The nuclear receptor co-repressor RIP140 controls the expression of metabolic gene networks

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Abstract
NRs (nuclear receptors) regulate the expression of specific gene networks in target cells by recruiting cofactor complexes involved in chromatin remodelling and in the assembly of transcription complexes. The importance of activating gene expression, in metabolic tissues, is well established, but the contribution of transcriptional inhibition is less well defined. In this review, we highlight a crucial role for RIP140 (receptor-interacting protein 140), a transcriptional co-repressor for NR, in the regulation of metabolic gene expression. Many genes involved in lipid and carbohydrate metabolism are repressed by RIP140 in adipose and muscle. The repressive function of RIP140 results from its ability to bridge NRs to repressive enzyme complexes that modify DNA and histones. In the absence of RIP140, expression from many metabolic genes is increased so that mice exhibit a lean phenotype and resistance to high-fat-diet-induced obesity and display increased glucose tolerance and insulin sensitivity. We propose that a functional interplay between transcriptional activators and the co-repressor RIP140 is an essential process in metabolic regulation.

Introduction
RIP140 (receptor-interacting protein 140) is a widely expressed co-repressor for NRs (nuclear receptors) that plays a crucial role in energy homeostasis by regulating metabolic functions in adipose tissue and muscle. It is also essential in the ovary for ovulation and female fertility. RIP140 seems to be capable of binding to most, if not all, members of the NR superfamily of ligand-dependent transcription factors, but its specific roles suggest that it is recruited to certain receptors at precise times to regulate these processes. Our goals are to identify the NR signalling pathways and the target genes that are repressed by RIP140 and elucidate the molecular mechanisms by which repression is controlled. In this review, we describe our current knowledge of the role of RIP140 in adipose tissue biology.

Function of RIP140 as a bridging protein between NRs and chromatin remodelling enzymes
The RIP140 protein is a nuclear protein that comprises 1158 amino acids. It contains domains and motifs that are required for its subcellular location, NR binding and repressive functions. RIP140 interacts with NRs by means of LXXLL motifs (where X is any amino acid) or NR boxes, which are short α-helical sequences that are necessary and sufficient for mediating the interaction. There are nine LXXLL motifs present in RIP140 and a tenth related motif (LYYML), which suggests some functional redundancy. Studies have shown that particular NRs have a clear preference for specific RIP140 NR boxes [1–3], and isolated fragments of RIP140 can interact either constitutively with an NR or only in the presence of ligand [4]. Given that numerous cofactors have the potential to bind to NRs via such LXXLL motifs, it is unclear how the recruitment is controlled and how it varies at different target genes and during different physiological responses. In addition to the ligand, the recruitment is likely to be regulated by the levels of different cofactors whose expression is subject to regulation during development or in response to physiological changes and/or by modifications brought about by other signalling pathways.

RIP140 contains four distinct repression domains, termed RD1 (repression domain 1)–RD4 [5], which are conserved across vertebrate species (see Figure 1). These domains act as scaffolds or platforms for the formation of different co-repressor complexes. The mechanism of repression for both RD1 and RD2 involves recruitment of HDAC (histone deacetylase)-modifying enzymes [6], but that of RD3 and RD4 is yet to be fully characterized. In the case of RD2, HDAC recruitment is mediated by the binding of CtBPs (C-terminal binding proteins), which is partially relieved by the HDAC inhibitor TSA (trichostatin A) [5], suggesting that CtBP is acting via HDAC-dependent and independent mechanisms. There are two family members, CtBP1 and CtBP2, with both distinct and overlapping functions that...
Figure 1 | Schematic representation of the repression domains, interaction motifs, and post-translational modifications of RIP140

Amino acid residues that are phosphorylated (+) or not (−) by the kinases MAPK, PKC (protein kinase C) or Ca2+/calmodulin-dependent protein kinase II (CaCal) are shown [10].

interact with RD2 by means of PXDLS (Pro-Xaa-Asp-Leu-Ser)-like motifs (Figure 1).

CtBP may serve dual functions as a co-repressor, since it may act as a dehydrogenase capable of binding both NAD+ and NADH and as part of a large multisubunit protein complex that contains a number of chromatin remodelling enzymes. Its recruitment to proteins containing PXDLS motifs is dependent on the catalytic residues and binding of NAD(H) [7]. The interaction of RIP140 with CtBP and therefore the repressive function of RD2 may also be modulated by post-translational modification. RIP140 is readily acetylated by p300 in vitro, but not by the other acetyl transferases P/CAF [p300/CREB (cAMP-response-element-binding protein)-binding protein] and GCN5 (positive general control of transcription-5) [8]. A major site for acetylation with functional consequences is Lys446, which when acetylated prevents the interaction of RIP140 with CtBP. In addition to this site, a further eight acetylated lysine residues have been identified in RIP140 that may be important in repressive function and subcellular localization [9] (see Figure 1).

The repressor is also phosphorylated, suggesting that other signalling pathways regulate its function. RIP140 may be subject to phosphorylation on as many as 11 different residues [10] (see Figure 1). Most of these are poorly characterized but RD1 seems to be regulated by MAPK (mitogen-activated protein kinase)-mediated phosphorylation on residues Thr202 and Thr207, which enhances repression by increased recruitment of HDAC3.

The relative levels of RIP140 are likely to be another key determinant in the recruitment of the co-repressor to NRs. Importantly, while the RIP140 gene is widely expressed in many tissues, it is localized in specific cell types and subject to developmental control. The highest levels of expression are detected in metabolic tissues including adipose tissue, liver and muscle and in the ovary [11]. RIP140 mRNA is transcribed from multiple promoters although the open reading frame is confined to a single exon [12]. Expression is regulated by a number of hormones including oestrogen [13], retinoic acid [14], progestin [15] and vitamin D [16]. It is also regulated during adipogenesis as exemplified in 3T3-L1 cells where there is a progressive increase in expression during differentiation in response to ERR (oestrogen-related receptor) signalling [11,17]. Preliminary work suggests that RIP140 gene expression is also subject to regulation.
in vivo during fasting and high-fat feeding, but the signalling pathways involved are yet to be determined.

RIP140 is a global suppressor of genes controlling carbohydrate and lipid metabolism

A major role for RIP140 has been demonstrated in the mature adipocyte, with no apparent role in adipogenesis. Ablation of RIP140 or depletion by siRNA (small interfering RNA) does not interfere with adipocyte differentiation but alters expression of several gene clusters required for many metabolic pathways. Analysis of gene expression profiles shows that RIP140 modulates the expression of genes encoding proteins involved in carbohydrate and fatty acid catabolic pathways [18]. Following RIP140 depletion, most of the enzymes encoding genes involved in the tricarboxylic acid cycle, glycolysis, fatty acid oxidation, oxidative phosphorylation and mitochondrial biogenesis are up-regulated. This is in contrast with the total complement of genes, where similar numbers of genes are up-regulated and down-regulated upon RIP140 depletion or ablation, indicating that RIP140 specifically represses gene networks required in metabolic processes.

Intriguingly, a number of genes that are subject to repression by RIP140 are also targets for transcriptional activation by NRs upon recruitment of PGC-1α (peroxisome-proliferator-activated receptor γ co-activator-1α). This co-activator, originally identified in BAT (brown adipose tissue) as a factor elevated in response to cold exposure, potently activates PPARγ (peroxisome-proliferator-activated receptor γ), TRβ (thyroid hormone receptor β) and ERRα [19,20] and is a major regulator of metabolic processes in many cell types. Post-translational modifications regulate its function and serve to integrate multiple signal transduction pathways in the control of gene expression [21]. Thus the relative expression of RIP140 and co-activators such as PGC-1 in combination with their functional regulation, controlled by other signalling pathways, is likely to be critical in the regulation of metabolic gene expression by NRs.

Interestingly, some genes normally restricted to expression in BAT are elevated in RIP140-null adipocytes from WAT (white adipose tissue) deposits, and these encode factors that are key mitochondrial proteins involved in energy dissipation. The expression of Ucp1 (uncoupling protein 1), Cidea (cell death-inducing DNA fragmentation factor a-like effector A) and Cpt1b (carnitine palmitoyl-CoA transferase 1b) is elevated in RIP140-null adipose tissue in vitro and in adipocytes in vivo, showing that the altered gene expression is a cell autonomous effect. Ucp1 is a member of the mitochondrial carrier family of proteins that upon activation uncouples respiration from ATP synthesis, resulting in an increased metabolic rate and the release of chemical energy in the form of heat. Cpt1b is a transporter for non-esterified fatty acids across the outer mitochondrial membrane. Cidea is a mitochondrial protein that is restricted to BAT in the mouse [22], but in the human is expressed in WAT and is down-regulated by obesity [23].

Chromatin immunoprecipitation and reporter gene assays show that RIP140 directly targets a characterized enhancer element in the Ucp1 promoter, which is known to bind NRs including PPARs, TR and RAR (retinoic acid receptor) [24]. The elevated Ucp1 expression in RIP140-null adipocytes in vitro and in vivo is not caused by altered expression of the p160 co-activators or PGC-1α, a key regulator of Ucp1 expression in BAT. PGC-1α expression was detected in differentiated adipocytes in vitro and in WAT deposits in vivo, albeit at lower levels than found in BAT [11,24]. The absence of RIP140 from adipocytes alters the balance of co-repressors and co-activators that may be critical for the expression of target genes such as Ucp1. In support of this, the converse has been shown in a recent study in which animals with a BAT double knockout for the p160 co-activators p/CIP [p300/CREB-binding protein]-interacting protein and SRC-1 (steroid receptor co-activator 1) displayed enhanced RIP140 binding to the Ucp1 promoter associated with reduced Ucp1 expression [25].

Role of RIP140 in adipose tissue biology

RIP140 is expressed at greater levels in WAT relative to BAT [11]. The amount of BAT and the expression of Ucp1 correlate with protection from obesity. Therefore the expression of genes in RIP140-null WAT, which are normally restricted to BAT, is likely to compromise the function of this tissue as a site of energy storage. Total oxygen consumption is significantly increased in RIP140-null mice [11], presumably as a consequence of energy dissipation in WAT resulting from expression of Ucp1 and Cpt1b. Further functional consequences of altered expression of mitochondrial genes include an increase in mitochondrial biogenesis upon RIP140 depletion, as shown by increased staining with the mitochondrial stain MitoTracker Red [18], and an associated increase in oxygen consumption, glucose conversion into carbon dioxide [18], and β-oxidation of fatty acids [24] by adipocytes in vitro.

Young RIP140-null mice fed a normal chow diet subjected to a glucose tolerance test display similar increases in blood glucose concentrations compared with wild-type mice [11,18]. In contrast, after a high-fat diet or at 8 months of age, RIP140-knockout mice exhibit significantly improved glucose tolerance. Null mice fed a high-fat diet also display increased insulin responsiveness with respect to lowering blood glucose. Insulin-stimulated glucose uptake is increased in RIP140-depleted adipocytes, mirroring an elevation of Glut4 expression. These in vitro and whole-body studies support a role for adipocyte expression of RIP140 in glucose metabolism, but also raise the question as to whether other tissues contribute to the responses detected. Skeletal muscle, in particular, may be an important site of RIP140 action and requires investigation as to whether it affects enhanced glucose tolerance and insulin-sensitivity in RIP140-null mice.
Summary
NRs play a crucial role in lipid and glucose homoeostasis by regulating the expression of gene networks in metabolic tissues. The importance of activating transcription of specific genes is well established, but recent work demonstrates that gene repression is also a critical process. The ability of an NR co-activator such as PGC-1α and an NR co-repressor such as RIP140 to control the transcription of metabolic gene networks, which include many common targets, provides a mechanism for maintaining energy homoeostasis as the metabolic state of an animal varies or its environment changes. Impaired expression of these cofactors or alterations in their function following perturbation of other signalling pathways are likely to contribute to metabolic disorders. Future research will aim to identify the genes that are regulated by RIP140 in metabolic tissues and to determine which NRs are repressed by RIP140, since the identification of key NR–RIP140 complexes may provide an opportunity for pharmacological intervention in the treatment of obesity and diabetes.

References

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