Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Stabilization of HDAC1 via TCL1-pAKT-CHFR axis is a key element for NANOG-mediated multi-resistance and stem-like phenotype in immune-edited tumor cells

Seon Rang Woo [a, b, c], Hyo-Jung Lee [a, b, c], Se Jin Oh [a, b, c], Suyeon Kim [a, b, c], Sang-Hyo Park [d], Jaeyoon Lee [e], Kwon-Ho Song [a, b, c, **], Tae Woo Kim [a, b, c, *]

[a] Department of Biochemistry & Molecular Biology, Korea University College of Medicine, Seoul, Republic of Korea
[b] Department of Biomedical Science, College of Medicine, Korea University College of Medicine, Seoul, Republic of Korea
[c] Translational Research Institute for Incurable Diseases, Korea University College of Medicine, Seoul, Republic of Korea
[d] School of Biosystem and Biomedical Science, Korea University, Seoul, Republic of Korea
[e] College of Science, College of Social Sciences and Humanities, Northeastern University, Boston, MA, USA

ARTICLE INFO

Article history:
Received 16 July 2018
Received in revised form
23 July 2018
Accepted 23 July 2018
Available online xxx

Keywords:
NANOG
HDAC1
CHFR
Immunotherapy
Chemoresistance
Immuneresistance

ABSTRACT

Cancer immunoediting enriches NANOG expression in tumor cells, resulting in multi-drug resistance and stem-like phenotypes. We previously demonstrated that these NANOG-associated phenotypes are promoted through HDAC1 transcriptional upregulation. In this study, we identified that NANOG also contributes to the stabilization of HDAC1 protein through the AKT signaling pathway. NANOG-AKT axis leads to phosphor-dependent inactivation of CHFR, an E3 ligase for HDAC1 protein, and thereby inhibiting the ubiquitin-mediated degradation of HDAC1. Furthermore, AKT inhibition disrupts HDAC1 WT-mediated phenotypes but had no effect on the phenotypes mediated by HDAC1 FM, a mutant that is unable to interact with CHFR. Critically, we applied a catalytic dead mutant, HDAC1-H141A, to uncover that HDAC1 confers immune-resistance, drug-resistance and stem-like phenotype in tumor cells through its catalytic activity. Collectively, our results establish a firm molecular link in immune-edited tumor cells among NANOG, AKT, CHFR, and HDAC1, identifying HDAC1 as a molecular target in controlling NANOGHIGH immune-refractory cancer.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Utilizing the immune system to combat tumor cells has been the central goal of immunotherapy [1]. However, despite immunotherapy's potential to revolutionize cancer treatment, the presence of immune-edited tumor cells limits its clinical application [2]. Immune selection contributes to the generation of cancer cells that have better survival advantages and eventually leads to the enrichment of cancer cells with stem-like properties [3]. Substantial effort to elucidate the molecular basis of these stem-like properties revealed that many of these molecular mechanisms have been linked to an epigenetic alteration of tumor cells [4].

Histone deacetylase (HDAC), an epigenetic modifier, plays an important role in regulating cell proliferation and differentiation [5]. Of the four classes of HDAC found in mammals, HDAC1 was found to be upregulated in relapsed tumor cells after treatments, while inhibition of the HDACs enhanced the anti-tumor effect of the treatment [6,7]. Despite the crucial roles of HDAC1 in tumorigenesis as well as the development of resistance against cancer therapy, the molecular mechanisms in the regulation of HDAC1 expression have not yet been extensively studied. Therefore, an in-depth understanding of the underlying molecular mechanisms in regulating HDAC1 expression is crucial for developing new strategies to combat against the multi-aggressive phenotypes of tumor cells.

Tracing one of the determinants of HDAC1 protein level among

* Corresponding author. Laboratory of Tumor Immunology, Korea University College of Medicine, Room 319, Moonsook Medical Hall, 73 Inchon-ro, Sungbuk-gu, Seoul, 02841, Republic of Korea.
** Corresponding author. Laboratory of Tumor Immunology, Korea University College of Medicine, Room 319, Moonsook Medical Hall, 73 Inchon-ro, Sungbuk-gu, Seoul, 02841, Republic of Korea.
E-mail addresses: kwonho@korea.ac.kr (K.-H. Song), twkim0421@korea.ac.kr (T.W. Kim).

https://doi.org/10.1016/j.bbrc.2018.07.118
0006-291X/© 2018 Elsevier Inc. All rights reserved.

Please cite this article in press as: S.R. Woo, et al., Stabilization of HDAC1 via TCL1-pAKT-CHFR axis is a key element for NANOG-mediated multi-resistance and stem-like phenotype in immune-edited tumor cells, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.118

tumor cells, studies have identified CHFR as a key regulator of the HDAC protein [8]. CHFR, an E3 ubiquitin-protein ligase, functions as checkpoint protein in the mitotic process by delaying entry into metaphase [9]. In cancer, CHFR is considered a tumor suppressor as it is frequently inactivated during tumorigenesis and participates in the process of cell cycle arrest [10]. Specifically, the biochemical function of CHFR is regulated in part by AKT signaling, which phosphorylates CHFR to promote mitotic progression [11]. However, despite the crucial role in regulating HDAC1, the underlying mechanism behind the inactivation of CHFR has not yet been extensively studied in immune-resistant tumor cells.

One of the key regulators in maintaining the tumor-initiating stem-like cells is NANOG, a homeodomain-containing transcription factor that's pivotal for tumorigenesis as well as cell potency [12,13]. Recently, we demonstrated that NANOG upregulates HDAC1 expression through promoter occupancy, thereby leading to a decrease of AcH3K14 and AcH3K27 [14]. In the study, we have found that HDAC1 protein level was remarkably increased by NANOG when compared to its mRNA level. The result indicates that NANOG also could affect post-transcriptional regulation of HDAC1. However, the underlying mechanisms responsible for NANOG-mediated regulation of HDAC1 protein have remained unclear.

In the present study, we demonstrate that NANOG stabilizes HDAC1 protein through the inactivation of CHFR, via NANOG-TCL1-pAKT axis and that the accumulation of HDAC1 results in CSC-like and multi-resistant phenotypes through its catalytic activity in immune-edited tumor cells.

2. Materials and methods

2.1. Cells

CaSki, HeLa and HEK293 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Generation of the immune resistant CaSki P3 and CaSki-NANOG cell lines were have been previously described [14]. All cells were grown at 37 °C in 5% CO_2 incubator/humidified chamber.

2.2. DNA constructs and site-directed mutagenesis

The p3 × FLAG CMV 7.1-NANOG and p3 × FLAG CMV 7.1-HDAC1 plasmids have been previously described [13,14]. p3 × FLAG CMV10 CHFR was kindly provided by Dr. Seol of Seoul National University. Site directed mutagenesis was performed using a QuickChange XL Site-directed Mutagenesis kit (Stratagene, San Diego, CA, USA) according to the manufacturer's instructions. For the generation of HDAC1 H141A construct, the following primer set was used: 5′-GGGCCTGCACGCTGCAAAGAAGTCCGAGGCATCTGGCT-3’ (forward) and 5′-GACTTCTTTGCAGCGTGCAGGCCCCCAGCCCAATTCACAG-3’ (reverse). For the generation of HDAC1 FM construct, the following primer set was used: F287Y, 5′-CCAAGTGTGTGGAATATGTCAAGAGCTTTAACCTGCCTATG-3’ (forward) and 5′-GTTAAAGCTCTTGACATATTCCACACACTTGGCGTGTCC-3’ (reverse); M297I, 5′-ACCTGCCTATGCTGATTCTGGGAGGCGGTGGTTACACCA-3’ (forward) and 5′- CCACCGCCTCCCAGAATCAGCATAGGCAGGTTAAAGCTC-3’ (reverse). For the generation of CHFR-TASA construct, the following primer sets: T39A, 5′-AAGCGGGAGTGGGCCATCGGGC GGAGA-3’ (forward) and 5′-TCTCCGCCCGATGGCCCACTCCCGCTT-3’ (reverse); S205A, 5′-AGAGCGTT CCTCCGCTTGTGGGTCTGGGG-3’ (forward) and 5′-CCCCAGACCCACAAGCGGAGGAACGCTCT-3’ (reverse). Mutations were verified by DNA sequencing.

2.3. siRNA constructs

Synthetic small interfering RNAs (siRNAs) specific for GFP, HDAC1, AKT, TCL1a and CHFR were purchased from Bioneer (Daejeon, Korea); Non-specific GFP (green fluorescent protein), 5′-GCAUCAAGGUGAACUUCAA-3’ (sense), 5′-UUGAAGUUCACCUUGAUGC-3’ (antisense); HDAC1, 5′-GAGUCAAAACAGAGGAUGA-3’ (sense), 5′-UCAUCCUCUGUUUUGACUC-3’ (antisense); AKT, 5′-GACAACCGCCAUCCAGACU-3’ (sense), 5′- AGUCUGGAUGGCGGUUGUC-3’ (antisense); TCL1a, 5′-GCCCUUAACCAUCGAGAUA -3’ (sense), 5′-UAUCUCGAUGGUUAAGGGC-3’ (antisense); CHFR, 5′-GAGAAGGGCAAGUUUAUCA-3’ (sense), 5′-UGAUAAACUUGCCCUUCUC-3’ (antisense).

2.4. Real-time quantitative RT-PCR

Real-time quantitative PCR was performed as described previously [15] with the following primer set; HDAC1, 5′-GGTCCAAATGCAGGCGATTCCT-3’ (forward) and 5′-TCGGAGAACTCTTCCTCACAGG-3’ (reverse); β-ACTIN, 5′-CATGTACGTTGCTATCCAGGC-3’ (forward) and 5′-CTCCTTAATGTCACGCACGAT-3’ (reverse). Relative quantification of the mRNA levels was performed using the comparative Ct method with β-ACTIN as the reference gene.

2.5. Western blot analysis

Western blot analysis was performed as described previously [16]. Primary antibodies against HDAC-1, AKT, phospho-AKT (S473) and TCL-1 (Cell Signaling Technology, Danvers, MA, USA); β-ACTIN and FLAG (Sigma Aldrich, St Louis, MO, USA); NANOG and CHFR (Abnova, Taipei, Taiwan); and HA (MBL, Nagoya, Japan) were used Western blotting followed by the appropriate secondary antibodies conjugated with horseradish peroxidase. Immunoreactive bands were developed with the chemiluminescence ECL detection system (Elpis Biotech, Daejeon, Korea), and signals were detected using a luminescent image analyzer (LAS-4000 Mini, Fujifilm, Tokyo).

2.6. In vivo ubiquitination assay

Cells were transfected with HA-tagged ubiquitin or empty vector. After 24 h, the cells were treated with 10 μM of MG132 for 8 h and lysed by incubation with two volumes of TBS containing 2% SDS at 95 °C for 10 min. After adding eight volumes of TBS containing 1% Triton X-100, lysates were sonicated and then immunoprecipitated with anti-HDAC1 antibody coupled to TrueBlot anti-mouse Ig IP beads. The beads were washed with TBS, eluted by boiling in SDS sample buffer and immunoblotted using anti-HA antibody.

2.7. Immunoprecipitation

To estimate phosphorylation of CHFR, HEK293 cells expressing the indicated constructs were cultured for 48 h, and whole-cell lysates were prepared with NP40 lysis buffer (50 mM Tris-HCL, pH 8.0, 5 mM EDTA, 150 mM NaCl, 1% NP-40) containing protease inhibitor. Immunoprecipitation was carried out by incubation with 1 μg of anti-FLAG (M185-3L, MBL) antibody or mouse IgG for 16 h. After incubation with TrueBlot anti-mouse Ig IP beads, the beads were washed with TBS, eluted by boiling in SDS sample buffer and immunoblotted using anti-AKT substrate antibody.

2.8. Apoptosis assay

For cisplatin-mediated apoptosis assay, cells were treated with 10 μM cisplatin for 24 h. For granzyme B-mediated apoptosis assay, recombinant human granzyme B (Enzo Life Sciences, Farmingdale, USA) was mixed with the BioPorter reagent (Sigma-Aldrich). Cells were treated with BioPorter-granzyme B complex for 4 h. The

Please cite this article in press as: S.R. Woo, et al., Stabilization of HDAC1 via TCL1-pAKT-CHFR axis is a key element for NANOG-mediated multi-resistance and stem-like phenotype in immune-edited tumor cells, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.118

Download English Version:

https://daneshyari.com/en/article/8956030

Download Persian Version:

https://daneshyari.com/article/8956030

Daneshyari.com