivator and thus may behave as a bifunctional coregulator. These findings raise the interesting possibilities that the corepressor versus coactivator activity of MTA1 may be influenced by MTA1 binding partners by posttranslational modifications of the protein, and by promoter context.

**What regulates the expression of MTA genes?** Although existing literature has identified a handful of downstream targets of MTA family proteins, knowledge of upstream regulators of the MTAs is limited. In this context, recent findings suggest that the expression of MTA3 in breast cancer cells is regulated by ERα and by...
AP1 and SP1 transcription factors (34,35). In contrast to MTA3, the MTA2 promoter is regulated by ETS-1 and SP1 (47). Expression of MTA1 is upregulated by heregulin beta 1, a ligand for the HER3 and HER4 receptors (26), as well as by hypoxia (38) (Fig. 2). Further, c-Myc regulates the expression of MTA1 by directly interacting with its promoter, and MTA1 is required for MYC-mediated transformation of fibroblast (48). Clearly, additional studies are needed to identify the pathways or signals that control the expression of MTA family members.

**Future Challenges and Concluding Remarks**

Recent developments in the area of MTA research have enriched our understanding of the role of this family of coregulators in transcriptional programs. Despite this progress, there are several urgent issues that deserve to be investigated in the future. These outstanding issues include the precise roles of MTA family proteins in NuRD complexes; the nature of posttranslational modifications that MTA family member are capable of undergoing; the biochemical and cellular basis of the dual coregulatory activity of MTA1; the upstream regulators of expression of MTA family members; the possibility that MTA proteins affect functions other than transcription; and are there NuRD-complex-independent functions of MTA family members? In addition to the pathogenic roles of the MTA family, we must understand the physiologic functions of these proteins, as they are widely expressed in normal tissues. Recent discoveries also raise the issue of whether this family of proteins should be renamed on the basis of structural motifs, as the current “metastasis-associated genes” nomenclature is not reflective of the physiologic functions of this family of proteins. In closing, the functional significance of the MTA family of coregulators is just beginning to emerge and may prove to have a profound influence on biologic processes in mammals.

**References**

Figure Legends

**Fig. 1. Schematic representation of components of NuRD and Sin3 complexes; and comparison of structural domains among MTA family members.** A, Composition of NuRD and Sin3 complexes. B, Schematic illustration of MTA1 protein. ZnF, zinc finger; BP1 and BP2 are bipartite nuclear localization signals; NLS-nuclear localization signal; AA, amino acids; PRO-rich, proline rich domain; Ac K626, lysine 626 acetylation. C, Comparison of various domains among the MTA family proteins. BAH, ELM and SANT domains located at the N-terminus are highly conserved whereas C-terminal region of MTA proteins is divergent. Identity (%), percentage of amino acid homology among MTA family members.

**Fig. 2. Emerging cellular targets of MTA family of proteins.** Upstream signals are shown by broken arrows and downstream signals are shown by solid arrows.

**Fig. 3. Deacetylation of nonhistones by MTA1/2/HDAC complex.** Although MTA2/HDAC and MTA1/HDAC complexes deacetylate p53 and HIF1α, but leads to opposing effects, i.e., destabilization and stabilization of p53 and HIF1α, respectively.
Figure 1
Figure 2

Diagram showing the interaction between various molecules and pathways such as HDAC, MTA1, MTA2, MTA3, ERα, and signaling pathways including ERα signaling, growth factors, hypoxia, and development in C. elegans.
Figure 3
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J. Biol. Chem. published online December 1, 2006

Access the most updated version of this article at doi: 10.1074/jbc.R600029200

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