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Loss of nucleolar localization of NAT10 promotes cell migration and invasion in hepatocellular carcinoma

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ABSTRACT

NAT10, a nucleolar acetyltransferase, participates in a variety of cellular processes including ribosome biogenesis and DNA damage response. Immunohistochemistry staining showed that cytoplasmic and membranous NAT10 is related to the clinical pathologic characteristics in human cancer tissues. However, the mechanism about how NAT10 translocates from the nucleolus to cytoplasm and membrane is unclear. Here, we obtain a NAT10 deletion mutant localizing in cytoplasm and membrane. Bioinformatics analysis showed that residues 68–75 and 989–1018 are two potential nuclear localization signals (NLS) of NAT10. GFP-NAT10 deletion mutant (Δ989-1018) predominantly translocates into cytoplasm with faint signal retained in the nucleolus, while GFP-NAT10(Δ68-75) still remains in the nucleolus and nucleoplasm, indicating residues 989–1018 is the main nucleolar localization signal (NuLS). GFP-NAT10-D3, with both fragments (residues 68–75 and 989–1018) deleted, completely excludes from the nucleolus and translocates to cytoplasm and membrane. Therefore, complete NuLSs of NAT10 should include residues 68–75 and 989–1018. The cytoplasmic and membranous NAT10 mutant (Flag-NAT10-D3) colocalizes with α-tubulin in cytoplasm and with integrin on cell membrane. Importantly, Flag-NAT10-D3 promotes α-tubulin acetylation and stabilizes microtubules. Consequently, Flag-NAT10-D3 promotes migration and invasion in hepatocellular carcinoma (HCC) cells. Statistical analysis of immunohistochemistry staining of NAT10 in HCC tissues demonstrates that the cytoplasmic NAT10 is correlated with poorer prognosis compared with nuclear NAT10, while the membranous NAT10 predicts the poorest clinical outcome of the patients. We thus provide the evidence for the function of cytoplasmic and membranous NAT10 in the metastasis and prognosis of HCC patients.

1. Introduction

N-acetyltransferase 10 (NAT10, also called hALP), a member of GNAT family, was firstly reported to activate telomerase activity by up-regulating hTERT expression [1]. And a truncated NAT10 is found to acetylate histones [1]. DNA damage induced by H_2O_2 treatment activates NAT10 expression, and ectopic NAT10 improves cell survival [2]. In addition, NAT10 coordinates with hsSUN1, contributing to proper chromosome de-condensation at the end of mitosis [3]. Moreover, NAT10 localizes in the midbody and acetylates α-tubulin. Depletion of NAT10 leads to impaired cytokinesis and G2/M cell cycle arrest [4]. Thus, NAT10 regulates cell cycle through various mechanisms. We previously found NAT10 activates rRNA transcription through binding and acetylating UBF [5]. Autoacetylation of NAT10 is required for its function in acetylating UBF and activating rRNA transcription [6]. NAT10 also acetylates rRNA to facilitate rRNA processing [7,8]. Yeast homolog of NAT10 participates in the tRNA acetylation and small ribosomal subunit biogenesis [9]. Recently, we reported that NAT10 stabilizes p53 through promoting MDM2 degradation under normal conditions and acetylates p53 to activate transcription of p53 upon DNA

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damage [10].

Moreover, NAT10 promotes cell growth in ovarian cancer [11]. We found NAT10 is upregulated in HCC tissues and promotes tumorigenesis of HCC through acetylating mutant p53 [12]. Recent study reported that a NAT10 inhibitor, Remodelin, ameliorates laminopathies through correcting abnormalities of nuclear architecture [13]. Inhibition of NAT10 using Remodelin weakened doxorubicin resistance in breast cancer cells [14].

NAT10 has been found to translocate to cytoplasm and membrane in human cancers. For instance, cell membranous NAT10 is detected at the invasive front of cancer tissues in colorectal carcinoma (CRC) [15]. Furthermore, the membranous NAT10 correlates with clinical TNM stages of CRC [15]. In addition, the cytoplasmic and membranous NAT10 negatively correlates with tumor differentiation in HCC [16]. These findings suggest that subcellular distribution of NAT10 plays important roles in cancer progression. However, how NAT10 translocates from the nucleolus to cytoplasm and cell membrane remains unknown.

Localization of nuclear or nucleolar proteins is determined by nuclear localization signal (NLS) or specific nucleolar localization signal (NuLS) [17,18]. The consensus NLS contains a stretch of 4–8 basic amino-acids, while there is no consensus NuLS sequences as the NuLS of each nucleolar protein is related with its function such as RNA-binding [19]. Loss of NLS/NuLS of certain proteins often changes their functions. For instance, the NuLS is required for p14/p19Arf to stabilize MDM2 [20]. The NuLS in MSP58 is essential for its function in promoting cell proliferation [21]. Through cytoplasmic translocation, nucleophosmin acquires ability to facilitate apoptosis [22]. In response to cellular stress, several nucleolar proteins including p14/p19Arf, nucleophosmin, nucleolin and some ribosomal proteins translocate from nucleolus to nucleoplasm to activate p53 by inhibiting HDM2 [10,23–30]. Therefore, unravelling of the mechanisms about translocation of cancer-related nucleolar proteins will provide insight in the understanding of their functions in cancer development.

In present study, we identified the NuLSs of NAT10. Loss of the complete NuLSs in NAT10 leads to cytoplasmic and cell membranous localization. NuLSs-deleted NAT10 facilitates α-tubulin acetylation, stabilizes microtubule and promotes HCC cell migration. We thereafter uncovered the function of cytoplasmic and cell membranous NAT10 in cancer cells and evaluated the clinical significance of cytoplasmic and cell membranous NAT10.

2. Materials and methods

2.1. Cell culture and transfections

HeLa, SMMC-7721, HepG2, Huh7 and MHCC-97H cell lines were maintained in DMEM supplemented with 10% FBS at 37 °C in a humidified atmosphere containing 5% CO2. Cells were transfected with plasmid DNA by using Lipofectamine® 2000 (Invitrogen, CA, USA) according to the manufacturer's protocol.

2.2. Plasmids and antibodies

GFP- or Flag-tagged NAT10 and NAT10 deletion mutants (D1 to D3) were cloned into pEGFP-C2 or pCI-neo. Anti-Flag (F1804) antibody was purchased from Sigma-Aldrich. Anti-Integrin (α5) antibody, anti-α-tubulin (HRP) antibody, anti-α-tubulin antibody and anti-acetylated-α-tubulin (K40) antibody were purchased from Abcam. Anti-UBF antibody was purchased from Santa Cruz biotechnology. Anti-acetyl-lysine antibody was purchased from Merck Millipore.

2.3. Immunofluorescence assay

Cells were seeded on coverslips and fixed with 4% paraformaldehyde. After permeabilization and blocking, cells were incubated with primary antibodies overnight at 4 °C. Cells were incubated with FITC/TRITC-conjugated secondary antibodies. Finally, cells nuclei were stained with DAPI. Images were acquired using confocal microscope (Leica TCS SP8).

2.4. Preparation of cellular fractions

Cellular fraction was prepared as described previously [10]. Briefly, cytoplasmic lysis was prepared in lysis Buffer A (10 mM Tris-HCl pH 7.4, 10 mM KCl, 2 mM MgCl2, 0.05% Triton™ X-100, 1 mM DTT, 1 mM EDTA and proteinase inhibitors). Nuclear proteins were extracted in High Salt Buffer (20 mM HEPES pH 7.9, 420 mM NaCl, 0.2 mM EDTA, 1.5 mM MgCl2, 0.5 mM DTT, and 10% glycerol with protease inhibitors).

2.5. In vivo acetylation experiment

In vivo acetylation was performed as described previously [10]. Cell lysates were prepared in Buffer A supplemented with protease inhibitors and HDAC inhibitors (10 mM NIA and 500 nM TSA). Immunoprecipitation was performed with anti-acetyl-lysine antibody. Immunoprecipitates were subjected to immunoblot with anti-UBF or anti-α-tubulin (HRP) antibody.

2.6. Tubulin partitioning experiment

Cells were placed on ice as described previously [31]. Cellular soluble proteins were extracted with PEMG buffer (100 mM PIPES, 1 mM EGTA, 1 mM MgSO4, 1 mM GTP, pH 6.8, 0.1% Triton X-100 and 4 M glycerol). The remaining polymeric (cytoskeletal) fraction was dissolved in 0.5% SDS and 25 mM Tris (pH 6.8). Fractions were then subjected to immunoblot with anti-α-tubulin antibody.

2.7. Wound-healing migration experiment

Cells were plated in 6-well plates and scraped by sterile 0.2 ml pipette tips as described previously [32]. Cells were maintained in medium contained 0.5% FBS and photographed at different time points. Images were acquired using a microscope.

2.8. Transwell chamber migration and invasion assay

Transwell assays were performed as described previously [32]. SMMC-7721 or HepG2 cells in serum-free DMEM medium were seeded in Millicell (Merck Millipore). The migrated cells on the transwell membrane were photographed under a microscope and counted in five random fields after fixation and staining. For the invasion assay, Matrigel-coated Millicell (Merck Millipore) was used.

2.9. Real-time quantitative PCR

Real-time qPCR was performed using the ABI 7500/7500 Fast Real-Time PCR systems (Applied Biosystems). The human β-Actin were used as internal controls. All real-time data were analyzed by comparative Ct method and normalized to β-Actin. PCR primers for pre-RNA: 5′-GAACGGTGGTGTGTCGTTC-3′ and 5′-GCGTCTCGTCTCGTCTCACT-3′, for β-Actin: 5′-ATCGTCCACCG-CAAATGCTTCTA-3′ and 5′-AGCCATGCCAATCTCATCTTGTT-3’ [6].