

The Multiple Endocrine Neoplasia Type 1 (MEN1) Tumor Suppressor Regulates Peroxisome Proliferator-Activated Receptor γ -Dependent Adipocyte Differentiation[∇]

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Menin, the product of the *MEN1* (multiple endocrine neoplasia type 1) tumor suppressor gene, is involved in activation of gene transcription as part of an MLL1 (mixed-lineage leukemia 1)/MLL2 (KMT2A/B)-containing protein complex which harbors methyltransferase activity for lysine 4 of histone H3 (H3K4). As MEN1 patients frequently develop lipomas and peroxisome proliferator-activated receptor γ (PPAR γ) is expressed in several MEN1-related tumor types, we investigated regulation of PPAR γ activity by menin. We found that menin is required for adipocyte differentiation of murine 3T3-L1 cells and PPAR γ -expressing mouse embryonic fibroblasts. Menin augments PPAR γ target gene expression through recruitment of H3K4 methyltransferase activity. Menin interacts directly with the activation function 2 transcription activation domain of PPAR γ in a ligand-independent fashion. Ligand-dependent coactivation, however, is dependent on the LXXLL motif of menin and the intact helix 12 of PPAR γ . We propose that menin is an important factor in PPAR γ -mediated adipogenesis and that loss of PPAR γ function may contribute to lipoma development in MEN1 patients.

Inactivating germ line mutations of the *MEN1* gene are causal to multiple endocrine neoplasia type 1 (MEN1), an inherited tumor syndrome with high penetrance and a variable expression. MEN1 is characterized by the combined occurrence of tumors in the parathyroid glands, pancreas and duodenum, pituitary glands, and adrenal glands as well as neuroendocrine tumors. Other manifestations include angiofibromas, collagenomas, and lipomas (5). The mechanism of lipoma formation in MEN1 patients is currently unknown.

Peroxisome proliferator-activated receptor γ (PPAR γ) is a member of the PPAR nuclear receptor (NR) family (28). Polyunsaturated fatty acids, eicosanoids, components of oxidized low-density lipoproteins, and nitro-oleic acid are natural low-affinity ligands for PPAR γ . Synthetic thiazolidinediones, such as pioglitazone and rosiglitazone, have high affinities for PPAR γ . Two isoforms of PPAR γ exist, with the PPAR γ 1 isoform being expressed in several tissues, including the pituitary gland and pancreatic islets (10, 36), while expression of the PPAR γ 2 isoform is largely restricted to adipose tissue. Both PPAR γ isoforms heterodimerize with the retinoid X receptors (RXRs) to bind to PPAR-responsive elements (PPREs) in promoters and regulate transcription of target genes (46). Ectopic expression of PPAR γ in mouse fibroblasts can result in full adipo-

genesis (47). Mutations in the *PPARG* gene are causative of an inherited form of partial lipodystrophy (41). Furthermore, PPAR γ has been shown to have antiproliferative effects in adipocytes, by inhibiting DNA binding of the cell cycle transcription factor E2F/DP (3). In conclusion, PPAR γ is a key regulator in adipocyte differentiation, which acts at the level of gene transcription.

Menin is the product of the *MEN1* tumor suppressor gene. Menin is expressed ubiquitously as a nuclear protein and can take part in many cellular processes, including transcription regulation, DNA replication, and DNA repair (29). As a transcriptional regulator, menin is involved in both repression and activation of transcription. Through association with a histone-deacetylating complex, menin acts as a corepressor for JunD-mediated transcription (21). Menin was demonstrated to attenuate transcription of the *hTERT* gene, possibly by this mechanism (30). Activation of transcription by menin also involves histone modifications. Menin is an integral component of MLL1 (KMT2A) or MLL2(KMT2B) protein complexes which have histone methyltransferase (HMT) activity specific for lysine 4 of histone H3 (H3K4) (17, 55). Trimethylation of this residue (H3K4me3) is associated with active transcription (39). Several downstream effector proteins reading the H3K4me3 mark via plant homeodomain fingers such as BPTF/NURF and TFIID are involved in transcription activation (50, 53). The menin-HMT complex is an activator of several *Hox* genes and the *p18^{Ink4c}* and *p27^{Kip1}* cyclin-dependent kinase inhibitor genes (17, 32, 55). Analysis of genome-wide DNA occupancy revealed that menin is located at 5' regions of many active genes and also at 3' regions and intergenic loci (1, 40).

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Promoter recruitment of menin depends on interactions with DNA sequence-specific transcription factors: for example, via JunD in the repressing complex or via Smad transcription factors as an activator (43). Furthermore, promoter association may be stabilized by the low-affinity sequence-independent interaction of menin with DNA (24). The menin-HMT complex was recently demonstrated to be recruited to the *c-Myc* gene enhancer by interacting with the C-terminal activation domain of β -catenin (42). A complex containing MLL1(KMT2A) and possibly menin was found to couple E2F function to H3K4me3 (49). Previously, we have demonstrated that menin is able to coactivate estrogen receptor α (ER α)- and vitamin D receptor (VDR)-mediated transcription. In this case, menin recruits H3K4 HMT activity to the estrogen-responsive *TFF1/PS2* gene (9).

Approximately 30% of MEN1 patients develop lipomatous tumors (8). In addition, it has been shown that PPAR γ 1 is expressed in MEN1-associated tumor types, such as pituitary adenomas and neuroendocrine tumors (15, 31). We hypothesized that deficient differentiation dependent on PPAR γ plays a role in lipoma tumorigenesis and that menin is involved in this differentiation. Therefore, to further explore the role of menin as a coactivator for NRs and to determine the biological relevance of these interactions, we examined PPAR γ as a potential menin-interacting protein. In this report, we show that menin is indeed a transcriptional coactivator for PPAR γ and that menin is important for PPAR γ -dependent transcription and adipocyte differentiation. Menin can recruit histone H3K4 trimethylation to PPAR γ target genes, through a direct interaction with the activation function 2 (AF2) domain of PPAR γ . These results extend the role of menin as a coactivator for NR-mediated transcription to PPAR γ and provide new insight into the tissue-specific manifestations of MEN1.

MATERIALS AND METHODS

Patient material. Normal adipose tissue (from paracolic, pararenal, breast, and peritoneal origin) and lipoma samples (five intramuscular lipomas, one subcutaneous lipoma, one osteochondrolipoma, and one soft tissue lipoma) were obtained from the tissue bank of the Department of Pathology/UMCU Biobank and used in accordance with the hospital scientific committee regulations and the code "Proper Secondary Use of Human Tissue," as installed by the Federation of Biomedical Scientific Societies (www.federa.org).

Plasmids and siRNA oligonucleotides. Construction of vectors B42-PPAR γ , B42-RXR α , Gal4-RXR α , Gal4-PPAR γ (AF2) (containing AF2), Gal4-PPAR γ (AF1), glutathione *S*-transferase (GST)-PPAR γ (AF2), GST-PPAR γ (AF2-L468A/E471A) (containing AF2 with L468A and E471A mutations), pBabeHygroMenin, pBabeHygroMeninL22R, pEG202NLS-menin, pMSCV-PPAR γ 2, pSG5-HA-CARM1, and pSG5-SRC1e has been described previously (7, 9, 12, 17–19, 37, 52). The menin expression vector pCDNA3.1 M+ was a gift from G. Weber. The menin L22R, LLW44 (mutated residues in *italic*), and Gal4-PPAR γ (AF2-L468A/E471A) mutations were introduced by site-directed mutagenesis. Control (D-001810-10), mMEN1#1 (D-042675-02), mMEN1#2 (D-042675-04), and mPPARG (J-040712-05) small interfering RNA (siRNA) oligonucleotides were purchased from Dharmacon.

Antibodies and immunoblotting. The following antibodies were used: anti- α -tubulin (CP06; Calbiochem), anti-Gal4 (SC-510; Santa Cruz), anti-GST (SC-138; Santa Cruz), anti-H3K4me3 (ab-8580; Abcam), antimenin (A300-105A; Bethyl), anti-PPAR γ (SC-7196 [Santa Cruz] or C26H12.#2435 [Cell Signaling Technology]), anti-FABP4 (SC-18661; Santa Cruz), and antihemagglutinin (anti-HA) (3F10; Roche). Immunoblotting was carried out as described before (9).

Cell lines and stable and transient protein expression. Cos7 (African green monkey kidney) cells and murine 3T3-L1 cells were routinely cultured in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin. Generation of wild-type and *MEN1*^{-/-} (*MEN1* T/T) mouse embryonic fibroblasts (MEFs)

has been described previously (4). MEFs were kindly provided by C. Zhang. MEFs were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 100 μ M β -mercaptoethanol.

For polyclonal PPAR γ -expressing MEF cell lines, wild-type and *MEN1*^{-/-} MEFs were infected with retroviruses collected from 293T Phoenix cells that had been transiently transfected with pMSCV-PPAR γ 2 or pMSCV and were maintained under selection of 2 μ g/ml puromycin.

For menin reexpression studies, *MEN1*^{-/-} MEFs expressing PPAR γ were infected with viruses obtained from Phoenix cells transiently transfected with pBabeHygro, pBabeHygroMenin, and pBabeHygroMeninL22R. Cells were grown in medium containing 2 μ g/ml puromycin and 500 μ g/ml hygromycin, and monoclonal cell lines were isolated.

Luciferase reporter experiments were carried out as described previously (9). DNA (750 ng per well, in a 12-well format) was transfected using FuGene 6 reagent (Roche). Transfection mixtures consisted of 200 ng luciferase reporter, 25 ng pCMV-*Renilla*, 5 ng Gal4-PPAR γ (AF2), 25 ng Gal4-PPAR γ (AF1), or 10 ng Gal4-PPAR γ (AF2-L468A/E471A) vector and supplemented with a maximum of 520 ng pCDNA3.1 M+ and/or empty pCDNA3 plasmid. Twenty-four hours after transfection, the medium was changed to medium containing the appropriate ligand—1 μ M rosiglitazone (in dimethyl sulfoxide [DMSO]), 1 μ M 9-*cis*-retinoic acid (in ethanol)—or vehicle. Luciferase and *Renilla* activities were measured 24 h afterwards. Total cell lysates were used for immunoblotting.

Cell differentiation and siRNA transfection assays. Differentiation of 3T3-L1 cells was performed as described previously (18). For siRNA experiments, cells were grown to 80% confluence and transfected with the indicated siRNA oligonucleotides using Dharmafect 3 reagent. siRNA transfections were repeated every 3 days during the differentiation assay. Polyclonal MEFs expressing PPAR γ were seeded at 5×10^3 cells/ml in a 24-well plate. Cells were grown in medium containing 1 μ M rosiglitazone. The medium was changed every 2 days. After 10 days, cells were subjected to oil red O staining. Monoclonal *MEN1*^{-/-} MEF cell lines were grown to confluence. After 2 days, 1 μ M rosiglitazone was added to the medium, which was refreshed every 2 days for 14 days before cells were subjected to oil red O staining.

Protein expression and purification. The expression and purification of baculovirus-expressed menin have been described previously (9). Plasmids encoding GST, GST-PPAR γ (AF2), and GST-PPAR γ (AF2-L468A/E471A) were transformed into the *Escherichia coli* BL21-Codon Plus strain. Cells were grown at 30°C with shaking. Expression and lysis procedures have been described previously (2).

Yeast two-hybrid analysis. EGY48 cells were transformed with the B42-PPAR γ (AF2) constructs and the indicated LexA-menin constructs. Cells were grown overnight at 30°C in 2% galactose–1% sucrose-containing SC medium lacking the appropriate amino acids and in the presence of vehicle, 1 μ M rosiglitazone, and 1 μ M 9-*cis*-retinoic acid. Lysates were prepared, and LacZ activity was determined by a liquid β -galactosidase assay, as described previously (2).

Protein binding experiments. Soluble lysates containing equivalent amounts of GST, GST-PPAR γ (AF2), and GST-PPAR γ (AF2-L468A/E471A) were bound to glutathione agarose beads (Sigma) for 3 h at 4°C in binding buffer (50 mM Tris-HCl [pH 8.0], 10% glycerol, 140 mM NaCl, 10 mM MgCl₂, 1 mM NaF, 1 mM Na₃VO₄, 0.5 mM dithiothreitol, and protease inhibitors). After washing with binding buffer, purified menin protein (6 μ g/ml) was added. Binding experiments with GST and GST-PPAR γ (AF2) were carried out in the presence of 100 nM rosiglitazone or DMSO for 3 h at 4°C. Glutathione beads were washed three times with excess binding buffer, and eluted proteins were analyzed by immunoblotting.

Analysis of PPAR γ target gene expression. RNA from differentiated and undifferentiated 3T3-L1 cells was isolated using the RNeasy kit (Qiagen). Two hundred fifty nanograms of total RNA was used for cDNA synthesis using random primers. mRNA levels were analyzed by quantitative PCR on a Chromo4-equipped PCR cycler (Bio-Rad) and normalized against a standard reference cDNA.

A 1 μ M concentration of rosiglitazone or DMSO was added to the MEF medium 6 h prior to cell harvesting. Total RNA was extracted using the RNeasy kit (Qiagen) and subsequently treated with DNase. After homogenization, RNA from human samples was isolated using the RNeasy lipid tissue kit (Qiagen). Two hundred nanograms of total RNA was used for cDNA synthesis using oligo(dT). The following PCR primers were used: h β -actin-F (AGAAAATCTGGCA CCACACC), h β -actin-R (AGAGGCGTACAGGGATAGCA), hMEN1-F (GCT CTGGCTGCTCTATGACC), hMEN1-R (TAGAGGGTGAGTGGGCTCTGG), hPPARG-F (GCTGGCCTCCTTGATGAATA), hPPARG-R (TTGGGCTCCA TAAAGTCAC), hFABP4-F (AACCTTAGATGGGGGTGTCC), hFABP4-R

(GTGGAAGTGACGCCTTTCAT), hAQP7-F (GGAAGGTCAGCAGGAGC AC), hAQP7-R (GGTGCTCAGCCAATGAGG), mB-actin-F (GATCTGGCA CCACACCTTCT), mB-actin-R (GGGGTGTGTAAGGTCTCAAA), mFabp4-F (GAAAACGAGATGGTGACAAGC), mFabp4-R (TTGTGGAAGTCACGCCT TT), mAqp7-F (GGCTTCTCCCTTCTCTAGTTT), mAqp-R (AAGGCCACTG AGGAAGTCATT), mP18-F (CTGGAGTTCAGGCTGATGT), mP18-R (GCAGGCTGTGTGCTTCATAA), mP27-F (AACTAACCCGGGACTTG GAG), mP27-R (CCAGGGGCTTATGATTCTGA), mMen1-F (CGATCTT CACTGACTCTTTGG), mMen1-R (AGGTCTGCCAAGTTCCTA GC), mPparg-F (GATGGAAGACCCTCGCATT), and mPparg-R (AACC ATTGGGTCAGCTCTTG).

ChIP experiments. The chromatin immunoprecipitation (ChIP) procedure followed has essentially been described previously (9), with the following adjustments. Subconfluent cultures of MEF cells were treated with 1 μ M rosiglitazone or DMSO for 6 h. Cells were cross-linked by addition of 1% formaldehyde in phosphate-buffered saline for 10 min at 37°C. Glycine was added to a final concentration of 125 mM. After lysis, the cell lysate was sonicated three times for 30 s in a Bioruptor (Diagenode), yielding DNA fragments of about 200 bp. After reverse cross-linking and proteinase K treatment, DNA was purified by phenol-chloroform-isoamyl alcohol treatment (twice), followed by a chloroform-isoamyl alcohol cleaning and ethanol precipitation. Binding of DNA was assessed by quantitative PCR and normalized against input samples from the same experiment. The following primers were used: Fabp4(PPRE)-F (GACAAAGGCAGAAATGCACA), Fabp4(PPRE)-R (AATGTCAAGCATCTGGGAAC), Fabp4(ORF)-F (CAG CTAAAGGGACCGAGATG), Fabp4(ORF)-R (TCGCATTCTCAACAATC AGG), Aqp7(PPRE)-F (CTTGGCTTAAGTGGCATGGG), Aqp7(PPRE)-R (CCAGGGTGGCTGTGAGTTCT), Myo-F (CACCTGAGACCCTGGAT AA), and Myo-R (GCAGCATGTTGTCCCTTCTT).

RESULTS

Expression of the PPAR γ target gene *FABP4* is reduced in sporadic lipomas. To investigate our hypothesis that PPAR γ -dependent transcription is affected in lipomas, we carried out a reverse transcription-PCR (RT-PCR) analysis on a series of four normal adipose tissue samples and eight sporadic lipomas. We measured mRNA levels of total *PPARG*, *MEN1*, and β -actin genes as well as two known PPAR γ target genes: those coding for fatty acid binding protein 4 (*FABP4*/*Ap2*) and aquaporin 7 (*AQP7*) (22, 46). We found that whereas *PPARG*, *MEN1*, and *AQP7* mRNA levels did not differ significantly between these groups (using a two-tailed Student's *t* test), relative *FABP4* mRNA levels were reduced significantly ($P = 0.004$) in lipomas (Fig. 1). From these observations, we suggest that loss of PPAR γ -dependent gene expression may be a feature of sporadic lipomas. In addition, we show that *MEN1* is expressed in adipose tissue.

Menin promotes PPAR γ -dependent adipocyte differentiation. Based on the results shown above, we decided to further explore a potential role for menin in adipocyte differentiation. Unfortunately, no collection of MEN1 patient-derived lipomas is available to us, because of the rarity of the disease and the infrequent need to perform surgery on lipomas. Therefore, we chose to study the relevance of menin for the biological function of PPAR γ using two different controlled mouse cell systems. First, we studied adipocyte differentiation of 3T3-L1 murine fibroblasts. We used siRNA oligonucleotides directed against *MEN1* and as a control against *Pparg* to determine the importance of menin for adipogenesis. Knockdown of menin levels showed a clear reduction in adipogenic potential of the 3T3-L1 cells compared to cells treated with the control siRNA, as illustrated by oil red O staining of triglycerides at day 6 of differentiation (Fig. 2A and B). Both knockdown of menin and PPAR γ resulted in reduced protein levels of the adipocyte

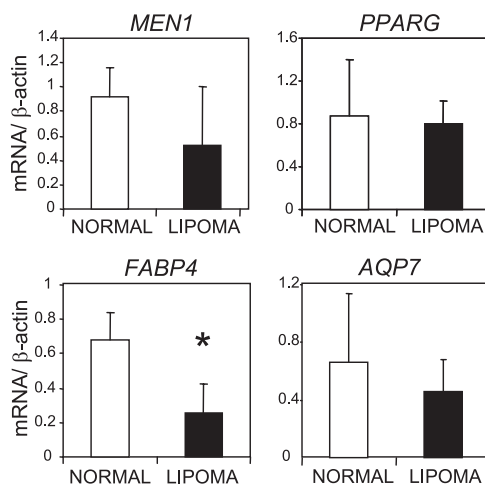


FIG. 1. Expression of the PPAR γ target gene *FABP4* is reduced in sporadic lipomas. mRNA levels of *MEN1*, *PPARG*, *FABP4*, and *AQP7* were determined by quantitative RT-PCR in four normal adipose tissue samples (white columns) and eight sporadic lipomas (black columns). The values shown are averages and standard deviations of mRNA levels normalized against β -actin mRNA. The asterisk indicates statistical significance ($P = 0.004$).

marker *FABP4* (Fig. 2B). To investigate whether the effect of menin knockdown on adipocyte differentiation results from an altered transcription, mRNA expression was examined in the knockdown cells by quantitative RT-PCR. This showed that in cells treated with siRNAs against *Men1*, *Fabp4* mRNA levels were lower, irrespective of *Pparg* mRNA levels (Fig. 2C). These findings suggest that menin is involved in transcription regulation of the *Fabp4* gene.

The second cell system used were the MEFs as they have the capacity to differentiate into adipocytes upon ectopic expression of PPAR γ (47). Wild-type and *MEN1*^{-/-} MEFs were first retrovirally transduced with wild-type mouse PPAR γ 2 (mPPAR γ 2). Both polyclonal cell lines were treated with rosiglitazone. We noticed that *MEN1*^{-/-} MEFs failed to undergo adipogenesis compared to wild-type MEFs as visualized by oil red O staining (Fig. 3A). To exclude that this was due to differences between the cell lines unrelated to the *MEN1* gene status, the wild-type *MEN1* gene was reexpressed in the *MEN1*^{-/-} PPAR γ -expressing cells and monoclonal cell lines were generated (Fig. 3B). Additionally, cell lines expressing a *MEN1* gene missense mutation derived from a MEN1 patient with a lipoma were established (Fig. 3B) (51). This mutation leads to a change from leucine to arginine at position 22 (hereafter, menin L22R). Menin protein levels were found to be similar to those of the wild-type MEF cell line (data not shown). After 14 days of treatment with rosiglitazone, marked lipid accumulation was detected in menin-expressing cells as opposed to cells expressing menin L22R or the empty vector (vector)-transfected cells (Fig. 3C). Furthermore, we noted that introduction of wild-type menin reduces the proliferation rate of *MEN1*^{-/-} MEFs (data not shown). These results show that the presence of wild-type menin is important for proper PPAR γ -dependent adipogenesis in this MEF system.

PPAR γ target gene expression is regulated by menin. To investigate further how menin affects PPAR γ function in the

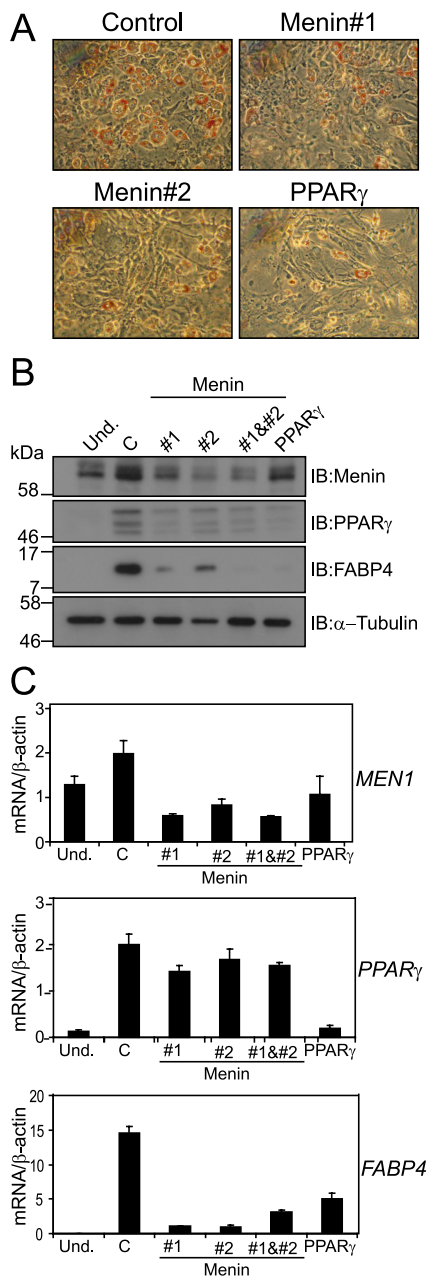


FIG. 2. Knockdown of menin interferes with differentiation of murine 3T3-L1 cells. (A) 3T3-L1 cells were transfected with the indicated siRNA oligonucleotides. After 6 days of differentiation, oil red O staining was performed to assess lipid accumulation. (B) Immunoblot (IB) analysis of global menin, PPAR γ (the Cell Signaling Technology antibody recognizes multiple forms of PPAR γ), and FABP4 levels in undifferentiated (Und.) cells and siRNA-treated cells was performed. α -Tubulin was used as a loading control. The positions of molecular mass markers, with their respective molecular masses in kilodaltons, are indicated next to the blots. (C) RT-PCR analysis of total mRNA levels of *Men1*, *Pparg*, and *Fabp4* in siRNA-treated 3T3-L1 cells. The values shown represent averages and standard deviations. mRNA levels were normalized against β -actin mRNA.

MEF system, we studied the expression of the PPAR γ target genes, *Fabp4* and *Aqp7*. Monoclonal cell lines expressing similar levels of menin and PPAR γ were selected for this analysis. Cells were treated for 6 h with rosiglitazone or DMSO and

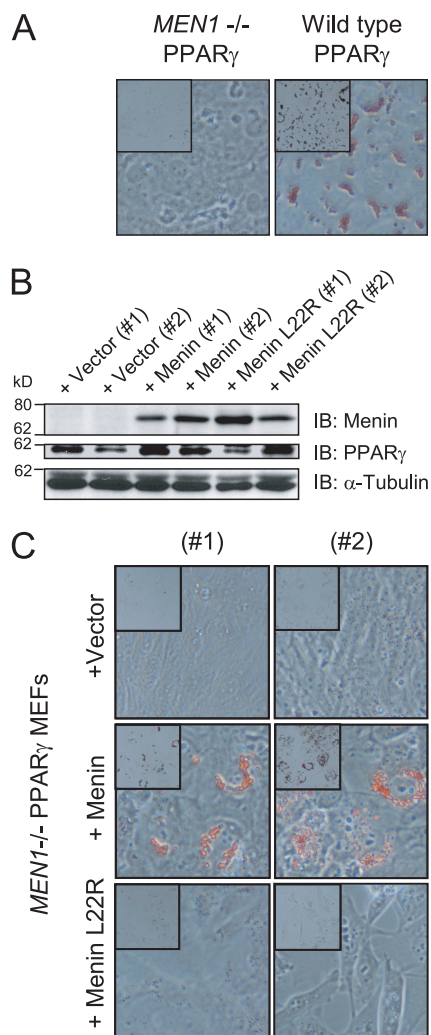


FIG. 3. Menin is required for PPAR γ -dependent adipocyte differentiation. (A) Polyclonal *MEN1*^{-/-} and wild-type MEF cells that ectopically express PPAR γ were treated with 1 μ M rosiglitazone for 10 days. Lipid vesicle formation was assessed by oil red O staining (40 \times magnification, inset represents 10 \times magnification). (B) Menin and menin L22R were reexpressed in *MEN1*^{-/-} MEFs expressing PPAR γ . The presence of menin and PPAR γ (SC antibody recognizes PPAR γ 2 specifically) was visualized by immunoblotting (IB) using α -tubulin as a loading control in total cell lysates of two monoclonal cell lines carrying the empty vector, two cell lines reexpressing wild-type menin, and two cell lines expressing menin L22R that had been treated with 1 μ M rosiglitazone for 14 days. The positions of molecular mass markers, with their respective molecular masses in kilodaltons, are indicated next to the blots. (C) Oil red O staining of the cell lines described in panel B.

subsequently subjected to quantitative RT-PCR analysis. Compared to cells expressing wild-type menin, ligand-induced mRNA levels of *Fabp4* and *Aqp7* were found to be lower in cells expressing menin L22R or the cells carrying only the vector (Fig. 4A).

The genes for p18^{Ink4c} (*Cdkn2c*) and p27^{Kip1} (*Cdkn1b*) are well-established menin target genes, which can restrict cell proliferation (32). Moreover, p18^{Ink4c} was found to be upregulated in mouse fibroblasts that ectopically expressed PPAR γ (34). We hypothesized that mRNA levels of *Cdkn2c* and *Cdkn1b* would be higher in menin-expressing cells. However,

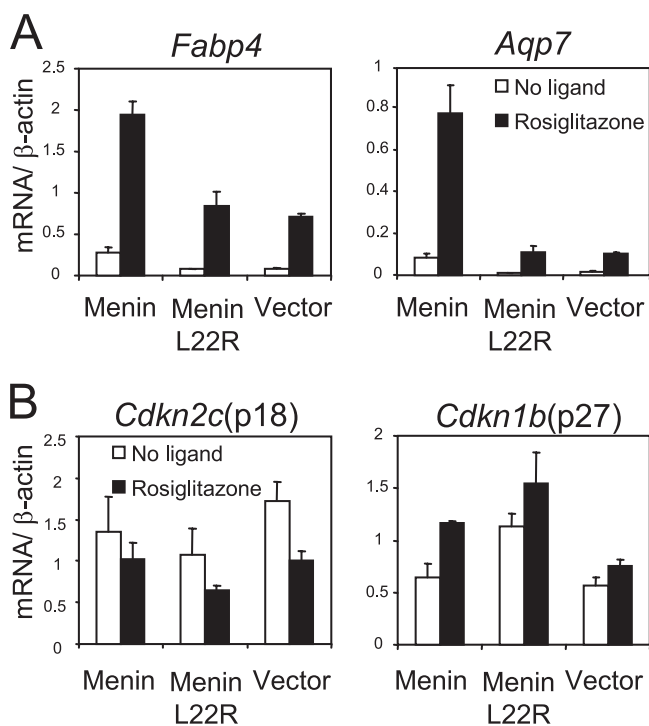


FIG. 4. Menin regulates transcription of PPAR γ target genes. (A) The menin (no. 1), menin L22R (no. 2), and vector (no. 1) cell lines were treated with DMSO (white columns) or 1 μ M rosiglitazone (black columns) for 6 h. mRNA levels of *Fabp4* and *Aqp7* were measured by quantitative RT-PCR. Averages and standard deviations relative to β -actin mRNA are shown from a representative experiment performed in triplicate. (B) Relative levels of *p18/Cdkn2c* and *p27/Cdkn1b* mRNA were determined in the same experiment.

expression of both genes did not differ between these cell lines, and treatment with rosiglitazone did not clearly affect transcription of these genes (Fig. 4B). RT-PCR analysis of expression of the above-mentioned genes in polyclonal *MEN1*^{-/-} and wild-type MEF cell lines ectopically expressing PPAR γ yielded similar results (data not shown). Altogether, these experiments show that in the absence of menin the expression of PPAR γ target genes is downregulated and that in our system *p18^{GlnK4c}* and *p27^{Kip1}* are probably not involved in adipocyte differentiation.

Histone H3K4 trimethylation on PPAR γ target genes is largely dependent on menin. Upon ligand stimulation, PPAR γ target genes show increased H3K4 trimethylation levels (44, 45). To determine the H3K4me3 status of PPAR γ target genes in the presence or absence of menin, we performed ChIP experiments in the monoclonal *MEN1*^{-/-} MEF cell lines ectopically expressing PPAR γ . We used antibodies directed at menin, PPAR γ , or the trimethylated form of histone H3K4 to study their presence on the *Fabp4* and *Aqp7* genes (Fig. 5A). The heterochromatic myoglobin (*Myo*) gene was used as an internal negative control. In both wild-type menin- and menin L22R-reexpressing cells, menin could be detected on the PPRES of the *Fabp4* and *Aqp7* genes after cells had been treated with rosiglitazone for 6 h (Fig. 5B, upper panels). No menin recruitment could be detected at the transcription start site of *Fabp4* (data not shown). In Fig. 5B (middle panels), we

show that when ligand is present approximately equal quantities of PPAR γ are recruited. However, both menin L22R-expressing and vector control cells showed a clear reduction of ligand-induced H3K4me3 levels in the open reading frame of *Fabp4* and at the transcription start site of *Aqp7* (Fig. 5B, lower panels). From these data, we conclude that both wild-type menin and mutant menin colocalize with PPAR γ at PPRES and that menin is needed for recruitment of adequate H3K4 trimethyltransferase activity to two different PPAR γ target genes.

To ascertain that menin recruitment on PPAR γ -responsive promoters is dependent on PPAR γ , we performed additional ChIP experiments. Wild-type MEFs expressing endogenous menin were used that had been infected with PPAR γ 2 or vector control viruses. As shown in Fig. 5C (upper panels), menin is recruited exclusively to the *Fabp4* and *Aqp7* promoters when liganded PPAR γ is present (Fig. 5C, middle panels). In the absence of PPAR γ , H3K4 trimethylation levels are severely reduced (Fig. 5C, lower panels). These experiments show that PPAR γ is required for bringing the menin HMT complex to PPAR γ target genes.

Menin can interact directly with the AF2 domain of PPAR γ in a ligand-independent fashion. We previously found that menin can bind to ER α in a ligand-dependent manner (9). To investigate whether menin and PPAR γ can interact directly, we carried out yeast two-hybrid and GST pull-down assays. In yeast two-hybrid assays, we found a ligand-independent interaction between wild-type menin fused to LexA and B42-coupled PPAR γ (AF2). Menin contains one potential LXXLL NR interaction motif (amino acids 263 to 267; LLWLL). Mutation of this motif disrupts the canonical NR coactivator interaction (16). Interestingly, both the LLWAA and L22R menin mutants retained PPAR γ binding (Fig. 6A). In GST pull-down experiments, purified wild-type menin was incubated with equal amounts of a GST-PPAR γ (AF2) fusion protein. Consistent with the yeast two-hybrid result, menin could bind to GST-PPAR γ (AF2), albeit with low affinity (Fig. 6B). In fact, in this assay we reproducibly found a small decrease of the interaction in the presence of ligand. Also in this assay, the menin-PPAR γ interaction did not depend on the presence of ligand. The L468A E471A double mutant in helix 12 in the AF2 domain of PPAR γ does not bind to the coactivators CBP and SRC1, but is still able to bind ligand (14). This L468A E471A mutation—hereafter PPAR γ (AF2mut)—was introduced into the GST-PPAR γ (AF2) expression plasmid. As shown in Fig. 6B, menin was able to bind to a GST-PPAR γ (AF2mut) protein, indicating that the integrity of helix 12 of PPAR γ is not required for menin binding. PPAR γ binds to PPRES in a heterodimeric form with RXR isoforms. To determine if menin can bind to RXR, we performed yeast two-hybrid assays. Coexpression in yeast of LexA-menin and B42-fused RXR α revealed an interaction between menin and RXR α , which is increased in the presence of its ligand (Fig. 6C). These findings suggest that besides by binding to PPAR γ , menin can potentially also be recruited to PPRES via liganded RXR α .

Two patient-derived missense mutations leading to leucine-to-proline substitutions at positions 264 or 267 in menin severely disrupt the capacity of menin to bind to ER α (9). These mutations are both in the predicted NR interaction motif, leading to LPWLL and LLWLP, respectively (mutated residue

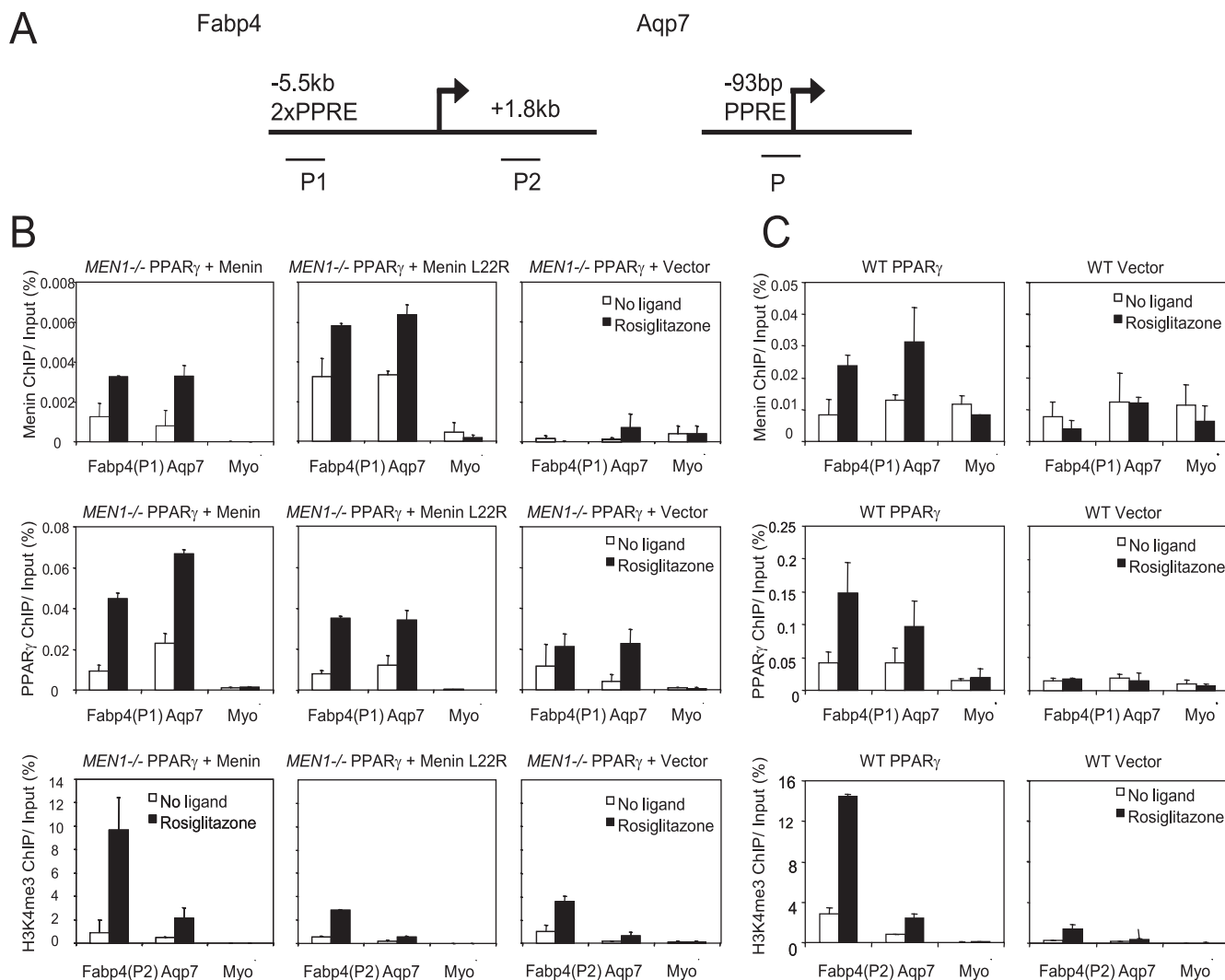


FIG. 5. Menin recruits H3K4 trimethylation activity to PPAR γ target genes. (A) Schematic representation of the *Fabp4* and *Aqp7* genes. *Fabp4* contains two PPREs in an upstream enhancer element. For ChIP analysis, two primer pairs were used: P1 for amplifying the region of the PPREs for studying menin and PPAR γ occupancy and P2 in the open reading frame to measure H3K4me3. The PPRE of *Aqp7* is located close to the transcription start site; one primer pair was used for ChIP analysis. (B and C) Cell lines used for mRNA expression analysis (B) and polyclonal wild-type (WT) MEFs expressing either PPAR γ or vector (C) were used. Cells were stimulated for 6 h with 1 μ M rosiglitazone or DMSO. Averages of immunoprecipitated DNA, as a percentage of input, and standard errors of the means of two independent experiments are shown. (B and C, upper and middle panels) Menin and PPAR γ recruitment was assessed on the PPREs of *Fabp4* and *Aqp7*. (B and C, lower panels) H3K4me3 levels were measured in the open reading frame of *Fabp4* and near the transcription start site of *Aqp7*. The myoglobin (Myo) gene was used as an internal negative control. (B, upper right panel) The *MEN1^{-/-}* vector cell line that does not express menin serves as a negative control for the menin antibody. (C, right panels) The wild-type MEF cells that do not express exogenous PPAR γ serve as a control for PPAR γ in menin recruitment.

in italic), and have more severe structural consequences than the LLWAA mutation. In a yeast two-hybrid setup, both the L264P and L267P mutants showed reduced menin binding (Fig. 6D). From these observations, we conclude that the interaction between menin and PPAR γ is not a classical LXXLL-helix 12 interaction. Rather, a larger domain of menin which includes LLWLL may be required for menin-PPAR γ binding.

Menin enhances PPAR γ -dependent transcription. To study in more detail the mechanism of coactivation of PPAR γ by menin, we performed luciferase assays. To this end, Cos7 cells were transiently transfected with increasing amounts of MEN1 expression vector together with the expression vector for a

fusion protein containing the AF2 domain of PPAR γ fused to the DNA binding domain of the yeast Gal4 activator. A thymidine kinase promoter-luciferase construct with five Gal4 binding sites was used as a reporter gene. Overexpression of menin increased ligand-induced transcription up to approximately threefold, depending on the amount of menin that was cotransfected (Fig. 7A). This is similar to the effect of overexpression of menin on ligand-dependent transcription driven by a Gal4-ER α fusion protein (9). We performed similar experiments using a Gal4-RXR α fusion protein expression vector and found that menin can also coactivate 9-*cis*-retinoic acid-induced transcription (data not shown). To assess the specificity of menin for liganded PPAR γ , the MEN1 expression vector

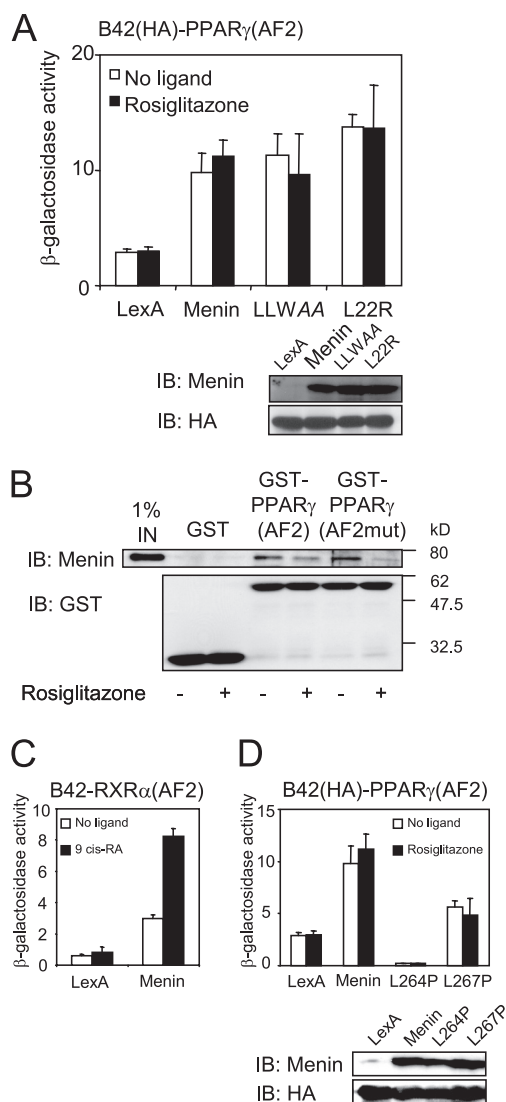


FIG. 6. Menin and PPAR γ can interact directly. (A) Mutations resulting in LLWAA and L22R menin mutants were introduced in the pEG202-LexAMenin yeast expression vector. EGY48 cells transformed with the indicated LexA constructs and B42-HA-PPAR γ (AF2) were treated with 1 μ M rosiglitazone or DMSO for 12 h. Cells were lysed, and β -galactosidase activity was determined and normalized for the protein content of the lysates. Averages and standard deviations of a representative experiment performed in triplicate are shown. Expression levels of the menin proteins and the levels of B42-HA-PPAR γ (AF2) were verified by immunoblotting (IB). (B) Glutathione beads were coated with purified GST, GST-PPAR γ (AF2), or GST-PPAR γ (AF2mut) proteins and incubated with purified recombinant menin in the absence or presence of 100 nM rosiglitazone. Retention of menin protein was determined by immunoblotting. (C) Together with a LexA-menin expression construct, a B42-fused ligand binding domain of RXR α was transformed into EGY48 cells. Cells were incubated with 1 μ M 9-*cis*-retinoic acid (9 *cis*-RA) or vehicle for 12 h, and β -galactosidase activities were determined. (D) Two patient-derived mutations, L264P and L267P, were introduced into the LexA-menin fusion plasmid and transformed for a yeast two-hybrid experiment as described.

was cotransfected with a Gal4-PPAR γ (AF1) expression plasmid. Gal4-PPAR γ (AF1)-dependent transcription was not affected by menin (Fig. 7B). The coactivation of AF2 observed by menin was also similar to the effect of two known PPAR γ

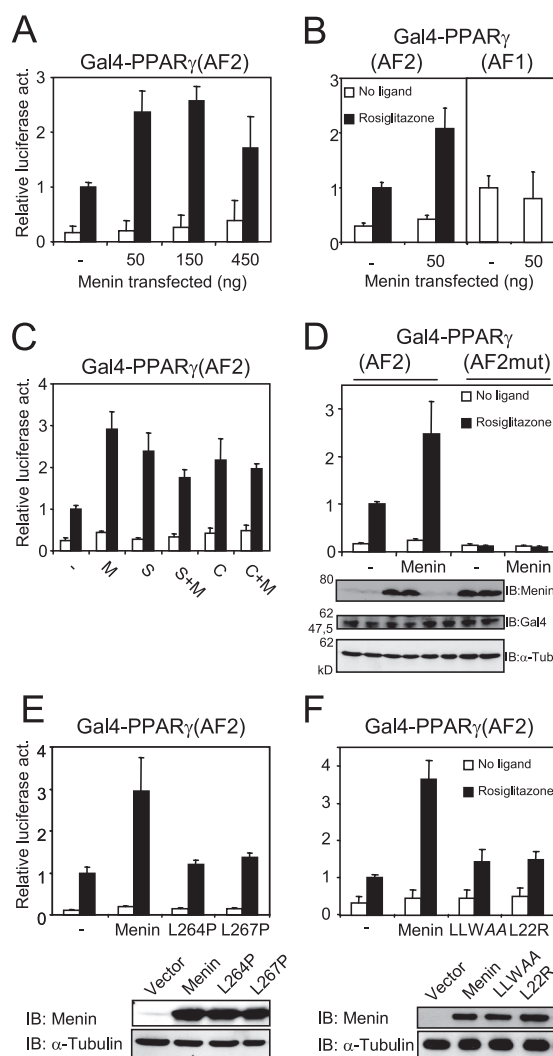


FIG. 7. Menin coactivates PPAR γ -mediated transcription in a ligand-dependent fashion. (A) Cos7 cells were transiently transfected with a 5 \times GalTK-luciferase construct and an expression plasmid for Gal4-PPAR γ (AF2) and treated with 1 μ M rosiglitazone or DMSO for 24 h. Increasing amounts of menin were cotransfected. Firefly luciferase readings were normalized against *Renilla* luciferase, which served as an internal control. Columns represent averages and standard deviations of two independent experiments performed in triplicate. All luciferase assays shown were performed in this fashion. (B) Menin was overexpressed in cells cotransfected with Gal4-PPAR γ (AF2) and in cells coexpressing Gal4-PPAR γ (AF1). (C) Along with 50 ng of the menin (M) expression plasmid, cells were transfected with 450 ng of pSG5-SRC1e (S), 450 ng pSG5-HA-CARM1 (C), or a combination of these factors and menin. (D) A Gal4-PPAR γ (AF2) construct bearing two mutations in helix 12 (L468A E471A) was transiently transfected into Cos7 cells. Protein levels of menin, Gal4-PPAR γ (AF2), and α -tubulin (α -Tub.) were determined by immunoblotting (IB). Lanes on the gel correspond to the columns shown in the panel above. (E) Expression plasmids encoding the L264P and L267P menin mutants were cotransfected with Gal4-PPAR γ (AF2). Menin protein levels were determined by immunoblotting with α -tubulin as a loading control. (F) The LLWAA and L22R mutations were also introduced in the MEN1 expression plasmid and transfected into Cos7 cells for luciferase assays. Protein levels were determined as in panel E.

coactivators, SRC1 and CARM1 (Fig. 7C) (35, 54). In this setup, we did not detect synergy between menin and these known coactivators (Fig. 7C).

We introduced the L468A E471A double mutation into the Gal4-PPAR γ (AF2) vector. As shown in Fig. 7D, Gal4-PPAR γ (AF2mut) does not activate transcription upon addition of ligand. Overexpression of menin did not affect luciferase activity driven by this PPAR γ mutant (Fig. 7D). Cotransfection of the menin L264P and L267P mutants did not result in any coactivation (Fig. 7E). The menin LLWAA and L22R mutations were also introduced into the MEN1 expression vector. Strikingly, menin LLWAA could also not stimulate rosiglitazone-induced transcription (Fig. 7F). Finally, as expected, menin L22R, the mutant that failed to recruit HMT activity to the *Fabp4* and *Aqp7* gene promoters (Fig. 5D) or restore PPAR γ -driven adipogenesis (Fig. 3C), was also not able to coactivate PPAR γ (AF2) in the luciferase setup (Fig. 7F).

We conclude from these results that menin acts as a coactivator for the AF2 function of PPAR γ to an extent that is comparable to those of other well-established coactivators. The integrity of helix 12 of PPAR γ as well as the LLWLL motif in menin is required for coactivation. The MEN1 lipoma-derived menin L22R mutant failed to coactivate PPAR γ -dependent transcription.

DISCUSSION

The hallmark of the MEN1 syndrome is the development of multiple tumors in endocrine organs. A large proportion of MEN1 patients also develop lipomas (8). Our results link the function of menin to the nuclear receptor PPAR γ . This receptor has been reported to be expressed in liposarcomas (malignant lipomatous tumors), and treatment of cultured liposarcoma cells with the synthetic PPAR γ ligand pioglitazone led to increased differentiation (48). To our knowledge, no study has addressed yet the role of PPAR γ or menin in lipoma development. In a series of sporadic lipomas, we found that expression of the PPAR γ target gene *FABP4* was lower than that of normal adipose tissue (Fig. 1). As PPAR γ is involved in adipocyte differentiation and proliferation, it is tempting to speculate that loss of PPAR γ signaling plays a role in lipoma development. Inactivation of menin, which functions as a PPAR γ coactivator, could in this way play a direct role in loss of PPAR γ -dependent differentiation, resulting in MEN1 lipomas. Possibly, *MEN1* control of cell proliferation also contributes to this. In sporadic lipomas, inactivation of this pathway may occur through alternative mechanisms, since we found no significant differences in *MEN1* and *PPARG* levels between normal and lipomatous tissues (Fig. 1).

Besides its expression in lipomas, several studies have shown that PPAR γ is expressed in MEN1-related tumors, like pituitary tumors and neuroendocrine tumors (15, 31) and also neuroendocrine tumors of the thymus in MEN1 patients (K. M. A. Dreijerink and H. T. M. Timmers, unpublished results). In addition, pioglitazone has been shown to inhibit proliferation of a lung neuroendocrine tumor cell line (13), while rosiglitazone treatment of mice that had been subcutaneously injected with pituitary adenoma cells led to attenuation of tumor growth (15). Furthermore, the recent finding that menin plays a role in suppression of adaptive pancreatic β -cell

proliferation in obese mice, in which PPAR γ is also involved, raises the interesting possibility that the interaction of menin and PPAR γ plays a role in this process as well (20, 38). Taken together, the relevance of the menin-PPAR γ interaction could move beyond that of a role in lipoma development.

Menin and adipocyte differentiation. Our findings provide more insight into the most intriguing question pending for MEN1: how do germ line mutations in a ubiquitously expressed gene cause an endocrine tumor phenotype? The *Cdkn2c* gene (encoding p18^{Ink4c}) and the *Cdkn1b* gene (encoding p27^{Kip1}) are targets of the menin-HMT complex (32). These cyclin-dependent kinase-inhibiting genes are inhibitors of cell proliferation. Mice lacking either p18^{Ink4c}, p21^{CIP1/Waf1}, or p27^{Kip1} develop tumor phenotypes that show significant overlap with the manifestations of the MEN1 syndrome (11). However, why these knockout mice display endocrine tissue-specific phenotypes is not clear. Expression levels of *Cdkn2c* and *Cdkn1b* were not clearly affected in our MEF system, rendering it unlikely that deregulation of these genes contributes to the effect of menin on adipocyte differentiation. We found that murine 3T3-L1 cells and embryonic fibroblasts lacking functional menin are defective in PPAR γ -dependent adipocyte differentiation. Reexpression of menin in *MEN1*^{-/-} MEFs restored the adipogenic potential (Fig. 3). In this study, we used a system in which adipocyte differentiation is maximally dependent on PPAR γ activity. Previously, Sowa et al. showed that transient knockdown of menin in multipotential mouse mesenchymal stem cells did not affect bone morphogenic protein 2 (BMP-2)-induced adipocyte differentiation (43). This indicates that menin is involved in selective differentiation pathways.

Recruitment of H3K4-HMT complexes by menin. Menin is an integral component of HMT complexes that contain MLL1(KMT2A) or MLL2(KMT2B) (17, 55). Our results extend the evidence that menin can be tethered to DNA through interaction with NRs, which results in increased H3K4me3 levels on target genes.

In *MEN1*^{-/-} cells, we found that ligand-induced H3K4 trimethylation on the *Fabp4* and *Aqp7* genes is reduced but not completely absent (Fig. 5B). The identification of several other protein complexes that can recruit HMT activity directed at H3K4 to NRs raises the possibility of redundancy of these complexes. Mo et al. reported that an MLL4(KMT2D)-HMT complex, which lacks menin, can coactivate ER α -mediated transcription (33). Furthermore, the ASCOM complex, which contains the NR coactivator activating signal cointegrator 2 (ASC-2), MLL3(KMT2C), and MLL4(KMT2D), which also lacks menin, can recruit HMT activity to the retinoic acid receptor (RAR) and the liver X receptor (LXR) (26, 27). Similar to menin-HMT, the ASCOM complex was recently demonstrated to regulate adipogenesis and H3K4me3 on a PPAR γ target gene (25). MLL3 knockout cells showed partial loss of H3K4me3 on the *FABP4* gene. Therefore, other H3K4me3 complexes could very well be responsible for the remaining H3K4me3 observed on the PPAR γ target genes in the *MEN1*^{-/-} MEFs (Fig. 5B). Loss of menin in tumors of MEN1 patients does not lead to global loss of H3K4 trimethylation in these tumors, indicating that menin plays a gene-specific function (R. A. Varier, J. A. Kummer, and H. T. M. Timmers, unpublished results).

We found that a MEN1 lipoma-derived mutant, menin L22R, retained the ability to bind to PPAR γ in a yeast two-hybrid assay (Fig. 6A) but could not coactivate PPAR γ in a luciferase assay (Fig. 7F). Consistent with these findings, we could detect colocalization of menin L22R with PPAR γ on the PPREs of the *Fabp4* and *App7* genes (Fig. 5B). Rosiglitazone-induced H3K4me3 levels at these genes, however, were found to be reduced (Fig. 5B). Hughes et al. showed that menin L22R can be incorporated into an enzymatically active MLL complex (17). Our results demonstrate that menin L22R bound to PPAR γ on DNA does not recruit HMT activity. Possibly, this is due to a conformational change in menin L22R, which prevents menin from recruiting HMT activity in the chromatin context.

Menin interacts with PPAR γ . We studied the interaction between menin and the AF2 domain of PPAR γ . Using yeast two-hybrid and GST pull-down experiments, we found that this interaction is not dependent on the presence of ligand (Fig. 6A and B). In the liganded situation, the affinity of the interaction seemed slightly weaker than without ligand (Fig. 6B). This is in contrast to the interactions of menin with ER α and RXR (Fig. 6C), which are ligand dependent and represent classical NR coactivator interactions (9). Unlike a mutation of ER α (AF2) helix 12 (9), a mutation of helix 12 of PPAR γ (AF2) did not affect the interaction with menin (Fig. 6B). Based on structural differences within the ligand binding domains, NRs can be divided into two classes (6). These structural differences between the AF2 domains of PPAR γ (which is a class II receptor) and those of ER α and RXR (which are class I receptors) could be related to differential binding to menin. In support of this, yeast two-hybrid studies with menin and the ligand binding domain of VDR, which also belongs to the class II receptors, showed a similar ligand-independent binding (K. M. A. Dreijerink and H. T. M. Timmers, unpublished results).

The role of the LLWLL (amino acids 263 to 267) motif of menin for PPAR γ function is not entirely clear. Whereas the LLWAA mutant affects the coactivator function of menin, binding to AF2 of PPAR γ is not disturbed (Fig. 6A and 7F). In contrast, the MEN1 patient-derived mutants LPWLL (L264P) and LLWLP (L267P) are defective both for PPAR γ binding and the coactivator function (Fig. 6D and 7E). Interestingly, the PPAR γ interaction pattern of menin resembles that of another known PPAR γ coactivator, CBP. CBP also does not interact with PPAR γ in a classical manner as it is also able to bind to PPAR γ irrespective of the presence of ligand. Nuclear magnetic resonance studies revealed that the segment within CBP required for the interaction with PPAR γ is larger than the LXXLL motif only (23). Possibly, the PPAR γ interaction function of menin also extends beyond the LLWLL motif, as indicated by the patient-derived mutations. It would be interesting to obtain a structural model for the domain within menin that interacts with PPAR γ .

In conclusion, we demonstrate that menin is a direct coactivator for PPAR γ -mediated transcription by recruiting HMT activity to PPAR γ target genes. The lipoma-derived menin L22R mutant, which failed to recruit HMT activity, is defective in supporting the capacity of PPAR γ to induce adipogenesis. Our results couple PPAR γ -dependent adipocyte differentiation to MEN1-related lipoma development and possibly other tissue-specific manifestations of MEN1. The functional linkage

of PPAR γ and menin could form the basis for future therapeutic interventions for MEN1 patients.

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REFERENCES

- Agarwal, S. K., S. Impey, S. McWeeney, P. C. Scacheri, F. S. Collins, R. H. Goodman, A. M. Spiegel, and S. J. Marx. 2007. Distribution of menin-occupied regions in chromatin specifies a broad role of menin in transcriptional regulation. *Neoplasia* 9:101–107.
- Albert, T. K., H. Hanzawa, Y. I. Legtenberg, M. J. de Ruwe, F. A. van den Heuvel, M. A. Collart, R. Boelens, and H. T. Timmers. 2002. Identification of a ubiquitin-protein ligase subunit within the CCR4-NOT transcription repressor complex. *EMBO J.* 21:355–364.
- Altiock, S., M. Xu, and B. M. Spiegelman. 1997. PPAR γ induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. *Genes Dev.* 11:1987–1998.
- Bertolino, P., I. Radovanovic, H. Casse, A. Aguzzi, Z. Q. Wang, and C. X. Zhang. 2003. Genetic ablation of the tumor suppressor menin causes lethality at mid-gestation with defects in multiple organs. *Mech. Dev.* 120:549–560.
- Brandi, M. L., R. F. Gagel, A. Angeli, J. P. Bilezikian, P. Beck-Peccoz, C. Bordi, B. Conte-Devolx, A. Falchetti, R. G. Gheri, A. Libroia, C. J. Lips, G. Lombardi, M. Mannelli, F. Pacini, B. A. Ponder, F. Raue, B. Skogseid, G. Tamburrano, R. V. Thakker, N. W. Thompson, P. Tomassetti, F. Tonelli, S. A. Wells, Jr., and S. J. Marx. 2001. Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J. Clin. Endocrinol. Metab.* 86:5658–5671.
- Brelivet, Y., S. Kammerer, N. Rochel, O. Poch, and D. Moras. 2004. Signature of the oligomeric behaviour of nuclear receptors at the sequence and structural level. *EMBO Rep.* 5:423–429.
- Chen, D., H. Ma, H. Hong, S. S. Koh, S. M. Huang, B. T. Schurter, D. W. Aswad, and M. R. Stallcup. 1999. Regulation of transcription by a protein methyltransferase. *Science* 284:2174–2177.
- Darling, T. N., M. C. Skarulis, S. M. Steinberg, S. J. Marx, A. M. Spiegel, and M. Turner. 1997. Multiple facial angiofibromas and collagenomas in patients with multiple endocrine neoplasia type 1. *Arch. Dermatol.* 133:853–857.
- Dreijerink, K. M., K. W. Mulder, G. S. Winkler, J. W. Hoppener, C. J. Lips, and H. T. Timmers. 2006. Menin links estrogen receptor activation to histone H3K4 trimethylation. *Cancer Res.* 66:4929–4935.
- Dubois, M., F. Pattou, J. Kerr-Conte, V. Gmyr, B. Vandewalle, P. Desreumaux, J. Auwerx, K. Schoonjans, and J. Lefebvre. 2000. Expression of peroxisome proliferator-activated receptor gamma (PPAR γ) in normal human pancreatic islet cells. *Diabetologia* 43:1165–1169.
- Franklin, D. S., V. L. Godfrey, D. A. O'Brien, C. Deng, and Y. Xiong. 2000. Functional collaboration between different cyclin-dependent kinase inhibitors suppresses tumor growth with distinct tissue specificity. *Mol. Cell. Biol.* 20:6147–6158.
- Ge, K., M. Guermah, C. X. Yuan, M. Ito, A. E. Wallberg, B. M. Spiegelman, and R. G. Roeder. 2002. Transcription coactivator TRAP220 is required for PPAR γ 2-stimulated adipogenesis. *Nature* 417:563–567.
- Goke, R., A. Goke, B. Goke, W. S. El-Deiry, and Y. Chen. 2001. Pioglitazone inhibits growth of carcinoid cells and promotes TRAIL-induced apoptosis by induction of p21waf1/cip1. *Digestion* 64:75–80.
- Gurnell, M., J. M. Wentworth, M. Agostini, M. Adams, T. N. Collingwood, C. Provenzano, P. O. Browne, O. Rajanayagam, T. P. Burris, J. W. Schwabe, M. A. Lazar, and V. K. Chatterjee. 2000. A dominant-negative peroxisome proliferator-activated receptor gamma (PPAR γ) mutant is a constitutive repressor and inhibits PPAR γ -mediated adipogenesis. *J. Biol. Chem.* 275:5754–5759.
- Heaney, A. P., M. Fernando, and S. Melmed. 2003. PPAR-gamma receptor ligands: novel therapy for pituitary adenomas. *J. Clin. Investig.* 111:1381–1388.
- Heery, D. M., E. Kalkhoven, S. Hoare, and M. G. Parker. 1997. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* 387:733–736.
- Hughes, C. M., O. Rozenblatt-Rosen, T. A. Milne, T. D. Copeland, S. S.

- Levine, J. C. Lee, D. N. Hayes, K. S. Shanmugam, A. Bhattacharjee, C. A. Biondi, G. F. Kay, N. K. Hayward, J. L. Hess, and M. Meyerson. 2004. Menin associates with a trithorax family histone methyltransferase complex and with the *hoxc8* locus. *Mol. Cell* **13**:587–597.
18. Jeninga, E. H., O. van Beekum, A. D. van Dijk, N. Hamers, B. I. Hendriks-Stegeman, A. M. Bonvin, R. Berger, and E. Kalkhoven. 2007. Impaired peroxisome proliferator-activated receptor gamma function through mutation of a conserved salt bridge (R425C) in familial partial lipodystrophy. *Mol. Endocrinol.* **21**:1049–1065.
 19. Kalkhoven, E., J. E. Valentine, D. M. Heery, and M. G. Parker. 1998. Isoforms of steroid receptor co-activator 1 differ in their ability to potentiate transcription by the oestrogen receptor. *EMBO J.* **17**:232–243.
 20. Karnik, S. K., H. Chen, G. W. McLean, J. J. Heit, X. Gu, A. Y. Zhang, M. Fontaine, M. H. Yen, and S. K. Kim. 2007. Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. *Science* **318**:806–809.
 21. Kim, H., J. E. Lee, E. J. Cho, J. O. Liu, and H. D. Youn. 2003. Menin, a tumor suppressor, represses JunD-mediated transcriptional activity by association with an mSin3A-histone deacetylase complex. *Cancer Res.* **63**:6135–6139.
 22. Kishida, K., I. Shimomura, H. Nishizawa, N. Maeda, H. Kuriyama, H. Kondo, M. Matsuda, H. Nagaretani, N. Ouchi, K. Hotta, S. Kihara, T. Kadowaki, T. Funahashi, and Y. Matsuzawa. 2001. Enhancement of the aquaporin adipe gene expression by a peroxisome proliferator-activated receptor gamma. *J. Biol. Chem.* **276**:48572–48579.
 23. Klein, F. A., R. A. Atkinson, N. Potier, D. Moras, and J. Cavarelli. 2005. Biochemical and NMR mapping of the interface between CREB-binding protein and ligand binding domains of nuclear receptor: beyond the LXXXLL motif. *J. Biol. Chem.* **280**:5682–5692.
 24. La, P., A. C. Silva, Z. Hou, H. Wang, R. W. Schnepf, N. Yan, Y. Shi, and X. Hua. 2004. Direct binding of DNA by tumor suppressor menin. *J. Biol. Chem.* **279**:49045–49054.
 25. Lee, J., P. K. Saha, Q. H. Yang, S. Lee, J. Y. Park, Y. Suh, S. K. Lee, L. Chan, R. G. Roeder, and J. W. Lee. 2008. Targeted inactivation of MLL3 histone H3-Lys-4 methyltransferase activity in the mouse reveals vital roles for MLL3 in adipogenesis. *Proc. Natl. Acad. Sci. USA* **105**:19229–19234.
 26. Lee, S., D. K. Lee, Y. Dou, J. Lee, B. Lee, E. Kwak, Y. Y. Kong, S. K. Lee, R. G. Roeder, and J. W. Lee. 2006. Coactivator as a target gene specificity determinant for histone H3 lysine 4 methyltransferases. *Proc. Natl. Acad. Sci. USA* **103**:15392–15397.
 27. Lee, S., J. Lee, S. K. Lee, and J. W. Lee. 2008. Activating signal cointegrator-2 is an essential adaptor to recruit histone H3 lysine 4 methyltransferases MLL3 and MLL4 to the liver X receptors. *Mol. Endocrinol.* **22**:1312–1319.
 28. Lehrke, M., and M. A. Lazar. 2005. The many faces of PPARgamma. *Cell* **123**:993–999.
 29. Lemos, M. C., and R. V. Thakker. 2008. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. *Hum. Mutat.* **29**:22–32.
 30. Lin, S. Y., and S. J. Elledge. 2003. Multiple tumor suppressor pathways negatively regulate telomerase. *Cell* **113**:881–889.
 31. Messenger, M., C. Carriere, X. Bertagna, and Y. de Keyser. 2006. RT-PCR analysis of corticotroph-associated genes expression in carcinoid tumours in the ectopic-ACTH syndrome. *Eur. J. Endocrinol.* **154**:159–166.
 32. Milne, T. A., C. M. Hughes, R. Lloyd, Z. Yang, O. Rozenblatt-Rosen, Y. Dou, R. W. Schnepf, C. Krankel, V. A. Livolsi, D. Gibbs, X. Hua, R. G. Roeder, M. Meyerson, and J. L. Hess. 2005. Menin and MLL cooperatively regulate expression of cyclin-dependent kinase inhibitors. *Proc. Natl. Acad. Sci. USA* **102**:749–754.
 33. Mo, R., S. M. Rao, and Y. J. Zhu. 2006. Identification of the MLL2 complex as a coactivator for estrogen receptor alpha. *J. Biol. Chem.* **281**:15714–15720.
 34. Morrison, R. F., and S. R. Farmer. 1999. Role of PPARgamma in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18(INK4c) and p21(Waf1/Cip1), during adipogenesis. *J. Biol. Chem.* **274**:17088–17097.
 35. Nolte, R. T., G. B. Wisely, S. Westin, J. E. Cobb, M. H. Lambert, R. Kurokawa, M. G. Rosenfeld, T. M. Willson, C. K. Glass, and M. V. Milburn. 1998. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature* **395**:137–143.
 36. Occhi, G., N. Albiger, S. Bertucchi, M. Gardiman, M. Scanarini, R. Scienza, A. Fassina, F. Mantero, and C. Scaroni. 2007. Peroxisome proliferator-activated receptor gamma in the human pituitary gland: expression and splicing pattern in adenomas versus normal pituitary. *J. Neuroendocrinol.* **19**:552–559.
 37. Pijnappel, W. W., H. F. Hendriks, G. E. Folkers, C. E. van den Brink, E. J. Dekker, C. Edelenbosch, P. T. van der Saag, and A. J. Durston. 1993. The retinoid ligand 4-oxo-retinoic acid is a highly active modulator of positional specification. *Nature* **366**:340–344.
 38. Rosen, E. D., R. N. Kulkarni, P. Sarraf, U. Ozcan, T. Okada, C.-H. Hsu, D. Eisenman, M. A. Magnuson, F. J. Gonzalez, C. R. Kahn, and B. M. Spiegelman. 2003. Targeted elimination of peroxisome proliferator-activated receptor γ in β cells leads to abnormalities in islet mass without compromising glucose homeostasis. *Mol. Cell. Biol.* **23**:7222–7229.
 39. Ruthenburg, A. J., C. D. Allis, and J. Wysocka. 2007. Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Mol. Cell* **25**:15–30.
 40. Scacheri, P. C., S. Davis, D. T. Odom, G. E. Crawford, S. Perkins, M. J. Halawi, S. K. Agarwal, S. J. Marx, A. M. Spiegel, P. S. Meltzer, and F. S. Collins. 2006. Genome-wide analysis of menin binding provides insights into MEN1 tumorigenesis. *PLoS Genet.* **2**:e51.
 41. Semple, R. K., V. K. Chatterjee, and S. O'Rahilly. 2006. PPAR gamma and human metabolic disease. *J. Clin. Investig.* **116**:581–589.
 42. Sierra, J., T. Yoshida, C. A. Joazeiro, and K. A. Jones. 2006. The APC tumor suppressor counteracts beta-catenin activation and H3K4 methylation at Wnt target genes. *Genes Dev.* **20**:586–600.
 43. Sowa, H., H. Kaji, L. Canaff, G. N. Hendy, T. Tsukamoto, T. Yamaguchi, K. Miyazono, T. Sugimoto, and K. Chihara. 2003. Inactivation of menin, the product of the multiple endocrine neoplasia type 1 gene, inhibits the commitment of multipotential mesenchymal stem cells into the osteoblast lineage. *J. Biol. Chem.* **278**:21058–21069.
 44. Steger, D. J., M. I. Lefterova, L. Ying, A. J. Stonestrom, M. Schupp, D. Zhuo, A. L. Vakoc, J.-E. Kim, J. Chen, M. A. Lazar, G. A. Blobel, and C. R. Vakoc. 2008. DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. *Mol. Cell. Biol.* **28**:2825–2839.
 45. Takada, I., M. Mihara, M. Suzawa, F. Ohtake, S. Kobayashi, M. Igarashi, M. Y. Youn, K. Takeyama, T. Nakamura, Y. Mezaki, S. Takezawa, Y. Yogiashi, H. Kitagawa, G. Yamada, S. Takada, Y. Minami, H. Shibuya, K. Matsumoto, and S. Kato. 2007. A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR-gamma transactivation. *Nat. Cell Biol.* **9**:1273–1285.
 46. Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. 1994. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* **8**:1224–1234.
 47. Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* **79**:1147–1156.
 48. Tontonoz, P., S. Singer, B. M. Forman, P. Sarraf, J. A. Fletcher, C. D. Fletcher, R. P. Brun, E. Mueller, S. Altioh, H. Oppenheim, R. M. Evans, and B. M. Spiegelman. 1997. Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor gamma and the retinoid X receptor. *Proc. Natl. Acad. Sci. USA* **94**:237–241.
 49. Tyagi, S., A. L. Chabes, J. Wysocka, and W. Herr. 2007. E2F activation of S phase promoters via association with HCF-1 and the MLL family of histone H3K4 methyltransferases. *Mol. Cell* **27**:107–119.
 50. Vermeulen, M., K. W. Mulder, S. Denissov, W. W. Pijnappel, F. M. van Schaik, R. A. Varier, M. P. Baltissen, H. G. Stunnenberg, M. Mann, and H. T. Timmers. 2007. Selective anchoring of TFIIID to nucleosomes by trimethylation of histone H3 lysine 4. *Cell* **131**:58–69.
 51. Vortmeyer, A. O., R. Boni, E. Pak, S. Pack, and Z. Zhuang. 1998. Multiple endocrine neoplasia 1 gene alterations in MEN1-associated and sporadic lipomas. *J. Natl. Cancer Inst.* **90**:398–399.
 52. Winkler, G. S., K. W. Mulder, V. J. Bardwell, E. Kalkhoven, and H. T. Timmers. 2006. Human Ccr4-Not complex is a ligand-dependent repressor of nuclear receptor-mediated transcription. *EMBO J.* **25**:3089–3099.
 53. Wysocka, J., T. Swigut, H. Xiao, T. A. Milne, S. Y. Kwon, J. Landry, M. Kauer, A. J. Tackett, B. T. Chait, P. Badenhorst, C. Wu, and C. D. Allis. 2006. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* **442**:86–90.
 54. Yadav, N., D. Cheng, S. Richard, M. Morel, V. R. Iyer, C. M. Aldaz, and M. T. Bedford. 2008. CARM1 promotes adipocyte differentiation by coactivating PPARgamma. *EMBO Rep.* **9**:193–198.
 55. Yokoyama, A., Z. Wang, J. Wysocka, M. Sanyal, D. J. Auferio, I. Kitabayashi, W. Herr, and M. L. Cleary. 2004. Leukemia proto-oncoprotein MLL forms a SET1-like histone methyltransferase complex with menin to regulate *Hox* gene expression. *Mol. Cell. Biol.* **24**:5639–5649.