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Analysis of the subcellular localization of the human histone methyltransferase SETDB1

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ABSTRACT

SET domain, bifurcated 1 (SETDB1) is a histone methyltransferase that methylates lysine 9 on histone H3. Although it is important to know the localization of proteins to elucidate their physiological function, little is known of the subcellular localization of human SETDB1. In the present study, to investigate the subcellular localization of hSETDB1, we established a human cell line constitutively expressing enhanced green fluorescent protein fused to hSETDB1. We then generated a monoclonal antibody against the hSETDB1 protein. Expression of both exogenous and endogenous hSETDB1 was observed mainly in the cytoplasm of various human cell lines. Combined treatment with the nuclear export inhibitor leptomycin B and the proteasome inhibitor MG132 led to the accumulation of hSETDB1 in the nucleus. These findings suggest that hSETDB1, localized in the nucleus, might undergo degradation by the proteasome and be exported to the cytosol, resulting in its detection mainly in the cytosol.

1. Introduction

Post-translational modifications of histones are important mechanisms that regulate chromatin structure and gene expression. Histone tails are targets of post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation [1,2]. Because these epigenetic alterations play important roles in development and diseases [3,4], epigenetic regulatory enzymes are important therapeutic targets [5].

The activities of histone-modifying enzymes are also regulated by post-translational modifications [6], as well as by protein–protein interactions [7,8] and cell cycle-dependent processes [9]. The subcellular location of these enzymes is also important for regulating their function. For example, the class III NAD^+-dependent histone deacetylase, sirtuin 1 (SIRT1), is expressed predominantly in the cytoplasm of neural precursor cells. However, under differentiation conditions, SIRT1 is transiently translocated into the nucleus and enhances neuronal differentiation by inhibiting the expression of Hes1 [10]. Another example is protein arginine methyltransferase 5 (PRMT5), a type II PRMT enzyme. This enzyme mediates the repression of a set of target genes with the transcriptional repressor B lymphocyte-induced maturation protein 1 (BLIMP1) by dimethylation of arginine 3 on histone H2A and/or H4 (H2A/H4R3me2s) in germ cells [11]. However, when the BLIMP1-

Abbreviations: CRM1, chromosome region maintenance 1; EGFP, enhanced green fluorescent protein; H3K9, lysine 9 on histone H3; HMT, histone methyltransferase; LMB, leptomycin B; mAb, monoclonal antibody; NES, nuclear export signal; NLS, nuclear localization signal; SETDB1, SET domain, bifurcated 1.

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PRMT5 complex translocates from the nucleus to the cytoplasm during embryogenesis, H2A/H4R3me2s modifications are decreased and epigenetic reprogramming of germ cells occurs.

SET domain, bifurcated 1 (SETDB1) is a histone methyltransferase (HMT) that methylates lysine 9 on histone H3 (H3K9) [12]. The enzymatic activity of SETDB1, in association with MBD1-containing chromatin-associated factor 1 (MCAF1), converts H3K9me2 to H3K9me3 and represses subsequent transcription [8,13]. *SETDB1* is amplified in cancers such as melanoma and lung cancer, and increased expression of SETDB1 promotes tumorigenesis in a zebrafish melanoma model [14,15]. In addition, SETDB1 is required for endogenous retrovirus silencing during early embryogenesis [16], inhibition of adipocyte differentiation [17], and differentiation of mesenchymal cells into osteoblasts [18]. Although it is important to know the localization of proteins to elucidate their physiological function, little is known of the subcellular localization of human SETDB1. In this study, we investigated the subcellular localization of hSETDB1 in cultured cells and found that it exists mainly in the cytoplasm.

2. Materials and methods

2.1. Plasmid construction

The full-length coding sequence of human SETDB1 was obtained by the polymerase chain reaction (PCR) with a HEK293 cDNA and cloning into the pcDNA3 vector (Invitrogen, Carlsbad, CA, USA) to generate an expression plasmid (pcDNA3-hSETDB1). The enhanced green fluorescent protein (EGFP) fragment was obtained by PCR from the pBI-EGFP vector (Clontech, Mountain View, CA, USA) and cloned into pcDNA3-hSETDB1 (termed pcDNA3-EGFP-hSETDB1). Deletion constructs (pcDNA3-EGFP-hSETDB1(1–680) and pcDNA3-EGFP-hSETDB1(681–1291)) were generated by PCR using pcDNA3-hSETDB1 as the template. All constructs were verified by sequencing.

2.2. Antibodies

Mouse monoclonal antibodies (mAbs) Z4601 (IgG2b) and Z4606 (IgG2a), directed against hSETDB1, were raised in our laboratory by immunizing separate mice with recombinant baculovirus displaying gp64-fusion proteins containing amino acids 1025–1080 of human SETDB1 [19,20]. The following antibodies were purchased: mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mAb (MAB374, Millipore, Billerica, MA, USA), goat polyclonal anti-heat shock protein 60 (HSP60) (N-20, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and mouse mAb anti-Lamin B2 (ab8983, Abcam, Cambridge, United Kingdom).

2.3. Cell culture, transfections, and treatments

HeLa human cervical carcinoma cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Nacalai Tesque, Kyoto, Japan) containing 10% fetal bovine serum (FBS) (Biowest, Nuaillé, France), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Nacalai Tesque). Cells were transfected with the hSETDB1 expression plasmid using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. For treatment with the nuclear export inhibitor leptomycin B (LMB), cells were cultured in medium containing either 50 nM LMB or vehicle (ethanol) for 9 h. For treatment with the proteasome inhibitor MG132, cells were treated with either 1 μM MG132 or vehicle (dimethyl sulfoxide) for 9 h.

2.4. Generation of a stable hSETDB1-expressing cell line

HeLa cells were transfected with pcDNA3-EGFP-hSETDB1 using Lipofectamine 2000. The cells were cultured in medium containing 700 μg/mL G418 (Nacalai Tesque) and G418-resistant clones were isolated. The clones were further screened by assessing the expression of EGFP using fluorescence microscopy and the expression of EGFP-hSETDB1 proteins using western blot analyses.

2.5. Fluorescence microscopy

The subcellular localization of EGFP fusion proteins in transfected cells was visualized with a FSX100 inverted fluorescence microscope (Olympus, Tokyo, Japan). For immunofluorescence assays, cells were fixed in 4% paraformaldehyde in phosphate-buffered saline and permeabilized with 0.1% Triton X-100. The cells were incubated with a primary antibody against hSETDB1

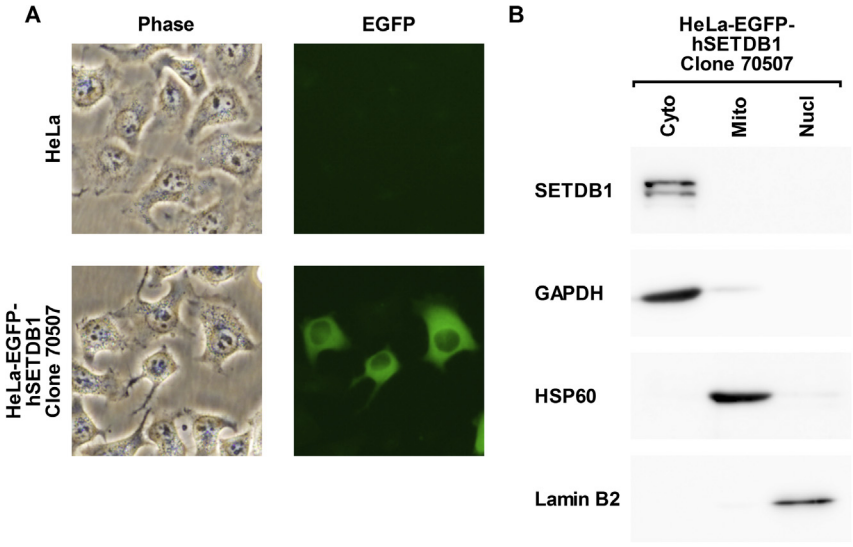

Fig. 1. Intracellular distribution of EGFP-fused hSETDB1 protein in HeLa cells. (A) HeLa cells constitutively expressing the EGFP-hSETDB1 fusion protein were used for phase contrast (left panels) and fluorescence (right panels) microscopy. The images show the same fields. (B) Expression of EGFP-hSETDB1 protein in the various cell fractions of HeLa cells was assessed by western blotting. GAPDH, HSP60, and Lamin B2 were used as markers for the cytoplasmic (Cyto), mitochondrial (Mito), and nuclear fractions (Nucl), respectively.