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Research Article

Silencing of OSBP-related protein 8 (ORP8) modifies the macrophage transcriptome, nucleoporin p62 distribution, and migration capacity

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ABSTRACT

ORP8 is an oxysterol/cholesterol binding protein anchored to the endoplasmic reticulum and the nuclear envelope, and is abundantly expressed in the macrophage. We created and characterized mouse RAW264.7 macrophages with ORP8 stably silenced using shRNA lentiviruses. A microarray transcriptome and gene ontology pathway analysis revealed significant alterations in several nuclear pathways and ones associated with centrosome and microtubule organization. ORP8 knockdown resulted in increased expression and altered subcellular distribution of an interaction partner of ORP8, nucleoporin NUP62, with an intranuclear localization aspect and association with cytoplasmic vesicular structures and lamellipodial edges of the cells. Moreover, ORP8 silenced cells displayed enhanced migration, and a more pronounced microtubule cytoskeleton than controls expressing a non-targeting shRNA. ORP8 was shown to compete with Exo70 for interaction with NUP62, and NUP62 knockdown abolished the migration enhancement of ORP8-silenced cells, suggesting that the endogenous ORP8 suppresses migration via binding to NUP62. As a conclusion, the present study reveals new, unexpected aspects of ORP8 function in macrophages not directly involving lipid metabolism, but rather associated with nuclear functions, microtubule organization, and migration capacity.

Introduction

Oxysterols are 27-carbon oxygenated derivatives of cholesterol that arise from cholesterol via enzymatic or non-enzymatic oxidative processes or as side products of cholesterol biosynthesis [1–3]. These compounds are emerging as signaling lipids with impacts on several aspects of cell regulation [4,5]. Oxysterol binding protein (OSBP) was identified by Taylor and co-workers [6,7] as a cytosolic

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ER, Endoplasmic reticulum; Exo70, Exocyst complex protein of 70 kDa; GST, Glutathione-S-transferase; NPC, Nuclear Pore Complex; NUP62, Nucleoporin p62; ORP, OSBP-related protein; OSBP, Oxysterol-binding protein; ORD, OSBP-related (ligand binding) domain; ShRNA, Short hairpin RNA

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high-affinity receptor for oxysterols, and the rabbit and human OSBP cDNAs were cloned by the group of Brown and Goldstein [8,9]. Increasing interest has been attracted by OSBP after the discovery that families of proteins with sequence homology to the ligand binding domain of OSBP exist in eukaryotic organisms from baker's yeast to man [10–12]. The human and mouse genomes contain 12 OSBP-related genes [11–13]. ORP8 is a member of the mammalian OSBP-related protein (ORP) family. It localizes to the endoplasmic reticulum (ER) via a C-terminal 19-amino acid segment predicted to traverse the membrane bilayer [14]. ORP8 is expressed in many tissues including liver, kidney, spleen and brain, but especially abundantly in the macrophage, including those in human coronary atherosclerotic lesions [14]. In the human THP-1 macrophage, ORP8 was reported to modulate ABCA1 mRNA and protein expression, with an impact on cholesterol efflux [14]. Our recently published results revealed the nuclear pore component NUP62 as an interaction partner of ORP8 [15].

The nuclear pore complex (NPC) is a dynamic gateway between the nucleus and the cytoplasm that mediates nucleocytoplasmic transport via interaction with numerous soluble transport factors [16]. In vertebrates, the NPC is a structure composed of multiple copies of nearly 30 different nucleoporins (NUPs) [17–19]. NUP62 is a glycosylated phenylalanine-glycine repeat (FG)-Nup located in the central channel of the NPC. The N-terminal FG-rich region of NUP62 serves as a docking site for NTF2 (nuclear transport factor 2) [20,21], which is the transport receptor for Ran, a small GTPase that provides directionality to the nuclear transport process [22]. The C terminus of NUP62 is predicted to adopt a coiled-coil structure and to facilitate the anchoring of NUP62 to the NPC [23,24]. The C-terminus of NUP62 has been shown to interact with the transport receptor importin-β in vitro [25] and to mediate interaction with the NPC proteins NUP58, NUP54, and NUP45 that are parts of the NUP62 complex [26,27]. The C-terminal part of NUP62 also contains the determinant(s) for interaction with ORP8, and the ORP8 C-terminal region interacts with NUP62 [15].

Increasing evidence suggests important functions for NPC proteins apart from NPC [28–30]. Recent publications showed NUP62 interaction with the filamentous actin-capping protein CapG, localizing to the midbody ring at the end of abscission [28]. The same group reported that NUP62 interacts directly with the exocyst component Exo70 and colocalizes with Exo70 at the leading edge of migrating cells. NUP62 cycles between the plasma membrane and the perinuclear recycling compartment, binds the N-terminal domain of Exo70 through its coiled-coil domain, and plays an important, yet poorly understood role in cell migration [29]. Moreover, it was demonstrated that NUP62 forms a complex with NUP98 and NUP50 that interacts predominantly with active genes in the nucleoplasm in Drosophila [30].

Here we report, in the RAW264.7 murine macrophage model stably silenced for ORP8, novel and unexpected aspects of ORP8 function not directly involving lipid metabolism. We show that ORP8 silencing affects mRNA levels of several nuclear and centrosome/microtubule associated gene ontology pathways. We further demonstrate that the ORP8 interaction partner NUP62 has an abnormal distribution in cells silenced for ORP8, and that these cells exhibit enhanced migration and altered microtubule organization. Our findings suggest that the endogenous function of ORP8 in the macrophage involves regulation of nuclear functions, microtubule cytoskeleton, and cell motility.

Materials and methods

Antibodies and other reagents

The rabbit ORP8 antibody was described in [14]. Rabbit polyclonal antibody against β-actin was from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies (mab) against Nucleoporin p62 were from BD Transduction Lab (San Jose, CA) and Proteintech (Chicago, IL), mab against lamin A/C was from Cell Signaling (Beverly, MA), mab against β-tubulin (TUB 2.1) from Sigma-Aldrich (St. Louis, MO), and anti-6xHis was from Proteintech. Antibodies against Exo70 were a kind gift from Dr. Charles Yeaman (Carver College of Medicine, Univ. of Iowa).

shRNA lentiviruses and siRNAs

shRNA lentiviruses were purchased from Sigma-Aldrich MISSION® TRC-Mm 1.0 (Mouse) shRNA library; shORP8.4 corresponds to TRCN 0000105248 and shORP8.5 to TRCN 0000105249. Non-Target shRNA lentivirus particles (SHC002V) were used as a control. Silencer Select® siRNA against mouse Nup62 (cat. no. 4390771) and a non-targeting control (cat. no. 4390846) were obtained from Life Technologies/Ambion (Carlsbad, CA).

Cell culture

Mouse RAW264.7 macrophages were cultured in Dulbecco's Modified Minimal Essential Medium (DMEM) supplemented with 10% FBS, 10 mM Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, or in macrophage serum free medium (M-SFM) supplemented with macrophage colony-stimulating factor (M-CSF; Sigma-Aldrich; 10 ng/ml). Lentiviral transduction was performed according to the manufacturer's (Sigma-Aldrich) instructions, and the selection was carried out using puromycin (4 μg/ml). For siRNA-mediated Nup62 silencing, shNT or shORP8.5 RAW264.7 macrophages cultured in serum-containing medium were transfected for 48 h with control or Nup62 siRNAs by using the HiPerfect® reagent (Qiagen, Valencia, CA), followed by total RNA isolation and quantitative real-time PCR analysis or trans-filter migration assays (see below).

Quantitative RT-PCR

Quantitative real-time PCR (qPCR) analysis of the ORP8 mRNA was performed as described earlier [14] with the following primers: ORP8-forward TCCATCCTTTGGAGCAGTCT, ORP8-reverse TCCGCTTAAGGAGACTGGAA; housekeeping gene 36B4-forward CATGCTCAACATCTCCCCCTT, 36B4-reverse GGGAAGGTGTAATCCGTCTCCACAG. qPCR analysis of selected target gene mRNAs (*Ranbp1*, *Stmn1*, *Mcm2*, *Asf1b*, *Sfrs1*, *Ddx1*, *Tmem48*, *Nup107*, *Brca1*) was carried out by using Taqman Gene Expression Assays (Life Technologies/Applied Biosystems, Foster City, CA), with *Gapdh* as a housekeeping reference, and a 7000 Sequence Detection System (Applied Biosystems).

Western blotting

Cells were lysed in buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 10% glycerol, 0.5% NP-40) containing protease