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Regulation of Nampt expression by transcriptional coactivator NCOA6 in pancreatic β-cells

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ABSTRACT

Nuclear receptor coactivator 6 (NCOA6) is a transcriptional coactivator and crucial for insulin secretion and glucose metabolism in pancreatic β-cells. However, the regulatory mechanism of β-cell function by NCOA6 is largely unknown. In this study, we found that the transcript levels of nicotinamide phosphoribosyltransferase (Nampt) were decreased in islets of NCOA6$^{+/-}$ mice compared with NCOA6$^{+/+}$ mice. Moreover, NCOA6 overexpression increased the levels of *Nampt* transcripts in the mouse pancreatic β-cell line NIT-1. Promoter analyses showed that transcriptional activity of the *Nampt* promoter was stimulated by cooperation of sterol regulatory element binding protein-1c (SREBP-1c) and NCOA6. Additional studies using mutant promoters demonstrated that SREBP-1c activates *Nampt* promoter through the sterol regulatory element (SRE), but not through the E-box. Using chromatin immunoprecipitation assay, NCOA6 was also shown to be directly recruited to the SRE region of the *Nampt* promoter. Furthermore, treatment with nicotinamide mononucleotide (NMN), a product of the Nampt reaction and a key NAD$^+$ intermediate, ameliorates glucose-stimulated insulin secretion from NCOA6$^{+/-}$ islets. These results suggest that NCOA6 stimulates insulin secretion, at least partially, by modulating Nampt expression in pancreatic β-cells.

1. Introduction

Nicotinamide adenine dinucleotide (NAD) is an electron carrier that enables living cells to generate ATP during oxidative phosphorylation. Recent studies have demonstrated that NAD also contributes to a number of important signaling pathways and transcriptional regulation in mammalian cells [1]. Mechanically, NAD is involved in both cellular redox reactions as an essential coenzyme and in NAD-dependent enzyme reactions as a substrate. Prokaryotes and lower eukaryotes synthesize NAD *de novo* via quinolinic acid and by the salvage pathway via nicotinic acid, whereas, mammals predominantly use nicotinamide as a precursor for NAD biosynthesis [2]. Nicotinamide phosphoribosyltransferase (Nampt) is a rate-limiting enzyme in the NAD biosynthetic pathway of mammalian cells that converts nicotinamide to nicotinamide mononucleotide (NMN), which is then directly converted to NAD by nicotinamide/nicotinic acid mononucleotide adenylyltransferase [2,3].

Nampt is ubiquitously expressed in nearly all tissues and cells, and influences the activity of NAD-consuming enzymes as a regulator of intracellular NAD levels [4,5]. To date, many studies suggest that Nampt is involved in a wide variety of physiological functions [5–8]. Particularly, Nampt-mediated NAD biosynthesis is reported to be cardinal for maintaining NAD levels in pancreatic islets and for normal glucose-stimulated insulin secretion (GSIS) from β-cells [8]. Despite pleiotropic roles of Nampt, regulation of Nampt expression remains poorly understood.

Nuclear receptor coactivator 6 (NCOA6), also known as activating signal cointegrator-2 (ASC-2), nuclear receptor coregulator (NRC), and peroxisome proliferator-activated receptor interacting protein (PRIP), is a transcriptional coactivator for many transcription factors [9,10]. Recently, NCOA6 was shown to be involved in insulin secretion from pancreatic β-cells and glucose metabolism [11]. In this study, we found that Nampt expression was decreased in NCOA6-deficient β-cells. In addition, defective insulin secretion

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from NCOA6$^{+/-}$ islets was compensated by NMN treatment. Furthermore, we investigated the regulatory mechanism of *Nampt* transcription by NCOA6. Our results identified sterol regulatory element binding protein-1c (SREBP-1c) as a stimulatory transcription factor of *Nampt* in pancreatic β-cells. The SREBP-1c-mediated *Nampt* transcription was further enhanced by NCOA6. Thus, the present study demonstrates that NCOA6 is a coactivator that stimulates *Nampt* transcription and insulin secretion from β-cells.

2. Materials and methods

2.1. Isolation of mouse pancreatic islets and cell culture

Islets were isolated by collagenase digestion and differential centrifugation through Ficoll gradients from 24-week-old NCOA6$^{+/-}$ or wild-type mice using previously described procedures [11]. After isolation, islets were incubated in 11 mM glucose RPMI 1640 medium containing 10% fetal bovine serum (FBS). The mouse pancreatic β-cell line NIT-1 was maintained in 11 mM glucose DMEM supplemented with 10% FBS. All animal protocols were approved by the Institutional Animal Care and Use Committee of the Asan Institute for Life Sciences, Asan Medical Center (Approval No. 2011-12-088), and were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1996). Mice were maintained in a temperature-controlled facility with a 12 h light/12 h dark cycle and provided *ad libitum* access to water and regular rodent chow diet.

2.2. Isolation of total RNA and quantitative RT-PCR

Total RNA was extracted from mouse pancreatic islets or NIT-1 cells using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Purified RNA was reverse-transcribed by M-MLV reverse transcriptase (Promega, Madison, WI). The levels of specific transcripts were measured by quantitative RT-PCR (qRT-PCR) using a LightCycler 480 system (Roche, Basel, Switzerland). Quantitative expression analyses of target genes were performed using the $2^{-\Delta\Delta CT}$ method. The following oligonucleotide primers were used for qRT-PCR: mouse *Nampt*, 5′-ATTGCTCCTTCAAGTGCAGCTAT-3′ and 5′-CATTAACCCCAAGGCCATTG-3′; mouse *Sirt1*, 5′-GGCCGCGGATAGGTCCATAT-3′ and 5′-TTCGAGGATCGGTGCCAAT-3′; mouse *NCOA6*, 5′-CCCCACATGTGCAGAGCAT-3′ and 5′-TCCATGGCCCGAGAAGTG-3′ and mouse *RPS29*, 5′-CGCAAATACGGGCTGAACA-3′ and 5′-GCCTATGTCCTTCGCGTACTG-3′. *RPS29* was used as an internal control for quantitative analysis.

2.3. Immunoblot analysis

For immunoblot analysis, 100 μg of lysate protein was resolved by electrophoresis on 7.5% polyacrylamide gels under denaturing conditions and then transferred on to polyvinylidene difluoride membranes (Millipore, Billerica, MA). Blots were incubated with primary antibodies against Nampt (NBP1-52877, 1:500 dilution; Novus), Flag (F3165, 1:1000 dilution; Sigma, St. Louis, MO), and tubulin (T9026, 1:1000 dilution; Sigma) followed by incubation with horseradish peroxidase-labeled secondary antibodies. The membranes were developed using an enhanced chemiluminescence substrate. Protein bands with chemiluminescence signals were visualized by the Chemi-Smart system (Vilber Lourmat, Marne La Vallée, France).

2.4. Construction of Nampt promoter-luciferase genes and luciferase assay

The −616/+127 *Nampt* promoter region was amplified from mouse genomic DNA by PCR and used as a template to construct the related deletion promoter-luciferase reporters. Deletion and site-specific mutations within the −616/+127 promoter were created using PCR cloning strategies. The −616/+127 *Nampt* promoter and corresponding deletion constructs −316/+127, −267/+127, and −219/+127 were amplified using unique forward primers: 5′-CGGGGTACCACCGCAAGCAGCTC-3′ (−616), 5′-CGGGGTACCCCACGCCCCTCAG-3′ (−316), 5′-CGGGGTACCGGCTTTAGGCCCG-3′ (−267), 5′-CGGGGTACCGTCCTCCTCAGTCC-3′ (−219), and the common reverse primer 5′-CCGCTCGAGATGTTGAAGTCGGCTT-3′ (+127). Two mutant promoters were prepared using the following primer pairs: E-box mutant, 5′-AGACGAAGGTGCGTGGCTCCTTGG-3′ and 5′-AGCCACGCACCTTCGTCTCCCTGT-3′; and sterol regulatory element (SRE) mutant, 5′-CTGGTGACATAACTCCAACAAGACTCT-3′ and 5′-TTGGAGTTATGTCACCAGCTAAGGCC-3′. Plasmid constructs were verified by DNA sequencing to confirm identity. For luciferase reporter assay, NIT-1 cells were plated into 24-well plates 24 h before transfection and transfected with *Nampt* promoters, SREBP-1c expression vector and NCOA6 expression vector using Lipofectamine 2000 reagent (Invitrogen, Grand Island, NY) according to the manufacturer's protocol. pActin-βgal plasmids were included in each transfection experiment as a control for transfection efficiency. Luciferase activity was then measured using a Centro LB 960 luminometer (Berthold Technology, Bad Wildbad, Germany), and values were normalized to β-galactosidase activity.

2.5. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were performed as described previously with minor modifications [12]. For ChIP assay of the endogenous *Nampt* promoter, NIT-1 cells were directly fixed with 1% formaldehyde for 15 min at room temperature. Soluble chromatin from cells was prepared by sonication (VCX-600 sonicator, Sonics & Materials, Newtown, CT) and precleared by protein G-agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA). Precleared supernatants were immunoprecipitated with anti-NCOA6 (mouse ascites) or immunoglobulin G (IgG) antibodies (Abcam, Cambridge, UK) for 12 h at 4 °C. Immunoprecipitated DNA was then purified using phenol–chloroform extraction and analyzed by conventional PCR. For ChIP assay of the SRE mutant *Nampt* promoter, HEK293T cells were transfected with −616/+127 wild-type promoter or SRE mutant promoter constructs, incubated overnight, and then fixed with 1% formaldehyde as above. The oligonucleotide primers used for ChIP-PCR analyses were as follows: *Nampt* SRE region, 5′-GCAAGCAGCTCCTCTGATT-3′ and 5′-TAAAGCCGACCGTCACC-3′; *Nampt* exon 11 region, 5′-GGCCTTAACCAAGTTGTTTG-3′ and 5′-CTTCTTAAAATCTTGAGGTGC-3′.

2.6. Measurement of GSIS from mouse islets

After islet isolation from 24-week-old wild-type and NCOA6$^{+/-}$ mice, islets were incubated in 11 mM glucose RPMI 1640 medium overnight in the absence or presence of 50 μM NMN and then transferred to 5.5 mM glucose RPMI 1640 medium. These islets were washed twice with Krebs-Ringer/bicarbonate-HEPES buffer (KRBH) containing 2% bovine serum albumin (BSA). Fifty islets were added to each well of a 12-well plate and preincubated in KRBH containing 2% BSA for 1 h. After an additional hour of incubation with 2.5 mM glucose, supernatants were collected and used to determine basal insulin levels. The same islets were stimulated with 25 mM glucose KRBH buffer for 1 h and were then assayed for stimulatory insulin levels. Insulin concentrations in the supernatant were measured by radioimmunoassay (rat insulin kit from LINCO Research Inc., Charles, MO). The cell pellet was assayed for DNA content to normalize insulin secretion by islet mass.

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