Biochimica et Biophysica Acta 1843 (2014) 2592–2602

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Histone H3K4 methyltransferase Mll1 regulates protein glycosylation and tunicamycin-induced apoptosis through transcriptional regulation

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ARTICLE INFO

Article history:
Received 24 February 2014
Received in revised form 20 June 2014
Accepted 23 June 2014
Available online 28 June 2014

Keywords:
Histone H3K4 methylation
Mll1
Tunicamycin
Apoptosis
UPR
Protein glycosylation

ABSTRACT

Disrupting protein glycosylation induces ER (endoplasmic reticulum) stress, resulting in the activation of UPR (unfolded protein response) pathways. A key function of the UPR is to restore ER homeostasis, but prolonged or unsolved ER stress can lead to apoptosis. MLL1 (Mixed Lineage Leukemia 1, also named ALL-1 or HRX), a histone H3K4 methyltransferase in mammals, plays important roles in leukemogenesis, transcriptional regulation, cell cycle and development. Here, we find that *Mll1* deficiency enhances UPR and apoptosis induced by the glycosylation inhibitor TM (tunicamycin). The abnormal regulation of the UPR appears to be caused by a defect in protein glycosylation. Furthermore, Mll1 directly binds to the promoters of *H6pd*, *Galnt12* and *Ugp2*, which regulates H3K4 trimethylation and the subsequent expression of these genes. The knockdown of *H6pd*, *Galnt12* or *Ugp2* enhances TM-induced apoptosis in *Mll1*$^{+/+}$ MEF cells, whereas the ectopic expression of these proteins inhibits TM-induced apoptosis in *Mll1*$^{-/-}$ MEF cells. Together, our data suggest that the maturation of glycoproteins in the ER is subject to regulation at the epigenetic level by a histone methyltransferase whose abnormality can lead to cancer and developmental defects.

1. Introduction

Almost all secreted and membrane-bound proteins are first co-translationally translocated into the lumen of the ER as unfolded polypeptide chains. These nascent polypeptides are glycosylated by a common N-linked oligosaccharide. Once properly folded, polypeptides exit from the ER to the Golgi apparatus for extensive modification. Glycosylation is also a part of the quality-control machinery in the ER that monitors the conformations of glycoproteins and determines their destinations. The perturbations that disrupt the nucleotide-sugar-precursor pool or glycosyltransferase activities result in glycosylation deficiency and induce the activation of intracellular signal transduction pathways collectively named, the UPR [1–3].

Studies of the UPR mechanisms in mammalian cells have identified three major ER-resident transmembrane proteins that act as stress sensors to transmit the signals, including IRE1 (Inositol-Requiring Protein-1), ATF6 (Activating Transcription Factor-6) and PERK (Protein kinase RNA (PKR)-like ER Kinase) [2,4–8]. Both IRE1 and PERK are type I transmembrane serine/threonine protein kinases. Once activated, these kinases undergo oligomerization-dependent autophosphorylation and initiate intracellular signal transduction [9,10]. Transcription factors activated by all three ER stress transducers (i.e., XBP1(s), ATF4 and p50ATF6) collaborate to activate the UPR target genes [6,11–16].

The initial goal of the UPR is to adapt cells to the altered proteostasis in the ER by reducing misfolded proteins. However, if ER stress persists and cannot be resolved, certain UPR effectors can switch roles from survival promoters to apoptotic inducers. Prolonged ER stress promotes apoptosis by activating CHOP, NOXA and JNK pathways [17–20]. ER stress-induced cell death is thought to contribute to the pathogenesis of a number of diseases, such as diabetes, renal diseases, neurodegenerative diseases, cancer and atherosclerosis [21].

MLL1 is one of the mammalian homologs of *Saccharomyces cerevisiae* Set1 (SET domain-containing 1), which was characterized as a histone H3 lysine 4 (H3K4) methyltransferase [22,23]. Trimethylation on histone H3K4 at promoter regions is highly correlated with active transcription; thus, the post-translational modification is considered a key regulator for gene transcription [24–26].

MLL1 was first identified and linked to leukemia in 1991 [27]. Chromatin translocation resulting in the fusion of the *MLL1* gene with up to 100 different genes leads to blockade of hematopoietic differentiation and the induction of leukemia [28,29].

Though *Mll1* deficiency leads to embryonic lethality, MEFs (mouse embryonic fibroblasts) derived from *Mll1*$^{-/-}$ mice grow normally [29].

Abbreviations: H6pd, hexose-6-phosphate dehydrogenase; Galnt12, UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 12; Ugp2, UDP-glucose pyrophosphorylase 2; IRE1, Inositol-Requiring Protein-1; ATF6, Activating Transcription Factor-6; PERK, Protein kinase RNA (PKR)-like ER Kinase; LAMP2, Lysosomal-Associated Membrane Protein 2; Dpagt1, dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase); TG, thapsigargin; BFA, Brefeldin A; 2-DG, 2-deoxy-d-glucose

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http://dx.doi.org/10.1016/j.bbamcr.2014.06.013
0167-4889/

MLL colocalizes with RNA polymerase II on the promoter regions of actively transcribed genes, where histone H3K4 trimethylation occurs [30,31]. However, only approximately 5% of the actively transcribed genes have reduced H3K4 trimethylation in *Mll1*$^{-/-}$ MEF cells in comparison with wild-type cells [32].

Studies on MLL1 over the past decades have suggested that MLL1 is involved in many different important cellular pathways. It is reported that MLL1 participates in cell cycle regulation [33–37] and that its expression level is tightly regulated by the two E3 ligase complexes SCF^{SKP2} and APC^{CDC20} [38,39]. MLL1 also regulates circadian gene transcription [40]. We previously reported that MLL1 selectively regulates the activation of NF-kappa B downstream genes stimulated by TNF-alpha and LPS [41]. From our gene expression profile data, we found that *Mll1* deficiency affects the transcription of genes in other cellular pathways [41], indicating that Mll1 may regulate more cellular processes in addition to the above-mentioned processes.

To study other biochemical pathways regulated by Mll1, we compared the cytotoxicity of *Mll1* knockout MEF cells to that of wild-type cells exposed to different chemical inhibitors. We found that *Mll1*$^{-/-}$ MEF cells are particularly sensitive to TM-induced cell death. Moreover, these cells exhibit enhanced UPR activation. *Mll1* deficiency enhances the inhibition of protein glycosylation by TM in the ER. We further showed that Mll1 regulates the expression of a set of glycosyltransferases and related enzymes, thereby affecting cellular sensitivity to TM.

2. Materials and methods

2.1. Cell lines and cell culture

Mll1$^{+/+}$, *Mll1*$^{-/-}$ and F-MLL1 MEF cell lines were kindly provided by Dr. Jay L. Hess (University of Pennsylvania). Cells were cultured in complete DMEM (Dulbecco's modified Eagle's medium) (Gibco) supplemented with 10% fetal bovine serum (HyClone) and 1× penicillin/streptomycin (HyClone).

2.2. Constructs, antibodies, siRNA and chemicals

The pRK-FLAG-H6pd plasmid was constructed by ligating PCR-amplified mouse cDNA into the SalI and NotI sites of the pRK-FLAG vector [42]. The pcDNA5-Galnt12 and pcDNA5-Ugp2 plasmids were constructed by ligating PCR-amplified mouse cDNA into the restriction digested BamHI and XhoI sites of the pcDNA5 vector (Invitrogen).

Antibodies used in this study include FLAG antibody (Sigma); actin antibody (CWBIO); LAMP2, JNK and p-IRE1 (S724) antibodies (Epitomics); IRE1, BiP, CHOP, p-PERK (T980), p-SAPK/JNK (T183/Y185) and caspase-3 antibodies (Cell Signaling Technology); H3K4 trimethylation antibody (Millipore); WDR5 antibody (Upstate); ASH2L antibody (BETHYL); H6PD, GALNT12 and UGP2 antibodies (Abcam).

The siRNAs against Mll1 (−GGACAAGAGTAGAGAGAGA−), H6pd (−GCCAGAAGCTCATGGACAA−), Galnt12 (−CGGAAAGAAATACGCTATA−), Ugp2 (−GGATCTAACTGTTCAGCAA−), B4Galnt4 (−GGTTTGGGTTCTATAAATA−), Glt8d2 (−CAACATGACTGAATGGAAA−), Wdr5 siRNA (−GAUGAAAGUGUGAGGAUAU−) and Ash2l siRNA (−AAGCAAAGACCCAGAAGAA−) were synthesized by GenePharma.

TM (Calbiochem), TG (Calbiochem), A23187 (Abcam), BFA (Tocris Bioscience) and 2-DG (Sigma) were stored as powders and dissolved in dimethylsulfoxide (DMSO).

2.3. MTT assay and Western blotting analysis

Cell viability was performed by the MTT assay as previously described [43]. Briefly, cells were split at 1×10^3 per well in a 96-well plate. After 24 h of culture, cells were treated with drugs for 48 h. MTT (25 μg) was added to each well, and the cells were then incubated for 4 h at 37 °C. The medium with the formazan sediment was dissolved in 50% DMF and 30% SDS (pH 4.7). The absorption was read at 570 nm. Data represent the average ± SD of at least three independent experiments performed in triplicate.

For the Western blotting assays, cells were harvested and then lysed in 1× SDS loading buffer (60 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.01% bromophenol blue). The cell lysates were boiled at 95 °C for 5 min and subjected to sonication. Proteins were resolved on an SDS-PAGE gel and transferred onto NC polyvinylidene difluoride membranes (Millipore). The blots were blocked with 5% dry milk in TBS containing Tween 20 (0.1%) and incubated overnight at 4 °C in blocking buffer containing primary antibodies. The blots were then incubated with horseradish peroxidase-conjugated secondary antibodies in blocking buffer and developed using the ECL Western Blotting Detection Reagent was bought from Millipore. Experiments were repeated at least three times. Data shown are representative experiments.

2.4. RNA preparation and quantitative RT-PCR

qRT-PCR was performed as previously described [41]. Total RNA was extracted using an RNA extraction kit (Yuanpinghao) according to the manufacturer's manual. For quantitative RT-PCR, cDNA was synthesized using a first-strand cDNA synthesis kit (Toyobo). Real-time PCR was performed using the SYBR green PCR Master mixture (Bio-Rad). β-Actin was used to normalize the amount of each sample. The following primers were used for qRT-PCR: *H6pd* F-agatgtaccgtgtggatcat, R-ccttgcggttctgatctc; *Ugp2* F-tgagtttgtcatggaagtca, R-gatttccaccagtctcagtt; *Galnt12* F-gtgctgaccttcctagactg, R-ggactccttctcgtggat; *Dpagt1* F-aggacctcaacaagctcag, R-gatgaagcagaagaggatga; *BiP* F-ctattcctgcgtcggtgt, R-aggagtgaaggccacatac; *Chop* F-gacagagccagaataacagc, R-tgtggtggtgtatgaagatg; *Atf4* F-gctatggatgatggcttg, R-ttctccaacatccaatct; *Gadd34* F-ggctcagattgttcaaagc, R-ctttctcagcgaagtgtacc; *Orp150* F-ccaaggaggctactctgtt, R-tacagacatcacagccaatg; *B4galnt4* F-cactctttcctcacactcgt, R-atccggagtccatagttctt; and *Glt8d2* F-ttacccctctgctcaagacat, R-ttcttccggtagtccagata. The results shown are representative of three independent experiments performed in triplicate. Data represent means ± SD.

2.5. Transient transfection

MEF cells were plated into 6-cm dishes for 14 h before transfection. Transient transfections with plasmids or siRNAs were carried out in MEF cells with Lipo2000 (Invitrogen). After transfection for 24 h, cells were separated into 6-well or 96-well plates and treated with TM for the indicated times and then analyzed by the MTT assay, Western blotting or qRT PCR.

2.6. Glycoproteins isolation and detection

Cells were treated with or without TM, harvested and lysed in high-salt lysis buffer (20 mM Hepes pH 7.4, 10% glycerol, 0.35 M NaCl, 1 mM MgCl2, 0.5% Triton X-100, 1 mM DTT) with proteinase inhibitors. Proteins were precipitated with saturated ammonium sulfate and dissolved in Concanavalin A Sepharose binding buffer (0.5 M NaCl, 2 mM MgCl2, 2 mM MnCl2 and 2 mM CaCl2). The glycoproteins were isolated with Con A Sepharose 4B (Sigma) according to the manufacturer's instructions and resolved on an SDS-PAGE gel. The gel was stained using Pierce Silver Stain Kit (Thermo). Experiments were repeated at least three times. Data shown are representative experiments.

2.7. Alexa Fluor 594 Conjugate of wheat germ agglutinin (WGA) staining

MEF cells were plated on glass coverslips in 12-well tissue culture plate. After 20 h incubation, cells were treated with or without TM (50 ng/mL) for 24 h. After being washed with pre-warm HBSS (8 g/L NaCl, 0.4 g/L KCl, 1 g/L glucose, 60 mg/L KH_2PO_4, 47.5 mg/L Na_2HPO_4, 0.35 g/L $NaHCO_3$, pH 7.2), cells were incubated with 12.5 μg/mL Alexa Fluor 594-WGA (Invitrogen) in HBSS for 45 min at 37 °C. Cells were