Nuclear Receptor Corepressors and PPARγ

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The nuclear receptor corepressors NCoR and SMRT repress gene transcription by recruiting a histone deacetylase complex. Their roles in PPARγ action have been controversial. Recent evidence, however, suggests that NCoR and SMRT repress PPARγ-mediated transcriptional activity on specific promoters in the adipocyte. In addition, by repressing PPARγ action, these corepressors inhibit the ability of adipocyte differentiation to proceed. A further understanding of corepressor action in the adipocyte will provide insight into the balance of forces regulating adipogenesis, insulin sensitivity, and Type 2 diabetes mellitus.

Abbreviations: NCoR: nuclear corepressor protein; PPARγ: peroxisome proliferator-activated receptor γ; RAR: retinoic acid receptor; SMRT: silencing mediator of retinoid and thyroid hormone receptors; TR: thyroid hormone receptor; T2D: thiazolidinedione

Introduction

The nuclear receptor corepressors were initially identified as nuclear proteins recruited by the thyroid hormone receptor (TR) and retinoic acid receptor (RAR) isoforms to mediate ligand-independent repression. More recently, they have been shown to modulate the transcriptional activity of a wide variety of transcription factors. The two main nuclear receptor corepressors are the nuclear receptor corepressor protein (NCoR) [Horlein et al., 1995] and the silencing mediator of retinoid and thyroid hormone receptors (SMRT) [Chen and Evans, 1995]. Nuclear hormone receptors (NHRs) generally bind NCoR and SMRT in the absence of ligand or the presence of antagonists. These interactions are mediated by CoRNR box sequences (I/L-x-x-I/V-I) in the interacting domains of NCoR and SMRT [Hu and Lazar, 1999; Nagy et al., 1999; Perissi et al., 1999]. Sequences within and outside these CoRNR box motifs regulate the specificity of interactions between corepressors and NHRs [Cohen et al., 2001; Makowski et al., 2003]. The binding of ligand to the NHR results in a conformational change in the receptor, leading to loss of corepressor binding and subsequent recruitment of coactivators.

NCoR and SMRT are expressed ubiquitously, and they function in vitro as corepressors of gene transcription. However, their exact physiologic roles in distinct tissues remain relatively undefined, in part due to a lack of suitable animal models. An NCoR knock-out mouse has been developed, but NCoR deficiency was found to cause embryonic lethality [Jepson et al., 2000]. The use of cortical progenitor cells from NCoR -/- mice showed that NCoR was important in inhibiting the differentiation of neural stem cells into astrocytes [Hermanson et al., 2002]. However, the ability of NCoR and SMRT to modulate differentiation in other tissues remains relatively unexplored. Recently, we and other groups have found that NCoR and SMRT play an important role in the adipocyte. We have focused on the role of these corepressors in inhibiting adipocyte differentiation, which appears to occur via repression of peroxisome proliferator-activated receptor γ (PPARγ) activity.

PPARγ and transcriptional regulation

PPARγ is a member of the nuclear hormone receptor (NHR) superfamily of transcription, and exists as two isoforms which differ only in their A/B domains: PPARγ1 and PPARγ2. PPARγ2 is specifically expressed in adipocytes, and PPARγ response elements are found in a number of adipocyte-specific genes, including aP2, phosphoenolpyruvate carboxykinase, acyl-CoA synthetase, and lipoprotein lipase. Similar to other NHRs, the PPARγ ligand-binding domain binds multiple classes of coactivators. PPARγ, particularly the PPARγ2 isoform, is considered to be the key regulator of adipocyte differentiation [Rosen et al., 2002]. Recent knock-out experiments have clarified the roles of PPARγ in vivo. Knock-out of PPARγ in adipose tissue results in decreased adipocyte number, and decreased plasma levels of leptin and adiponectin [He et al., 2003]. Interestingly, these mice exhibit insulin resistance in fat and liver, but not in muscle, and have normal glucose tolerance and systemic insulin sensitivity in the basal state. In contrast, muscle-specific PPARγ -/- mice develop excess adiposity and systemic insulin resistance [Huever et al., 2003; Norris et al., 2003]. These data suggest that PPARγ action is more complex than previously understood, and that PPARγ plays important roles in tissues other than the adipocyte. However, the adipocyte represents a target tissue that is uniquely dependent on PPARγ action; PPARγ is required for adipogenesis, and the differentiated adipocyte appears to depend on PPARγ for its survival [Imai et al., 2004].

PPARγ and nuclear corepressors

There have been limited studies of corepressor recruitment by PPARγ, and early results were conflicting. Some studies suggested that PPARγ might not recruit NCoR or SMRT in the presence of DNA response elements [Zamir et al., 1997]. Another report suggested that PPARγ could recruit the corepressor SMRT, but mainly in the presence of epidermal growth factor (EGF) [Lavinsky et al., 1998]. Later work showed that PPARγ was able to recruit nuclear receptor corepressors in cells...
Recently, we have shown that NCoR and SMRT down-regulate PPARγ-mediated transcriptional activity in 3T3-L1 cells, a fibroblast cell line that retains the ability to differentiate into adipocytes in the appropriate hormone milieu [Yu et al., 2005]. We used RNA interference to down-regulate NCoR or SMRT levels in 3T3-L1 cells, and examined the ability of these cells to undergo adipogenesis. Interestingly, when stimulated with insulin, dexamethasone, and isobutylmethylxanthine, these cells exhibited increased expression of adipocyte-specific proteins as compared to wild-type cells. Moreover, these cells exhibited enhanced lipid droplet formation, as measured by Oil Red O staining. The cells were next stimulated to differentiate in a thiazolidinedione (TZD)-dependent differentiation cocktail. TZDs serve as ligands for PPARγ, and are used clinically to increase insulin sensitivity in the treatment of Type 2 diabetes mellitus [Olefsky, 2000]. Interestingly, cells deficient in NCoR or SMRT expressed an increased level of adipocyte-specific proteins when stimulated by TZDs [Yu et al., 2005]. Thus, these data show that NCoR and SMRT modulate adipogenesis, most likely via their ability to repress PPARγ action. Moreover, the relative cellular levels of corepressors and coactivators affect the ability of PPARγ ligands to induce adipocyte differentiation.

Other groups have also recently examined the ability of NCoR and SMRT to repress PPARγ transcriptional activity in 3T3-L1 cells. Guan et al., showed that NCoR and SMRT are both recruited to PPARγ, but this process is promoter-specific [Guan et al., 2005]. When PPARγ is recruited to the aP2 promoter, it does not recruit corepressors; instead PPARγ is bound to coactivators even in the presence of ligand. In contrast, when PPARγ is recruited to the glycerol kinase promoter, it recruits nuclear receptor corepressors and represses gene transcription in the absence of ligand. While the presence of ligand does not cause a shift in cofactor recruitment by PPARγ on the aP2 promoter, ligand results in release of corepressors and recruitment of coactivators on the glycerol kinase promoter. These data suggest that corepressors affect only a fraction of PPARγ-responsive genes. An area of active research is to identify which PPARγ-responsive genes are corepressor-dependent and which are not.

PPARγ, corepressors, and negative regulation

The standard view of PPARγ-mediated transcription involves an increase in gene transcription in the presence of ligands such as TZDs. However, there are many genes that are negatively regulated by PPARγ ligands. Negative regulation by PPARγ and other NHRs is poorly understood. Early work into negative regulation by PPARγ focused on leptin gene regulation, as it is known that PPARγ agonists down-regulate leptin mRNA levels [Zhang et al., 1996]. Interestingly, it was found that the putative PPARγ response element in the leptin promoter was not involved in negative regulation and it was hypothesized that PPARγ functionally antagonized C/EBPα to decrease transcription in response to TZDs [Hollenberg et al., 1997]. However, the role of corepressors in this process was not investigated.

More recently, Pascual et al., investigated the ability of PPARγ to down-regulate the inducible nitric oxide synthase (iNOS) gene in macrophages [Pascual et al., 2005]. These authors found that PPARγ ligands cause SUMOylation of the PPARγ ligand-binding domain. This process targets PPARγ to NCoR-containing complexes, and decreases the ability of p50 and p65 complexes to recruit coactivators. Thus, DNA-associated NCoR may recruit sumoylated PPARγ to down-regulate gene transcription in response to TZDs on promoters that do not contain classical PPARγ / RXR binding sites. Interestingly, while NCoR was important in negative regulation of the iNOS promoter, SMRT was ineffective, suggesting there may be corepressor specificity in terms of negative regulation by PPARγ [Pascual et al., 2005]. While this work was performed in macrophages, these results shed insight into potential ways that corepressors might also influence PPARγ-mediated negative regulation in adipocytes.

The paradox of the PPARγ heterozygous knock-out

TZDs serve as high affinity ligands for PPARγ receptors, and their effects on insulin sensitivity are thought to be dependent on this activity. Since PPARγ-/- mice die in utero, PPARγ +/- heterozygotes have been used to study the effects of PPARγ on insulin sensitivity. It was hypothesized that a reduction in PPARγ number would reduce insulin sensitivity. Paradoxically, PPARγ +/- mice have enhanced insulin sensitivity [Miles et al., 2000]. One potential explanation is that PPARγ recruits corepressors as well as coactivators. TZDs might increase insulin sensitivity via the recruitment of coactivators, whereas a decrease in PPARγ number would increase insulin sensitivity via a decrease in corepressor action [Miles et al., 2000].

As noted above, knock-out of PPARγ in adipose tissue results in decreased adipocyte number [He et al., 2003]. Interestingly, adipocyte-specific PPARγ knock-out mice exhibit normal systemic insulin sensitivity in the basal state, but are susceptible to insulin resistance induced by high-fat feeding [He et al., 2003]. In contrast, muscle-specific PPARγ -/- mice exhibit systemic insulin resistance even in the basal state [Hevener et al., 2003; Norris et al., 2003]. Thus, the in vivo function of PPARγ is complex, and further work will clarify the role of PPARγ in distinct tissues, including its relationship with NCoR and SMRT.
Corepressors and PPARγ

**Conclusion**

Increasing evidence suggests that the corepressors NCoR and SMRT play an important role in adipocyte differentiation and PPARγ transcriptional activity. It appears that only a subset of PPARγ-responsive genes is corepressor-dependent (see Figure 1). In addition, corepressors may also mediate negative regulation by PPARγ. Future work will delineate the roles of NCoR and SMRT in the adipocyte and determine which genes are regulated by corepressor activity. Currently, modulation of PPARγ transcriptional activity by TZDs is used as a mainstay of treatment for Type 2 diabetes mellitus [Olefsky, 2000]. However, TZDs are associated with side effects such as weight gain and edema. We hypothesize that alterations in corepressor activity might also allow for the modulation of PPARγ activity in the adipocyte and may represent an alternative or complementary therapeutic approach to the TZD class of medication.

![Figure 1. Corepressors modulate PPARγ activity via distinct mechanisms](image)

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