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Cytosolic functions of MORC2 in lipogenesis and adipogenesis

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ABSTRACT

Microrchidia (MORC) family CW-type zinc finger 2 (MORC2) has been shown to be involved in several nuclear processes, including transcription modulation and DNA damage repair. However, its cytosolic function remains largely unknown. Here, we report an interaction between MORC2 and adenosine triphosphate (ATP)-citrate lyase (ACLY), an enzyme that catalyzes the formation of acetyl-coA and plays a central role in lipogenesis, cholesterogenesis, and histone acetylation. Furthermore, we demonstrate that MORC2 promotes ACLY activation in the cytosol of lipogenic breast cancer cells and plays an essential role in lipogenesis, adipogenesis and differentiation of 3T3-L1 preadipocytic cells. Consistently, the expression of MORC2 is induced during the process of 3T3-L1 adipogenic differentiation and mouse mammary gland development at a stage of increased lipogenesis. This observation was accompanied by a high ACLY activity. Together, these results demonstrate a cytosolic function of MORC2 in lipogenesis, adipogenic differentiation, and lipid homeostasis by regulating the activity of ACLY.

1. Introduction

Microrchidia (MORC) family CW-type zinc finger 2 (MORC2), also known as ZCWCC1, ZCW3, and KIAA0852, is one of the members of the MORC protein superfamily [1,2]. The MORC proteins share conserved features, including a GHKL (gyrase, hsp90, histidine kinase, and MutL)-ATPase domain [3–5] at the amino-terminus, which functions in restriction-modification systems [2], a CW-type zinc finger (ZF-CW) domain, a nuclear localization signal (NLS), and coiled-coil domains at the carboxy-terminus [6]. According to their ZF-CW domain architectures, MORC proteins have been classified into two subfamilies; MORC1 and MORC2 are assigned to subfamily I, whereas MORC3 and MORC4 belong to subfamily IX [6]. Current evidence suggests that MORC1 is expressed specifically in male germ cells and regulates male gametogenesis [7]. In contrast, MORC3 is ubiquitously expressed in mammalian cells and induces cellular senescence through regulating p53 activity [8]. MORC4 is highly expressed in a subset of diffuse large B-cell lymphoma patients and in B-cell lymphoma cell lines, thus acting as a potential lymphoma biomarker [9]. Notably, MORC2 is the least characterized member of the family.

The evolutionary, contextual, and gene neighborhood studies on prokaryotic MORC proteins have predicted a role for eukaryotic MORCs in chromatin remodeling [2]. In support of this notion, recent studies pointed out that the members of the MORC protein family are conserved regulators of heterochromatin condensation and gene silencing [10]. Consistently, MORC2 has been shown to transcriptionally repress gene expression [11] and promote chromatin remodeling in response to DNA damage [12]. However, the biochemical and biological roles of MORC2 in mammalian cells remain largely unknown.

To uncover new functions of MORC2, we recently conducted an immunoprecipitation/mass spectrometry (IP/MS) analysis and identified the ATP citrate lyase (ACLY) as one of the MORC2 binding partners. ACLY is a 121-kDa enzyme that catalyzes the formation of acetyl-coenzyme A (CoA) and oxaloacetate from citrate and CoA, with the hydrolysis of ATP to ADP and phosphate [13]. Cytosolic acetyl-coA is the requisite building block for several important biosynthetic pathways, including lipogenesis and cholesterogenesis. Consequently, ACLY is a key player in the conversion of glucose (exported as citrate from the mitochondria) to fatty acids. Acetyl-CoA is also required for acetylation reactions that modify proteins, such as histone acetylation [14]. In addition, ACLY activity is required for growth factor-induced increases in nutrient metabolism to the regulation of nuclear histone acetylation and gene expression [15] and is essential for fetal growth and development [16].

Alterations of ACLY activity and/or expression have been observed in different pathological conditions. In this context, it has been documented that ACLY expression is upregulated in different types of cancers, such as lung, prostate, bladder, breast, liver, stomach and colon cancers [17–23]. Furthermore, ACLY-dependent production of acetyl-CoA for lipogenesis is important for the proliferation of glycolytically converted

Abbreviations: MORC2, Microrchidia family CW-type zinc finger 2; ZF, zinc finger; IP, immunoprecipitation; ATP, adenosine triphosphate; ACLY, ATP citrate lyase; IBMX, 3-isobutyl-1-methylxanthine; GST, glutathione-S-transferase; RIPA, radio-immunoprecipitation assay; MDH, malate dehydrogenase; ACC, acetyl-coA carboxylase; FAS, fatty acid synthase; HMGCR, 3-hydroxyl-3-methylglutaryl-coA reductase; GSK-3, glycogen synthase kinase-3; PKA, cAMP-dependent protein kinase

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tumor cells [24,25] and its inhibition suppresses proliferation of certain types of tumor cells [17,24,25]. In addition, there is increasing evidence of the importance of ACLY in many metabolic disorders, such as diabetes [26], pancreatic cell stress and apoptosis [27], obesity [28], hepatic steatosis [29], and hypocitraturia [30].

Given the critical role of ACLY in many critical biological processes, and its implication in myriad human diseases, we examined the biological consequences of the interaction between MORC2–ACLY identified by IP/MS assay. Interestingly, we found that MORC2 interacts with ACLY specifically in the cytosol of lipogenic breast cancer cells and regulates ACLY activity. Changes in MORC2 expression levels were associated with changes in the lipogenic capacity of the cells. Moreover, we demonstrate an essential role of MORC2 in adipocyte differentiation at the levels of lipogenesis and number of adipocytes. These results open an interesting new line of research about the control of ACLY activity as well as lipogenesis, and identify MORC2 as an important component in lipogenesis, adipocyte differentiation and metabolism.

2. Materials and methods

2.1. Cell culture and treatments

MCF-7, ZR-75-1 and 3T3-L1 cells were obtained from American Type Culture Collection (Manassas, VA). MCF-7 and ZR-75-1 cells were cultured in Dulbecco's Modified Eagle's Medium/F-12 (DMEM/F-12) (Atlanta Biologicals Inc., GA), supplemented with 10% fetal bovine serum, and 3T3-L1 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% calf bovine serum. All media were additionally supplemented with 1× antibiotic–antimycotic solution (Life Technologies, Grand Island, NY). All cell cultures were maintained in a humidified 5% CO_2 atmosphere at 37 °C. For those experiments in which 3T3-L1 cells were treated with the adipogenic differentiation cocktail in the presence of actinomycin D or cycloheximide, cells were first pre-incubated with 10 μg/ml cycloheximide or 0.5 μg/ml actinomycin D. Subsequently, cells were washed with PBS and then placed into a medium containing insulin, 3-isobutyl-1-methylxanthine (IBMX), dexamethasone and either cycloheximide or actinomycin D at the same working concentration for 24 h. After incubation, the cells were collected and lysed for Western blot analysis.

2.2. Antibodies and reagents

Sources of antibodies were as follows: rabbit anti-MORC2 (for detection of human MORC2) and anti-T7 were purchased from Bethyl Laboratories (Montgomery, TX); goat anti-MORC2 (for detection of mouse MORC2), rabbit anti-cyclin D1, anti-progesterone receptor (PR) and anti-HDAC2 antibodies were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA); rabbit anti-phospho-ACLY, anti-ACLY, anti-phospho-ACC, anti-ACC, and anti-FAS were obtained from Cell Signaling Technology (Danvers, MA); rabbit anti-AP2 was obtained from Abcam (Cambridge, MA); and mouse anti-α-tubulin, anti-vinculin, anti-actin, and normal rabbit IgG were obtained from Sigma-Aldrich (St. Louis, MO). All primary antibodies were used as according to manufacturer's instructions. Horseradish peroxidase-conjugated secondary antibodies were from GE Healthcare (Piscataway, NJ), and enhanced chemiluminescence (ECL) reagents were from Amersham Biosciences (Piscataway, NJ). Potassium citrate, ATP, CoA, malate dehydrogenase, nicotinamide adenine dinucleotide (NADH), $MgCl_2$, Tris–HCl, DTT, actinomycin D, cycloheximide, insulin, and IBMX were from Sigma-Aldrich. Dexamethasone was purchased from G Biosciences (St. Louis, MO).

2.3. Expression vectors, recombinant proteins, siRNAs, and transfections

The expression vector encoding ACLY was generously provided by Kathryn E. Wellen (University of Pennsylvania, Philadelphia, PA) and has been previously described [15]. To generate various deletion ACLY expression vectors, the cDNA fragments corresponding to amino acid residues 1–300 (region 1), 300–630 (region 2), 630–850 (region 3), and 850–1092 (region 4) were amplified by PCR using the primers listed in Table 1. Each fragment was ligated into the *EcoRI-XbaI* sites of pEF6/V5-HIS vector (Invitrogen, Carlsbad, CA). *In vitro* translation was performed using the TNT® Quick Coupled Transcription/Translation Systems (Promega, Madison, WI). T7-MORC2, glutathione-S-transferase (GST)-MORC2 N-terminal (63–718 amino acids) and C-terminal (718–1032 amino acids) expression plasmids have been previously described [12]. All GST recombinant proteins were expressed in *Escherichia coli* strain *BL21* (DE3) (Stratagene, La Jolla, CA) and subsequently purified using the Glutathione Sepharose 4B batch method (GE Healthcare, Piscataway, NJ). Plasmid transfections were carried out using FuGENE HD Transfection Reagent (Roche Applied Science, Indianapolis, IN) according to manufacturer's instructions. Specific siRNAs targeting mouse or human MORC2 or control siRNAs were obtained from Thermo Fisher Scientific (Rockville, MD). The transfection of siRNA was performed twice at 24-h intervals with Oligofectamine™ reagent (Invitrogen) according to the manufacturer's protocol.

2.4. Quantitative real time PCR (qPCR)

Total RNA was isolated from cultured cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol, and 2 μg of extracted RNA was converted to cDNA using the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen). The resultant cDNA was subjected to qPCR by using the iQTM SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA) on an iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories). The values for specific genes were normalized to human or mouse actin housekeeping controls. Mean values are displayed ± standard deviations. The primers used for qPCR are listed in Table 2. All qPCR primers were synthesized in Sigma-Aldrich.

2.5. Western blot and immunoprecipitation

Protein extracts were prepared by lysing the cells in radioimmunoprecipitation assay (RIPA) buffer containing 50 mM Tris–HCl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1× protease inhibitor cocktail (Roche Applied Science), and 1× phosphatase inhibitor cocktail I and II (Sigma-Aldrich). Nuclear and cytoplasmic extracts were prepared as described previously [31]. Protein concentrations were determined using Bio-Rad DC Protein Assay reagents (Bio-Rad Laboratories). Cell extracts were then resolved by SDS-PAGE, transferred to nitrocellulose membranes, and incubated with the indicated antibodies. Detections were performed using the ECL reagents. For immunoprecipitation (IP) analysis, total 1 mg of protein materials was incubated with 1 μg of primary antibody overnight at 4 °C on a rocket platform, followed by incubation with total 50 μl of protein A/G PLUS-agarose (Santa Cruz Biotechnology) or Trueblot IP beads (eBioscience, San Diego, CA) for 2 h at 4 °C. The immunoprecipitates were collected by centrifugation in a microcentrifuge at 6000 rpm for 5 min. The supernatant was discarded, whereupon the pellet was washed with Nonidet P-40 (NP40) buffer (50 mM Tris–HCl, pH 8.0,

Table 1
List of primers used for amplification of the ACLY protein regions (R1–R4).

Primer	Sequence	Regions
ACLY_F1	5′-CGC GAA TTC ATG TCA GCC AAG GCA ATT TCA-3′	R1
ACLY_R1	5′-CGC TCT AGA TCA TTC ATT GAC ACC TCC AAG-3′	
ACLY_F2	5′-ACC GAA TTC ATG CTG GCG AAT TAC GGG GAA-3′	R2
ACLY_R2	5′-CGC TCT AGA TCA ACC AGT ATT CCC GAT CTT-3′	
ACLY_F3	5′-ACC GAA TTC ATG GGA ATG CTG GAC AAC ATC-3′	R3
ACLY_R3	5′-CGC TCT AGA TCA GGT GAT GGG CAT GCC CGC-3′	
ACLY_F4	5′-ACC GAA TTC ATG GAG GTC TTC AAG GAG GAG-3′	R4
ACLY_R4	5′-CGC TCT AGA TTA CAT GCT CAT GTG TTC TGG-3′	