Mechanisms Regulating Adipocyte Expression of Resistin

Running title: Adipocyte resistin gene expression

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ABSTRACT

Resistin, also known as Adipocyte Secreted Factor (ADSF) and FIZZ3, is a mouse protein with potential roles in insulin resistance and adipocyte differentiation. The resistin gene is expressed almost exclusively in adipocytes. Here we show that a proximal 264 base pair fragment of the mouse resistin promoter is sufficient for expression in adipocytes. Ectopic expression of the adipogenic transcription factor C/EBPα was sufficient for expression in non-adipogenic cells. C/EBPα binds specifically to a site which is essential for expression of the resistin promoter. Chromatin immunoprecipitation (ChIP) studies of the endogenous gene demonstrated adipocyte-specific association of C/EBPα with the proximal resistin promoter in adipocytes but not preadipocytes. C/EBPα binding was associated with recruitment of coactivators p300 and CBP and a dramatic increase in histone acetylation in the vicinity of the resistin promoter. The antidiabetic thiazolidinedione (TZD) drug rosiglitazone reduced resistin expression with an ED$_{50}$ similar to its Kd for binding to PPARγ. Other TZD- and non-TZD PPARγ ligands also downregulated resistin expression. However, no functional PPARγ binding site was found within 6.2 kb of the transcriptional start site suggesting that if PPARγ is involved, it is either acting at a long distance from the start site, in an intron, or indirectly. Nevertheless, rosiglitazone treatment selectively decreased histone acetylation at the resistin promoter without a change in occupation by C/EBPα, CBP, or p300. Thus, adipocyte-specificity of resistin gene expression is due to C/EBPα binding, leading to recruitment of transcriptional coactivators and histone acetylation characteristic of an active chromatin environment. TZD reduces resistin gene expression at least in part by reducing histone acetylation associated with binding of C/EBPα in mature adipocytes.
INTRODUCTION

Adipose tissue is increasingly recognized as a dynamic tissue that serves functions other than storage of energy in the form of triglycerides (1). Lack of adipose tissue causes hyperlipidemia, insulin resistance, and type 2 diabetes (2). Excess adipose tissue is more common among humans in industrialized societies, and is also associated with insulin resistance and diabetes (3). These functions of adipose tissue are mediated in part by secreted products, including fatty acids as well as numerous protein products (4).

Adipocytes are highly differentiated cells, and numerous genes are expressed specifically or predominantly in fat cells (5). These include transcription factors, metabolic enzymes, structural proteins, and secreted proteins. Transcription factors implicated in adipogenesis include basic-leucine zipper-containing C/EBP family members and the nuclear receptor peroxisome proliferator activated receptor γ (PPARγ) (6,7). C/EBPβ and δ are transiently induced during adipogenesis (8), and are involved in the upregulation of C/EBPα and PPARγ (9,10), which remain expressed in mature adipocytes. C/EBPα and PPARγ are both able to induce adipogenesis, in part by inducing each other's expression (11). Recent studies indicate that PPARγ can induce adipogenesis in cells lacking C/EBPα (12,13), whereas C/EBPα is insufficient for adipogenesis in the absence of PPARγ (14). Nevertheless, numerous adipocyte-specific genes contain binding sites for C/EBPα as well as PPARγ (15).

Resistin, also known as ADSF and FIZZ3, is a recently described protein whose expression is adipocyte-specific in the mouse (16-18). Resistin belongs to a family that in the mouse includes two other members called Resistin-Like Molecules (RELMs) and Found in Inflammatory Zone proteins (18,19). Although other RELM/FIZZ family members exhibit tissue-specific expression, resistin is the only one of these to be
expressed in adipocytes. The function of resistin is not well understood, but there is evidence that it plays a role in obesity-related insulin resistance as well as in adipocyte differentiation (16, 17).

Little is known about the adipocyte-specific determinants of resistin gene expression. Here we show that the mouse resistin promoter contains a C/EBPα binding site that is necessary and sufficient for expression. Binding of C/EBPα in adipocytes is associated with recruitment of coactivators CBP and p300, and abundant acetylation of histones at the resistin promoter. Multiple classes of PPARγ ligands, as well as RXR ligands, downregulate resistin gene expression. This is associated with reduced histone acetylation without change in C/EBPα or coactivator recruitment. These results suggest that positive regulation of resistin regulation is due to C/EBPα, while negative regulation by PPARγ ligands involves a different mechanism converging on histone acetylation at the resistin promoter.

**METHODS**

**Isolation of the resistin gene.** Resistin promoter fragments was isolated from a BAC clone by PCR and subcloned into pGL2-enhancer vector.

**Transfection studies.** Transient transfection of 293T cells using lipofectamine and luciferase reporter assays were performed as described(20). 3T3-L1 adipocytes were transfected by electroporation. Day 5 3T3-L1 adipocytes (10^7 cells) were trypsinized and resuspended in media without serum and electroporated at 960 µF, 150V. After electroporation, the cells were then replated. Ligands were added to cells after adherence. Luciferase and beta-galactosidase reporter assays were done after 48 hour treatment.
Electrophoretic mobility shift assays. Gel shift assays were performed as previously described (21).

Chromatin immunoprecipitation (ChIP) assays. The method of Shang et al (22) was modified as follows. 3T3L1 cells were grown to confluence in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum. Two days post confluence, cells were either collected as described below (as preadipocytes) or incubated with differentiation media (dexamethasone, IBMX, insulin) for 48 hours as described (11). Day 7 adipocytes were treated with 1 µM rosiglitazone or DMSO vehicle and collected at various time points after treatment. Cells were collected by washing twice with PBS and cross-linking with 1% formaldehyde in PBS at 37 for 10 minutes. Cells were then rinsed twice with ice-cold PBS, centrifuged for 4 min at 700g, and resuspended in lysis buffer (1%SDS, 5mM EDTA, 50 mM TrisHCl, pH 8.1). Following a 20 minute incubation on ice, samples were sonicated at 15 second pulses 3 times on ice. The lysates were centrifuged at 14000g for 10 min and the collected supernatant was diluted in buffer (1% triton X-100, 2mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.1) with protease inhibitors (Roche Molecular Biochemicals, Indianapolis, IN). Samples were precleared with 2 µg sheared salmon sperm DNA and 45 ul protein A- sepharose beads for 2 hours. Immunoprecipitation with the following antibodies was performed overnight: CEBPα, p300, normal rabbit IgG, CBP (Santa Cruz Biotechnology, Santa Cruz, CA), acetylated Histone H3, acetylated Histone H4, acetylated Histone H3 (lys 9), acetylated Histone H4 (lys 8) (Upstate Biotechnology, Inc, Lake Placid, NY). Samples were then incubated with 45 µl protein A- sepharose beads for 1 hour followed by 10 minute sequential washes in TSE I (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20 mM Tris HCl, 150 mM NaCl), TSE II (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20 mM Tris HCl, 500 mM NaCl), buffer III (0.25% M LiCl, 1% NP-40, 1% deoxycholate 1mM EDTA, 10 mM Tris HCl), and TE buffer. Precipitates were then extracted by incubating with elution buffer
(1% SDS, 0.1 M NaHCO3) at 65 C for 6 hours to overnight. DNA fragments were purified with Qiagen PCR purification kit and eluted with 50 µl EB. 2 to 10 µl of purified sample were used in a 29 cycles of PCR. Primers surrounding the resistin transcription start site, with the following sequence: 5' gtc ttg gct cct agc ctt gc; 5' gtt gac ttc tgg ccc atc c. Primers for the 36B4 control gene were: 5' cct cgt tgg agt gac atc g; 5' ggt gtt ctt gcc cat cag c.

RESULTS

The proximal resistin promoter is sufficient for adipocyte specific expression. To understand the regulation of mouse resistin expression, the mouse resistin gene was isolated and its 5' flanking region plus 40 basepairs of the first exon were fused to a luciferase reporter gene (Figure 1a). A luciferase reporter containing 3510 basepairs of 5' flanking DNA supported expression in 3T3-L1 adipocytes (Figure 1b), but not in non-adipocytic 293T embryonal kidney cells (Figure 1c). Moreover, a construct containing only the most proximal 224 basepairs of 5' flanking sequence was sufficient for adipocyte expression.

The adipogenic transcription factor C/EBPα induces expression of the resistin promoter in non-adipocytic cells. C/EBPα and PPARγ are adipogenic transcription factors that frequently transactivate adipocyte-specific genes. We therefore tested the ability of these factors to stimulate expression from the resistin promoter in 293T cells. Expression of PPARγ did not increase expression of any of the resistin-luciferase reporter genes in the presence or absence of the thiazolidinedione ligand rosiglitazone (data not shown). By contrast, expression of C/EBPα led to a robust increase in the activity of the resistin promoter (Fig. 2). The magnitude of the stimulation of transcription by C/EBPα was similar for all constructs tested. Thus a construct containing 5' flanking 224
basepairs of the resistin transcriptional start site was sufficient for C/EBPα-induced expression.

The proximal resistin promoter contains a functional C/EBP binding site. The sequence of the proximal resistin promoter is shown in Fig. 3a. Inspection of the sequence identified a putative C/EBP binding site centered 56 base pairs from the transcriptional start site of the resistin mRNA. We studied the properties of the wild type sequence as well as one containing a 4 bp substitution in the middle of the putative C/EBP binding site (Fig. 3b). Electrophoretic mobility shift analysis showed that C/EBPα bound to the wild type resistin promoter (Fig. 3c). This binding could be supershifted by anti-C/EBPα antiserum, and was competed by cold competitor DNA containing the wild type but not a mutated sequence (Fig. 3c). The importance of this C/EBPα binding site for resistin expression was tested by comparing the transcriptional activity of the wild type resistin promoter with that of a promoter bearing the mutation in the C/EBPα binding site. In contrast to the wild type promoter, the mutant promoter was inactive in 293T cells cotransfected with C/EPBα (Fig. 3d) and also inactive in 3T3-L1 adipocytes expressing endogenous C/EBPα (Fig. 3e). Together, these results implicate C/EBPα as necessary and sufficient for stimulation of transcription from the resistin promoter.

Endogenous C/EBPα is bound to the resistin promoter in adipocytes, in association with coactivators and local histone hyperacetylation. The transfection and gel shift studies used to implicate C/EBPα in the regulation of resistin expression utilize recombinant, overexpressed C/EBPα with an artificial reporter gene. The role of endogenous C/EBPα was investigated by chromatin immunoprecipitation (ChIP) analysis of the resistin promoter in 3T3-L1 preadipocytes and adipocytes. In this procedure, chromatin is isolated and subjected to crosslinking and shearing of the DNA prior to
immunoprecipitation with antibodies against specific proteins. Association of the protein of interest with the resistin promoter was assessed by PCR using primers specific for the resistin promoter (Fig. 4a) after reversal of crosslinking. By this analysis, endogenous C/EBPα was clearly associated with the resistin promoter in adipocytes but not preadipocytes (Fig. 4b). p300 has been shown to function as a potent coactivator of C/EBPα (23). Indeed, p300 as well as the closely related coactivator CBP were found to be recruited to the resistin promoter specifically in adipocytes (Fig. 4b). We next investigated histone acetylation, since p300 and CBP both contain intrinsic histone acetyltransferase (HAT) activity which is critical to their coactivation function (24,25). Acetylation of histone H3 and histone H4 was also found to be dramatically increased in the region of the resistin promoter (Fig. 4b). By contrast, non-specific antibodies did not precipitate the resistin promoter sequences in preadipocytes or in adipocytes. Moreover, the increased histone acetylation was specific to the resistin gene because acetylation of histone H3 (as well as H4, data not shown) in the vicinity of the constitutively active 36B4 promoter was indistinguishable in preadipocytes compared with adipocytes (Fig. 4c). Using specific antibodies, we found that the robust increase in acetylation of H3 and H4 was due to acetylation of multiple lysine residues including lysines 9 and 14 of H3 and lysines 8 and 12 of H4 (Fig. 4d). Together, these results suggest that the functional C/EBPα binding site identified in transfection and gel shift studies is an endogenous binding site for C/EBPα, which recruits HAT-containing coactivators leading to hyperacetylation and activation of the resistin gene promoter.

Resistin gene expression is downregulated by multiple TZD and non-TZD PPARγ ligands, as well as RXR ligands. We next explored the mechanism by which rosiglitazone downregulates resistin gene expression. Many of the effects of rosiglitazone are mediated by PPARγ but, unfortunately, fat cells lacking PPARγ are not available, and might be impossible to generate given the requirement of PPARγ for
adipogenesis (14,26-28). Therefore, we addressed the potential role of PPARγ in other ways. First we compared the ED_{50} for downregulation of resistin gene expression by rosiglitazone in 3T3-L1 cells with the Kd of PPARγ for rosiglitazone binding. The ED_{50} for rosiglitazone downregulation was approximately 50 nM, very similar to that for upregulation of the substantiated PPARγ target, aP2 (Fig. 5a, b). This is similar to the Kd of rosiglitazone binding to PPARγ (29,30). In addition, TZDs other than rosiglitazone, such as pioglitazone and troglitazone were effective at downregulating resistin gene expression (Fig. 5c). Moreover, FMOC-L-leucine, a PPARγ ligand which is structurally unrelated to TZDs (31), also markedly downregulated resistin expression in 3T3-L1 cells (Fig. 5d). We explored the effects of RXR agonists, which activate the PPARγ/RXR heterodimer (32); multiple RXR agonists downregulated resistin gene expression, while an RAR-specific ligand had little effect (Fig. 5d). All of these data are consistent with the possibility that PPARγ mediates the effect of rosiglitazone. This effect could be direct, i.e., due to PPARγ binding to the resistin promoter, or could also be indirect, involving induction of a protein or proteins that repress resistin expression. Indeed, the latter possibility is suggested by the relatively lengthy time course of resistin downregulation, with half-maximal reduction of resistin mRNA levels observed after approximately 24h of exposure of 3T3-L1 cells to rosiglitazone (Fig. 5e).

Rosiglitazone treatment reduces histone acetylation at the resistin promoter. Given the ability of C/EBPα to activate the resistin promoter in transiently transfected 293T cells, we investigated the ability of rosiglitazone to regulate the resistin promoter. However, using the constructs shown in Fig. 1a as well as another containing 6.2 kb of flanking sequence, only a modest decrease in transcription from the resistin promoter, never more than 30%, was observed in the presence of rosiglitazone and transfected PPARγ (plus or minus C/EBPα, data not shown). By contrast, rosiglitazone downregulates resistin gene expression by greater than 50%, generally 80-90% as judged
by Northern analysis (17,33; and see Fig. 5). We therefore evaluated the effects of rosiglitazone on the resistin promoter in 3T3-L1 adipocytes using the ChIP assay.

Up to 48h of rosiglitazone treatment had little effect on total histone H3 and H4 acetylation in the vicinity of the resistin promoter (data not shown). Remarkably, rosiglitazone treatment reduced acetylation of Lys9 of histone H3 and Lys8 of histone H4 at the resistin promoter, without significantly altering acetylation of Lys 14 of H3 or Lys 12 of H4 (Fig. 6a). Consistent with the time course of the rosiglitazone-induced reduction in gene expression, this effect was observed at 24 and 48 hours of rosiglitazone treatment (Fig. 6a) but not at 5 hours (data not shown). C/EBPα binding to the resistin promoter was unaffected by rosiglitazone (Fig. 6a). We considered the possibility that ligand binding to PPARγ could "squelch" transcription by recruiting p300 and CBP away from the promoter as has been suggested to explain negative regulation of AP1 activity by nuclear receptor ligands (34). However, we observed no change in CBP or p300 recruitment to the resistin promoter in the presence of rosiglitazone (Fig. 6b).

**DISCUSSION**

**Adipocyte specificity of resistin gene expression.** Adipocyte specificity is a hallmark of resistin gene expression in the mouse. C/EBPα and PPARγ are the two major adipogenic factors that have been implicated as direct regulators of numerous adipocyte-specific genes. Recent studies have demonstrated that PPARγ can induce adipogenesis in the absence of C/EBPα, whereas C/EBPα is unable to induce adipogenesis in the absence of PPARγ, thereby establishing PPARγ as a master regulator of adipogenesis (14). The present studies clearly suggest that C/EBPα binds to and activates the proximal resistin promoter *in vitro*, in transfected cells, and most importantly, in the endogenous situation in fat cells. A 224
bp segment of the resistin promoter contains the C/EBPα binding site and is sufficient for expression in adipocytes. By contrast, PPARγ does not directly activate resistin gene constructs containing up to 6.2 kb of 5' flanking sequence. Thus PPARγ appears not to be directly required for expression of the resistin promoter, although it is possible that the resistin gene contains a PPARγ responsive element, either further 5' or in an intron, that was not included in the promoter constructs analyzed here. Even if PPARγ does not directly activate the resistin gene, it is likely that PPARγ promotes resistin expression by inducing C/EBPα during normal adipocyte differentiation. Indeed, constitutively active PPARγ induces both C/EBPα and resistin expression in the course of adipocyte differentiation (35).

Although it is unusual for adipocyte-specific genes not to be directly upregulated by liganded PPARγ, there does appear to be a subset of adipocyte genes that rely on C/EBPα for expression. Interestingly, none of these is required for the adipocyte phenotype but, rather, all play a role in adipocyte function. These include the genes involved in adipocyte insulin sensitivity, such as insulin receptor and insulin-receptor substrate 1 (12) as well as the insulin responsive glucose transporter (GLUT4) (36), as well as adipocyte secreted factors leptin (37-39) and ACRP30/adiponectin (40). Unlike resistin, leptin, and ACRP30/adiponectin, C/EBPα expression is not restricted to adipocytes, and thus it will be important for future studies to address the mechanisms restricting the expression of resistin, as well as other C/EBPα-dependent adipocytes transcripts. Interestingly, like resistin, leptin gene expression in adipocytes is dependent upon C/EBP (37-39) and is downregulated by TZDs (39,41-43) despite the apparent lack of a PPARγ binding site in the leptin promoter (39). The fact that TZDs downregulate both resistin and leptin gene expression provide further evidence against a direct role of PPARγ in the upregulation of both of these genes during adipogenesis.
The putative human homologue of mouse resistin is only 53% identical at the amino acid level, and although it is expressed in human adipose tissue, the level of its expression has been noted to be considerably less than that observed for the gene encoding mouse resistin in mouse adipose tissue (44-47). Consistent with this, the promoter sequence of the putative human resistin gene is remarkably divergent from that of the mouse gene reported here, with little or no similarity (K. Tyler, X. Hu, H. B. Hartman, and M. A. Lazar, unpublished observations). Thus the gene regulatory sequences have diverged tremendously. Since there are two other genes related to resistin (i.e., three Resistin/RELM/FIZZ family genes) in the mouse, and only two members of this family identified thus far in humans, it is possible that there exists a closer relative to resistin in the human genome that has yet to be discovered. In any case, the differing primary amino acid sequences and expression patterns of the mouse and human genes, explained by the divergent regulatory sequences, are suggestive of different functions for these proteins.

**Coactivators and histone hyperacetylation of the resistin gene promoter.** Our studies have demonstrated that the tails of histones H3 and H4 are hyperacetylated in the region of the resistin promoter. Histone hyperacetylation is recognized as a mechanism of gene activation in many cell types (48,49). This histone hyperacetylation is most likely mediated, at least in part, by the p300 and CBP coactivators that we have shown to associate with the resistin promoter in adipocytes. Importantly, these molecules have intrinsic HAT activity (24,25) and are validated coactivators of C/EBPα (23). Further analysis with antibodies specific for different acetylation sites revealed that lysine sites 8, 9, 12, 14 were all acetylated upon gene activation. These data agree with in vitro studies showing that CBP/P300 can acetylate these sites (25,50,51), which have been specifically implicated in transcriptional activation (52-56). To our knowledge, this is the first example of specific histone hyperacetylation accompanying gene activation in adipocytes.
Downregulation of resistin gene expression by TZDs. Resistin was identified as a gene whose expression was downregulated by TZDs in 3T3-L1 adipocytes (17). A number of observations presented herein suggest that TZD downregulation of resistin expression is mediated by PPARγ: 1) the ED50 for downregulation of resistin expression by rosiglitazone is similar to the ED50 for induction of aP2 by rosiglitazone; 2) the ED50 for down regulation of resistin expression by rosiglitazone is on the order of 100 nM, similar to the Kd of rosiglitazone binding to PPARγ (29,30); 3) multiple TZDs downregulate resistin expression; and 4) the non-TZD PPARγ ligand FMOC-L-leucine also downregulates resistin expression. However, rosiglitazone and PPARγ separately or together did not markedly downregulate expression of the resistin promoter in 293T cells, and we have not been able to demonstrate PPARγ binding to the proximal resistin promoter (data not shown). It is possible that the putative PPARγ negative response element in the resistin gene is outside of the region contained in the promoter constructs we have studied or that another adipocyte factor (perhaps induced by PPARγ) represses resistin transcription.

As for other nuclear receptor ligands, the mechanism of negative regulation of gene expression by TZDs is not as straightforward as transcriptional activation (61). TZDs interfere with the transcriptional activity of C/EBP proteins as well as another basic-leucine zipper transcription factor, AP-1, on other target genes (62,63). The ability of nuclear receptor ligands to inhibit the activity of AP-1 has been previously suggested to be due to "squelching" of CBP and/or p300 (34). However, our results indicate that this is unlikely.
to be the mechanism by which TZDs reduce resistin gene expression, since we observe that occupancy of the resistin promoter by CBP and p300 is unchanged by rosiglitazone treatment.

Although the mechanism by which TZDs downregulate resistin expression is elusive, ChIP analysis of the endogenous resistin promoter demonstrated reduced histone acetylation at a subset of lysines in the tails of histones H3 and H4. The reduced acetylation of lysine 9 in histone H3 is consistent with recent observations correlating deacetylation of lysine 9 with transcriptional repression (52). Interestingly, acetylation at sites lysine 14 of histone H3 and lysine 12 of histone H4 were unchanged by rosiglitazone treatment. The histone code theory postulates that the pattern of modifications of specific residues in histone tails serves as a code to determine the recruitment of different co-factors (56). Thus it is likely that the specific differences in acetylation fine-tune the level of resistin expression. While CBP and p300 have not been shown to preferentially acetylate specific lysine residues, it is possible that one or more histone deacetylases (HDACs) might target lysine 9 of histone H3 and lysine 8 of histone H4. We have thus far been unable to identify specific HDACs recruited to the resistin promoter by rosiglitazone (data not shown), but this remains a possibility as novel HDACs continue to be discovered (64,65).

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REFERENCES

FIGURE LEGENDS

**Figure 1.** The mouse resistin promoter is active in adipocyte cells. a. Schematic representation of the mouse resistin promoter and various luciferase (luc) reporter constructs. b. Resistin promoter activity in 3T3-L1 adipocytes. c. Resistin promoter activity in 293T cells.

**Figure 2.** The mouse resistin promoter is activated by C/EBPα in 293T cells. C/EBPα was co-transfected with luciferase reporter constructs into 293T cells.

**Figure 3.** Transcription from the resistin promoter required a C/EBP binding site in the proximal promoter. a. Sequence of mouse resistin near the transcription start site. The C/EBP binding site, TATA box and transcription start site are underlined and labeled. b. Schematic representation of the wild type (wt) and C/EBP binding site mutation (mut) reporter constructs. c. C/EBPα binds to the resistin promoter. Electrophoretic mobility shift assay using ^32^P-labeled fragment of resistin promoter. "Shift" denotes migration of fragment supershifted by C/EBP antibody. d. C/EBPα increases the transcription of the wild type but not the mutant promoter reporter in transiently transfected 293T cells. e. Wild type but not mutant resistin promoter is active in transiently transfected 3T3-L1 adipocytes.

**Figure 4.** Chromatin immunoprecipitation assays of resistin promoter in preadipocyte and adipocytes. a. Schematic of resistin promoter. Arrows indicate the PCR primers used to evaluate ChIP samples. b. ChIP analysis for C/EBPα, p300, CBP, and acetylated (Ac) histone H3 and H4 at the resistin promoter. ChIP protocol was performed on preadipocytes and adipocytes as described in Methods. c. ChIP analysis for acetylated histone H3 at the
36B4 promoter. d. ChIP analysis for acetylated lysines 9 and 14 (K9 and K14) of histone H3, and acetylated lysines 8 and 12 (K8 and K12) of histone H4.

**Figure 5.** Downregulation of resistin expression by ligands for PPARγ and RXR. a. Dose-dependent downregulation of resistin and upregulation of aP2 by rosiglitazone. Northern analysis of resistin, aP2, and 36B4 loading control. The rosiglitazone concentrations are, from left to right, 0, 0.002, 0.01, 0.02, 0.1, 0.2, 0.5, 2, 10, 100 µM. b. Quantitative analysis of the results shown in a, by phosphorimaging. c. Multiple TZDs downregulate resistin expression. Rosi=rosiglitazone, Pio=pioglitazone, Trog=troglitazone. The concentration of each ligand is 1 µM. d. The non-TZD PPARγ ligand FMOC-L-Leucine and RXR ligands downregulate resistin expression. The concentrations FMOC-L-Leucine ("FMOC-Leu") are 10 and 100 µM. RAR ligand is BMS453 (66). RXR ligands: 1=BMS649 (identical to SR11237 (67,68)); 2=BMS749 (69); 3=HX630 (70); 4-HX600 (70). RAR and RXR ligands were used at concentration of 10 µM. e. Time course of resistin downregulation by rosiglitazone.

**Figure 6.** Effect of rosiglitazone on histone acetylation and factor association with the resistin promoter. a. Differentiated adipocytes were treated with rosiglitazone for 24 and 48 h, then ChIP analysis was performed for acetylated lysines 9 and 14 (K9 and K14) of histone H3, and acetylated lysines 8 and 12 (K8 and K12) of histone H4, as well as C/EBPα. b. ChIP analysis for CBP and p300 in differentiated adipocytes, and after 48h treatment with rosiglitazone or vehicle.
Figure 1

Mouse Resistin Gene

-3510/ +40

-939 / +40

-224/ +40

3T3-L1 Adipocytes

293T Cells

Fold Increase over pGL2

Fold Increase over pGL2
Figure 2

293T Cells

Fold Activation

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Figure 3

a

\[-224\text{ CTACAGGTGAAGTCTTGCTCCTAGCCTTGCCCCTCCCACCATGGTCCCTGGTGTATCT}\]
\[\text{CCAGACAACGTCTCTGAGAAAAACAAATCCTTATGTAGAAGGGGTGAGCAGGAGGGAAAA}\]
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b

\[-224 /+40 (wt)\]
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\[-224 /+40 (mut)\]
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C/EBP site
TATTA
Luc

b

C/EBP site
TATTA
Luc

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Shift
C/EBP\(\alpha\)

Resistin promoter (-224 to +40)

293T Cells

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3T3-L1 Adipocytes

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Fold Activation (% of wild type)

Fold Activation (% of wild type)
Figure 4

(a) Schematic diagram of the Resistin gene promoter region showing the C/EBP binding site (red), TATA box (yellow), and ATG start codon (green). The promoter region is indicated by arrows.

(b) Western blot analysis of C/EBPα, p300, and CBP proteins in Pread and Ad samples.

(c) Western blot analysis of Ac-H3 and Input proteins in Pread and Ad samples for the 36B4 gene.

Figure 6

a

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Mechanisms regulating adipocyte expression of resistin
Helen B. Hartman, Xiao Hu, Keala X. Tyler, Chiraj K. Dalal and Mitchell A. Lazar

J. Biol. Chem. published online March 18, 2002

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