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A-type lamin-dependent homo-oligomerization for pY19-Caveolin-2 to function as an insulin-response epigenetic regulator

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ABSTRACT

Association of Caveolin-2 in the inner nuclear membrane specifically with A-type lamin is crucial for the maintenance of its Tyr-19 phosphorylation to promote insulin-response epigenetic activation at the nuclear periphery. Here, we identify that pY19-Caveolin-2 in the inner nuclear membrane exists as homo-oligomeric forms and the A-type lamin is required for sustenance of its oligomeric status. Oligomerization-defective and hence pY19-dephosphorylated monomeric Caveolin-2 in the inner nuclear membrane is unable to carry out Caveolin-2-mediated epigenetic activation of *Egr-1* and *JunB* genes and transactivation of Elk-1 and STAT3 in response to insulin. The homo-oligomeric pY19-Caveolin-2 localizes in and recruits epigenetic modifiers to the A-type lamin-enriched inner nuclear membrane microdomain for the epigenetic activation. Our data show that A-type lamin-dependent Caveolin-2 homo-oligomerization in the inner nuclear membrane microdomain is a precondition for pY19-Caveolin-2-mediated insulin-response epigenetic activation at the nuclear periphery.

1. Introduction

Caveolins, synthesized in the endoplasmic reticulum (ER), form small oligomers containing 7–10 Caveolins (i.e. the 8S oligomers). The small oligomers are translocated to the Golgi by COPII-dependent transport [1,2]. Plasma membrane (PM)-targeted Caveolin-2 (Cav-2), which is anterograde-transported from the Golgi by microtubule-dependent Rab6, forms large homo-oligomers (i.e. the 70S oligomers) in the noncaveolar PM microdomain, which requires intact actin cytoskeleton [3]. The homo-oligomeric Cav-2 is an essential component of the noncaveolar PM microdomain in which Cav-2 recruits insulin receptor and initiates insulin signaling cascade in response to insulin [3].

Similar to PM lipid microdomains, a nuclear lipid microdomain composed of phosphatidylcholine, sphingomyelin, cholesterol and proteins in the inner nuclear membrane (INM) has been shown to function as a platform for several important nuclear processes such as RNA synthesis [4,5]. It has been reported that A-type lamin-enriched microdomain, distinct from the B-type lamin-enriched microdomain, exists within the INM and provides an environment for the regulation of gene expression [6]. However, biochemical and structural information for the specific components and functions of the INM microdomains remains largely unknown.

In contrast to the anterograde trafficking of Cav-2 to the PM, Cav-2 in the Golgi is translocated to the INM by Rab6-mediated Golgi-to-ER retrograde trafficking in response to insulin [7]. Insulin-induced Tyr-19 phosphorylation is essential for the INM targeting [8,9] and the INM-integrated pY19-Cav-2 regulates insulin-response epigenetic activation of *Egr-1* and *JunB* genes via specific interaction with the A-type but not B-type lamin [10]. However, lamin-type specific INM microdomain localization and oligomeric status of Cav-2 in the INM have not been identified, and role of Cav-2 oligomerization in the INM for insulin-response epigenetic regulation by pY19-Cav-2 at the nuclear periphery remains unaddressed.

We investigated the oligomeric status and A-type lamin-enriched microdomain localization of Cav-2 in the INM, and tested whether its oligomerization in the INM microdomain regulates pY19-Cav-2-mediated insulin-response epigenetic activation. Our data show that the specific association with A-type lamin is required for the sustenance of homo-oligomeric forms of Cav-2 to maintain Tyr-19 phosphorylation, thereby recruiting epigenetic modifiers in the A-type lamin-enriched INM microdomain to facilitate insulin-response epigenetic activation at the nuclear periphery.

Abbreviations: ER, endoplasmic reticulum; PM, plasma membrane; Cav-2, Caveolin-2; INM, inner nuclear membrane; STAT3, signal transducer and activator of transcription 3; PTP1B, protein tyrosine phosphatase 1B; H3K9me3, histone H3 lysine 9 trimethylation; H3K9ac, histone H3 lysine 9 acetylation; H3K18ac, histone H3 lysine 18 acetylation; AcH3, acetylated histone H3; Hirc-B, human insulin receptor-expressed rat 1 fibroblast; HEK, human embryonic kidney; WT, wild type; siRNA, small interfering RNA; qRT, quantitative reverse transcription; ChIP, chromatin immunoprecipitation; TSSs, transcription start sites.

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2. Materials and methods

2.1. Materials

Antibodies and reagents used were as follows: Cav-2 (BD 610685; 1/250), Cav-1 (BD 610406; 1/1000), lamin A/C (BD 612162; 1/1000), signal transducer and activator of transcription 3 (STAT3) (BD 610190; 1/2500) and Ku-70 (BD 611892; 1/500) antibodies (BD Transduction Laboratories); emerin (sc-15378; 1/200), GFP (sc-9996; 1/200), calnexin (sc-11397; 1/200), actin (sc-1616; 1/200), GCN5 (sc-20698; 1/200), p300 (sc-585; 1/200), protein tyrosine phosphatase 1B (PTP1B) (sc-1718; 1/200), histone H3 (sc-10,809; 1/200) and histone H1 (sc-8030; 1/200) antibodies (Santa Cruz Biotechnology); pY19-Cav-2 (ab3417; 1/500), histone H3 lysine 9 trimethylation (H3K9me3) (ab8898; 1/1000), histone H3 lysine 9 acetylation (H3K9ac) (ab32129; 1/1000) and histone H3 lysine 18 acetylation (H3K18ac) (ab1191; 1/1000) antibodies (Abcam); acetylated histone H3 (AcH3) (06-599; 1/1000) and RNA polymerase II antibodies (05-623; 1/1000) (Millipore); FLAG® M2 (F 1804; 1/500), TRITC- (T5268) and HRP-conjugated anti-mouse-IgG (A4416) and anti-rabbit-IgG (A6154) antibodies, DAPI (D8417), and sodium ortho-vanadate (S6508) (Sigma); human insulin (Eli Lilly).

2.2. Cell culture

Human insulin receptor-expressed rat 1 (Hirc-B), Cav-2 shRNA-stable Hirc-B [3] and human embryonic kidney (HEK) 293T cells were routinely cultured in DMEM plus 10% FBS in a 5% CO_2 incubator at 37 °C and tested for contamination as described [10,11].

2.3. Plasmids

Full-length wild type (WT) Cav-2 cDNA (NM_131914) and Cav-1 cDNA (NM_001753.4) were subcloned into pcDNA3 vectors. Tyrosine- and palmitoylation-defective point mutants, Y19/27A and C109/122/145A (3CA), respectively, were generated by PCR mutagenesis using mutated oligonucleotides [9,12]. Oligomerization domain deletion mutants, Δ47–86-Cav-2 and Δ61–101-Cav-1 were generated by using the WT pcDNA-Cav-2 and pcDNA-Cav-1 as template via EZchange site-directed mutagenesis kit (Enzynomics, Daejeon, Korea). The resulting entry vectors of WT and mutants were converted into self-constructed FLAG tagging destination expression vector (pDS_FLAG-XB vector) or GFP tagging destination expression vector (pEGFP-N1 vector). All expression vectors were verified by sequencing.

2.4. Velocity gradient centrifugation

Sucrose velocity gradient centrifugation was performed as described previously [2,3]. pcDNA-Cav-2-, FLAG-Δ47–86-Cav-2-, pcDNA-Cav-2 and FLAG-Cav-1- or FLAG-Δ61–101-Cav-1-, FLAG-Cav-1-, FLAG-Δ61–101-Cav-1-, Δ47–86-Cav-2-GFP and FLAG-Cav-1- or FLAG-Δ61–101-Cav-1-, FLAG-3CA-Cav-2-, or FLAG-Y19/27A-Cav-2-expressing HEK293T cells were lysed with 0.5% Triton X-100 lysis buffer (0.5% Triton X-100, 100 mM NaCl, 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, and protease inhibitors) for 20 min at room temperature. The purified nuclear fractionation as described below was incubated with 2% Triton X-100 lysis buffer (2% Triton X-100, 20 mM Tris-HCl, pH 7.5, 280 mM NaCl, 10 mM NaF, 1 mM sodium ortho-vanadate, 5 μg/ml aprotinin, 3 μg/ml pepstatin, 5 μg/ml leupeptin, 1 mM EDTA, and 1 mM DTT) or 2% Triton X-100 lysis buffer containing 1% SDS or 8 M urea for 30 min at room temperature. The total cell lysates and nuclear lysates were centrifuged at 12,000 rpm for 10 min at 4 °C and the supernatant was loaded onto 10–40% linear sucrose gradients containing 0.5% Triton X-100 lysis buffer and 2% Triton X-100 lysis buffer or 2% Triton X-100 lysis buffer containing 1% SDS or 8 M urea, respectively. After centrifugation in a SW41 Ti rotor (Beckman Instruments) at 38,000 rpm for 255 min at 4 °C, fourteen 720 μl fractions were collected from the top to the bottom. An equal volume from each gradient fraction was separated by SDS-PAGE and subjected to immunoblot analysis.

2.5. Immunoprecipitation

Cav-2-GFP and FLAG-Cav-1- or FLAG-Δ61–101-Cav-1-, and Δ47–86-Cav-2-GFP-, 3CA-Cav-2-GFP-, or Y19/27A-Cav-2-GFP- and FLAG-Cav-1-expressing HEK293T cells were lysed in immunoprecipitation buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA, 0.2 mM sodium ortho-vanadate, 0.2 mM PMSF, and 0.5% Nonidet P-40) containing 60 mM *n*-octylglucoside (Calbiochem) [8]. The lysates were incubated overnight with anti-GFP antibody and with 30 μl of protein G plus Agarose (Calbiochem) further for 4 h at 4 °C. The immunocomplexes were collected by centrifugation at 12,000 rpm for 10 min at 4 °C and washed three times in ice-cold immunoprecipitation buffer. The immunoprecipitates were resuspended in 20 μl of 2 × SDS-PAGE sample buffer and analyzed by immunoblotting.

2.6. Immunoblot analysis

Equal amounts of total cell lysates and nuclear lysates and equal volume from each sucrose velocity gradient fraction described below were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore). Membranes were blocked for 2 h in 2% non-fat dry milk in TBS, 0.1% Tween 20 (TBS-T) at room temperature and incubated overnight at 4 °C in primary antibody in 2% BSA in TBS-T, followed by three washes in TBS-T. The membranes were incubated for 1 h at room temperature in HRP-conjugated secondary antibody in 2% non-fat dry milk in TBS-T, washed three times in TBS-T and developed using a Luminata™ Crescendo western HRP substrate (Millipore).

2.7. Confocal microscopy and image analysis

Cells were fixed and immunostained as described [10]. Fluorescent images were obtained using an Olympus Fluoview 1000 confocal microscope attached to IX-81 inverted microscope equipped with PlanApo 60×/1.40 or 100×/1.35 oil immersion objective lens (Olympus). FV10-ASW software (Olympus) was used to merge the images from GFP, TRITC, and DAPI. Colocalization of H3K9me3 or AcH3 with DAPI was quantitated by using the Colocalization Finder Plugin of Image J (National Institutes of Health, Bethesda, MD, USA). Quantitative analysis of insulin-induced INM targeting of Cav-2 was calculated by counting cells in which Cav-2-GFP or Δ47–86-Cav-2-GFP was localized at the INM. The localization was judged by the appearance of the nuclear ring pattern.

2.8. Nuclear fractionation

Nuclei purification was performed as described [10,13]. Cells were scraped with hypotonic lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 1 mM sodium ortho-vanadate, 5 μg/ml aprotinin, 3 μg/ml pepstatin, 5 μg/ml leupeptin, 1 mM EDTA, and 1 mM DTT) and homogenized using 10 strokes of a Dounce homogenizer and then centrifuged at 1000 rpm for 3 min at 4 °C. The crude nuclear pellet was resuspended in nuclear isolation buffer (0.5% Nonidet P-40, 10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 1 mM sodium ortho-vanadate, 5 μg/ml aprotinin, 3 μg/ml pepstatin, 5 μg/ml leupeptin, 1 mM EDTA, and 1 mM DTT) and incubated on ice for 5 min, and nuclei were pelleted by centrifugation at 1000 rpm for 3 min at 4 °C to yield the purified nuclear fraction.