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MTA1 regulates higher-order chromatin structure and histone H1-chromatin interaction *in-vivo*

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ABSTRACT

In the current study, for the first time, we found that metastasis-associated gene 1 (MTA1) was a higher-order chromatin structure organizer that decondenses the interphase chromatin and mitotic chromosomes. MTA1 interacts dynamically with nucleosomes during the cell cycle progression, prominently contributing to the mitotic chromatin/chromosome structure transitions at both prophase and telophase. We showed that the decondensation of interphase chromatin by MTA1 was independent of Mi-2 chromatin remodeling activity. H1 was reported to stabilize the compact higher-order chromatin structure through its interaction with DNA. Our data showed that MTA1 caused a reduced H1-chromatin interaction *in-vivo*. Moreover, the dynamic MTA1-chromatin interaction in the cell cycle contributed to the periodical H1-chromatin interaction, which in turn modulated chromatin/chromosome transitions. Although MTA1 drove a global decondensation of chromatin structure, it changed the expression of only a small proportion of genes. After MTA1 overexpression, the up-regulated genes were distributed in clusters along with down-regulated genes on chromosomes at parallel frequencies.

Abbreviations: MTA1, metastasis-associated gene 1; NuRD, nucleosome remodeling and histone deacetylase; NRL, nucleosome repeat length; HATs, histone acetyltransferases; HDACs, histone deacetyases; DE, differentially expressed; GO, gene ontology; CoIP, co-immunoprecipitation; MBD3, methyl-CpG binding domain protein 3; HMG, high mobility group; Pol II, RNA polymerase II.

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1. Introduction

In eukaryotes, genomic DNA molecules are hierarchically packaged into chromatin fibers. The basic unit of chromatin is the nucleosome, which consists of 147 base pairs (bp) of DNA wrapped in approximately 1.7 superhelical turns around the core histone octamer (Luger et al., 1997). The linear repeating array of nucleosomes comprises the primary chromatin structure, and interactions between nucleosomes stabilized by linker histone H1 drive the formation of a more condensed 30 nm fiber (Horn and Peterson, 2002; Tremethick, 2007), which can be further folded into increasingly compacted structures. Higher-order chromatin structure refers to the hierarchically condensed chromatin structures above the primary linear nucleosomal arrays, ranging from the less compacted 30 nm fiber (the first level of higher-order compaction) to the highly compacted metaphase chromosome architecture.

Chromatin undergoes various structural hierarchy during a cell cycle. Histone modifications lead to chromatin structure alteration, hence influencing DNA replication and transcription. Linker histone H1 or its variants, such as H5, plays a major role in stabilizing both the intermediate and extensively folded nucleosomal arrays (Carruthers et al., 1998; Hizume et al., 2005; Thoma et al., 1979). Histone H1 promotes and stabilizes the folding of linear nucleosomal arrays into higher-order chromatin structures (Allan et al., 1980; Bednar et al., 1998; Hamiche et al., 1996; Thoma et al., 1979; Travers, 1999). Binding of histone H1 to nucleosomal arrays affects the nucleosome repeat length (NRL) (Fan et al., 2003, 2005; Pearson et al., 1984; Woodcock et al., 2006), restricts mobility of nucleosomes (Pennings et al., 1994), and promotes chromatin folding; meanwhile, loss of H1 disables normal 30-nm chromatin fibers formation (Routh et al., 2008; Thoma et al., 1979), leads to decondensation of chromatin (Bates et al., 1981; Fan et al., 2005), and then facilitates DNA transcription. In view of the pivotal role of H1 on stabilizing higher-order chromatin structure, any factor that affects the association of H1 with chromatin will strikingly impact the compaction of chromatin and hence DNA-dependent processes.

ATP-dependent nucleosome remodeling and core histone modifications were reported to be other important factors affecting the high-order structure regulation (Varga-Weisz and Becker, 2006). Histone hyperacetylation loosens the compaction of chromatin structure, whereas its hypoacetylation condenses it (Ahringer, 2000). Nucleosome remodeling and histone deacetylation (NuRD) complex contains two components playing opposite roles in chromatin remodeling by ATPase Mi-2 subunit and chromatin condensing by histone deacetylase HDACs subunit. However, as a whole complex, NuRD generally displays the transcriptional repression property of HDACs, in which Mi-2 disrupts nucleosomes to facilitate the accessibility of HDACs to DNA (Wang and Zhang, 2001; Xue et al., 1998).

MTA1 was reported to be overexpressed in a variety of tumors and strongly promoted the malignant properties especially invasion and metastasis abilities of cancer cells (Toh and Nicolson, 2009). MTA1 is a nucleosome-bound protein (Nair et al., 2013). It has been described as an integral subunit of NuRD, providing clues to the chromatin-modifying roles of MTA1 (Nicolson et al., 2003). However, its actual role in chromatin structure regulation is relatively unknown. Since MTA1-containing NuRD is predominantly a transcriptional repressor, it is expected that MTA1 may work as a chromatin condensor. Herein, we attempted to assess the actual effect of MTA1 on chromatin and chromosome structure *in-vivo*, analyze the role of MTA1 in transcriptional regulation, and explore the possible underlying mechanisms. Unexpectedly, we found that MTA1 produced an overall chromatin decondensation instead of the expected condensation.

2. Materials and methods

2.1. Cell culture and transfection

The cell lines HEK293, NCI-H446, Hep3B, and HCT-8 were obtained from the American Type Culture Collection (ATCC). The cell lines HaCaT, Ishikawa, and SF767 were maintained in our laboratory as described previously (Boukamp et al., 1988; Nishida, 2002; Wyrick et al., 1997). All cell lines were cultured in Dulbecco's modified Eagle's medium (BIOROC, China) supplemented with 10% fetal bovine serum at 37 °C in a 5% CO2 standard incubator. DNA transfection was carried out using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions.

2.2. Plasmid construction and stable cell line establishment

The complete human MTA1 CDS was cloned by RT-PCR using the positive clones as template screened by *in-situ* plaque hybridization with the human lung phage library. pEGFP-MTA1 were constructed by inserting the complete human MTA1 CDS into the pEGFP-C2 vector (Clontech, USA), in which GFP tag was fused to the N terminus of MTA1. The EGFP tag fused to the C terminus of MTA1 was ligated after Xho I digestion and then inserted into the pCDNA3.1 vector (Life Technologies, USA) to yield the pCDNA3.1-MTA1-EGFP vector; MTA1-flag was constructed by PCR and inserted into the retroviral vector pMX-IRES-GFP. All sequences were verified by DNA sequencing.

The pMX-MTA1-flag-IRES-GFP was used to package retroviral particles and infect HCT116 colon cells to obtain stable MTA1-expressing cell lines, out of which HCT116-M1 and HCT116-M3 were identified with different overexpression levels and used in the present study. The HCT116 cell line with MTA1 stably knocked down was established by G418 selection after transfection of shRNA targeting the GACCCTGCTGGCAGATAAA sequence in HCT116 cells.

2.3. Antibodies and immunoprecipitation

Antibodies against MTA1 and histone deacetylase (HDAC)-2 (ab51832) were purchased from Abcam (USA); anti-Fibrillarin (#2396) and anti-phospho-Histone H3 (Ser10) (#3458) antibodies were purchased from Cell Signaling