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P300 binds to and acetylates MTA2 to promote colorectal cancer cells growth

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ABSTRACT

MTA2 is a member of metastasis associated family, which is highly expressed in several solid tumors and associated with tumor cells migration and invasion. Here, we report that MTA2 is acetylated at K152 and histone acetyltransferase p300 binds to and acetylates MTA2. Furthermore, mutation of the MTA2 acetylation site inhibits the growth of colorectal cancer cells and migration and invasion of Rat1 fibroblasts. These results reveal a novel post-translational regulation of MTA2 by the way of p300-dependent acetylation, which is important for tumor cells growth and migration and provides a potential target for clinical cancer research.

1. Introduction

Colorectal cancer is the third most commonly diagnosed cancer in the world and it is the fourth most common cause of cancer death after lung, stomach, and liver cancer [1]. Globally more than 1 million people were clinically diagnosed colorectal cancer every year [2] resulting in about 715,000 deaths as of 2010 up from 490,000 in 1990 [3].

Distant metastasis is the major cause of death for cancer patients, which is regulated by multiple pathways and involves several molecules events [4,5]. However, mechanisms of metastasis in colorectal cancer are still under investigation. Metastasis-associated tumor gene family (MTA) has three members: MTA1, MTA2 and MTA3. MTA1 over expressed in many solid tumors, including breast, esophageal, pancreatic, hepatocellular carcinoma [6], and correlated with cancer cell invasion and migration [7,8]. MTA2 has high homology with MTA1 in amino acid sequence, and it is also a component of nucleosome remodeling and histone deacetylase (NuRD) complex [9,10]. These results suggested that MTA2 may also play an important role on cancer cell growth and migration. Currently, the biological function of MTA2 in colorectal cancer has not been fully elucidated.

In present study, we focused on the biological function of MTA2's post-translational modification in colorectal cancer and reported that MTA2 is acetylated at K152 and histone acetyltransferase p300 binds to and acetylates MTA2. Furthermore, mutation of the MTA2 acetylation site inhibits the growth of colorectal cancer cells and migration and invasion of Rat1 fibroblasts.

2. Materials and methods

2.1. Cell lysis and immunological procedures

Cells were lysed in an NP40 buffer containing 50 mM Tris pH 7.5, 150 mM NaCl, 0.5% Nonidet P-40, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM Na_3VO_4 and 1 mM PMSF for 30 min and cell lysate was centrifuged at 13,000 rpm for 15 min at 4 °C. The supernatant was subjected to immunoprecipitation with Flag beads and then western analysis. Western blot was performed according to standard methods. Antibodies specific to Flag (Sigma), HA (Santa Cruz), Myc (Cell Signaling), β-actin (Sigma), MTA2 (Santa Cruz), p300 (Santa Cruz), pan anti-acetylated-lysine (Cell Signaling) and p300 siRNA (Santa Cruz) were purchased commercially.

2.2. P300 Knockdown

P300 Knockdown was carried out by using siRNA oligonucleotides synthesized from Genepharma, Shanghai. siRNA oligonucleotides were diluted with RNase free water to a concentration of 20 μM. 12.5 μl of siRNA was transfected using Lipofectamine 2000 (Invitrogen) and Opti-MEM (Invitrogen) according to the manufacturer's instructions. Cells were harvested 72 h after transfection. The knockdown efficiency was verified by straight Western blot of cell lysate. P300 siRNA sequence: 5′-AACAGAGCAGUCCUGGAUUAG-3′ [11,12].

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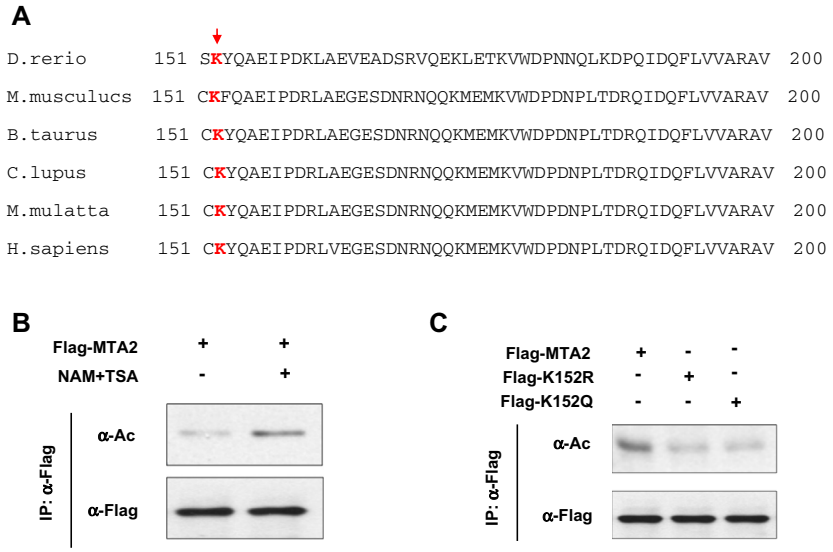

Fig. 1. MTA2 is acetylated at K152. (A) Amino acid sequence alignment of MTA2. 50 amino acids (151–200) from human (Hs; Homo sapiens) and five other vertebrates, are aligned. (B) MTA2 is acetylated. Flag-MTA2 was transfected into 293T cells followed by treatment with TSA and NAM for 8 h, and MTA2 acetylation and protein levels were analyzed by Western blot with indicated antibody, respectively. (C) K152 is the major acetylation site of MTA2. Wild-type MTA2 and K152 mutants were expressed in HEK293T cells, affinity purified and acetylation levels were analyzed by Western blot.

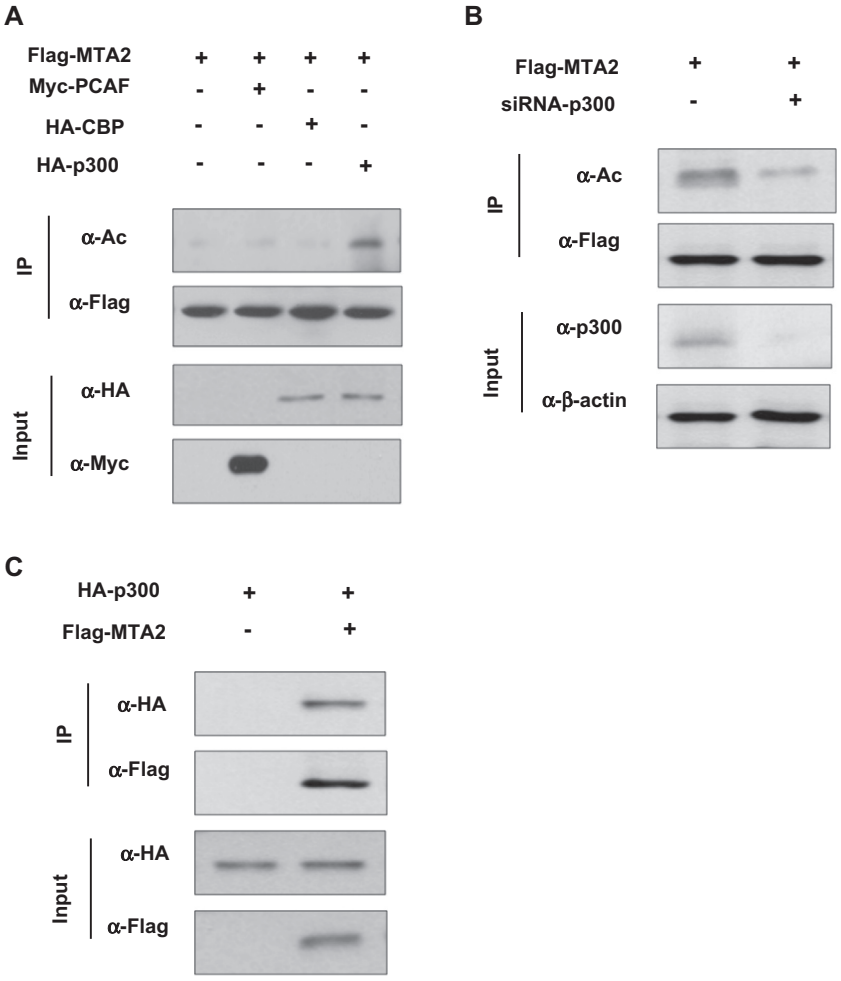

Fig. 2. P300 binds to and acetylates MTA2. (A) Over expression of p300 promotes acetylation of MTA2. Flag-MTA2 was co-expressed in HEK293T cells with three acetyltransferases (Myc-PCAF, HA-CBP and HA-p300), respectively as indicated. Flag-MTA2 proteins were purified with Flag beads and analyzed by Western blot. (B) Knocking down p300 reduces the acetylation level of MTA2. Acetylation levels of over expressed Flag-MTA2 in HEK293T cells with or without p300 knockdown using siRNA were analyzed by immunopurification with Flag beads and Western blot with acetylation antibody. (C) P300 interacts with MTA2. Flag-MTA2 was co-expressed in HEK293T cells with HA-p300, immunoprecipitated and analyzed by Western blot.

2.3. Cell proliferation analysis

5×10^4 HCT116 cells either express wild type or K152R mutant of MTA2 stablely were seeded in triplicate in 6-well plates, and cell numbers were counted every 24 h over a four-day period. Each count was carried out in triplicate.

2.4. Cell migration and invasion assays

Cell migration and invasion assays were carried out using Boyden chambers as described previously [13].

3. Results and discussion

3.1. MTA2 is acetylated at K152

Protein function can be regulated by different post-translational modifications, such as phosphorylation, ubiquitination and acetylation. Acetylation has been emerged as an important modification recently. It was reported that many important metabolic enzymes are modified and regulated by acetylation [14–16]. In addition to metabolism, diverse cellular processes, such as chromatin remodeling, cell cycle, splicing, nuclear transport, and actin nucleation can all be regulated by acetylation. Choudhary et al. did a systematic study of the mammalian acetylome, which identified 3600 acetylation sites on 1750 proteins [17]. Among these proteins, MTA2 was found to be acetylated at K152 in the mass spectrometry analysis of this study. Importantly, MTA2 K152 is highly conserved among all vertebrates (Fig. 1A). To confirm its acetylation, Flag-tagged MTA2 was ectopically expressed in HEK293T cells and immunoprecipitated. Western blotting with pan anti-acetylated-lysine antibody (abbreviate for α-Ac in the figures) confirmed that MTA2 was indeed acetylated and its acetylation level was significantly enhanced after treatment with trichostatin A (TSA, an inhibitor of histone deacetylase HDAC I and II) and nicotinamide (NAM, an inhibitor of the SIRT family deacetylases) for 8 h (Fig. 1B). To further confirm this finding, we generated two mutants (K152R and K152Q, with different charges) and compared their acetylation levels to wild-type MTA2 via Western blot with pan anti-acetylated-lysine antibody. We found that the acetylation levels of both mutants were significantly lower than wild type MTA2 (Fig. 1C), confirming that MTA2 is acetylated and that K152 is the main acetylation site of MTA2.

3.2. P300 binds to and acetylates MTA2

Given the localization of MTA2, we tested the effect of co-expression of three nuclear acetyltransferases (PCAF, CBP and p300) on the acetylation level of MTA2. Result demonstrated that over expression of p300 can notablely increase the acetylation level of MTA2, while PCAF and CBP have little effect (Fig. 2A), which indicated that p300 maybe the acetyltransferase of MTA2. To confirm this hypothesis, we knocked down p300 with siRNA in HEK293T cells expressing Flag-MTA2 and examined its effect on acetylation level of MTA2. Consistent with the over expression data, knocking down p300 significantly reduced MTA2 acetylation level (Fig. 2B). To further confirm the notion, we studied the interaction between Flag-MTA2 and HA-p300. Immunoprecipitation and Western blot analysis demonstrated that p300 can bind to MTA2 (Fig. 2C), supporting the notion that p300 is the acetyltransferase of MTA2. Taken together, p300 binds to and acetylates MTA2.

3.3. Mutation of the MTA2 acetylation site inhibits the growth of colorectal cancer cells

To determine whether the acetylation regulation of MTA2 is important for colorectal cancer cells growth, we first set up HCT116 cell lines which stablely express wild-type MTA2 or the acetylation resistant mutant K152R, respectively (Fig. 3A). Then we checked the proliferation rate of these two colorectal tumor cell lines. We found that HCT116/MTA2 wild-type cells proliferated 2.1-fold as fast as the HCT116/MTA2 K152R mutant cells (Fig. 3B), demonstrating a growth advantage was conferred by the acetylation regulation of MTA2 by p300 to the colorectal cancer cells.

3.4. Mutation of the MTA2 acetylation site inhibits the migration and invasion of Rat1 fibroblasts

To further determine the functional significance of acetylation regulation of MTA2, we assessed the role of MTA2 acetylation in migration and invasion. We next studied the biological characteristics of Rat1 fibroblasts expressing MTA2 or MTA2 K152R mutant, respectively (Fig. 4A). Interestingly, over expression of wild-type MTA2, showed much higher motility (Fig. 4B) and invasiveness (Fig. 4C) as compared with cells over expressing MTA2 K152R mutant, demonstrating a promotion of migration and invasion of MTA2 acetylation, which further indicates that the acetylation of MTA2 is critical for tumor metastasis.

It was reported that both mouse and human MTA2 were regulated by transcription factor specificity protein 1 (Sp1) [18,19]. However, the biological function of posttranslational regulation of MTA2 in tumor cell growth and metastasis is still unclear. In the present study, we found that p300 binds to and acetylates

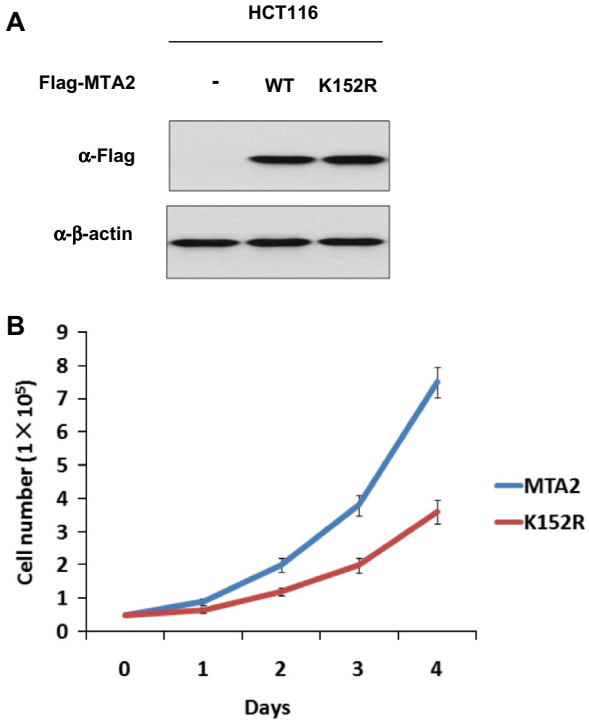

Fig. 3. Mutation of the MTA2 acetylation site inhibits the growth of colorectal cancer cells. (A) Expression of MTA2 and the MTA2 K152R mutant in HCT116 cells. Whole cell lysates from stable cell lines expressing MTA2 and MTA2 K152R were prepared and analyzed by Western blot. (B) Mutation of MTA2 K152 inhibits the growth of colorectal cells. 5×10^4 wild-type MTA2 and MTA2 K152R mutant cells were seeded in each well. Cell numbers were counted every 24 h over a four-day period. Error bars represent ± SD for triplicate experiments.

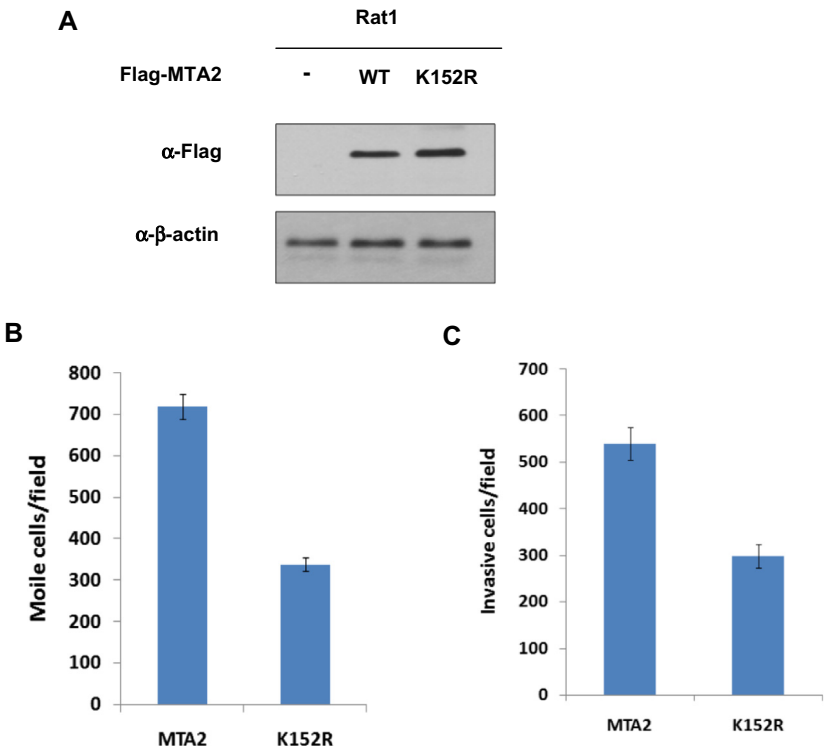

Fig. 4. Mutation of the MTA2 acetylation site inhibits the migration and invasion of Rat1 fibroblasts. (A) Expression of MTA2 and the MTA2 K152R mutant in Rat1 fibroblasts. Whole cell lysates from stable cell lines expressing MTA2 and MTA2 K152R were prepared and analyzed by Western blot. (B) The effect of MTA2 acetylation site mutation on cell migration in Rat1 cells. Five-thousand Rat1 cells expressing wild-type MTA2 and MTA2 K152R stablely were loaded onto an insert of Boyden chamber with a pore size of 8 mm. (C) The effect of MTA2 acetylation site mutation on cell invasiveness in Rat1 cells.

MTA2. Mutation of MTA2 acetylation site inhibits the growth of colorectal cancer cells and migration and invasion of Rat1 fibroblasts. These findings demonstrate an important role of acetylation regulation of MTA2 in tumor growth and metastasis and provide a potential target for clinical cancer research and treatment.

4. Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

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