Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

GATA3 acetylation at K119 by CBP inhibits cell migration and invasion in lung adenocarcinoma

Xueying Li [a], Jiaqi Jin [a], Siyuan Yang [a], Weizhi Xu [a], Xianbin Meng [b], Haiteng Deng [b], Jun Zhan [a], Shan Gao [c, **], Hongquan Zhang [a, *]

[a] Department of Anatomy, Histology and Embryology, MOE Key Laboratory of Carcinogenesis and Translational Research, State Key Laboratory of Natural and Biomimetic Drugs, Peking University Health Science Center, Beijing, 100191, China
[b] MOE Key Laboratory of Bioinformatics, School of Life Sciences, Tsinghua University, Beijing, 100084, China
[c] CAS Key Laboratory of Bio-medical Diagnostic, Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou, 215163, China

ARTICLE INFO

Article history:
Received 28 January 2018
Accepted 13 February 2018
Available online xxx

Keywords:
GATA3
Acetylation
CBP
Cell migration
Cell invasion
Lung adenocarcinoma cells

ABSTRACT

GATA3 is a transcriptional factor involved in the development of multiple organs. Post translational modifications of GATA3 are critical to its function. Here, we report that GATA3 interacts with and is acetylated by the acetyltransferase CBP. Class I deacetylases HDAC1, HDAC2 and HDAC3 deacetylate GATA3. The major acetylated site of GATA3 in lung adenocarcinoma cells was determined at lysine 119 (AcK119). Functionally, GATA3-acetylation mimics K119Q mutant was found to inhibit lung adenocarcinoma cell migration and invasion with concomitant downregulation of EMT-controlling transcriptional factors Slug, Zeb1 and Zeb2. Taken together, we demonstrated that GATA3 acetylation at lysine 119 by CBP hinders the migration and invasion of lung adenocarcinoma cells.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

The GATA family, consisting of a series of transcription factors, has been reported to play vital roles in development [1,2]. In vertebrates, six GATA factors have been discovered, and in the nematode *caenorhabditis elegans* there can be as much as eleven, while only three or four were identified in Drosophila [3,4]. The GATA family members can specifically bind to (A/T) GATA (A/G) DNA sequence, and also contain specific domains, including transactivation domains and zinc fingers [1,2].

GATA3, an important member of GATA factors, is firstly reported to be pivotal in T-cell differentiation and thymocyte development [5–7]. Furthermore, critical roles of GATA3 in development of different human organs have been discovered as well [8–12]. GATA3 can regulate breast cancer progression. The expression level of GATA3 is generally high in well differentiated breast cancers and cancers with estrogen receptor (ER)-positive and lymphoid node-negative [13–16]. Some key genes involved in breast cancer progression can also be regulated by GATA3 [17]. GATA3 acts as a suppressor in breast cancer metastasis, for it can mediate the reversal of epithelial-mesenchymal transition and thus promotes epithelial differentiation of invasive cancer cells [18,19]. Further, GATA3 is also involved in the development of nervous system [20], kidney [21], and skin [22].

Several post-translational modifications (PTM) of GATA3 have been found. The phosphorylation of GATA3 can regulate IFNγ expression in memory Th2 cells [23]. GATA3 methylation at arginine 261 regulates the transactivation of interleukin-5 gene in T helper 2 cells [24]. In addition, GATA3 ubiquitination by ubiquitin ligase SCFFBXW7α leads to its degradation [25]. The KRRLSA motif of GATA3, which is located between the two zinc fingers, can be modified by acetylation and phosphorylation. The hypoacetylated KRR mutant (KRR-AAA) of GATA3 was found to function in a dominant-negative manner [26]. Furthermore, the lysine 305 in KRR (K305) as well as K293 and K347 nearby could be acetylated by P300. This potent negative form is indeed resulted from the acetylation deficiency at K305 and acetylation inhibition in the

* Corresponding author. Laboratory of Molecular Cell Biology and Tumor Biology, Department of Anatomy, Histology and Embryology, Peking University Health Science Center, #38 Xue Yuan Road, Beijing, 100191, China.
** Corresponding author.
E-mail addresses: Gaos@Sibet.ac.cn (S. Gao), Hongquan.Zhang@bjmu.edu.cn (H. Zhang).

https://doi.org/10.1016/j.bbrc.2018.02.120
0006-291X/© 2018 Elsevier Inc. All rights reserved.

Please cite this article in press as: X. Li, et al., GATA3 acetylation at K119 by CBP inhibits cell migration and invasion in lung adenocarcinoma, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.02.120

neighboring lysines (K293 and K347). GATA3 acetylation in immune system clearly explained T-cell survival and homing [27]. However, no GATA3 acetylation with human cancers so far has been known. Although lysine acetylation was firstly identified on histone that regulates gene transcription [28,29], acetylation of some non-histone proteins has been recently reported to be vital in the regulation of cancer progression, including p53, STAT3, EZH2 and HOXB9 [30–34].

In this report we identified that GATA3 can be acetylated at lysine 119 by acetyltransferase CBP. We demonstrated that GATA3-AcK119 contributes to the inhibition of lung adenocarcinoma cell migration and invasion. Our findings clearly established the regulatory role of GATA3 acetylation in lung adenocarcinoma cell progression.

2. Materials and methods

2.1. Cell culture, transfection and treatment

Human embryonic kidney cell line HEK-293T was cultured in DMEM. Human lung adenocarcinoma cell line H1299 was cultured in RPMI1640.10% fetal bovine serum, 100 units/ml penicillin and 100 mg/ml streptomycin were added into the medium and cells were cultured at 37 °C with 5% CO_2. Polyethylenimine reagent (PEI) was used for plasmid transfection. The histone deacetylase (HDAC) inhibitor TSA and class III sirtuin (SIRT) inhibitor nicotinamide were purchased from Sigma. TSA was added at a final concentration of 3 μM for 12 h before harvest. Nicotinamide was added 5 mM for 12 h before harvest.

2.2. Constructs

To generate the FLAG tagged GATA3, full-length GATA3 cDNA was amplified by polymerase chain reaction (PCR) and subcloned into 3×FLAG vector (Sigma). The FLAG-GATA3 mutants were generated by the QuikChange Site-Directed Mutagenesis Kit (Stratagene). To generate the GST-fusion proteins of GATA3, the cDNA sequence for the N240 (1–240), N300 (1–300), N370 (1–370) and 300C (300–444) domains were amplified by PCR and subcloned into pGEX-4T-1 vector (GEHealthcare, USA).

2.3. Antibodies

Acetylated-Lysine (Cell Signaling Technology #9441), GATA3 (Cell Signaling Technology #5852), FLAG (Sigma F1804), HA (Abcam ab9110).

2.4. Co-immunoprecipitation and western blotting

Cells lysates were prepared using NP40 lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP40, 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM sodium butyrate) containing protease inhibitors on ice for 30 min. For co-immunoprecipitations, lysates from cells transfected with FLAG-tagged plasmid were incubated with anti-FLAG M2 beads (Sigma) at 4 °C for 2 h. After washed with lysis buffer for three times, the immunoprecipitated complexes were resolved by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with the indicated antibodies.

2.5. In vitro acetylation assay

GST-fusion GATA3 were incubated with HA-CBP purified from HEK293T cells, in 50 μl acetyltransferase assay buffer (50 mM Tris–HCl, pH 8.0, 10% glycerol, 0.1 mM EDTA and 1 mM dithiothreitol) with 20 μM acetyl CoA at 30 °C for 2 h. Reaction mixtures were resolved by SDS-PAGE and analyzed with indicated antibodies.

2.6. Glutathione S-Transferase (GST) pull-down

GST-fused constructs were expressed in BL21 Escherichia coli induced with IPTG (Isopropyl-β-D-thio-galactoside) and purified with Glutathione Sepharose 4B (GE Healthcare). The cellular supernatants were pre-cleared with Glutathione-Sepharose 4B beads, and incubated with beads containing GST fusion proteins at 4 °C overnight. After washed for 5 times, the beads were resuspended with 2×SDS loading buffer, boiled for 10min and analyzed by western blotting.

2.7. Cell migration and invasion assays

Cell migration assays: Cells were digested, resuspended and counted. 1×10^5 cells were added to the top chamber of 24-well. The lower wells contain 20% FBS. After cultivating for 6 h at 37 °C, the migrated cells were fixed with 4% formaldehyde and stained by crystal violet.

Cell invasion assays: 1×10^5 cells were added to the top chamber of 24-well coated with Matrigel. The lower wells contain 20% FBS. After 48 h cultivating at 37 °C, cells invaded to the lower surface were fixed and stained. The number of the cells were counted and analyzed.

2.8. Statistical analysis

Statistical calculations were analyzed by Prism 5 (GraphPad). Results were expressed as mean ± SEM. Student's t-test was applied for a single comparison of two groups. * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$. Survival analyses were obtained from Kaplan-Meier analysis.

3. Results

3.1. GATA3 is acetylated at residue K119 by CBP

GATA3 is important in development and differentiation. GATA3 can be acetylated by P300 in T cells and affects T cell survival and homing [27]. However, there were no reports about the acetylation of GATA3 in human cancer cells. To this end, we detected whether GATA3 is acetylated in human lung adenocarcinoma cells. First, we identified that endogenous GATA3 can be co-immunoprecipitated by an anti-acetylated-lysine antibody, suggesting that endogenous GATA3 is an acetylated protein in living cells (Fig. 1A). Second, FLAG-GATA3 was co-immunoprecipitated with anti-FLAG M2 beads and examined with an anti-acetylated-lysine antibody. Results showed that exogenous GATA3 can also be acetylated in H1299 cells (Fig. 1B). To identify which acetyltransferase may acetylate GATA3, we co-transfected FLAG-GATA3 with a variety of acetyltransferases including CBP, P300, PCAF and MOF in H1299 cells. Interestingly, GATA3 could only be acetylated by CBP (Fig. 1C). Moreover, GATA3 was acetylated by CBP in a dose-dependent manner (Fig. 1D). Further, we performed *in vitro* acetylation assays using GST-GATA3 purified from *Escherichia coli* and HA-CBP purified from HEK293T cells. Results showed that GST-GATA3 was indeed acetylated *in vitro* by CBP (Fig. 1E). Taken together, GATA3 can be acetylated by CBP both *in vivo* and *in vitro*.

To identify the acetylation sites of GATA3, we co-transfected FLAG-GATA3 with HA-CBP into H1299 cells. We enriched FLAG-GATA3 with anti-FLAG M2 beads and then FLAG-GATA3 was analyzed by LC-MS/MS. As a result, five acetylated lysine residues

Please cite this article in press as: X. Li, et al., GATA3 acetylation at K119 by CBP inhibits cell migration and invasion in lung adenocarcinoma, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.02.120

Download English Version:

https://daneshyari.com/en/article/8294015

Download Persian Version:

https://daneshyari.com/article/8294015

Daneshyari.com