

## **MATERIALS AND METHODS**

### **Cell culture**

Human embryonic kidney 293T cells, HeLa cells and CHO cells were cultured in DMEM containing 10% fetal bovine serum (FBS, Gibco) at 37 °C in a 5% CO<sub>2</sub> humidified atmosphere. Human APL cell line NB4 cells were cultured in RPMI-1640 medium supplemented with 10% FBS.

### **Plasmids and antibodies**

The expression plasmid of wild-type PML-RARA was constructed by inserting the cDNA of PML-RARA into the pcDNA-3Flag vector with designed primers (PML-RARA-forward: 5'-CggAATTCATggAgCCTgCACCCg, and reverse: 5'-CCgCTCgAgTCACgggggAgTgggTggCCg). The mutation constructs of PML-RARA-S214L, A216T, L217F and S220G were generated by site-directed mutagenesis PCR with Taq Plus Master Mix (Vazyme Code: P212-03, Vazyme Biotech Co., Ltd, Nanjing, China) using the following primers:

S214L-forward: 5'-gCTgTgCTgCCTgTgCgCgCTCCTTgAC,

S214L-reverse: 5'-gTCAAaggAgCgCgCACAggCAgCACAgC;

A216T-forward: 5'-CTgCTCgTgCACgCTCCTTgACAgCAgC,

A216T-reverse: 5'-gCTgCTgTCAAaggAgCgTgCACgAgCAg;

L217F-forward: 5'-CgTgCgCgTTCCTTgACAgCAgC,

L217F-reverse: 5'-gCTgCTgTCAAaggAACgCgCACg;

S220G-forward: 5'-gCTCCTTgACggCAgCCACAgTgAgCTC,

S220G-reverse: 5'-gAgCTCACTgTggCTgCCgTCAAggAgC, respectively.

The introduction of mutation was verified by DNA sequencing. Anti-Flag monoclonal antibody was purchased from Sigma (M2, Sigma-Aldrich (Shanghai) Trading Co., Ltd) and anti- $\beta$ -actin Mouse Monoclonal antibody (BE0036-100) was from EASYBIO (Beijing, China).

### **In vivo SUMOylation assay**

HEK293T or CHO cells were transfected with the indicated plasmids and the SUMOylation level of distinct PML-RARA mutants were detected by *in vivo* pull-down assay as previously described (Zhang et al., 2014).

### **Streptavidin agarose affinity assay**

CHO cells transfected with wild-type or mutants of PML-RARA were treated with 10  $\mu$ M Biotin-As for 2 h, and then cells were harvested and lysed in 8 M urea buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 8 M urea). The supernatant was collected and incubated with streptavidin agarose beads (SA100-04, Life technology, 5791 Van Allen Way, Carlsbad, California, USA) overnight at 4 °C. After washing four times with the urea buffer, the streptavidin beads were resuspended in 30  $\mu$ l SDS-PAGE loading buffer. The binding of arsenic with PML was analyzed by western blot with indicated antibodies.

### **Subcellular Fraction**

The subcellular fraction was performed as described previously (Goto et al., 2011). HEK293T cells were transfected with the indicated PML-RARA plasmids. At 24 h after transfection, cells were treated with 2  $\mu$ M  $AS_2O_3$  for 2 h and then lysed in RIPA buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP-40, 0.2 mM PMSF, and EDTA-free protease inhibitor cocktail) and fractionated into supernatants and pellets by centrifugation at 12,000 rpm for 15 min. The soluble or insoluble fraction was collected to a new tube and equal volume loading buffer was added, respectively. Samples were boiled at 96  $^{\circ}C$  for 10 min and then analyzed by western blot with the indicated antibodies.

### **Immunofluorescence microscopy**

The subcellular localization of PML-RARA or PML-RARA mutants in response to  $AS_2O_3$  treatment was detected as previously described (Bai et al., 2014). Images were visualized with a confocal laser scanning microscope (Leica TCS SP5) and processed with the ZEN software. DAPI was used to stain the cell nuclei.

### **REFERENCES**

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