Retinoic acid-related orphan receptors α and γ: key regulators of lipid/glucose metabolism, inflammation, and insulin sensitivity

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INTRODUCTION

In the past 50 years, the occurrence of obesity has greatly increased worldwide in both adults and children and has become a major health-care concern in many countries. In the United States 30% of the population is considered obese, while more than 66% of adults and almost 17% of children and adolescents are overweight (Browning et al., 2004; Ogden et al., 2012). Obesity is associated with an increased risk of several pathologies, including type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease (NAFLD). Accumulating evidence indicates that networks regulating lipid metabolism and inflammation are highly integrated and play a critical role in the development of these pathologies (Hotamisligil, 2006; Donath and Shoelson, 2011; Ouchi et al., 2012). Obesity leads to a systemic state of low-grade inflammation, particularly involving adipose tissue, that is causally involved in the development of insulin resistance and related diseases. Blood levels of free fatty acids (FFAs) are elevated in obesity and through their interaction with Toll-like receptor 4 (TLR4) FFA induce proinflammatory pathways in macrophages and other cell types that may promote insulin resistance (Samuel and Shulman, 2012). Recent studies demonstrated that retinoic acid-related orphan receptors (RORs) are among many factors that through their modulation of immune responses and lipid/glucose homeostasis regulate the development of inflammation, metabolic syndrome, and insulin resistance (Jetten, 2009; Solt and Burris, 2012).

RORs AND PROTEINS

The RORs alpha, beta, and gamma (RORα–γ or NR1F1–3) constitute a subfamily of nuclear receptors that function as ligand-dependent transcription factors (Jetten, 2004, 2009; Solt and Burris, 2012). RORs exhibit a domain structure typical of nuclear receptors and contain an ‘N’-terminal domain, the function of which has not yet been clearly defined, a highly conserved DNA-binding domain (DBD) consisting of two zinc finger motifs, a LBD, and a hinge domain spanning the DBD and LBD. By using different promoters and/or alternative splicing each ROR gene produces several isoforms that vary only in their N-terminal region. Some of these isoforms exhibit a distinct tissue-specific pattern of expression and control different genes and biological processes. RORs regulate transcription by binding as monomers to ROR response elements (RORE), which consist of the core sequence “AGGTCA” preceded by an A/T-rich sequence, in the regulatory region of target genes. The activation function (AF-2), localized at the C-terminus within the LBD of RORs, is involved in the recruitment of co-activators or co-repressors that mediate the transcriptional activation or repression by RORs. Recent studies have identified a number of (anti)agonists that interact with the LBD of ROR and either activate or inhibit ROR transcriptional activity (Kallen et al., 2002; Huh and Littman, 2012; Solt and Burris, 2012). Interaction with agonists induces a conformational change in the LBD that allows release of the co-repressor complex and promotes assembly of a co-activator complex that mediates the transcriptional activation of target genes.
activation by ROR, while the inverse happens for antagonists. These observations not only indicated that RORα function as ligand-dependent transcription factors, but also suggested that ROα might be potential therapeutic targets to treat disease.

**RORs as regulators of several immune processes**

ROα and ROγ are important regulators of several diverse immune functions. ROγ-deficient mice lack lymph nodes and Peyer’s patches indicating that it is essential for lymph node development (Kurohara et al., 2000; Sun et al., 2000). Recent studies demonstrated that ROα and ROγ are required to develop a key role in T cell lineage determination (Ivanov et al., 2006; Yang et al., 2008; Jetten, 2009). The ROγ isomeric variant in particular, and to a lesser extent ROα, is required for the differentiation of naïve T cells into interleukin 17 (IL-17) producing T helper 17 (Th17) cells. IL-17A expression is directly regulated by ROαs through their interaction with ROαs in the IL17 promoter (Yang et al., 2008). Proinflammatory Th17 cells and Th17 have been implicated in several autoimmune diseases and other inflammatory disorders. Deficiency in ROγ or both ROαs/γ receptors has been shown to greatly inhibit the generation of Th17 cells and the development of experimental encephalomyelitis in mice. In addition, mice deficient in ROα or ROγ displayed a diminished susceptibility to allergen-induced lung inflammation and collagen-induced arthritis (Jaradat et al., 2006; Tälty et al., 2007) and polymorphisms in ROα have been associated with increased susceptibility to asthma (Ramasesray et al., 2012). A recent study identified a role for ROαa in the generation of natural helper (NH) cells (Halim et al., 2012). ROα-deficient, but not ROγ-deficient, mice lack NH cells. NH cell-deficient mice generated by ROα-deficient bone marrow transplantation exhibited normal Th2 cell responses, but failed to develop acute lung inflammation in response to a proallergic allergen. These findings might at least in part explain the reduced susceptibility to allergen-induced lung inflammation in ROα-deficient mice (Jaradat et al., 2006).

An increased Th17 response has been reported to correlate with white adipose tissue (WAT)-associated inflammation and the development of obesity in obese mice (Ahmed and Gaffen, 2010; Berton et al., 2012). Whether inhibition of Th17 differentiation plays a role in the protection ROγ or ROγ-deficient mice against diet-induced insulin resistance needs further study. ROα or ROγ have also been implicated in the regulation of thyroptosis. Loss of ROα or ROγ results in accelerated apoptosis of distinct positive thymocytes, while ROα deficiency significantly reduces the generation of single positive thymocytes (Kurebayashi et al., 2000; Sun et al., 2000; Dzhabaglova et al., 2004).

**RORα in diet- and age-induced obesity**

Study of staggerer (ROαγ/sγ) mice, a natural mutant strain containing a deletion in the ROαγ gene that results in loss of ROαγ expression, indicated that ROαγ plays a critical role in the control of lipid metabolism and the development of insulin resistance in obese mice and patients. These investigations showed that ROαγ/sγ mice are protected against age- and diet-induced obesity and the development of several obesity-linked pathologies, including adipose tissue-associated inflammation, hepatosteatosis, and insulin resistance (Kang et al., 2011; Lui et al., 2011). ROαγ/sγ mice fed a high fat diet (HFD) gain relatively less weight and exhibit a significantly lower total body fat index compared to wild-type (WT) litters on a HFD. Similarly, male ROαγ/sγ mice were also protected against age-induced obesity. Adipose tissue is the main site of storage of excess energy that is stored in the form of triglycerides in single large lipid droplets. The reduced adiposity in ROαγ/sγ mice was largely related to smaller adipocyte size due to diminished deposition of triglycerides.

ROαγ, particularly the ROαγ isoform, has been shown to be highly expressed in WAT and to be induced during differentiation of 3T3-L1 preadipocytes (Austin et al., 1998). Overexpression of ROαγ in preadipocytes inhibits adipocyte differentiation (Ducet et al., 2009; Oboka et al., 2009). This appears to be mediated through a direct interaction of ROαγ with CCAAT/enhancer-binding protein β (C/EBPβ) that results in the inhibition of the recruitment of the co-activator CBP and repression of C/EBPβ transcriptional activity. These studies suggest that ROαγ has a negative regulatory role in adipocyte differentiation. This function, however, does not explain the reduced adiposity observed in ROαγ-deficient mice.

Obesity is a consequence of an imbalance between energy intake and expenditure (Glass and Olefsky, 2012; Samuel and Shulman, 2012). However, the decrease in diet-induced adiposity in ROαγ/sγ mice was found not to be due to reduced food intake or increased fecal lipid excretion. Indirect calorimetric analysis showed that VO2, VCO2, and heat generation were significantly enhanced in ROαγ/sγ mice on a HFD (Kang et al., 2011). This suggested that elevated energy expenditure might at least in part be responsible for the reduced weight gain and resistance to hepatosteatosis and insulin insensitivity in ROαγ/sγ mice.

**RORα and WAT-associated inflammation**

In addition to functioning as the main site of storage of extra energy in the form of triglycerides derived from food intake, white adipocytes produce a variety of endocrine hormones, including leptin, adiponectin, resistin, and retinol-binding protein 4 (RBP-4) which regulate food intake, lipid metabolism, and inflammation (Hotamisligil, 2006; Guilherme et al., 2008; Glass and Olefsky, 2012). Leptin and adiponectin promote insulin sensitivity, while resistin and RBP4 have the opposite effect and impair insulin sensitivity. It is now well-recognized that obesity is associated with a chronic state of low grade, systemic inflammation and that this is an important contributory factor in the development of insulin resistance (Hotamisligil, 2006; Odegard and Chawla, 2008; Nishimura et al., 2009; Glass and Olefsky, 2012). Progressive infiltration of various immune cells, including macrophages and CD8+ effector T lymphocytes, in WAT lead to increased release of proinflammatory cytokine- and chemokines. In addition to the accumulation of bone marrow-derived macrophages, there is also a shift from anti-inflammatory "alternatively activated" (CD11c+CD206+) M2 macrophages to proinflammatory "classically activated" (CD11c+CD206−) M1 macrophages (Sun et al., 2011; Glass and Olefsky, 2012), which in advanced obesity aggregate into crown-like structures (CLS) surrounding necrotic adipocytes. Recent studies indicated that CD8+ T cells are critical in promoting recruitment of macrophages in WAT in obesity (Weisberg et al., 2003; Odegard and Chawla, 2008; Nishimura et al., 2009; Glass and Olefsky, 2012). Progressively infiltrated macrophages and CD8+ T lymphocytes, in WAT lead to increased release of proinflammatory cytokine- and chemokines. In addition to the accumulation of bone marrow-derived macrophages, there is also a shift from anti-inflammatory "alternatively activated" (CD11c+CD206+) M2 macrophages to proinflammatory "classically activated" (CD11c+CD206−) M1 macrophages (Sun et al., 2011; Glass and Olefsky, 2012), which in advanced obesity aggregate into crown-like structures (CLS) surrounding necrotic adipocytes. Recent studies indicated that CD8+ T cells are critical in promoting recruitment of macrophages in WAT in obesity (Weisberg et al., 2003; Odegard and Chawla, 2008; Nishimura et al., 2009; Glass and Olefsky, 2012).
et al., 2009). In addition, a reduction in anti-inflammatory T regulatory (Treg) cells and an increase in proinflammatory Th17 response further stimulate WAT-associated inflammation (Figure 1).

Deficiency of RORα greatly inhibits diet-induced adipose tissue-associated inflammation in mice (Kang et al., 2011; Lau et al., 2011). This is indicated by the greatly reduced infiltration of macrophages and CD8+ T lymphocytes in WAT of RORα-/- mice fed a HFD. This was further supported by the significant reduction in the formation of CLS and the expression of several macrophage markers, such as F4/80, Mac-2, macrophage expressed 1 (Mpeg1), and macrophage scavenger receptor 1 (Msr1), in WAT of RORα-/- mice. Moreover, the relative percentage of proinflammatory M1 macrophages was significantly diminished in RORα-/- WAT. This was supported by flow cytometric analysis and the much lower levels of CD11c expression. The reduced inflammation in RORα-/- WAT is further indicated by gene expression profiling showing a greatly reduced expression of a large number of chemokines, including Ccl2, Ccl8, Ccl3, and Ccl7, the chemokine receptors Ccr3, Ccr5, and Ccr7, the proinflammatory cytokines Tnfα and Il-6, the interleukin 1 receptor antagonist (Il1rn), osteopontin (Opn), Ccr5, serum amyloid 3 (Saa3), and several TLRs and metalloproteinases in WAT of RORα-/- mice compared to their WT counterparts (Nomiyama et al., 2007; Bertola et al., 2009; Kiefer et al., 2011; Kodama et al., 2012). These observations suggest that suppression of several proinflammatory genes and pathways in RORα-/- WAT is causally linked to the reduced inflammation (Figure 1). Future studies have to determine what the primary effects are by which RORα regulate the expression of these genes.

**RORα AND HEPATOSTEATOSIS**

Obesity is associated with increased prevalence of NAFLD, which is characterized by elevated lipid accumulation in hepatocytes (Fabbrini et al., 2010). NAFLD develops when the rate of fatty acid uptake and synthesis and subsequent esterification to triglycerides is greater than the rate of fatty acid oxidation and secretion. Advanced NAFLD progresses into increased inflammation and hepatotoxicity. Several studies showed that compared to WT mice hepatic triglyceride levels are considerably reduced in RORα-/- mice fed a HFD or aging male RORα-/- mice (Raspe et al., 2001; Lau et al., 2008; Kang et al., 2011). These observations indicated that RORα-/- mice are protected against the development of age- and diet-induced hepatosteatosis. Gene expression profiling revealed that the expression of a number of lipogenic genes was
significantly reduced in the liver of ROβα/− mice fed a HFD. Expression of Srebplc and fatty acid synthase (Fas), key regulators for lipogenesis, was reduced in liver of ROβα/− mice. In addition, the expression of several genes involved in the main pathway of triglyceride synthesis, including glycerol-3-phosphate acyltransferase (Gpam or Gpat1) and acyl-glycerol-3-phosphate acyltransferase 9 (Apg9p9) and Mgst1, which is part of an alternative pathway of triglyceride synthesis, were significantly diminished in ROβα/− liver (Kang et al., 2011). The hepatic expression of the cell death-inducing DFFA-like effectors a and c (Cidea and Cidea) and perilipin 2 (Plin2 or Adip), which play a critical role in the regulation of lipid storage, lipid droplet formation, and lipolysis (Gong et al., 2009; Greenberg et al., 2011), was also suppressed in ROβα-deficient mice. ROβα has been reported to activate Plin2 transcription directly through interaction with ROREs in the Plin2 promoter (Kang et al., 2011). Recently, the expression of throboblast growth factor (Fgf21), an important regulator of lipid/glucose metabolism, was found to be directly regulated by ROβα in hepatocytes (Wang et al., 2010c). Together these observations suggest that the protection against hepatosteatosis in ROβα/− mice is related to reduced expression of many genes involved in promoting lipogenesis and triglyceride storage, some of which are directly regulated by ROβα (Figure 1).

ROβα and Insulin Resistance

Both adipose-associated inflammation and hepatosteatosis have been linked to the pathogenesis of insulin resistance in obesity (Guilherme et al., 2006; Donath and Shoelson, 2011; Samuel and Shulman, 2012), although a cause-effect relationship not always exists between hepatosteatosis and diabetes (Sun and Lazar, 2013). The phenotypic differences observed between WT and ROβα/− mice fed a HFD are consistent with this correlation. ROβα/− mice, which are protected against obesity, hepatosteatosis, and WAT-associated inflammation, exhibited a significantly reduced susceptibility to diet-induced insulin resistance and glucose intolerance compared to obese WT mice (Lau et al., 2008; Kang et al., 2011). In humans, two studies have revealed a connection between ROβαa, obesity, and type 2 diabetes. A rearrangement resulting in disruption of human ROβ1 was found to be associated with severe obesity (Klar et al., 2005), while a recent GWAS study showed an association between a single nucleotide polymorphism in ROβα (rs7164773) and increased risk for type 2 diabetes in the Mexican Mestizo population (Gambou-Melendez et al., 2012).

Many inflammatory and lipogenic genes, including Plin2, Il1rn, Opm, Cd44, and Cidea, that are down-regulated in ROβα/− mice have been reported to also regulate insulin sensitivity. Plin2 null mice displayed reduced hepatic lipid accumulation and improved insulin sensitivity and glucose tolerance in an ob/ob background (Chang et al., 2010). Il1rn, one of the genes most dramatically repressed in WAT of ROβα/− mice (Kang et al., 2011), has been reported to be highly up-regulated in obese humans and to regulate insulin sensitivity (Iuge-Aubry et al., 2003; Somm et al., 2006). Similarly, Opm expression was found to be elevated in obesity, while Opa deficiency was shown to inhibit obesity-induced inflammation and insulin resistance (Bertola et al., 2009; Kiefer et al., 2011). Deficiency in Cd44, a receptor for Opm, also results in improved insulin sensitivity (Kodama et al., 2012) suggesting a role for the Opm/Cd44 pathway in the control of insulin sensitivity. Mice deficient in Cidea or Cidea, which play a role in lipid storage, are protected from diet-induced obesity and display improved insulin sensitivity (Gong et al., 2009). Thus, the down-regulation of several genes, including Plin2, Il1rn, Opm, Cd44, and Cidea in ROβα/− mice may collaboratively be responsible for the improved insulin sensitivity through their interrelated effects on inflammation, adipogenesis, and lipid homeostasis (Figure 1).

In addition to adipose tissue and liver, the pancreas and the skeletal muscle also play important roles in energy homeostasis and insulin resistance. The pancreatic islets produce a number of hormones, including insulin and glucagon, that are critical in the regulation of lipid and glucose homeostasis (Saltiel and Kahn, 2001; Cryer, 2012). ROβα was shown to be selectively expressed in the glucagon-producing alpha cells; however, its role in these cells and its relationship to the phenotype observed in ROβα-deficient mice needs yet to be established (Muhlbaier et al., 2013). In skeletal muscle, ROβα has been reported to regulate the expression of a number of genes involved in lipid and carbohydrate metabolism (Lau et al., 2011). Ectopic expression of an ROβα mutant in skeletal muscle C2C12 cells reduced the expression of the lipogenic genes, sterol regulatory element-binding transcription factor 1 (Srebp3), Fas, and stearoyl-CoA desaturase 1 (Scd1), and genes involved in cholesterol efflux, such as ATP-binding cassette, subfamily A, member 1 (Abca1). Caveolin-5 (Cav5) and carnitine palmitoyltransferase-1 (Cpt1) were found to be directly regulated by ROβα. Changes in the expression of these genes may be in part responsible for the modulation of lipid and glucose homeostasis by ROβα.

In muscle, insulin stimulates glucose uptake by stimulating the translocation of Glut4 (Slc2a4) to the plasma membrane (Rose and Richter, 2005). This involves phosphorylation of the insulin receptor substrate 1 (IRS1), which leads to the activation of phosphatidylinositol 3-kinase (PI3K) and subsequently AKT, which then promotes Glut4 translocation. Recently, evidence was provided for a role of ROβα in PI3K-Akt signaling (Lau et al., 2011). Akt1/2 expression was up-regulated in skeletal muscle of ROβα/− mice and this correlated with an increase in the level of insulin-induced Akt phosphorylation, Glut4 expression, and glucose uptake. This stimulation in Akt signaling might at least in part account for the improved insulin sensitivity observed in ROβα/− mice.

ROβγ1 and Insulin Sensitivity

The ROγ gene generates two different isoforms, ROβγ1 and ROβγ2 (ROβγ2), that are expressed in a highly tissue-specific manner (Jetten, 2009). Expression of the ROβγ1 isoform is restricted to several peripheral tissues, including liver, adipose tissue, kidney, small intestines, pancreas, and skeletal muscle. Recent studies identified ROβγ1 as a negative regulator of adipocyte differentiation and a modulator of obesity-associated insulin resistance (Meissburger et al., 2011; Tinahones et al., 2012). In obese ROβγ−/− mice, the number of adipocytes was increased (hypertrophy), while adipocyte size was reduced. Fasting blood insulin levels were shown to be significantly lower in diet-induced obese...
ROγ−/− mice and in ROγ−/− ob/ob double knockout mice and mice displayed improved insulin sensitivity. In addition, ROγ−/− adipocytes were highly insulin sensitive leading to improved control of circulating FFA. These observations are consistent with a recent study showing that, opposed to adipose hypertrophy, obese patients with adipose tissue hyperplasia (many small adipocytes) exhibit better glucose and lipid profiles and might be less susceptible to developing insulin resistance (Hoffstet et al., 2010) and with data showing that in human patients the level of ROγ−/− expression positively correlated with adipocyte size and insulin resistance (Meissburger et al., 2011; Tinahones et al., 2012). Up to now, no association has been established between ROγ polymorphisms and susceptibility to insulin resistance in humans. However, in cattle, a single polynucleotide polymorphism in ROγ has been linked to increased adiposity (Burendse et al., 2007). These observations suggest that the loss or potentially the inhibition of ROγ might protect against insulin resistance and type 2 diabetes.

In addition to adipose tissue, regulation of lipid and glucose metabolism in other tissues, including liver, pancreas, and skeletal muscle might be part of the mechanism by which ROγ modulates insulin sensitivity. In skeletal muscle, ROγ has been reported to regulate the expression of genes associated with lipid and carbohydrate metabolism as well as the production of reactive oxygen species (Raichur et al., 2007). A recent study revealed that ROγ is selectively expressed in insulin-producing pancreatic β cells, however, its role in β cells and how this relates to the modulation of insulin sensitivity by ROγ has yet to be established (Mühlbauer et al., 2013). Further study is required to understand the modulation of lipid/glucose homeostasis and insulin sensitivity by ROγ.

**CONNECTION BETWEEN RORs, CIRCADIAN RHYTHM, AND METABOLIC SYNDROME**

It has been well established that many behavioral and physiological activities display circadian rhythms that are regulated by endogenous clocks (Acker and Schibler, 2011; Bass, 2012; Mohanek et al., 2012). At the molecular level the clockwork consists of an integral network of several interlocking positive and negative transcriptional and translational feedback loops that include the transcriptional regulators brain and muscle ARNT-like 1 (Bmal1), neuronal PAS domain protein 2 (Npas2), circadian locomotor output cyclosizes kaput (Clock), two cryptochrome proteins (Cry1, 2), and Circadian locomotor activity domain protein 2 (Cry2), which regulate the circadian expression of target genes involved in lipid/glucose metabolism.

Accumulating evidence suggests that disruption of circadian rhythm is closely associated with several pathologies, including sleep disorders, cancer and metabolic syndrome (Maury et al., 2010). Recent studies have established a strong link between the circadian clock machinery and the regulation of a number of metabolic pathways (Acker and Schibler, 2011; Bass, 2012). Bmal1, Clock, and Cry1 have been implicated in the regulation of glucose homeostasis and dysfunctions in these proteins lead to impaired glucose tolerance (Rudic et al., 2004; Zhang et al., 2010). Hepatic overexpression of Cry1 has been reported to improve insulin sensitivity in insulin-resistant db/db mice (Zhang et al., 2010). In addition, circadian oscillator components, such as Cry1, have been implicated in the regulation of immune responses (Castanon-Cervantes et al., 2010; Logan and Sarkar, 2012; Narasimamurthy et al., 2012). In Cry1−/−/Cry2−/− cells, NF-κB and protein kinase A (PKA) signaling pathways are constitutively activated resulting in elevated levels of circulating TNFs, IL-6, and IL-7 (Narasimamurthy et al., 2012).

A number of studies demonstrated that RORs play a role in the modulation of circadian behavior and clock gene expression (Sato et al., 2004; Ueda et al., 2005; Duez and Staels, 2010; Figure 2). Bmal1, Npas2, E1b-p44, and Cry1 transcription are directly regulated by ROγ and ROμ in several peripheral tissues through their interaction with ROEs in their regulatory regions (Crambley et al., 2010; Takeshita et al., 2011, 2012). ROγ1 appears to be the major RO isotype modulating the circadian expression of clock genes in peripheral tissues. ROγ1 itself exhibits a strong oscillatory pattern of expression in several peripheral tissues, including kidney, liver, pancreas, and adipose tissue, while ROγ exhibits only a weak circadian expression pattern (Mongrain et al., 2008; Takeshita et al., 2012; Mühlbauer et al., 2013). The ROγ1 gene is directly regulated by Bmal1/Clock heterodimers which interact with two successive E-boxes in the ROγ1 promoter (Mongrain et al., 2008; Takeshita et al., 2012). Recent studies have suggested that ROγ1 and ROμ might provide a link between the clock machinery and their regulation of metabolic genes (Takeshita et al., 2012; Figure 2). Data demonstrating that the circadian pattern of expression of a number of metabolic genes are regulated by clock proteins and ROs and observations showing that circadian expression of ROγ1 is controlled by the clock machinery suggested that ROs might function as downstream mediators in the mechanism by which clock proteins regulate the circadian expression of...
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acterization of the core mammalian clock component, NPAS2, as a

SUMMARY

The study of ROR-deficient mice has clearly demonstrated that RORα and RORγ are important in several physiological pro-
cesses, including the regulation of several immune responses, lipid/glucose homeostasis, and circadian rhythm. These studies revealed that loss of RORα protects against the development of diet- and age-induced obesity, hepatosteatosis, glucose intoler-
ance, and insulin resistance, while loss of RORγ protects against insulin resistance. These protective effects have been linked to suppression of the expression of multiple proinflammatory and metabolic genes. RORs regulate expression of some of these genes directly by binding ROREs in their regulatory region and in certain cases involves changes in their circadian pattern of expression. Although much progress has been made, what event or which ROR target genes are the primary driving force by which RORs influences WAT-associated inflammation, hepatosteatosis, and insulin resistance needs further study. With the increas-
ing evidence for an interrelationship between the controls of lipid/glucose metabolism, inflammation and circadian rhythm, RO
Rs might functions as intermediaries between the controls. With the discovery of ROR antagonists, RO
Rs may provide a novel therapeutic target in the management of various aspect of metabolic syndrome.

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metabolic genes (Sato et al., 2004; Akashi and Takumi, 2005; Guillaumond et al., 2005; Ueda et al., 2005; Crumbley et al., 2010; Duan and Staels, 2010; Takeda et al., 2011, 2012). This is supported by observations showing that RORs regulate the circadian pattern of expression of a number of genes involved in the lipid/glucose homeostasis, including PPARγ, subtilisin-25-hydroxycholesterol (25-OH), 7-dehydrocholesterol, 7α-hydroxycholesterol, which exhibit roles in lipogenesis, glycogenolysis, and/or chole-
terol synthesis (Kang et al., 2007; Crumbley et al., 2012; Takeda et al., 2012). Thus, RORs appear to be part of the mechanism that links the circadian clock to its regulation of lipid/glucose homeostasis, inflammation, and insulin resistance (Figure 2).

RORα as therapeutic targets for metabolic syndrome and insulin resistance

X-ray crystallography studies of the LBD of RORα identified the presence of cholesterol in the ligand-binding pocket of RORα (Kallen et al., 2002). Subsequent studies identified cholesterol sulfate, 7-dehydrocholesterol, and 25-hydroxycholesterol as RORα agonists (Kallen et al., 2004). All-trans retinoic acid and the synthetic retinoid, ALRT 1530 were reported to bind and function as antagonists for RORγ and RORβ, but not RORα (Stehlin-Gaon et al., 2003). Recently, ursoacid and several oxygenated steroids, including 7α-hydroxycholesterol (7α-OHC), 7β-hydroxycholesterol, 7-ketocholesterol, and 24S-
hydroxycholesterol, were shown to function as inverse agonists to both RORα and RORγ (Wang et al., 2010a; Xu et al., 2011), while 28-hydroxycholesterol and 22R-hydroxycholesterol acted as agonists (Jin et al., 2010). The LXR agonist Tg001317 and several other synthetic derivatives, including SR1001, were identi-
fied as RORα and RORγ inverse agonists. Diginon and several derivatives were identified as specific inhibitors for RORγ transcrip-
tional activity (Fujita-Sato et al., 2011; Huh et al., 2011). The RORγ (inverse) antagonists have been reported to repress the expression of ROR target genes and the activation of their promoter regulatory region by inhibiting the recruitment of co-
avtivators. Moreover, ROR antagonists have shown to inhibit Th17 cell differentiation and IL-17 production both in vitro and in vivo and to suppress the development of experimental autoimmune encephalomyelitis (Huh et al., 2011; Jetten, 2011; Solt et al., 2011). Therefore, antagonists for RORγ might be potential drugs for pharmacological intervention in the treat-
ment and suppression of several autoimmune diseases, includ-
ing multiple sclerosis, collagen-induced arthritis, rheumatoid arthritis, and asthma (Solt et al., 2010; Huh and Littman, 2012). Because of their role in regulating various features of metabolic syndrome, RORα and γ antagonists might also have beneficial effects in the management of obesity and insulin resistance.
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