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JAB1/CSN5 inhibits the activity of Luman/CREB3 by promoting its degradation

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ABSTRACT

Luman/CREB3 (also called LZIP) is an endoplasmic reticulum (ER)-bound transcription factor that has been implicated in the ER stress response. In this study, we used the region of Luman containing the basic DNA-binding domain as bait in a yeast two-hybrid screen and identified the Jun activation domain-binding protein 1 (JAB1) or the COP9 signalosome complex unit 5 (CSN5) as an interacting protein. We confirmed their direct binding by glutathione *S*-transferase pull-down assays, and verified the existence of such interaction in the cellular environment by mammalian two-hybrid and co-immunoprecipitation assays. Deletion mapping studies revealed that the MPN domain in JAB1 was essential and sufficient for the binding. JAB1 also colocalized with Luman in transfected cells. More interestingly, the nuclear form of Luman was shown to promote the translocation of JAB1 into the nucleus. We found that overexpression of JAB1 shortened the half-life of Luman by 67%, and repressed its transactivation function on GAL4 and unfolded protein response element (UPRE)-containing promoters. We therefore propose that JAB1 is a novel binding partner of Luman, which negatively regulates the activity of Luman by promoting its degradation.

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1. Introduction

Luman/CREB3 [1], also called LZIP [2], is identified through its association with herpes simplex virus (HSV)-related host cell factor 1 (HCF-1) [3,4]. The mode of interaction between Luman and HCF-1 is mimicked by the HSV-1 protein VP16, which has led to the hypothesis that Luman may play a role in the viral reactivation from latency [2,5,6]. Current data suggest that the primary cellular function of Luman may be in the endoplasmic reticulum stress response [2]. Luman has been implicated in HIV replication [7], dendritic cell maturation [8], breast cancer metastasis [9] and monocyte cell migration [10].

Luman is a cAMP response element (CRE)-binding protein that has four other closely related CREB3 family members, including CREB-H or CREB3-like 1 (CREB3L1) [11,12], BBF2H7/CREB3L2 [13], OASIS/CREB3L3 [14] and CREB4/AIbZIP/Atce1/Tisp40/CREB3L4 [15–19]. As transcript factors, they share a unique structural feature — a hydrophobic transmembrane domain that tethers them to the endoplasmic reticulum (ER). All CREB3 family members appear to play a role in the ER stress response or the unfolded protein response (UPR) [reviewed in 20,21], although with different tissue specificities. In the event of the UPR, these CREB3 proteins are believed to be cleaved and released from the ER by regulated intramembrane proteolysis, translocating into the nucleus and activating downstream target genes [8,13,16,19,22–24].

In addition to CRE, these CREB3 proteins differentially bind to various enhancer elements commonly found in the promoter region of UPR-related genes. Luman can bind the CAAT enhancer binding protein (C/EBP) element [1] and the Tax responsive element (TxRE) [25]. Recently, the ER-associated degradation (ERAD)-related protein Herp (homocysteine-induced ER protein) [26] or Mif1 [27] has been found to be a direct downstream target of Luman [23]. Luman induces cellular Herp expression during the UPR via transactivation of an ERSE-II enhancer element in the promoter. Luman may also induce another ERAD protein EDEM, through a UPRE-like element [28]. On the basis of viral mimicry, we have thus proposed that Luman may play a unique role in the ERAD that is fundamental to HSV lytic/latent replication cycle [23].

Here we report the identification of JAB1 (Jun activation domain-binding protein-1) as a cellular ligand of Luman using a yeast two-hybrid strategy. We verified the interaction between Luman and JAB1 with in vitro and in vivo assays. We found that JAB1 could repress the activation potential of Luman and decrease its protein stability.

2. Material and methods

2.1. Yeast two-hybrid screen

The Matchmaker 3 yeast two-hybrid system and a pre-transformed adult human brain Matchmaker cDNA library (BD Biosciences Clontech) were used in the screen. All experimental procedures

Abbreviations: ER, endoplasmic reticulum; ERAD, ER-associated degradation; HSV, herpes simplex virus; UPR, unfolded protein response; UPRE, unfolded protein response

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followed the manufacturer's instruction. Briefly, the bait plasmid pGBKT7/LU(AD-BD) was constructed by cloning a PCR fragment of Luman cDNA (a.a. 55–181) into the pGBKT7 vector. The bait plasmid was then transformed into the *Saccharomyces cerevisiae* MATα reporter strain AH109 using the small-scale LiAc protocol. For library screens, the AH109[pGBKT7/LU(AD-BD)] reporter strain was mated with *S. cerevisiae* MATa strain Y187, pre-transformed with an adult human brain Matchmaker cDNA library, and plated on SD/-Leu/-Trp/-Ade/-His minimal media supplemented with 10 mM 3-amino-1,2,4-triazole (3-AT). Plates were incubated at 30 °C for up to 16 days. Approximately 5×10^5 colonies were screened. Forty-three colonies were picked up and re-streaked onto to new SD/-Ade/-His/-Leu/-Trp plates, out of which 24 grew rapidly. Plasmids were recovered from these yeast strains, and were cotransformed back into the yeast strain AH109 with the bait plasmid pGBKT7/LU(AD-BD), and selected on SD/-Ade/-His/-Leu/-Trp plates. Among them, 7 clones were found to be positive and were subsequently sequenced, one of which contained JAB1 cDNA.

2.2. Cell culture and transfection

Human embryonic kidney (HEK)-293, COS7 and Vero cells were grown in Dulbecco's modified Eagle's medium [high glucose (4500 mg/L), 4 mM L-glutamine; HyClone] supplemented with 10% fetal bovine serum (Sigma) and 1% v/v penicillin/streptomycin. Cells were maintained at 37 °C in a humidified 5% CO_2 incubator and passaged every 2–3 days. Cells were transfected by the calcium phosphate precipitation method as previously described [6].

2.3. Plasmids

The full-length JAB1 cDNA was amplified using primers 5′-CTGAATTCCACACCCGGAAACCTAGC and 5′-GTACCTCGAGTATCAGATTTTGGGTAACT (attached *Eco*RI and *Xho*I restriction sites are underlined), and was cloned between *Eco*RI and *Xho*I sites of pcDNA3 with an N-terminal FLAG or HA epitope tag, yielding pFLAG-JAB1 and pHA-JAB1. The same strategy was used to clone the fragment into pGEX-KG (encoding a glutathione-S-transferase fusion protein at the N-terminus; gift from Gerry Weinmaster, University of California, Los Angeles) and pM1 (encoding an N-terminal GAL4 DNA binding domain fusion protein; gift from Ian Sadowski, University of British Columbia), which gave rise to pGEX-JAB1 and pM-JAB1.

JAB1 deletion mutant plasmids pHA-JAB1(1–195), pHA-JAB1(44–195), and pHA-JAB1(190–334) were constructed using the same cloning scheme as the full-length JAB1, and inserted into the *Eco*RI/*Xho*I sites of the pcDNA3 vector. The primer pairs for amplifying mutant JAB1 fragments a.a. 1–195, 44–195 and 190–334 were 5′-CTGAATTCAACGACAACTTCTCCGCTTCC/5′-GTGTCTCGAGTTAAGGTTTGTAGCCCTTTGG, 5′-GAGAATTCCCCTGGACTAAGGATCACCAT/5′-GTGTCTCGAGTTAAGGTTTGTAGCCCTTTGG and 5′-GTACCTCGAGTATCAGATTTTGGGTAACT/5′-CAGAATTCCCAAAGGGCTACAAACCT. The p5 × UPRE-luciferase plasmid [29] was a gift from Ron Prywes, Columbia University. Other plasmids used in this study have been described previously [23,28].

2.4. GST (glutathione S-transferase) pull-down

GST fusion proteins were produced in *Escherichia coli* strain BL21(DE3) (Novagen) and were purified using glutathione-sepharose beads (GE Healthcare) [1,5]. A rabbit reticulocyte in vitro transcription–translation system (TnT; Promega) was used to produce ^{35}S-labeled Luman, JAB1 and its mutants, and the GAL4 activation domain (AD) fused to GFP (as a negative control) according to the manufacturer's protocol. Protein concentrations of the bead slurry were calculated by comparison to BSA standards (Pierce). GST fusion proteins bound to glutathione-sepharose-4B beads were incubated for 1 h with in vitro ^{35}S-labeled protein in the binding buffer [140 mM NaCl, 50 mM Tris, pH 8.0, 2 mM Na3VO4, 5 mg/mL BSA and 0.5% (vol/vol) Ipegal CA 630]. Beads were collected by centrifugation, washed and re-suspended in 2× SDS sample buffer. The eluted protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and visualized on a Typhoon 9400 PhosphorImager (GE Healthcare).

2.5. Dual luciferase reporter assays

Assays were performed according to the Dual Luciferase Reporting System manual (Promega). In each assay, 30 ng of pRL-SV40 and 1 μg of a reporter plasmid were used in the cell transfection. All reporter plasmids were based on the pGL3-promoter plasmid (Promega), encoding a firefly luciferase gene downstream of the response element. The pRL-SV40 plasmid encodes *Renilla* luciferase gene under the control of the SV40 promoter, which was used as an internal control for transfection efficiency.

At 16 h post-transfection, media were changed and the cells were allowed to recover for 8 h. Cells were harvested, lysed, and used in the dual luciferase assays. Luciferase activity was measured using a Turner TD-20e Luminometer and calculated as relative luciferase activity (firefly luciferase/*Renilla* luciferase). Assays were independently repeated at least 3 times. Data are shown with standard errors.

2.6. Co-immunoprecipitation

HEK-293 cells in 35-mm dishes were transiently transfected with a total of 5 μg of plasmid DNA (the blank vector pcDNA3 was used as filler DNA in single transfections). Cells were treated with a proteasome inhibitor MG132 at a final concentration of 5 μM for the last 6 h prior to cell harvest. At 24 h post-transfection cells were washed with cold PBS and lysed in RIPA buffer (2.5 M NaCl, 0.5 M Tris pH 7.4, 0.5 M EDTA, 10% v/v Triton X-100, 1 mM PMSF and 0.2 mM Na_3VO_4) at 4 °C. Cell lysates were pre-cleared with Protein G beads, and were incubated with 1 μg of either an anti-FLAG (Sigma) or anti-Luman antibody (M13) for 21 h. After incubation, the beads were collected by centrifugation, washed with cold RIPA buffer and resuspended in sample buffer. For Western blotting of immunoprecipitates, an anti-Luman (M13) [6] or anti-FLAG (Sigma) antibody was used as primary antibodies at 1:1000 dilution. HRP-conjugated anti-rabbit IgG or anti-mouse IgG antibodies (Promega) were used as secondary antibodies at 1: 30,000. Blots were visualized using ECL Plus (GE Healthcare) on a Typhoon 9400 PhosphorImager (GE Healthcare). The results are representative of at least two independent experiments.

2.7. Confocal immunofluorescence microscopy

Cells were fixed in 4% formaldehyde, and permeabilized in 0.1% Triton X-100. The cover slips were incubated with 1:100–200 dilution of primary antibodies anti-JAB1 B-17 and anti-HA HA.11 (Santa Cruz Biotechnologies), then with a 1:400 dilution of Alexa594- or Alexa488-conjugated anti-mouse IgG antibody (Molecular Probes Inc.) and were mounted with 50% glycerol containing 500 pM 4′,6-diamidino-2-phenylinole (DAPI). Images were captured with a Hamamatsu ORCA-ER Digital Camera under a Leica DMRE confocal microscope.

2.8. [^{35}S]-methionine/cysteine pulse-chase

HEK-293 cells were seeded into 100-mm dishes one day prior to transfection. The cells were transfected with 20 μg of pFLAG-Luman only, or co-transfected with the pHA-JAB1 plasmid. Cells were split into 4 × 60-mm dishes at 16 h post-transfection. At 27 h post-transfection, cells were incubated in the pre-labeling media (DMEM without L-methionine and L-cysteine; Invitrogen) for 1 h. Cells were subsequently pulse-labeled for 1.5 h in pre-labeling media supplemented with a final concentration of 250 μCi/mL of [^{35}S]-methionine/cysteine

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