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ScienceDirect
Biochemical and Biophysical Research Communications 358 (2007) 475–482
BBRC
www.elsevier.com/locate/ybbrc

SUMO modification modulates the transrepression activity of PLZF

Ting-Ting Chao [a,b], Che-Chang Chang [b], Hsiu-Ming Shih [a,b,*]

[a] Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan, ROC
[b] Institute of Biomedical Sciences, Academia Sinica, 128, Sec. 2, Academia Road, Taipei 11529, Taiwan, ROC

Received 4 April 2007
Available online 4 May 2007

Abstract

Small ubiquitin-like modifier (SUMO) modification has recently been shown to associate with transcriptional regulation and nuclear body formation. Here, we show that transcription factor PLZF can be SUMO modified at lysine residue 242, 387 and 396. Converting these three SUMO acceptor Lys to Arg 3KR does not significantly affect PLZF nuclear body formation, which is distinct from the scenario of PML sumoylation in PML nuclear body formation. Furthermore, PLZF-3KR markedly reduced the transcriptional repression activity, correlating with a loss of PLZF-mediated growth suppression. These results reveal an important role of SUMO modification in PLZF-mediated transcriptional repression.

Keywords: SUMO modification; PLZF; Transcriptional repression; Nuclear body

Sumoylation, protein covalently modified by a small ubiquitin-like modifier (SUMO) peptide on the lysine residue, has recently emerged as an important posttranslational modification in regulating cellular processes [1,2]. Similar to ubiquitination, the process of sumoylation requires the E1-activating (SAE1/SAE2) and the E2-conjugating (Ubc9) enzymes [1,3]. Ubc9 catalyzes the formation of an isopeptide bond between the C-terminus of SUMO and the amino group of the target lysine. In general, sumoylation occurs on the lysine within a consensus motif ψKXE (where ψ is a large hydrophobic residue and X is any residue) [4]. Recent studies indicated that SUMO E3 ligases such as the PIAS family of proteins, RanBP2 and Pc2 function can enhance substrate sumoylation [1,5]. Conversely, sumoylated substrates can be de-conjugated by members of the SENP family isopeptidases [3,5].

With an increasing number of sumoylated factors being identified, sumoylation largely occurs in the nuclear compartment and most of identified SUMO substrates are known as transcription factors and co-regulators [1,6,7]. Sumoylation leads to transcriptional repression in most cases. Currently, the molecular mechanism by which conjugated SUMO elicits transcriptional repression largely remains unclear. We recently demonstrated that sumoylated transcription factors such as AR, GR, Smad4 and CBP coactivator can recruit a transcriptional corepressor Daxx for transcriptional repression [8–11]. Similarly, sumoylated p300 and Elk-1 were reported to recruit HDAC6 and HDAC2 for transcriptional repression [12,13], respectively. Besides transcriptional regulation, sumoylation is also important for some nuclear body formation. For instance, sumoylation is essential for the formation of PML nuclear body (PML-NB) [14–16], also known as the PML oncogenic domain (POD) or nuclear domain 10 (ND10). Sumoylation of PML was shown to be crucial for the recruitment of factors such as Daxx and Sp100 into PML-NBs [14,16]. More recently, sumoylated PML was reported to mediate the nucleation event of PML-NB formation through a network of noncovalent interaction between PML proteins [17].

The promyelocytic leukemia zinc-finger (PLZF), a member of the POK (BTB/POZ and *Kruppel*-like zinc-finger) protein family, consists of an N-terminal POZ domain

* Corresponding author. Address: Institute of Biomedical Sciences, Academia Sinica, 128, Sec. 2, Academia Road, Taipei 11529, Taiwan, ROC. Fax: +886 2 2782 7654.
E-mail address: hmshih@ibms.sinica.edu.tw (H.-M. Shih).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.04.157

mediating protein homo- and hetero-dimerization, a central repression domain RD2 and a C-terminal nine C2–H2 zinc-finger-like domains involved in DNA binding [18]. The POZ domain of PLZF can also recruits several transcriptional corepressors for transcriptional repression [19–23]. Recently, sumoylation of PLZF at K242 within RD2 domain was also reported to associate with transcriptional repression [24]. Furthermore, PLZF can form nuclear bodies distinct from PML-NBs [25]. Whether sumoylation is important for the nuclear body formation of PLZF remains unknown.

In the present study, we showed that K242, K387 and K396 are the SUMO acceptor sites of PLZF. Unlike sumoylation of PML for PML-NB formation, mutation of all three sumoylation sites (3KR) does not affect the formation of PLZF-NBs. Furthermore, PLZF-3KR markedly abrogated its transrepression activity on a *c-myc* promoter whereas K242R moderately reduced its transrepression potential, indicating the involvement of all three sumoylation sites in PLZF-mediated repression. Accordingly, suppression of cell growth mediated by PLZF transrepression was observed in WT- but not in 3KR-expressing cells. Together, these results reveal an important role of SUMO modification in PLZF-mediated transcriptional repression.

Materials and methods

Yeast two-hybrid screen and β-galactosidase assays. A yeast two-hybrid array screen using the LexA-PLZF as bait was performed as described previously [26]. Briefly, the L40 strain transformed with LexA-PLZF was mated with AMR70 strains, which have been transformed with each specific prey gene in the pACT2 vector, in 96-well plates. The resulting mated yeast cells were selected on a medium lacking histidine, leucine, and tryptophan then followed by X-gal assay. Quantitative β-gal assays of yeasts containing pairs of indicated bait and prey plasmids were determined by liquid culture according to the instructions of the Galacto-light Plus kit (Tropix).

Plasmid construction. pLexA-MST3, pGalAD-Ubc9, pGalAD-SUMO-1 and mammalian constructs expressing EGFP-SUMO-1gg and EGFP-SUMO-1aa were described previously [8,9,11]. The cDNA of SENP2 was inserted into the pEGFP-C2 vector (Clontech) to generate pEGFP-SENP2$_{WT}$. The pCMX-HA-PLZF construct was a gift from Dr. Ronald Evans. The cDNAs coding PLZF fragments 1–120, 121–456 and 457–673 were subcloned into the pCMX-HA vector to generate HA-tagged proteins. EGFP-SENP2$_{CS}$ and the KR mutants of HA-PLZF and HA-PLZF$_{121–456}$ were generated by the QuikChange site-directed mutagenesis kit (Stratagene). *c-myc*-Luc containing the 2.5-kb *c-myc* promoter linked to luciferase reporter gene was a gift from Dr. Daniel Tenen [27]. For Lenti-HA-PLZF-WT and 3KR, the cDNAs coding HA-PLZF-WT and 3KR were inserted into the pTY-EF1α vector, a gift from Dr. Huey-Kang Sytwu. All constructs were verified by DNA sequencing analyses.

Cell culture, transient transfection and luciferase reporter assays. For the culture and transfection of COS-1, HeLa and 293T cells were described previously [11]. U937 cells were cultured in RPMI supplemented with 10% FBS. For reporter gene assays, cells were transfected in 24-well plates with 1 μg of DNA, including the indicated reporter construct and expression vectors, and pTK-*Renilla* as indicator for normalization of transfection efficiency. Transfected cells were assayed for relative luciferase activity as described previously [9].

In vitro and in vivo sumoylation assays. In vitro sumoylation assay was performed as described previously [11] with *in vitro* synthesized ^{35}S-labeled PLZF proteins. For *in vivo* sumoylation, transfected COS-1 cells were lysed directly in 200 μl of a 1:3 dilution of buffer I (5% SDS, 150 mM Tris–HCl pH 6.7, 30% glycerol) and buffer II (25 mM Tris–HCl pH 8.2, 50 mM NaCl, 0.5% NP-40, 0.1% SDS, 0.1% azide) as described [28], supplemented with 5 mM DTT, complete protease inhibitor cocktail (Roche) and 10 mM NEM. Lysates were further boiled for 10 min and equal amount of proteins were subjected to Western analysis.

Western and immunofluorescence analyses. Western and immunofluorescence analyses were performed as described previously [8] except immunofluorescence images were visualized by Bio-Rad Confocal Microscope Radiance-2100.

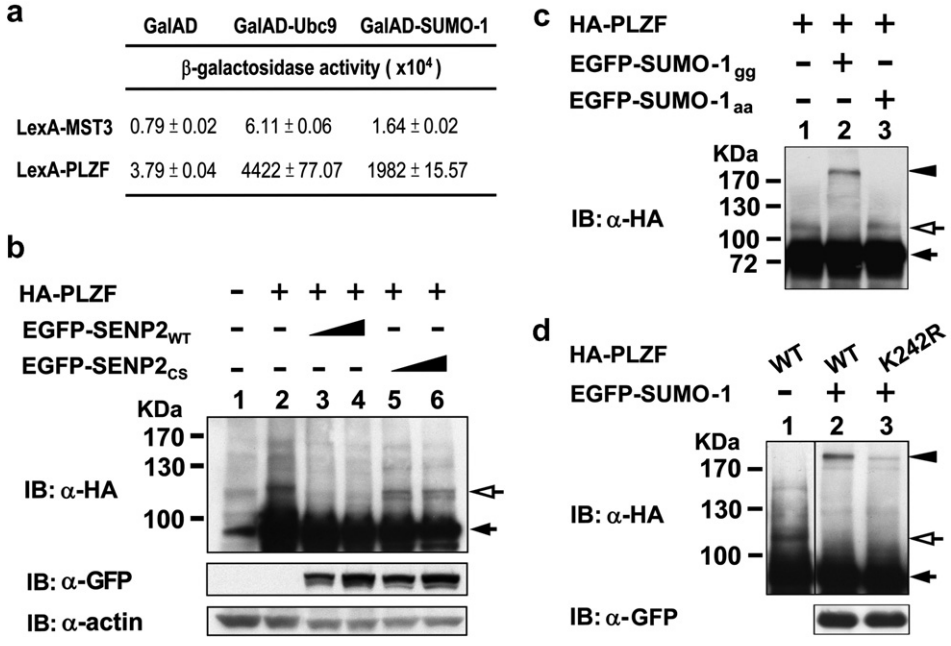

Fig. 1. SUMO modification of PLZF. (a) Yeast cells transformed with indicated baits and preys were subjected to quantitative β-galactosidase assays. The data represent means ± SD of three independent experiments. (b–d) Western blots showing SUMO-modified PLZF proteins from transfected COS-1 cells. *Open arrow* and *arrow* represent SUMO modified and un-modified PLZF proteins. *Arrowhead* indicates EGFP-SUMO-1 modified PLZF proteins.