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Sigma-1 receptor ablation impedes adipocyte-like differentiation of mouse embryonic fibroblasts

Huan Yang¹, Hongtao Shen², Jing Li², Kristin I. Stanford³, Lian-Wang Guo^{2,4,*}

¹Department of Surgery, University of Wisconsin, Madison WI 53705, USA.

²Department of Surgery, School of Medicine, University of Virginia, Charlottesville, VA 22908, USA

³Departments of Physiology & Cell Biology, College of Medicine; Davis Heart and Lung Research Institute, Wexner Medical Center. The Ohio State University, Columbus, OH 43210, USA.

⁴Robert M. Berne Cardiovascular Research Center, University of Virginia, Charlottesville, VA 22908, USA

Abstract

The sigma-1 receptor (Sig1R) is a unique ligand-operated endoplasmic reticulum (ER) protein without any mammalian homolog. It has long been a pharmacological target for intervention of psychiatric disorders, and recently garnered refreshed interest for its neuroprotective potential. Though reported to modulate various intracellular events, its influence on cell identity is little known. We explored a role for Sig1R in adipocyte differentiation.

We induced adipogenic differentiation of mouse embryonic fibroblasts (MEFs) with a differentiation medium. MEFs were isolated from *Sigmar1*^{-/-} and *Sigmar1*^{+/+} mice. The induced adipocyte-like phenotype was detected through Western blots of master transcription factors (PPAR γ , CEBPA, SREBP1, SREBP2), lipogenic proteins (FABP4, ACC1, ACAT2), and Oil-Red-O staining of lipids. We found that the induced upregulation of these proteins and lipid accumulation were severely mitigated in *Sigmar1*^{-/-} (vs *Sigmar1*^{+/+}) MEFs. Sig1R activation with a selective agonist (PRE084) increased Sig1R protein and further enhanced the induced adipocyte-like phenotype in *Sigmar1*^{+/+} MEFs. We also determined mouse body weight gain induced by high-fat diet for 6 months, which was impeded in *Sigmar1*^{-/-} (vs *Sigmar1*^{+/+}) male mice.

In summary, genetic ablation of Sig1R impairs, and agonist activation of Sig1R enhances adipocyte-like phenotype of induced MEFs. In vivo, Sig1R ablation impedes the body weight gain of male mice on high-fat diet. This study warrants further investigation of a previously unrecognized role for Sig1R in adipocyte differentiation.

*Corresponding author: Lian-Wang Guo, Ph.D., Department of Surgery, School of Medicine, University of Virginia, Charlottesville, VA 22908, USA, Tel: +1 614 292 5276. lg8zr@virginia.edu.

Competing interests

None

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Keywords

Sigma-1 receptor; mouse embryonic fibroblast; adipogenic induction; body weight gain

Introduction

The sigma-1 receptor (Sig1R) is a drug-binding site residing mostly in the endoplasmic reticulum (ER)^{1, 2}. Research on Sig1R has traditionally centered on its pharmacological targeting to treat psychotic disorders; some of the S1R ligands are in clinical use³. Recent pharmacological and genetic studies highlighted a potential in Sig1R modulation to combat neurodegenerative diseases⁴. Human genetics also pointed to a link between Sig1R mutations and familial motor neuron disease^{5, 6}. Efforts on mechanistic interpretation identified Sig1R-associated modulations of a wide variety of intracellular activities, ranging from ER stress, oxidative stress, inflammation, Ca²⁺ homeostasis, ion channel functions, GPCR signaling, to autophagy^{4, 7–10}. While most of these align with Sig1R being a molecular chaperone¹, the exact context-specific Sig1R molecular actions remain largely obscure.

Earlier work implicated a potential role for Sig1R in lipid trafficking or metabolism. Sig1R contains steroid binding domain-like sequences¹¹. It binds cholesterol and sphingolipids^{12–14} and may influence ER microdomain formation and ER-mitochondria interaction¹⁵. Aside from the physical interaction of Sig1R with lipid molecules, Sig1R regulates the stability of UDP-galactose:ceramide galactosyltransferase (CGaT)¹⁶. In addition, Sig1R was also found on lipid droplets as revealed by Sig1R-GFP subcellular fluorescence¹³. Since accumulation of lipid droplets is a prominent feature of adipocytes, we became curious as to whether Sig1R is involved in adipocyte differentiation.

Adipocytes play a major role in health and disease, especially with regard to their contribution to the development of obesity and diabetes¹⁷. These cells originate from adipose-derived stromal cells (ASC) or their equivalents in most tissues¹⁸. Adipogenesis involves orchestrated gene regulations and protein changes¹⁹. Peroxisome proliferator-activated receptor gamma (PPAR γ) and CCAAT-enhancer-binding protein alpha (CEBPA) are master transcription factors dictating late-stage adipocyte differentiation²⁰. The nuclear receptor PPAR γ is critical for both differentiation and phenotype maintenance of adipocytes.

Here we explored Sig1R's influence on adipocyte phenotype using mouse embryonic fibroblasts (MEFs) that were isolated from *Sigmar1*^{-/-} and *Sigmar1*^{+/+} mice. We found that the adipocyte-like phenotype induced by a differentiation medium, that is, increase of PPAR γ , CEBPA and other markers and lipid storage²⁰, were abated in the absence of Sig1R; however, agonist activation of Sig1R had an opposite effect. In vivo, Sig1R ablation reduced high fat diet-induced body weight gain in male mice. This previously unrecognized Sig1R-specific effect on induced adipocyte-like phenotype may inspire future studies on Sig1R as a potential player in adipogenesis.

Results

Sig1R ablation abates the upregulation of lipogenesis-associated proteins in MEFs induced for adipocyte differentiation

To investigate the possible involvement of Sig1R in adipogenic cell differentiation, we first determined the effect of Sig1R ablation on adipocyte marker proteins. We opted to use primary mouse embryonic fibroblasts (MEFs) for differentiation towards adipocyte-like cells – a well-established method applied in studies of adipogenesis²¹. MEFs were isolated from *Sigmar1*^{-/-} and littermate *Sigmar1*^{+/+} control mice, which were bred from the colonies established in our published studies²². MEF adipogenic induction was performed using the standard adipocyte differentiation medium²³, and the levels of lipogenic marker proteins were determined. As seen in Figure 1, Sig1R knockout was confirmed by Western blot. Interestingly, whereas the differentiation medium induced upregulation of FABP4, ACC1, and ACAT2 in *Sigmar1*^{+/+} MEFs, this upregulation did not occur in *Sigmar1*^{-/-} cells (Figure 1B). In contrast to the above three marker proteins, GOT2, a mitochondrial inner-membrane protein essential for the malate-aspartate shuttle in the glycolysis pathway, did not show appreciable changes across the four experimental conditions (Figure 1B). GOT2 thus conferred a negative control indicative of the functional specificity of Sig1R observed here. It is noted that in the basal growth medium (which does not induce adipogenic differentiation), MEFs from *Sigmar1*^{-/-} and *Sigmar1*^{+/+} did not exhibit a major difference in the levels of the three lipogenic proteins. This result indicated that these proteins were not disturbed in the basal condition without Sig1R, supporting a specific role for Sig1R in the induction of adipogenic differentiation.

Sig1R ablation blocks the upregulation of master transcription factors in MEFs induced for adipocyte differentiation

Western blot analysis further revealed that while PPAR γ and CEBPA were dramatically upregulated in *Sigmar1*^{+/+} MEFs by adipogenic induction, neither were upregulated in *Sigmar1*^{-/-} cells (Figure 1C). These two proteins are well established master transcription factors that drive adipocyte differentiation, both in development in vivo and in response to adipogenic differentiation of MEFs in vitro²⁰. CEBPA encodes an mRNA that contains alternative translation initiation sites resulting in two major protein isoforms: the fully translated CEBPA (P42), and an N-terminally truncated protein (P30) lacking the N-terminal transactivation domain. The upregulation of both CEBPA isoforms in response to induction of adipogenesis was blunted in the absence of Sig1R (Figure 1C). We also determined protein levels of SREBP1 and SREBP2, master transcription factors governing fatty acid and cholesterol synthesis, transport, and homeostasis, as well as active participants in adipocyte differentiation²⁴. SREBP1 and SREBP2 behaved similarly to PPAR γ and CEBPA.

Taken together, these data indicate that while increased protein production of PPAR γ , CEBPA, SREBPs, FABP4, ACC1, and ACAT2 were stimulated in the in vitro model of MEF adipogenic differentiation, this process was mostly averted in the absence of Sig1R. As these proteins are key factors in adipocyte differentiation and maintenance, our results suggest a novel role for Sig1R in induced adipogenesis.

Sig1R ablation prevents accumulation of Oil Red O-stained lipids in MEFs induced for adipocyte differentiation

After identifying the Sig1R influence on the marker proteins, we further characterized *Sigmar1*^{-/-} and *Sigmar1*^{+/-} MEFs after adipogenic induction for their adipocyte-like phenotype at the cellular level. We performed Oil Red O staining of lipids in intact cells, a standard approach to the characterization of adipocyte differentiation. As shown in Figure 2A, Oil Red O staining was intensified in *Sigmar1*^{+/-} cells after adipogenic induction, an effect not obviously seen in the *Sigmar1*^{-/-} MEF culture. This observation was corroborated with quantified data (Figure 2B and 2C). The Oil Red O staining was 6-fold higher in *Sigmar1*^{+/-} cells after adipogenic induction. However, no significant difference between the two medium conditions was seen in *Sigmar1*^{-/-} cells. Furthermore, the black/white images showed that there was no obvious change in cell density when compared between *Sigmar1*^{-/-} and *Sigmar1*^{+/-} cells in the differentiation medium (Figure 2D); rather, there were fewer oil-rich *Sigmar1*^{-/-} (vs *Sigmar1*^{+/-}) cells. Together, these data provide direct evidence for the inhibitory effect of Sig1R ablation on adipogenic-induced MEF differentiation.

Agonist activation of Sig1R enhances induced adipocyte-like differentiation of MEFs

Sig1R is best known as an ER-residing drug binding site, and highly Sig1R-selective agonists and antagonists have been developed and widely applied in clinical and preclinical studies¹⁰. Taking advantage of these known agonists and antagonists, we treated *Sigmar1*^{+/-} MEFs with the commonly used Sig1R-selective agonist PRE084 and antagonist BD1047 in order to further characterize the role for Sig1R in adipogenic differentiation (Figure 3). Agonist activation of Sig1R served as gain-of-function complementary to the above loss-of-function experiments using *Sigmar1*^{-/-} MEFs. Consistent with a general observation in the literature with different cell types²⁵, the treatment of MEFs with PRE084 increased Sig1R protein after adipogenic induction, thus validating the efficiency of this agonist used here. More importantly, while the protein levels of PPAR γ , CEBPA, SREBPs, FABP4, and ACC1 increased in response to adipogenic induction, treatment with PRE084 (vs vehicle) further markedly elevated these proteins (Figure 3). In contrast, treatment with the Sig1R-selective antagonist BD1047 did not produce this effect, indicative of the functional specificity of Sig1R agonist activation. No significant change was observed with GOT2, a result consistent with that from the *Sigmar1*^{-/-} MEF experiment (Figure 1), hence adding another layer of specificity to Sig1R's role in adipogenic differentiation. Moreover, further determination via Oil Red O staining indicated that the Sig1R agonist and antagonist significantly increased or decreased, respectively, lipid accumulation in response to adipogenic-induced differentiation (Figure 4).

Sig1R ablation impairs the body weight gain of male mice fed on high-fat diet

Up to this point, our in vitro loss- and gain-of-function data revealed a profound influence of Sig1R on the salient adipocyte-like, biochemical and cellular phenotypic parameters, including levels of master transcription factors, lipogenic proteins, and lipid accumulation (Figures 1–4). As adipocyte differentiation is a well-known contributor to body weight gain, we investigated the role of Sig1R in vivo in mice fed with a high-fat diet (HFD). When fed

on a normal diet, the *Sigmar1*^{-/-} animals were fertile and healthy without showing a difference in body weight compared to age-matched *Sigmar1*^{+/+} mice (Figure 5A), as previously shown²⁶. However, *Sigmar1*^{-/-} mice had a markedly lower average body weight compared to *Sigmar1*^{+/+} mice after 4 weeks on a HFD. This HFD-induced phenotype continued throughout to the end of the 6-month time course, and was observed in male, but not female mice (Figure 5B). This difference in body weight was independent of food intake; rather, the food intake normalized to net body weight gain was actually higher in *Sigmar1*^{-/-} mice compared to *Sigmar1*^{+/+} mice (Figure 5C).

In summary, the in vitro experiments showed profound influence of Sig1R genetic ablation or pharmacological activation on the adipocyte-like phenotype of MEFs under adipogenic induction with differentiation medium (Figure 1–4). The in vivo data revealed that when fed on HFD, body weight gain was impeded in *Sigmar1*^{-/-} vs *Sigmar1*^{+/+} male mice (Figure 5).

Discussion

Sig1R has been the target of intensive studies of medicinal chemistry in pursuit of Sig1R-selective compounds for intervention of psychiatric disorders⁹. However, the interpretation of Sig1R-specific molecular/cellular activities lagged behind²⁷. On the one hand, a pharmaceutical potential is enticing. On the other hand, there are no mammalian Sig1R homologs for deducing its functional pattern. In particular, Sig1R's influence on cell identity has been poorly explored¹⁴. In this regard, it is intriguing to observe the pronounced effect of Sig1R loss- or gain-of-function on adipocyte phenotype, which was not previously identified in published data.

Reports showed that a broad spectrum of intracellular events and signaling players were influenced by the expression level or ligand regulation of Sig1R; in most cases, the influence was moderate. In a general sense, Sig1R is deemed a pluripotent modulator which fits its character as a chaperone²⁵. In accordance, Sig1R knockout mice do not show overt phenotypes in normal conditions^{26, 28}. Likely for this sake, the biological importance of Sig1R has been overall underestimated, as partly reflected by the lack of knowledge regarding Sig1R in cell identity change or maintenance. It was therefore somewhat striking for us to see the profound effect of Sig1R ablation on induced adipocyte phenotype, as detected via the levels of lipogenic enzymes, adipogenic master transcription factors, and lipid accumulation.

Among these critical players in adipocyte differentiation, PPAR γ and CEBPA are master transcription factors that govern late-stage differentiation and phenotype maintenance, and they regulate each other¹⁹. A major target gene of PPAR γ is the intracellular lipid transporter FABP4 which is highly expressed upon adipocyte differentiation²⁴ as also observed here. While participating in adipogenic differentiation, SREBPs mainly control the transcription of lipogenic enzymes including ACC1 and ACAT2²⁴. ACC1 catalyzes carboxylation of acetyl-CoA to produce malonyl-CoA, a critical step in *de novo* fatty acid synthesis. ACAT2 is the chief enzyme responsible for the esterification of cholesterol. Fatty acids and esterified cholesterol are the major contents of lipid droplets. This string of events, transcription factor action $\rightarrow$ lipogenic enzyme production $\rightarrow$ lipid storage in droplets, is

well established in the literature²⁰. As such, that this process was overall markedly altered due to the absence of Sig1R is a significant observation.

Based on the aforementioned string of adipogenic events, it is reasonable to speculate that Sig1R influenced adipogenic differentiation of MEFs by altering the protein levels of master transcription factors. However, it has been notoriously challenging to explain the Sig1R-associated molecular mechanisms. One major hurdle is that aside from the evidence for Sig1R being a chaperone¹, the exact molecular function of Sig1R is largely unknown²⁷. A pioneering report showed that Sig1R knockdown in Chinese hamster ovary cells upregulated UDP-galactose:ceramide galactosyltransferase (CGalT)¹⁶. This finding may not be directly relevant to the current study, since the Sig1R negative regulation of CGalT does not explain its positive effect on lipogenic enzymes during MEF adipogenic differentiation. Another layer of complication exists; i.e., since Sig1R is an anti-stress signaling modulator, loss of the Sig1R protein *per se* can be an intracellular stress²². With this in mind, it remains an open question as to whether Sig1R influences the adipogenic proteins directly via a chaperone/client relationship or indirectly through intracellular signaling such as altered cellular stress levels. For the former, a body of work is available indicating that adipogenesis is regulated by chaperone proteins including HSP90²⁹, HSPA12A³⁰, and PGRMC2³¹. For the later, reports exist that ER stress and autophagy (where Sig1R plays an important role)^{1, 32} influence adipocyte differentiation^{33, 34}. In either case, it is worth further investigation especially given the magnitude of influence imposed either by Sig1R genetic depletion or its pharmacological activation.

In this study we opted to use the model of induced adipogenic differentiation with primary MEFs³⁵ directly isolated from *Sigmar1*^{-/-} and *Sigmar1*^{+/+} mouse embryos. By doing so we avoided a siRNA or CRISPR approach, which may not completely eliminate the Sig1R protein yet may result in off-target complications. Moreover, primary cells are more authentic than immortalized cell lines for interpreting endogenous cellular physiology²¹. In future experiments, the results from MEFs will be reevaluated using ASC isolated from *Sigmar1*^{-/-} and *Sigmar1*^{+/+} mice. On the other hand, permanent Sig1R depletion is possibly accompanied by compensatory signaling. To address this concern, we also performed instant activation of Sig1R with an agonist which complemented the permanent knockout setting. Importantly, the result from this gain-of-function approach agreed with that from using loss-of-function (knockout) cells. It is noteworthy that albeit showing an overall trend of mitigated differentiation (*vs* vehicle control), antagonist-treated cells did not exactly phenocopy knockout cells. The reason could be twofold. First, antagonist occupancy blocks the agonist binding in Sig1R but does not necessarily reduce the protein. Thus it may have a different functional consequence than the Sig1R knockout. Whereas antagonist binding promotes Sig1R oligomerization³⁶, the physical expulsion of the Sig1R protein may alter the ER membrane microdomain architecture¹⁵. Second, the putative endogenous agonists bind Sig1R with very low affinities (over 10 μ M)^{26, 37}. If Sig1R was not already endogenously activated before adding BD1047, an effect of the antagonist would not manifest. This may also explain the prominent effect of the exogenous high-affinity Sig1R agonist PRE084 which can readily compete with endogenous ligands for binding Sig1R. Nonetheless, by applying agonist activation of Sig1R which also raised endogenous Sig1R protein levels, we

were able to avoid ectopically overexpress Sig1R which disrupts the endogenous signaling and, if not properly folded, can cause extra stress⁵.

Reduced weight gain in male *Sigmar1*^{-/-} mice is another interesting result that has not been previously reported. It could be rationalized by impaired differentiation of *Sigmar1*^{-/-} MEFs to an adipocyte-like phenotype as observed here in vitro, since adipose tissue is an important contributor to body weight gain. We did not see significantly changed adipocyte marker levels (except for adiponectin) or cell size in *Sigmar1*^{-/-} (vs *Sigmar1*^{+/+}) mouse adipose tissues collected at the end of 6 months of HFD feeding (Figures S1 and S2). However, there are caveats in these results. First, the markers were detected using tissues which contained different cell types. Second, body weights were nearly plateaued at the end of 6 months, and the weight of *Sigmar1*^{-/-} mice appeared to “catch up” with that of *Sigmar1*^{+/+} mice. Thus, to attain conclusive comparison of adipogenesis between *Sigmar1*^{-/-} and *Sigmar1*^{+/+} animals, further investigation would be performed using adipocytes isolated from fresh adipose tissues, preferably at fast-growth earlier time points.

In a recent report³⁸, HFD-induced peripheral neuropathy was alleviated in *Sigmar1*^{-/-} mice, but no body weight data was available. In another study, treating mice with a Sig1R antagonist (BD1063) reduced overconsumption of palatable (high-sucrose) food during the first hour of feeding³⁹. This short-term effect appears to be more neuropsychiatry-related, an analogy being that Sig1R is a cocaine-binding site involved in drug addiction²⁶.

Interestingly, in a multiple-treatments meta-analysis to compare 15 antipsychotic drugs, haloperidol along with ziprasidone and lurasidone were the only ones not associated with significantly more weight gain than placebo in adult patients⁴⁰. Though a known Sig1R antagonist, haloperidol is not Sig1R selective^{12, 26}. Moreover, antipsychotic drugs often impact food intake⁴¹. Therefore, caution must be taken in the interpretation of the relationship between these drugs and body weight changes. In contrast to the pharmacological studies, in our long-term HFD feeding experiments showing lower *Sigmar1*^{-/-} vs *Sigmar1*^{+/+} body weights, we did not see reduced food intake of *Sigmar1*^{-/-} mice compared to the wild type animals over a 6-month period. Nevertheless, the well-documented importance of Sig1R in animal behaviors should be taken into account when thinking of Sig1R for metabolic intervention. Sex is another important variable. Sig1R is a sterol binding protein with some putative endogenous ligands being steroid hormones²⁵. However, body weight is also subject to other factors such as housing environment, type of HFD, bedding, or even the season when measurement is made. Therefore, the difference in body weight observed between male and female mice needs to be re-examined in continued experiments. As body weight is a complex issue directly related to obesity, our findings may open a potential avenue for future research into the utility of SigR1 as an anti-obesity therapeutic target.

Conclusion

The influence of Sig1R on cell identity is an underexplored area. We found that induced adipocyte-like phenotypic parameters of MEFs were down- and up-regulated, respectively, due to Sig1R ablation and its pharmacological activation. The observed effect is deemed Sig1R-specific because primary MEFs were isolated from *Sigmar1*^{-/-} and *Sigmar1*^{+/+}

mouse embryos. With many Sig1R-binding drugs in cache and ligand-bound Sig1R structures recently solved^{27, 42}, we hope this study would prod interest in further evaluating the potential of targeting Sig1R for modulation of adipogenesis.

Methods

Ethics statement

All animal procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals and were in compliance with the Institutional Animal Care and Use Committee with approved protocols. All surgeries were performed under isoflurane anesthesia (through inhaling, flow rate 2 ml/min), and all efforts were made to minimize suffering. Animals were euthanized in a chamber gradually filled with CO₂.

Breeding of *Sigmar1*^{-/-} and *Sigmar1*^{+/+} mice

A breeding pair of heterozygous *Opr1* (*Sigmar1*) mutant line, that is, *Opr1*^{Gt(IRESBetageo)33Lex} on a C57BL/6 J × 129 s/SvEv mixed background, were originally purchased from the Mutant Mouse Regional Resource Center (MMRRC, UC Davis, CA)²⁶. The colony of *Sigmar1*^{-/-} mice was established after back-crossing to C57BL/6 J mice for ten times to reach a relatively uniform background. Back-crossing was repeated from time to time for several more rounds to refresh the colony and avoid genetic shift. Genotyping was performed as we previously described²². Briefly, primers 5'-tctgagtacgtgctgtcttcg (985-5'), 5'-cagaaatctcagcccagtcg (985-3') and 5'-ataaacctcttgagttgcac (LTR-rev) were synthesized. The pair of 985-5' and 985-3' were used to amplify the *Sigmar1*^{+/+} allele (~200 bp PCR products), and the pair of 985-5' and LTR-rev were used to identify the *Sigmar1*^{-/-} allele (~400 bp PCR products). While selecting *Sigmar1*^{-/-} pups, littermate *Sigmar1*^{+/+} mice were identified for the use as wild type control animals.

Preparation of primary mouse embryonic fibroblasts (MEFs)

MEFs were prepared following the standard method that was previously reported³⁵. Briefly, embryos were carefully separated from the maternal tissues and yolk sack of *Sigmar1*^{+/+} and *Sigmar1*^{-/-} mice between day 12.5 and day 13.5 after coitus. The top of head (eye and above) and all innards of the embryos were removed. The rest part of the embryos were minced and digested with 0.05% trypsin/1 mM EDTA for 30~45 min at 37 °C on shaking. After centrifugation for 5 min at 1000 × *g*, the pellet was resuspended, and cells were cultured in the growth medium, that is, high glucose DMEM (Invitrogen) containing 10% (v/v) FBS (Sigma Aldrich, F2442) and 100 units/ml penicillin/streptomycin (Invitrogen). Aliquots of the MEF cells were snap frozen at passage 1 in liquid nitrogen. Experiments were performed at passage 3 or 4.

Induction of MEFs for adipocyte differentiation

We followed a standard protocol²³. MEFs were first cultured for 2 days in growth medium supplemented with 10 µg/ml insulin (Sigma Aldrich, I6634), 1 µM dexamethasone (Sigma Aldrich, D4902), 0.25 mM isobuthylmethylxanthine (Sigma Aldrich, I5879), and 10 µM troglitazone (Sigma Aldrich, T2573). At the end of this incubation (denoted as day 0), the culture was continued in the differentiation medium supplemented with 10 µg/ml insulin and

10 μ M troglitazone for another 8 days, with the medium changed every 2 days. For pharmacological treatment, the Sig1R agonist PRE084 (cat#0589, Tocris) or antagonist BD1047 (cat#0956, Tocris) were added upon changing the culture to the differentiation medium.

Oil Red O staining

To visualize lipid accumulation, cells were stained with the Oil Red O dye (Sigma Aldrich, 00626) 5 days after pro-adipogenic induction. Cells were washed 2x with PBS and fixed with 4% formaldehyde on ice for 30 min. The fixed cells were washed 3x with water, 1x with 60% isopropyl alcohol, and then stained for 30 min with the Oil Red O dye (0.6% in isopropyl alcohol mixed with water, 6:4 v/v). Stained cells were washed 1x with 60% isopropyl alcohol and 2x with water and then imaged through microscopy. Staining intensity was quantified using NIH ImageJ.

Western Blot Analysis

The assay was performed as we previously described²². Cells were collected at the time points indicated in the figure legends, and incubated on ice for 30 min in RIPA buffer (Thermo Fisher, Cat#89901) with protease inhibitor mixture added (Roche Applied Science, 11697498001). Protein concentration was determined by the Coomassie PlusTM protein assay (Thermo Scientific, 23236). The cell lysate samples were then subjected to 4–20% SDS-PAGE and transferred to PVDF membranes (Invitrogen, LC2002). Specific proteins were detected by incubating the PVDF membrane sequentially with primary antibodies and HRP-conjugated secondary antibodies, and then visualized with ECL plus Western blotting detection kit (GE Healthcare, RPN2132). Primary antibodies were used to detect FABP4 (Cell Signaling Technology, 2120), PPAR γ (Bethyl Laboratories, A304-461A), Sigma 1 receptor (B-5) (Santa Cruz Biotechnology, sc-137075), C/EBP α (D56F10) (Cell Signaling Technology, 8178S), ACC1 (Cell Signaling Technology, 4190S), ACAT2 (Bethyl Laboratories, A304-293A), GOT2 (Bethyl Laboratories, A304-356A), SREBP-1 (A-4) (Santa Cruz Biotechnology, sc-365513), SREBP-2 (1C6) (sc-13552, Santa Cruz Biotechnology), β -Actin(AC-74) (Sigma Aldrich, A2228100UL). The secondary antibodies were AffiniPure Donkey Anti-Mouse IgG (H+L) -HRP (Jackson ImmunoResearch, 715-035-150), and Donkey Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, 711-035-152).

High-fat diet feeding and measurement of body weight and food Intake

We used a 60 kcal% fat chow for HFD containing 60% fat, 20% carbohydrate and 20% protein (Research Diet, D12492) and 10 kcal% chow for control (normal diet). The chow and body weights were measured every time before changing chow. The chow was changed every week, and fragments or powder were avoided for accurate measurement of food intake. No fresh food was provided between two measurements as it could result in differences between groups.

Statistics

Prior to statistical calculation, data normality was assessed based on the Shapiro-Wilk normality test using Prism 6.0 software (GraphPad Software, San Diego, CA, USA). For multiple-condition comparison, normally distributed data values were subjected to statistical analysis using one-way ANOVA followed by Bonferroni post hoc test (or otherwise specifically stated in figure legend). Two-condition comparison was performed with Student t-test. Significance was set at $P < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Sigma-1 receptor loss- or gain-of-function has a pronounced effect on adipocyte phenotype.
- Sigma-1 receptor ablation impedes body weight gain of male mice fed with high fat diet.
- Our study warrants further investigation of a previously unrecognized role for the sigma-1 receptor in adipocyte differentiation.

Figure 1. Lower marker protein levels in *Sigmar1*^{-/-} (vs *Sigmar1*^{+/+}) MEFs induced for adipocyte differentiation

MEFs isolated from *Sigmar1*^{-/-} or *Sigmar1*^{+/+} mouse embryos were cultured in the growth medium (undifferentiated control) or in the differentiation medium for adipogenic induction, as described in Methods. The cells induced for 8 days were harvested for Western blot analysis.

A. Western blots. The replicate bands of each condition represent MEF samples from 3 different embryos. Densitometry was normalized to β -actin. The value for Sig1R in *Sigmar1*^{-/-} cells is the background on the blot; r.u., relative unit.

B and C. Quantification. Mean $\pm$ SD, n = 3 embryos as different sources of MEFs. Statistics: One-way ANOVA with Bonferroni test, *P<0.05, ***P<0.001.

Figure 2. Lower lipid storage in *Sigmar1*^{-/-} (vs *Sigmar1*^{+/+}) MEFs induced for adipocyte differentiation

MEFs isolated from *Sigmar1*^{-/-} or *Sigmar1*^{+/+} mouse embryos were cultured in the growth medium (undifferentiated control) or in the differentiation medium for adipogenic induction. Cell cultures induced for 8 days were used for Oil Red O staining.

A. Overview of Oil Red O-stained cells in culture dishes.

B. Microscopic images of Oil Red O-stained cells.

C. Quantification of staining intensity in B (using ImageJ). Mean $\pm$ SD, n = 3 embryos as different sources of MEFs; r.u., relative unit. Statistics: One-way ANOVA with Bonferroni test, ***P<0.001.

D.Black and white bright-field image of cell culture in the dish.

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Figure 3. Agonist-enhanced production of adipocyte-like phenotype marker proteins in *Sigmar1*^{+/+} MEFs induced for adipocyte differentiation

MEFs isolated from *Sigmar1*^{+/+} mouse embryos were cultured in the growth medium (control) or in the differentiation medium for adipogenic induction. Vehicle (DMSO) or Sig1R agonist (PRE084, final 0.5 μ M) or antagonist (BD1047, final 0.2 μ M) was included when changing to the differentiation medium. The cells induced for 8 days were harvested for Western blot analysis.

A. Western blots. The replicate bands of each condition represent MEF samples from 3 different embryos. Densitometry was normalized to β -actin.

B and C. Quantification. Mean $\pm$ SD, n = 3 embryos as different sources of MEFs. Statistics: One-way ANOVA with Bonferroni test, *P<0.05, ***P<0.001.

Figure 4. Agonist-enhanced lipid accumulation in *Sigmar1*^{+/+} MEFs induced for adipocyte differentiation

MEFs isolated from *Sigmar1*^{-/-} or *Sigmar1*^{+/+} mouse embryos were cultured in the differentiation medium for adipogenic induction. Cell cultures induced for 8 days were used for Oil Red O staining.

A. Microscopic images of Oil Red O-stained cells.

B. Quantification of staining intensity (ImageJ). Mean ± SD, n = 3 embryos as different sources of MEFs; r.u., relative unit. Statistics: One-way ANOVA with Bonferroni test, *P<0.05, ***P<0.001; ##P<0.01, ###P<0.001, both compared to the *Sigmar1*^{+/+} vehicle control (the first bar).

C. High-magnification images showing Oil Red O-stained lipid droplets.

Figure 5. Impeded body weight gain of *Sigmar1*^{-/-} (vs *Sigmar1*^{+/+}) male mice on high fat diet Male and female *Sigmar1*^{-/-} and *Sigmar1*^{+/+} mice at age of 8 weeks (referred to as 0 week on the plots) were put on high fat diet (HFD) for the following 6 months. Body weight and food intake were measured every two weeks.

A. Comparison of body weights between male *Sigmar1*^{-/-} and *Sigmar1*^{+/+} mice on normal diet. In parallel to the HFD diet, male mice of equivalent age were kept on normal diet. The plotted data were collected at the time point corresponding to 28 weeks in B. Statistics:

unpaired Student's t-test, no significance ($P>0.05$); $n = 6$ *Sigmar1*^{+/+} mice and 7 *Sigmar1*^{-/-} mice.

B. Time course of body weight recording throughout 28 weeks. Statistics: Repeated-measures 2-way ANOVA followed by multiple comparisons corrected by Sidak test; * $p<0.05$, ** $p<0.01$, *** $p<0.001$; $n = 6$ males and 4 females.

C. Time course of cumulative food intake, plotted as net food intake or intake normalized to net body weight gain at each time point.