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Proteomic changes induced by histone demethylase JMJD3 in TNF alpha-treated human monocytic (THP-1) cells

Amitabh Das [1], Nando Dulal Das [1], Kyoung Hwa Jung, Ji Hyun Park, Hyung Tae Lee, DalMuri Han, Mi Ran Choi, Sung Chul Kang, Young Gyu Chai *

Department of Molecular & Life Science, Hanyang University, Ansan, 426-791, Republic of Korea

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ABSTRACT

JMJD3, a Jumonji C family histone demethylase, plays an important role in the regulation of inflammation induced by the transcription factor nuclear factor-kappa B (NF-κB) in response to various stimuli. JMJD3 is a histone-3 lysine-27 trimethylation (H3K27me3) demethylase, a histone mark associated with transcriptional repression and activation of a diverse set of genes. The present study assessed stable JMJD3 knockdown (KD)-dependent proteomic profiling in human leukemia monocyte (THP-1) cells to analyze the JMJD3-mediated differential changes of marker expression in inflammatory cells. To analyze the protein expression profile of tumor necrosis factor-alpha (TNF-α)-stimulated JMJD3-kd THP-1 cells, we employed matrix-assisted-laser-desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). Additionally, Ingenuity Pathways Analysis (IPA) was applied to establish the molecular networks. A comparative proteomic profile was determined in TNF-α-treated both of JMJD3-kd THP-1 cells and THP-1 scrambled (sc) cells. The expression of tripartite motif protein (TRIM5), glutathione peroxidase (GPx), glia maturation factor-γ (GMFG), caspase recruitment domain family, member 14 (CARMA2), and dUTP pyrophosphatase were significantly down-regulated, whereas heat shock protein beta-1 (HspB1) and prohibition were significantly up-regulated in JMJD3-kd THP-1 cells. The molecular and signaling networks of the differentially expressed proteins in JMJD3-kd THP-1 cells were determined by IPA. The molecular network signatures and functional proteomics obtained in this study may facilitate the suppression of different key inflammatory regulators through JMJD3-attenuation, which would be crucial to evaluate potential therapeutic targets and to elucidate the molecular mechanism of JMJD3-kd dependent effects in THP-1 cells.

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1. Introduction

Histone modifications regulate gene transcription by affecting chromatin compaction, DNA accessibility, and recruitment of transcription machinery and regulators. Various modifications such as methylation, phosphorylation, acetylation, and ubiquitination occur at residues in the amino- and carboxyl-terminal tails of core histones in several different ways (Cheung et al., 2000; Berger, 2007). Among these, modifications by histone acetylation and methylation are the most common. Regulation of a variety of nuclear processes dedicated to the maintenance of active and silent states of gene expression require histone methylation, which is essential for cellular regulation, homeostasis, and fate determination (Cloos et al., 2008). Multiple lysine residues on histones, including H3K4, H3K9, H3K27, and H3K36, are methylated by histone methyltransferases and demethylated by histone demethylases. A large number of demethylases with the ability to demethylate specific histone lysine and arginine residues via amine oxidation, hydroxylation, or deamination have been discovered (Shi and Whetstine, 2007).

JMJD3, a JmjC family histone demethylase, erases H3K27me3, a histone mark associated with transcriptional repression and involved in lineage determination. H3K27me3 is crucial for gene silencing. To allow commitment and differentiation to various cell lineages, this silencing state can be altered through the activity of the JMJD3 demethylase. Importantly, NF-κB-induced JMJD3 up-regulation relates inflammation to the control of histone modification involved in lineage determination, differentiation, and tissue homeostasis, which may provide a connection between chronic inflammation and the associated alterations of

* Corresponding author at: Department of Molecular & Life Science, Hanyang University, 1271 Sa 3-dong, Ansan, Gyeonggi-do 426-791, Republic of Korea. Tel.: +82 31 400 5513; fax: +82 31 406 6316.
E-mail addresses: amitabhdas.kn@gmail.com (A. Das), nando.hu@gmail.com (N.D. Das), khjung2@gmail.com (K.H. Jung), b88424@gmail.com (J.H. Park), photod@naver.com (H.T. Lee), holystom@gmail.com (D. Han), miran.choi@hotmail.com (M.R. Choi), gujiju11@gmail.com (S.C. Kang), ygchai@hanyang.ac.kr (Y.G. Chai).
[1] These authors contributed equally to this work.

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Table 1
Up-regulated proteins in 2 h TNFα-treated JMJD3-kd THP-1 cells.

Protein name	Spot no.	THP1 sc	THP1 JMJD3kd	Ratio	Est'd *Z*	Access number
FA81A_HUMAN RecName: Full = Protein FAM81A	2524	1	64,919	64,919	2.43	gi74730462
WDR67 protein	4423	1	17,182	17,182	1.14	gi20072649
Hypothetical protein	1505	1	8322	8322	2.43	gi6599170
hCG2010950	1312	1	3850	3850	1.1	gi119578959
Keratin 10	4424	1	2400	2400	2.26	gi186629
Unnamed protein product	1722	1	528	528	2.43	gi194390376
Heat shock protein beta-1	5302	1	244	244	2.43	gi4504517
Deoxyribonucleoside 5′-monophosphate N-glycosidase isoform 1	2217	28	2235	79.82	0.94	gi5454002
Heat shock 27 kDa protein 1	4313	554	11,027	19.9	1.32	gi15126735
Beta actin variant	5510	8718	81,515	9.35	2.43	gi62897625
Prohibitin	5409	640	5129	8.01	2.43	gi46360168
Folate binding protein	2401	45,765	116,687	7.4	0.23	gi2565194
Unnamed protein product	2525	2402	15,816	6.58	1.43	gi16550217
Nucleoporin like 2, isoform CRA_d	6615	110	622	5.65	1.33	gi119614184
Tat binding protein 1, TBP-1 = transcriptional activator [human, Peptide, 439 aa]	3623	3335	14,581	4.37	2.43	gi263098
Zinc finger protein 716	5728	1278	4335	3.39	1.88	gi226529227
Interleukin 3 receptor, alpha	2611	19,382	64,919	3.349	1.43	gi57163101
Protein disulfide-isomerase A3 precursor	6714	36,596	95,179	2.6	2.43	gi21361657
Unnamed protein product	6510	9660	18,098	1.87	2.43	gi194376310

differentiation (De Santa et al., 2009). As histone methylation is a reversible dynamic process mediated by methyltransferases and demethylases, whether and how these processes are regulated in acute to chronic inflammatory responses has yet to be elucidated. H3K27 methylation, marked as a repressive histone modification, acts by recruiting polycomb complexes that identify a checkpoint regulator of inducible gene expression. It is reported that JMJD3 is up-regulated after LPS stimulation and can induce expression of a subset of genes, such as BMP2 and HOX genes, through H3K27 demethylase function (De Santa et al., 2007). Interestingly, this repressive histone modification by JMJD3 could be one of the targets of the anti-inflammatory signaling pathway and gene specific inhibition of inflammatory responses (Medzhitov and Horng, 2009).

Several histone modifications have been reported by the differential regulation of JMJD3 and NF-κB targeted genes. However, very little work has been done to determine the importance of proteomic profiling on JMJD3 in regulating NF-κB activity and other cellular processes. Because of JMJD3 induction in macrophages (De Santa et al., 2009) and monocytes (Das et al., 2012a,b) exposed to bacterial products and inflammatory cytokines such as TNF-α, we chose THP-1 cells to study the involvement ofJMJD3 in inflammatory conditions to determine whether the proteomic changes affect different signaling pathways such as, NF-κB signaling and other cytokine signaling pathways. Our primary objective was to compare the protein expression profile of TNF-α-treated JMJD3-attenuated THP-1 (JMJD3-kd THP-1) cells with that of THP1 sc cells using two-dimensional polyacrylamide gel electrophoresis (2D)/matrix-assisted-laser-desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). Recently, proteomic techniques have emerged as a powerful tool to relate broad-spectrum protein expression with specific cellular responses. In our study, a comparative protein expression signature was established for TNF-α-treated both of JMJD3-kd THP-1 cells and THP1 sc to identify novel molecular targets of the JMJD3-kd THP-1 cells in cytokine-stimulated inflammatory conditions. In particular, we detected differential expression of proteins including tripartite motif protein (TRIM5), glutathione peroxidase (GPx), Rho GDP-dissociation inhibitor 1 (RHOGDI1), nuclear distribution gene C (NudC), nudix hydrolase (NUDT5), glia maturation factor-γ (GMFG), caspase recruitment domain family, member 14 (CARMA2), dUTP pyrophosphatase, WDR67, heat shock protein beta-1 (HspB1), prohibition and folate binding protein (FBP) in TNF-α-treated JMJD3-kd THP-1 cells. Overall, the effects suggest that JMJD3 might be a therapeutic target with possible research and clinical value. In addition, the signaling pathways and molecular networks induced by 2 h of TNF-α treatment of JMJD3-kd THP-1 cells were determined by Ingenuity Pathway Analysis (IPA). These pathways have the potential to define the molecular targets associated with JMJD3-kd THP-1 cells. Notably, the molecular network characterized by IPA analysis showed that TNF-α-treated JMJD3-kd THP-1cells up-regulated heat shock protein and down-regulated TRIM5, protein disulfide isomerase, NudC for the involvement of interleukin 15, immunoglobulin, and hepatocyte nuclear factor 4, alpha (HNF4α), which is due to the JMJD3-attenuation that could affect the inflammatory condition in THP-1 cells. The top five diseases and disorders associated with down-regulated genes in TNF-α-treated JMJD3-kd THP-1 cells, as determined by IPA analysis, were inflammatory disease, respiratory disease, immunological disease, genetic disorder and hematological disease.

2. Materials and methods

2.1. Reagents

Cell culture medium RPMI 1640, fetal bovine serum (FBS) and penicillin–streptomycin (PS) liquid were purchased from Invitrogen (USA). TNF-α was purchased from Sigma–Aldrich (USA). The lentiviral vector-based JMJD3 short hairpin RNA (shRNA) and scrambled shRNA were synthesized for this work (available on request).

2.2. Establishment of JMJD3-kd THP-1 cells and treatments

Establishment of scrambled shRNA-transfected, THP1 sc and JMJD3 shRNA-transfected, and JMJD3-kd THP-1 cells was performed as described in our previous paper (Das et al., 2012a,b). Human monocytic THP-1 cells were originally obtained from the American Type Culture Collection (Manassas, VA). Cell cultures were maintained at sub-confluence in a 95% air, 5% CO_2 humidified atmosphere at 37 °C using RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% PS, glutamine, HEPES and 50 μM β-mercaptoethanol (Shanmugam et al., 2003). Both THP-1 sc and

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