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Post-translational regulation of RORγt—A therapeutic target for the modulation of interleukin-17-mediated responses in autoimmune diseases

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ABSTRACT

Retinoic acid-related orphan receptor gamma t (RORγt) is a nuclear receptor, which is selectively expressed by various lymphocytes. RORγt is critical for the development of secondary and tertiary lymphoid organs, and for the thymic development of the T cell lineage. RORγt has been extensively studied as the master transcription factor of IL-17 expression and Th17 cells, which are strongly associated with various inflammatory and autoimmune conditions. Given its essential role in promoting pro-inflammatory responses, it is not surprising that the expression of RORγt is tightly controlled. By its nature as a nuclear receptor, RORγt activity is also regulated in a ligand-dependent manner, which makes it an attractive drug target. In addition, multiple post-translational mechanisms, including post-translational modifications, such as acetylation and ubiquitinylation, as well as interactions with various co-factors, modulate RORγt function. Here we attempt a comprehensive review of the post-translational regulation of RORγt, an area that holds the potential to transform the way we target the RORγt/IL-17 pathway, by enabling the development of safe and highly selective modulators of RORγt activity.
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1. Introduction

Nuclear receptors (NRs) constitute a large family of transcription factors which regulate gene expression in a ligand-dependent manner [1]. The NR superfamily includes receptors for steroid hormones, such as the estrogen receptor (ER) or the glucocorticoid receptor (GR), receptors for nonsteroidal ligands, such as the retinoic acid receptor (RAR) or the thyroid hormone receptor (TR), as well as a number of receptors that bind various products of lipid metabolism, including fatty acids and prostaglandins [1]. A number of NRs (17 out of 48 human NRs) are so-called orphan receptors for which regulatory ligands have not been identified. Retinoic acid-related orphan receptor gamma (RORγ) belongs to the retinoid acid-related orphan receptor (ROR) subgroup. This subfamily consists of three members: RORα [2], RORβ [3], and RORγ [4], also referred to as NR1F1, NR1F2 and NR1F3 (according to the Nuclear Receptor Nomenclature Committee) or RORA, RORB and RORC (according to the Human Gene Nomenclature Committee) [1,5].

Although RORs were originally named based on their sequence homology to RARs, more recent evidence suggests that RORα and RORγ preferentially bind oxysterol derivatives but not retinoic acid [6–10]. Despite the progress in identifying physiological endogenous ligands for RORγ [11,12], it is still being referred to as an orphan receptor.

RORγt [13], an immune cell-specific isoform of RORγ, has attracted much attention as the key transcription factor of Th17 cells, mediating the expression of the pro-inflammatory cytokines IL-17A and IL–17F in both mouse and human [14]. Th17 cells and their cytokines have been associated with multiple inflammatory and autoimmune diseases. The various roles of RORγt in immune homeostasis and immunopathology have been the subject of several excellent reviews [14–21]. RORγt, by its nature as a ligand-dependent transcription factor, has become a prime target for pharmacological intervention to repress the function of Th17 cells and their downstream cytokines. In fact, several research groups have developed potent inverse agonists for RORγt (reviewed in [22–24]). A detailed understanding of how RORγt function is regulated has been critical in this process.

Here, we attempt to provide a comprehensive overview of our current knowledge of the different levels of post-translational regulation of RORγt activity, including recent progress in identifying endogenous ligands. We mainly focus on the rapidly evolving area of post-translational modifications (PTMs), such as acetylation and ubiquitinylation of RORγt, which might provide novel opportunities for pharmacological intervention.

2. RORγt expression and function

The *RORA*, *RORB*, and *RORC* genes have been mapped to human chromosome 15q22.2, 9q21.13, and 1q21.3, respectively. Murine and human RORγ share 88% amino acid sequence homology [25]. Each ROR gene produces several isoforms that are generated through a combination of alternative promoter usage and exon splicing (Fig. 1A). These isoforms differ only in their amino-terminal A/B domain. In humans, four different RORα isoforms, RORα1-4, have been identified, while only two isoforms, α1 and α4, are found in mice. The *RORB* and *RORC* genes each are expressed as two different isoforms [26–28]. In RORγt (RORc2, RORγ2) [13], the 24 N-terminal residues of RORγ, which are encoded by the first two exons, are replaced by three alternative residues encoded by a first exon specific to RORγt (Fig. 1B).

RORs and their isoforms differ in their tissue-specific expression and regulate distinct physiological processes and target genes (reviewed in [16,29]). RORα, although expressed in a variety of tissues, is most abundant in several regions of the brain, particularly the cerebellum and thalamus [30]. Accordingly, RORα-deficient mice display ataxia, which is correlated with severe cerebellar atrophy. In addition, RORα has been implicated in the regulation of a number of other physiological processes, including the development of the olfactory bulb, bone formation and in lipid metabolism [31]. RORβ expression is largely restricted to several regions of the brain, the retina, and pineal glands. RORβ-deficient mice develop retinal degeneration that results in blindness [32]. All three RORs have been implicated in the regulation of circadian rhythms [16,29,33,34].

While RORγ mRNA has been detected in several tissues including kidney, liver, lung, muscle, heart, and brain, RORγt expression is restricted to lymphoid tissues and a number of lymphoid cell types [4,26]. RORγt-deficient mice lack Peyer's patches, cryptopatches and isolated lymphoid follicles in the intestine as well as peripheral lymph nodes (Fig. 2A). The lack of these lymphoid structures is explained by the absence of lymphoid tissue inducer (LTi) cells in RORγt-deficient mice [35,36]. During lymphoid organ formation, stromal organizer cells express lymphotoxin-β receptor (LTβR), and LTi cells that seed the developing lymph node express lymphotoxin-α1β2. The interaction of the two results in an up-regulation of adhesion molecules and chemokines that facilitate the attraction and retention of additional haematopoietic cells at the site of developing secondary lymphoid organs. The expression of RORγt is required for the differentiation of LTi cells [35–37]. RORγt is also a critical regulator of thymopoiesis (Fig. 2A). In the absence of RORγt, mice exhibit severe thymic atrophy [38,39]. Expression of RORγt is induced at the transition from the double negative (DN) to the double positive (DP) stage of thymic T cell development. The absence of RORγt results in a dramatic decrease in the number of $CD4^+CD8^+$ DP and mature single positive (SP) $CD4^-CD8^+$ and $CD4^+CD8^-$ thymocytes. This is due to significantly increased apoptosis in thymic DP cells related to a dramatic reduction in the expression of the anti-apoptotic factor Bcl-xL [38,39]. Indeed, targeted expression of Bcl-xL under the control of the RORγt promoter in RORγt-deficient mice rescues DP thymocytes [38,40]. Whether RORγt regulates Bcl-xL directly or indirectly has yet to be established. Although RORγt-deficient mice appear initially healthy, by the age of 4 months about 50% of the mice succumb to thymic lymphomas [41]. It is currently not clear if lymphoma formation translates into other species. So far it has not been observed in a limited number of patients with RORγ/RORγt loss-of-function mutations [42].

Besides its developmental functions in the immune system, RORγt has attracted considerable interest as the master transcription factor of Th17 cells (Fig. 2A) and more broadly of IL-17-producing cells in general (reviewed in [17,43]). In fact, RORγt expression marks various innate and adaptive lymphoid subsets that express the pro-inflammatory cytokines IL-17A and IL-17F, including Th17 cells, γδ T cells, mucosal-associated invariant T (MAIT) cells, as well as in innate lymphoid type 3 cells (ILC3s) (Fig. 2B). In RORγt-deficient mice, IL-17 production is greatly diminished and RORγt/RORα double-deficient mice lack IL-17 production altogether [14,44]. Elevated IL-17 production has been

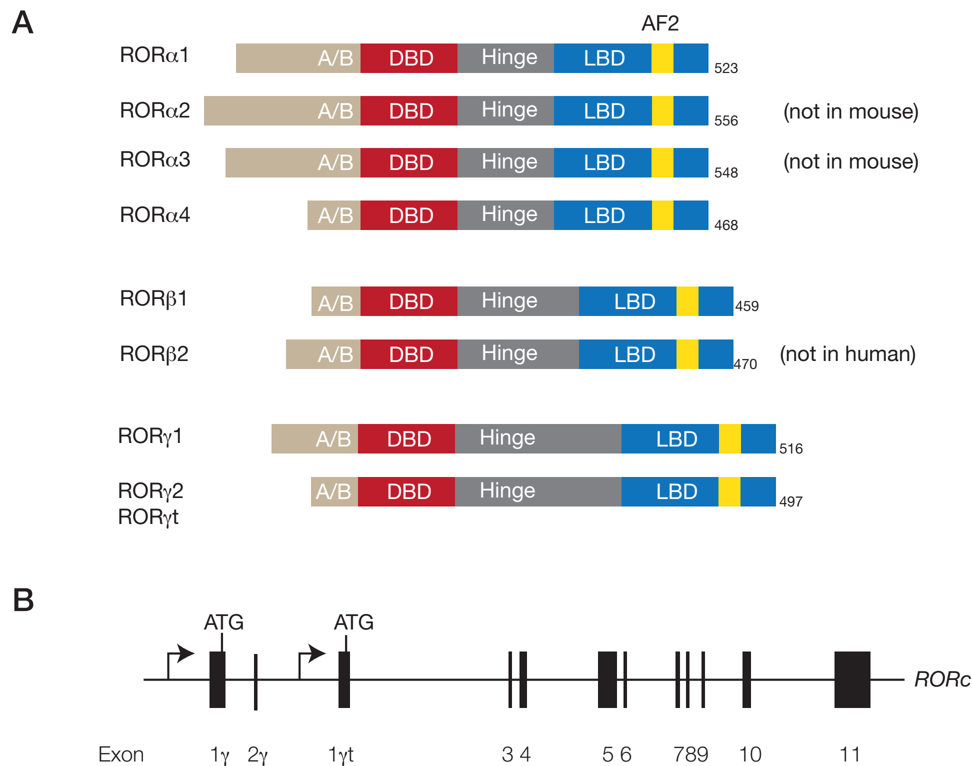

Fig. 1. Overview of the ROR family members.
(A) Schematic representation of the domain structure of the three ROR family members (α, β, γ) and their isoforms. The isoforms are generated by alternative promoter usage and/or alternative splicing in the variable A/B domain. The other regions of the proteins are the DNA-binding domain (DBD), the hinge region and the ligand-binding domain (LBD) containing the activation function 2 (AF2). The size of the protein in terms of number of amino-acids is indicated on the right. (B) Organization of the RORc locus. The usage of exons 1γ and 2γ produces the RORγ protein. These are replaced by a unique exon (exon 1γt) to produce the immune-specific isoform RORγt. As a result, the 24 first amino-acids of RORγ are replaced by 3 residues from exon 1γt.

associated with inflammatory and autoimmune conditions, such as rheumatoid arthritis (RA), multiple sclerosis (MS) and psoriasis (reviewed in [24,45,46]).

3. RORγt—a nuclear receptor

RORγt exhibits the typical structural architecture of all NRs, consisting of four major functional domains (Fig. 3A): a variable amino-terminal (A/B) domain containing the ligand-independent activation function 1 (AF1) helix, a DNA-binding domain (DBD), a flexible hinge domain, and a C-terminal ligand-binding domain (LBD) [1,47]. The DBD contains two highly-conserved zinc finger motifs involved in the recognition of DNA elements. RORs share a high degree of sequence homology within the DBD [48] and recognize common ROR response elements (ROREs) with a consensus sequence (WWCWAGGTCA, W = A or T) [25,49]. The P-box in the DBD, the loop between the last two cysteins within the first zinc finger, recognizes the core motif in the major groove of the DNA [50,51]. Additional residues immediately downstream of the second zinc finger, referred to as C-terminal extension (CTE), further determine the DNA binding specificity of RORs by making contact with the 5′-AT-rich segment of the RORE in the adjacent minor groove of the DNA. The A/B domain also influences the DNA-binding affinity of RORs, and likely accounts for the distinct binding specificities of individual ROR variants [27,49,50,52]. Unlike most NRs, which bind DNA as homodimers or heterodimers, RORs appear to bind their RORE as monomers [13,25,27,53,54].

The LBD, apart from its obvious role in ligand engagement, is critical for nuclear localization and, in other nuclear receptors, dimerization. It also contains the activation function 2 (AF2, also known as Helix 12) region responsible for providing an interface to recruit co-activator and co-repressor proteins. The LBD adopts a conserved three-layered fold of ~12 α-helices (H1-H12), with two or three β-strands forming a shorter sheet structure (Fig. 3B) [47]. H12 contains the AF2 consensus motif ΦΦXE/DΦΦ (where Φ is a hydrophobic amino acid and X is any amino acid), and is 100% conserved among RORs. In addition to the 12 prototypical helices, LBDs of RORs contain three additional helices, H2′, H3′, and H11′ [6,55]. The LBD of RORγ shares 48% and 46% sequence identity with those of RORα and RORβ, respectively [10]. A ligand-binding pocket resides inside the LBD, and forms part of its hydrophobic core (Fig. 3B). The structure of the RORγ LBD bound to the putative ligand 25-hydroxycholesterol (25-OHC) and the steroid receptor coactivator-2 (SRC-2) peptide has been determined [10]. The AF2 helix is stabilized via a hydrogen bond network between His479 and Tyr502, along with Gln487 and Ser507. The 25-hydroxyl group of 25-OHC makes a water-mediated hydrogen bond to Tyr502. This conformation of AF2/H12 forms a surface groove with helices H3, H4 and H5 into which the co-activator motif binds [10]. Hydrogen bonds from RORγ residues Lys336 and Glu504 to the co-activator further stabilize its binding and form the “charge clamp” [56]. At the other end of the ligand-binding pocket, near the C-terminus of helix 3 and helices 5 and 7, the 3β-hydroxyl group of 25-OHC makes a direct hydrogen-bonding interaction with Gln286 and a water-mediated hydrogen bond to Arg364. Similar to the paradigm in other NR structures, the interaction with the agonist ligand stabilizes AF2 in an active conformation that enables the recruitment of the co-activator [57–62].

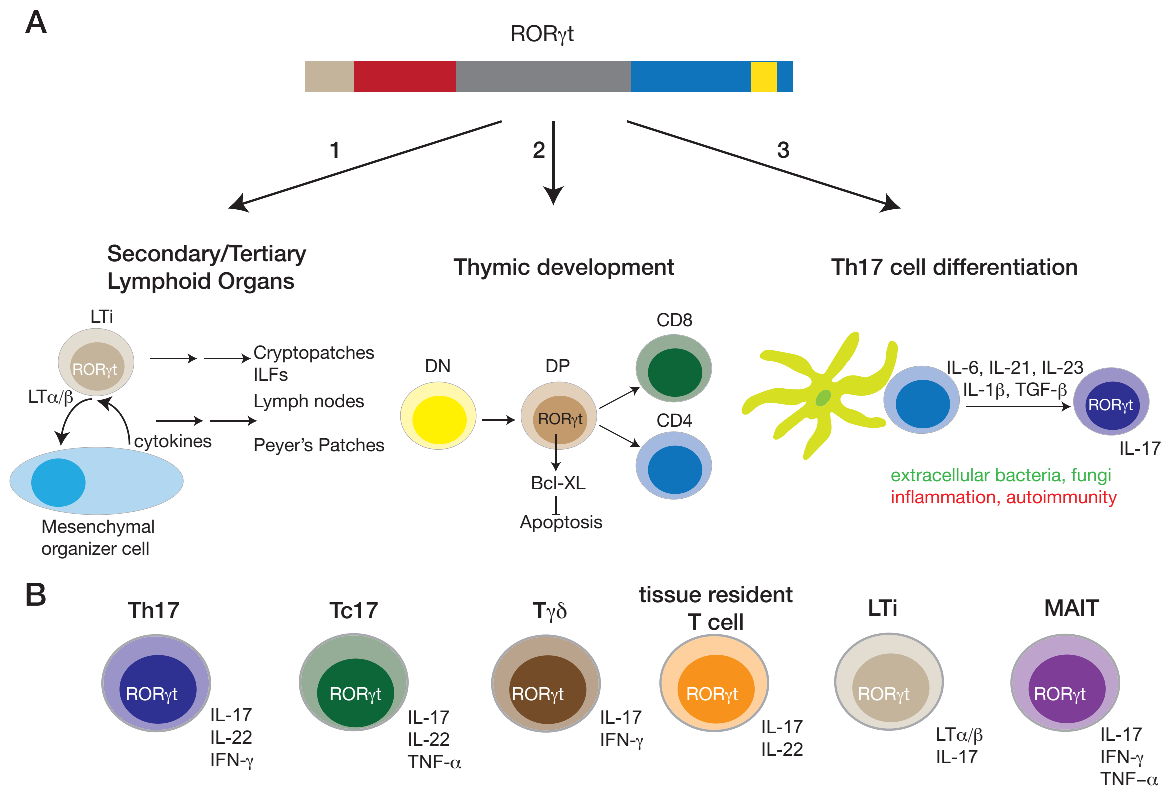

Fig. 2. RORγt biology.
(A) Biological functions of RORγt. 1. The development of LTi cells is dependent on RORγt expression. LTi cells are the source of lymphotoxin α/β that binds its receptor on the surface of mesenchymal organizer cells. This interaction between LTi and mesenchymal organizer cells is required for the development of secondary/tertiary lymphoid organs. 2. During thymocyte development, RORγt is expressed in DP cells and is required for the survival of these cells by controlling the expression of the survival factor Bcl-xL. 3. RORγt is the master transcription factor of Th17 cells, source of IL-17. RORγt is induced in naïve CD4T cells upon their activation by an antigen-presenting cell, in presence of a cocktail of cytokines (IL-6, IL-21, IL-23, IL-1β, TGF-β). Th17 cells have been shown to be required for immunity against extracellular bacterial infection and fungal infection. Excessive IL-17 production has been linked with inflammation and autoimmunity.
(B) RORγt-dependent immune cells and their cytokine production profiles. In addition to Th17 cells, several immune cells also express RORγt. These are Tc17 (a subset of CD8T cells), γδT cells (mainly located in skin and gut), tissue resident T cells, LTi and mucosal associated invariant T (MAIT) cells. Generally, these cells not only produce IL-17, but also IL-22 and/or IFN-γ. Additionally, LTi cells produce LTα/β and MAIT cells are a source of TNF-α.

4. Post-translational regulation of RORγt

4.1. Co-activators and co-repressors

NRs, including RORs, recruit co-regulators in order to modulate chromatin and either activate or repress target gene expression. These activities have been linked to interactions with general classes of co-activators or co-repressors (Fig. 4A). As discussed above, in the presence of an agonist, H12 forms a "charge clamp" in which a conserved glutamate in the AF2 helix and a conserved lysine in H3 make contact with the ends of a conserved helical LXXLL motif present in one or more components of most co-activator complexes (Fig. 4B). The leucine residues of the LXXLL helix pack into a specific hydrophobic pocket at the base of the charge clamp that stabilizes the interactions [57–63].

Binding of an inverse agonist, on the other hand, results in destabilization of the active conformation and in the disruption of the shape of the co-activator binding groove (Fig. 4B). Co-repressors interact with this conformation through an elongated helix with a LXX I/H IXXX I/L sequence. This extended helix can occupy the same hydrophobic pocket contacted by LXXLL motifs due to displacement of the AF2 helix [64–66]. Some co-repressors, such as RIP140 (receptor interaction protein 140) [67], contain LXXLL motifs and are recruited to agonist-bound receptors but function as repressors.

Co-activator complexes facilitate transcription by mediating epigenetic changes, such as the acetylation of histones to open chromatin (CBP and p/CAF complexes) and the repositioning of nucleosomes to increase accessibility (SWI/SNF complex); they also recruit core components of the transcriptional machinery (TRAP/DRIP/ARC complex). In contrast, co-repressors limit chromatin accessibility and recruit histone deacetylases (e.g. HDAC3) [58,68–71] (Fig. 4C). Several co-activators, including NCOA1 (SRC-1), NCOA2 (TIF2 or GRIP1), PGC-1α, p300, and CBP; as well as co-repressors NCOR1, NCOR2, RIP140, and NIX1 have been identified in co-complexes with ROR proteins [29,40,72–82]. At least in the case of RORα it was suggested that RORs can recruit different co-activator complexes in a target gene-specific manner [73]. Although RORγt has been shown to recruit steroid receptor co-activators (SRCs) [40], and in fact, peptides from SRC-1 or SRC-2 are routinely used in binding assays to screen for inverse agonists of RORγt, the specific co-factor landscape for RORγt as it is relevant to thymopoiesis or Th17 differentiation has not been well characterized to date.

4.2. Endogenous and synthetic ligands—RORγt as a drug target

In contrast to many other NRs, ectopic expression of RORγt is sufficient to induce transcriptional activity in various mammalian cell types, even without concomitant addition of exogenous agonists. This result initially seemed to suggest that RORγt was constitutively active in a ligand unbound state. However, RORγt completely fails to induce transcription when ectopically expressed in *Drosophila* cells, which are auxotrophic for poly-

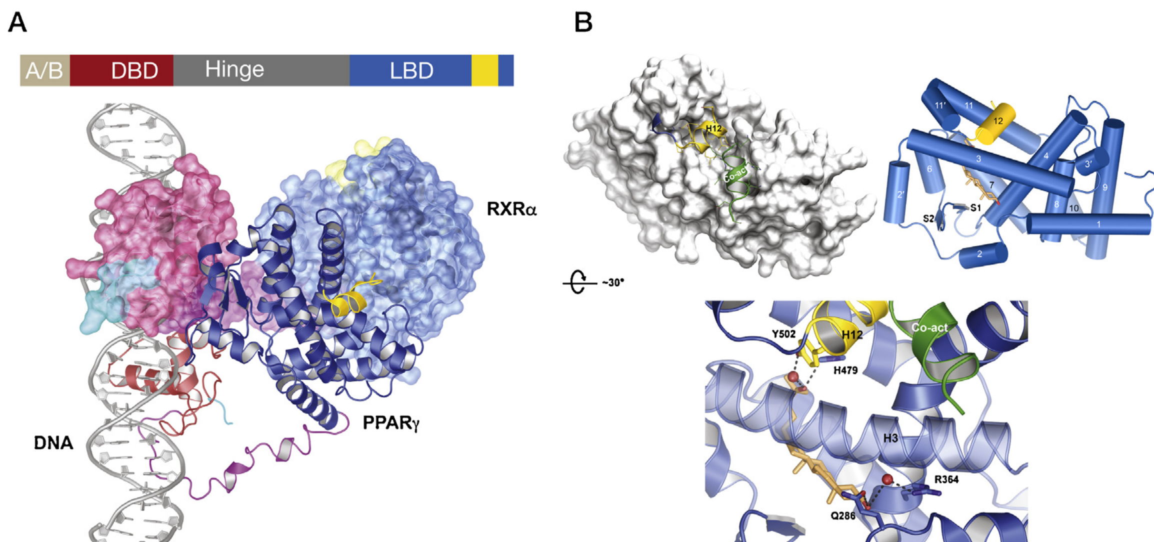

Fig. 3. Structure of RORγt.
(A) Crystal structure of an "intact" nuclear receptor heterodimer bound to duplex DNA. The RXRα protein (accession code: 3DZY) is depicted as a surface representation and the PPARγ protein is shown as a ribbon diagram. Structural domains of both proteins are colored according to the sequence diagram (note that the A/B domain was largely disordered in the structure). Colors: A/B in cyan, DBD in dark red, hinge in magenta, LBD in royal blue, H12 (AF2) in gold.
(B) Crystal structure of RORγ/RORγt ligand binding domain. In the top left panel, the molecular surface of the RORγ/RORγt monomeric LBD (accession code: 3L0L) reveals the grooves into which Helix 12 (gold, surface omitted) and the co-activator peptide (green) interact. The top right panel shows the crystal structure of the RORγ/RORγt LBD monomer, colors as in the sequence diagram, α helices and β sheets are numbered according to convention. The ligand, 25-hydroxycholesterol is shown with sand colored carbon atoms. Bottom panel: Binding of agonist ligands, like 25-hydroxycholesterol (sand carbons), into the binding site of RORγ/RORγt stabilizes the conformation of Helix 12 by hydrogen bonding to tyrosine 502 or stabilizing a hydrogen bond between that side chain and that of histidine 479. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

unsaturated fatty acids, retinoids, and sterols, when these cells are grown in serum-free media. Transcriptional activity can be restored by supplementing serum to the culture [10,12,83]. These findings strongly suggest that an endogenous ligand, which is ubiquitously present in mammalian cells, is indeed required for transcriptional activity.

4.2.1. Endogenous ligands

Attempts to directly detect the endogenous lipid ligand bound to RORγ have not been successful to date [12]. Cholesterol and cholesterol derivatives had been shown to bind RORα [6,8–10,55]. Indeed cholesterol and a number of its naturally occurring derivatives could restore RORγ activity when *Drosophila* cells grown in lipid-free chemically defined medium were supplemented with them. Cholesterol itself as well as 20α-hydroxycholesterol (20α-OHC), 22*R*-hydroxycholesterol (22*R*-OHC), 22*S*-hydroxycholesterol (22*S*-OHC) and 25-hydroxycholesterol (25-OHC) increased the recruitment of co-activator peptides in a dose-dependent manner [10]. Of note, cholesterol derivatives were up to 10-fold more potent than cholesterol itself. A separate screen of naturally occurring oxysterols identified 7β,27-OHC as a RORγt ligand. Addition of 7β,27-OHC to mouse Th17 cultures increased the number of IL-17A producing cells [84].

In order to identify endogenous ligands for RORγ, Santori et al. transfected mammalian cells with a RORγ reporter and probed multiple metabolic pathways either by the addition of 387 common metabolites or by co-transfection with 78 basal metabolic enzymes found in mammalian cells. Only enzymes of the cholesterol biosynthetic pathway were found to modulate RORγ activity (Fig. 5A). In addition, RORγ activity is lost in a squalene synthase-deficient cell line which cannot synthetize sterol lipids [12]. Combining over-expression, RNAi, and genetic deletion of metabolic enzymes, the authors arrive at the conclusion that RORγ ligands are cholesterol biosynthetic intermediates downstream of lanosterol and upstream of zymosterol. For instance, overexpression of CYP51, the enzyme that catalyzes the removal of the 14-methyl group after the formation of the canonical sterol nucleus, thus transforming lanosterol into FF-MAS, increases RORγ transcriptional activity [12]. Interestingly, mouse embryos deficient in CYP51 exhibit smaller lymph node anlagen, and have reduced numbers of LTi cells. Loss-of-function mutation of SC4MOL, an enzyme downstream of CYP51, reduces *in vitro* polarization into Th17 cells [12]. In an independent study, Hu et al. also found that inhibition of CYP51 by ketoconazole reduces IL-17 production from Th17 cells, without affecting RORγt expression *in vitro*. Ketoconazole also reduces IL-17 production from γδ T cells that had been stimulated with IL-1β/IL-23 to induce IL-17 production. Injection of anti-CD3 into mice results in elevation of IL-17 in the plasma. Treatment with the CYP51 inhibitor ketoconazole prior to anti-CD3 injection reduces IL-17 levels, whereas IFN-γ is not affected. Similarly, ketoconazole treatment of mice reduces skin inflammation and IL-17 expression in an imiquimod-induced psoriasis model [11]. Consistent with the findings reported by Santori et al., these data strongly suggest that sterols formed after the CYP51-mediated demethylation step in the cholesterol synthesis pathway function as endogenous RORγt agonists. However, Hu et al. found that both zymosterol and the further downstream desmosterol also increase co-activator recruitment and IL-17 production from Th17 cells [11]. More importantly, desmosterol (but not zymosterol) can be readily detected in Th17 cells, further suggesting that it could function as an endogeneous RORγt ligand. Like their 3-OH analogs, sulfated sterols, in particular desmosterol sulfate, can also be detected in Th17 cells, and are even more potent RORγt agonists than their 3-OH corresponding sterols [11]. The fact that the cholesterol derivatives 20α-OHC, 22R-OHC and 25-OHC have not been detected in Th17 cells does not formally exclude the possibility that they might be endogenous ligands. Taken together, these studies clearly demonstrate that intermediates in the cholesterol synthesis pathway indeed function as endogenous ligands for RORγt, although there is still some disagreement as to which ones are most critical. It is currently not clear whether different

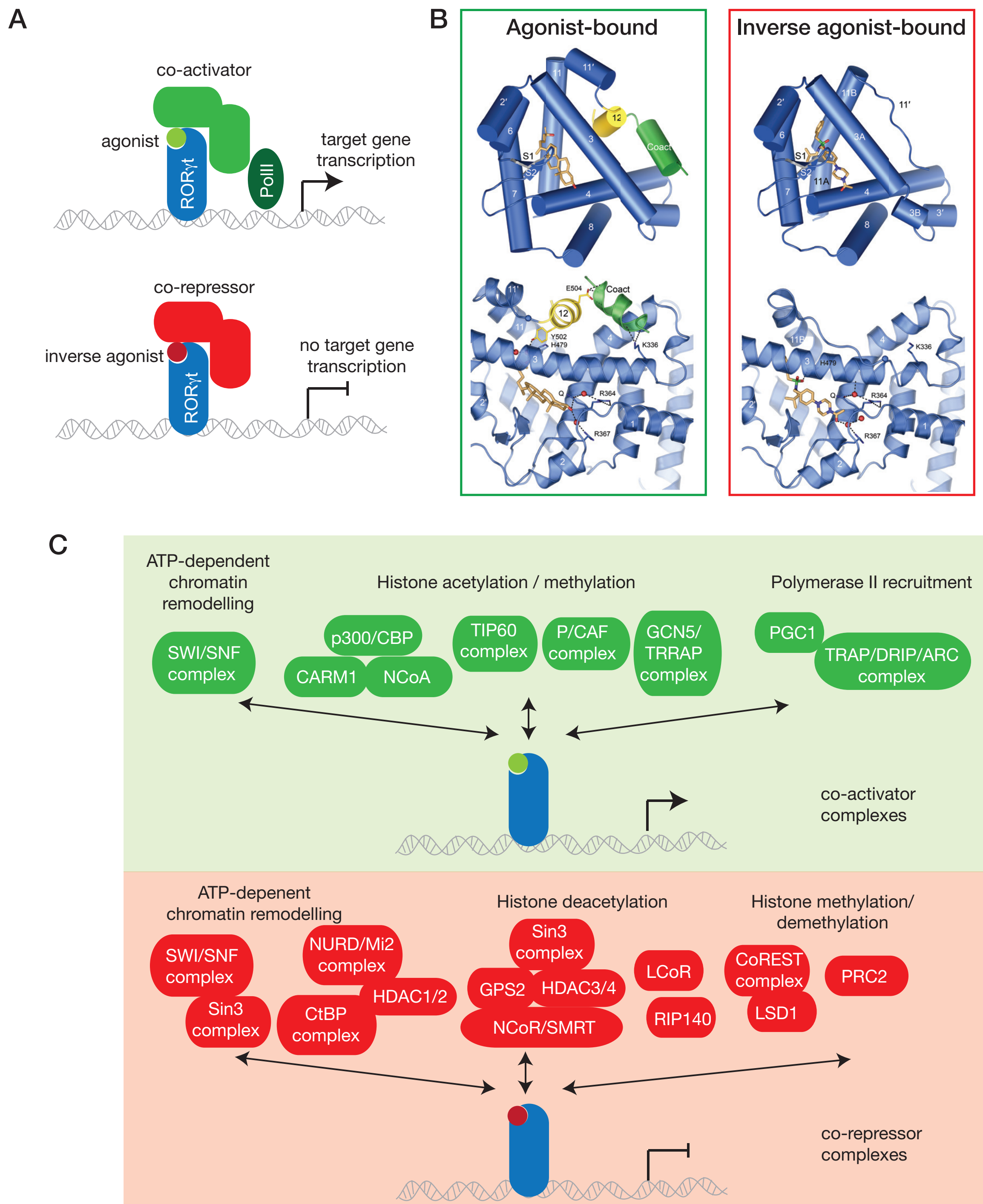

Fig. 4. Regulation of RORγt-mediated transcription.
(A) Mechanism of transcriptional regulation by RORγt. Binding of an agonist (top panel) to RORγt induces the recruitment of various co-activators (green) aimed at ultimately recruiting RNA polymerase II, inducing the transcription of target genes. In contrast (lower panel), in presence of an inverse agonist, co-repressors (red) are recruited to RORγt and the transcription of target genes is repressed.
(B) Structure of RORγ/RORγt LBD in an agonist-(green box) or inverse agonist-bound (red box) conformation. While Helix 12 likely samples a distribution of conformations absent a ligand, binding of an agonist ligand (25-hydroxycholesterol, sand carbons) can stabilize Helix 12 into the agonist conformation (left, accession code: 3L0L) and facilitate recruitment of co-activator proteins (green helices). 25-hydroxycholesterol stabilizes the conformation of H12 (gold helix) by a hydrogen bonding network that anchors Y502. The co-activator peptide (green helix) groove thus forms, and the charge clamp residues E504 and K336 hydrogen bond to the peptide. Alternatively, inverse agonist complex with a synthetic ligand bound blocks Y502 insertion into the hydrogen bonding network and thus destabilizes helix 11, resulting in its conversion into an extended linear conformation. Amino acid 498 was the last ordered residue of the structure, and that position is marked by a blue sphere in both panels for comparison. Key amino acid positions are noted, residue Q286 is marked solely by the letter "Q." (Some labels for structural elements were eliminated for clarity).
(C) Co-activator and co-repressor complexes involved in the regulation of NR-mediated transcription. Co-activator complexes (top panel) include proteins with different functions such as proteins involved in the remodeling of chromatin, histone acetylation/methylation and in the recruitment of the Polymerase II. In red, co-repressors contribute to chromatin remodeling, histone deacetylation and demethylation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

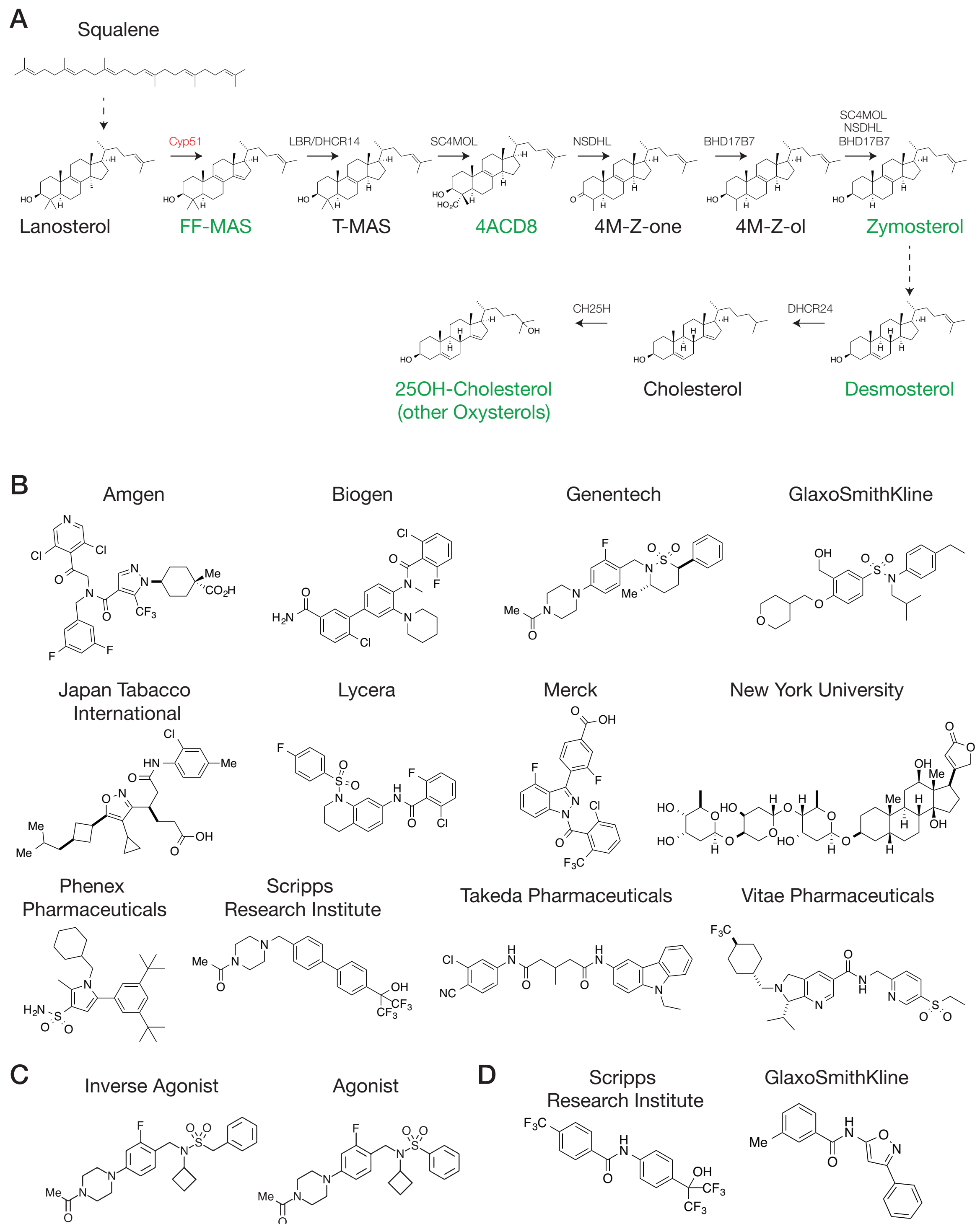

Fig. 5. RORγt ligands.
(A) RORγt endogenous ligands. Simplified representation of the cholesterol synthesis pathway showing in green the intermediates and cholesterol derivatives that have been reported to be RORγt endogenous ligands. Cyp51 (in red), the enzyme responsible of the conversion of lanosterol to FF-MAS, has been shown to be absolutely required for the generation of RORγt endogenous ligands.
(B) RORγt inverse agonists and antagonists. Several biotech/pharmaceutical companies as well as academic institutions have reported potent RORγt inverse agonists and antagonists: Amgen (WO 2015/129926), Biogen [181], Genentech [182], GlaxoSmithKline (WO 2015/061515), Japan Tabacco International (WO 2012/147916), Lycera (WO 2012/064744), Merck [92], New York University [83], Phenex Pharmaceuticals (WO 2012/139775), The Scripps Research Institute [183], Takeda Pharmaceuticals (WO 2013/042782), Vitae Pharmaceuticals (WO 2015/116904).
(C) Minor structural changes transform a RORγt inverse agonist into a RORγt agonist. Benzylsulfonamide (represented on the left) has been identified as a potent RORγt inverse agonist whereas phenylsulfonamide (represented on the right) behaves as a RORγt agonist [88].
(D) RORγt agonists. Several groups have reported RORγt agonists: The Scripps Research Institute [100], GlaxoSmithKline [101]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

endogenous ligands occupy the RORγt LBD depending on the activation and metabolic state of the cell. Interestingly, a recent report suggests that pathways that modulate the lipidome in Th17 cells indeed have the capacity to modify the transcriptional profile of RORγt and hence the pathogenicity of Th17 cells [85]. CD5L/AIM, a member of the scavenger receptor cysteine-rich superfamily known to regulate lipid metabolism by binding to fatty acid synthase in the cytoplasm of adipocytes [86], is expressed in non-pathogenic Th17 cells but down-regulated upon exposure to IL-23, which induces a more pro-inflammatory transcriptional program [85]. In fact, CD5L/AIM overexpression is sufficient to suppress RORγt-dependent transcription of IL-17 and IL-23 in Th17 cells. Conversely, loss of CD5L/AIM converts non-pathogenic Th17 cells into pathogenic cells that induce autoimmunity. Interestingly, non-pathogenic WT Th17 cells have a very different lipidome profile compared to CD5L-deficient Th17 cultured under non-pathogenic conditions or WT Th17 cells cultured under pathogenic conditions. CD5L/AIM decreases the level of polyunsaturated fatty acids (PUFA), affecting the expression of key cholesterol biosynthesis enzymes and, in turn, affecting the binding and activity of RORγt. Therefore, it appears that RORγt-expressing cells can respond to external signals by adjusting the abundance or the nature of endogenous ligands, in order to modulate the RORγt-dependent transcriptional profile.

4.2.2. Synthetic inverse agonists for RORγt

Given the prominent association of IL-17 production with inflammatory and autoimmune diseases, it is not surprising that there has been a strong interest in developing small molecule antagonists/inverse agonists targeting RORγt, in particular for the treatment of psoriasis [17,19,21,24]. It is desired to identify compounds that function as inverse agonists, meaning that they bind to RORγt and recruit co-repressors instead of co-activators in order to inhibit RORγt-dependent target gene transcription. Ligands may also disfavor co-activator recruitment by inducing a LBD conformational change by which the AF2 region is disordered and therefore cannot interact with either co-activator or co-repressor, such compounds are classified as antagonists [87]. It is noteworthy that all compounds targeting the LBD affect RORγt and RORγ alike.

Inverse agonists and antagonists of RORγt have been discovered by both screening and medicinal chemistry campaigns [23,83,88–92]. For instance, digoxin binding to RORγt in the ligand binding pocket prevents adoption of the agonist conformation of H12 by the compound protruding from the pocket between helices H3 and H11, thus antagonizing co-activator interaction [83]. Other synthetic ligands have been shown to disrupt the position of H11 and/or H12 and act as inverse agonists [23,83,88–92]. Another class of compounds, deemed allosteric ligands, have also been identified that can bind external to the ligand pocket and disrupt the interaction of the RORγt-LBD with steroid receptor coactivator-1 (SRC-1) cofactor peptide [92]. In this case, the RORγt LBD crystallized with the typical NR arrangement of helices 1–11, but with a novel position of H12. The putative allosteric pocket, absent in the classical NR-folding motif, is formed by helices 4, 5, 11 and the reoriented flexible H12. This antagonism mode is independent of and unaffected by ligand binding at the canonical ligand binding site [92]. Precisely how these different classes of compounds interfere with downstream RORγt functions is not well understood. Even when comparing two structurally related inverse agonists, Xiao et al. found that one compound disrupted RORγt binding to genomic DNA; whereas the other, more potent compound, affected transcriptional regulation without globally eliminating RORγt DNA binding [93]. The same study also suggests that RORγt, when bound by inverse agonists, can occupy additional DNA binding sites not normally bound in Th17 cells [93]. Co-activator binding assays and functional readouts, such as IL-17 production, which are routinely used for compound screens and validation, are not suited to reveal these mechanistic differences.

Over the past few years a large number of compounds have been identified by several groups (Fig. 5B) that are highly selective for RORγt over RORα and other NRs, and that suppress IL-17 production in various cell-based assays [83,91,94–98]. Several of these compounds have been tested in pre-clinical mouse models for MS, psoriasis or joint inflammation and result in reduced IL-17 production, decreased susceptibility, delayed onset and reduced disease severity [83,91,93,97–99]. VTP-43742, an inverse agonist for RORγt, developed by Vitae Pharmaceuticals is currently in phase II clinical trials for the treatment of psoriasis.

4.2.3. Synthetic agonists for RORγt

Ironically, several compounds that had been identified as inverse agonists, based on their capacity to interrupt the interaction between LBD and co-activator peptides in biochemical assays, function as agonists when tested on full-length RORγt in a cellular context, demonstrating imperfect translation from assay to *in vivo* outcome [100,101]. Interestingly, relatively small structural differences (Fig. 5C) can turn a RORγt inverse agonist (benzylsulfonamide) into a potent agonist (phenylsulfonamide) [88]. These findings also demonstrate that it is possible to enhance the "basal" (driven by endogenous ligand) activity of RORγt with synthetic agonists. Several groups have since reported compounds with agonist activity (Fig. 5D). These compounds elicit increased IL-17 production [88,100–102]. Although the role of IL-17 and Th17 cells in cancer is highly complex and contentious [103,104], it has been suggested that boosting IL-17 responses could be beneficial in certain settings for immunotherapy, for instance by shifting the balance between Th17 cells and regulatory T cells [104], which more recently led some groups to investigate to use of RORγt agonists in this context.

4.3. Post-translational modifications

Besides being regulated through ligand binding, various aspects of NR function are controlled or modulated by post-translational modifications (PTMs), including nuclear localization, protein stability, DNA-binding and transcriptional activity. The best-studied PTMs for NRs are phosphorylation, acetylation and ubiquitinylation, and the most data exist for the steroid receptors, estrogen receptor (ER), glucocorticoid receptor (GR) and androgen receptor (AR) [105,106]. In fact, PTMs in this particular class of NRs are linked to the pathophysiology of many diseases including cancers, diabetes, and obesity [105,106]. Our understanding of how RORγt is regulated by PTMs is still emerging. Its protein sequence reveals a multitude of residues that can potentially function as acceptors for PTMs. Indeed, proteomics studies have already identified a number of phosphorylation, acetylation and ubiquitinylation sites in RORγt (Fig. 6A).

4.3.1. Phosphorylation

Phosphorylation has been studied extensively for several NRs [106]. Phosphorylation by kinases that are associated with general transcription factors, such as Cdk7 within TFIIH, or that are activated in response to various signals, such as AKT, PKA, PKC or MAPK, can facilitate the recruitment of co-activators and, in doing so, cooperate with the ligand to enhance transcription activation. However, phosphorylation can also contribute to the termination of the ligand-induced response by decreasing ligand affinity, mediating dissociation from DNA or inducing NR degradation [105–107]. Surprisingly, despite the fact that multiple phosphorylation sites have been detected for RORγt, including S184, T204,

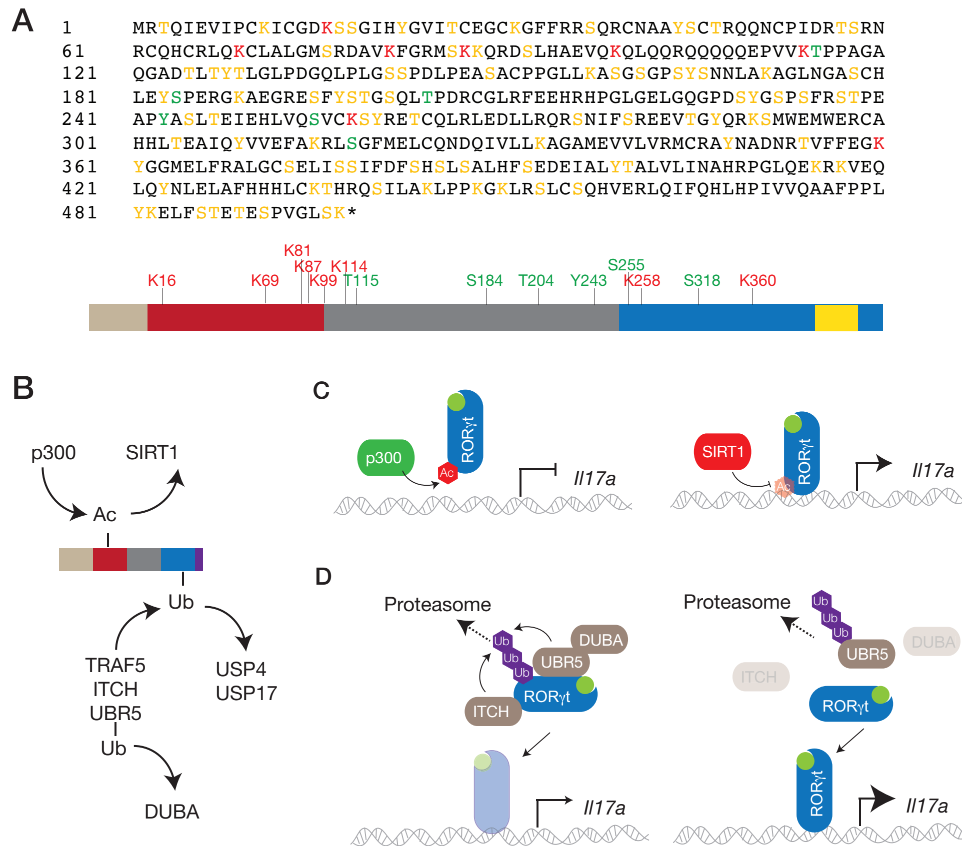

Fig. 6. Post-translational modifications of RORγt.
(A) The amino acid sequence of RORγt is shown. Represented in orange are all serine, threonine, tyrosine and lysine residues that could potentially be phosphorylated, acetylated or ubiquitinated. Residues shown in red have been demonstrated to be subject to ubiquitinylation or acetylation, residues in green have been shown to be phosphorylated. The positions of confirmed post-translational modifications are shown across the protein domains.
(B) Proteins mediating ubiquitinylation/acetylation of RORγt. The histone acetyltransferase p300 acetylates RORγt. This reaction can be reversed by the histone deacetylase SIRT1. E3 ubiquitin ligases, such as TRAF5, ITCH and UBR5, mediate RORγt ubiquitinylation. An additional step of this regulation has been revealed by the identification of DUBA as a deubiquitinase of UBR5. On the other hand, deubiquitinases, such as USP4 and USP17, act directly on RORγt.
(C) Effect of acetylation on RORγt-mediated transcription. Acetylation of RORγt by p300 impairs its DNA binding and consequently, transcriptional activity. The reverse reaction, mediated by SIRT1, a NAD+-dependent protein deacetylase, restores the ability of RORγt to bind DNA and to induce the transcription of target genes, such as *Il17a*.
(D) Regulation of RORγt by ubiquitinylation. The deubiquitinase DUBA interacts with UBR5 and protects it from proteasomal degradation (left panel). UBR5 and ITCH are E3 ubiquitin ligases that mediate RORγt ubiquitinylation and proteasomal degradation, reducing the transcriptional activity of RORγt. In the absence of DUBA and/or ITCH (as shown on the right), RORγt is stabilized, which enhances transcription (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Y243, S255 and S318, the functional consequences of RORγt phosphorylation await elucidation.

4.3.2. Acetylation

The recruitment of histone acetyltransferases (HATs) and histone deacetylases (HDACs) as co-factors regulating RORγt activity has been discussed above. However, like several other NRs, RORγt itself is modified by acetylation (Fig. 6B, C). Two recent studies report that p300 (KAT3B), but not other HATs, including the closely related CBP (KAT3A), acetylates RORγt when over-expressed in HEK293T cells [108,109]. In addition to modulating chromatin organization, p300 can regulate non-histone proteins, including nuclear transcription factors such as p53, NF-κB and FOXP3 [110,111]. Acetylation of these transcription factors modulates their transcriptional activity by altering their protein stability, subcellular localization and/or DNA-binding capacity [112]. Acetylation and ubiquitinylation often compete for the same lysine residues. Acetylation at these sites shields the protein from ubiquitin-mediated proteasomal degradation. Although RORγt might be stabilized to some extent through acetylation by p300 [109], it seems more convincing that acetylation in this case actually impairs DNA-binding and hence transcriptional activity [108]. Indeed, mass spectrometry and mutation studies confirmed that p300 acetylates RORγt at K69, K81, K99, and K112, within the DNA-binding domain. K69, K81, and K99 are predicted to be positioned near the DNA [108]. Naive CD4T cells transduced with K69/81/99Q (3K > Q) mutants mimicking a constitutively acetylated form of RORγt, fail to differentiate into Th17 cells and to produce IL-17A under either Th0 or Th17 polarizing conditions [108]. The histone deacetylase Sirtuin 1 (SIRT1) catalyzes the reverse reaction by deacetylating RORγt [108]. The sirtuins are NAD^{+}-dependent protein deacetylases that play critical roles in transcriptional regulation, cell cycling, replicative senescence, inflammation, and metabolism. In mammals, SIRT1 in particular acts as an epigenetic regulator that modulates the activity of several transcription factors important for immune function [113–115]. SIRT1 has been described as a negative regulator of regulatory T cell (Treg) function, via deacetylation of FOXP3, the signature transcription factor of Treg cells [116,117].

RORγt interacts with SIRT1 in both thymocytes and Th17 cells through its LBD. Co-expression studies demonstrated that wildtype SIRT1, but not a catalytically inactive H363Y mutant, deacetylates RORγt. Indeed, K81, K87/88, and K99 of RORγt are hyper-acetylated in SIRT1-deficient Th17 cells and thymocytes.

T cell–specific SIRT1 deletion or treatment with pharmacological SIRT1 inhibitors suppresses Th17 differentiation and protects mice from EAE [108]. These findings suggest that SIRT1 increases the transcriptional activity of RORγt, and reveal an unexpected pro-inflammatory role of SIRT1.

A second study suggests that HDAC1 can also deacetylate RORγt, at least when co-overexpressed *in vitro* [109]. Demonstration that this mechanism plays a role in Th17 cells has yet to occur.

4.3.3. Ubiquitinylation

Ubiquitinylation is a multi-step reversible process during which activated ubiquitin is transferred onto lysine residues of substrate proteins [118]. E3 ubiquitin ligases mediate the last step in this cascade, whereas deubiquitinylating enzymes function to remove ubiquitin from substrate proteins. Ubiquitin itself contains seven lysine residues which enables the construction of poly-ubiquitin chains. Depending on the chain topology, poly-ubiquitinylation either triggers substrate protein degradation by the proteasome (e.g. K48-linked poly-ubiquitinylation) or enables protein–protein interactions and signaling (e.g. K63-linked poly-ubiquitinylation), with additional new functions rapidly emerging [119]. Several recent reports have begun to shed light on the complex regulation of RORγt by ubiquitinylation by identifying several E3 ligases and deubiquitinases that target RORγt (Fig. 6B and D) [120–122].

TRAF5, a known signaling adaptor involved in CD40, NOD-like receptor (NLR), RIG-I like receptor (RLR) and IL-17 receptor (IL-17R) signaling pathways, interacts with and ubiquitinylates RORγt [122]. TRAF5 can function as an E3 ubiquitin ligase due to its N-terminal RING finger domain [123]. TRAF5 does not target RORγt for proteasomal degradation. Although the precise ubiquitinylation site has not been mapped, TRAF5 mediates K63-linked poly-ubiquitinylation of RORγt [122], presumably functioning to modulate its transcriptional activity. In fact, loss of TRAF5 in human Th17 cells down-regulates IL-17A and IL–17 F and even somewhat reduces RORγt levels [122].

In contrast, ITCH, a member of the HECT family of E3 ubiquitin ligases, was recently shown to limit IL-17 production by targeting RORγt for proteasomel degradation. ITCH-deficient mice develop spontaneous colitis at 6–8 months of age associated with increased IL-17A levels in mucosal tissues and elevated numbers of IL-17-producing cells in spleens and mesenteric lymph nodes. These mice also exhibit higher tumor burden and increased inflammation in a pre-clinical model of inflammation-induced colon cancer. ITCH and RORγt interact through their WW and PPXY motifs, respectively, and WT ITCH, but not a catalytic-dead mutant, mediates K48 poly-ubiquitinylation and hence proteasomal degradation of RORγt [121]. Another E3 ligase, UBR5, a member of the UBR box family, also interacts with RORγt in Th17 cells. Although the ubiquitin-linkage has not been determined, knockdown of UBR5 in Th17 cells drastically stabilizes RORγt protein and increases IL-17 production [120], suggesting that UBR5 indeed targets RORγt for proteasomal degradation.

Several deubiquitinases have been described to affect Th17 cell function or IL-17 signaling. USP18 has been found to regulate T cell activation and Th17 cell differentiation by deubiquitinylating the TAK1-TAB1 complex [124]. The ubiquitin-specific protease USP25 has been identified as a negative regulator of IL-17-mediated signaling and inflammation acting through the removal of ubiquitinylation on TRAF5 and TRAF6 [125]. Recently, two members of the USP family of deubiquitinases, USP17 and USP4, have been demonstrated to stabilize RORγt in co-overexpression studies [126,127]. USP17 decreases K48-linked poly-ubiquitinylation of RORγt at K360 and inhibits proteasome-dependent degradation. Knockdown of endogenous USP17 in Th17 cells decreases RORγt protein levels and expression of Th17-related genes, such as IL-17A and IL–17F [126]. The same group also described USP4, which is highly expressed in Th17 cells, as a deubiquitinase for RORγt. Similar to USP17, USP4 reduces K48-linked poly-ubiquitinylation of RORγt, at least upon over-expression [127]. The lysine residues that are targeted by USP4 have not been mapped. Again, knockdown of USP4 in Th17 cells decreases RORγt protein levels, and IL-17 transcription. The DNA-binding domain of RORγt is essential for its interaction with USP4 [127]. Interestingly, TGF-β together with IL-6 enhance USP4-mediated deubiquitinylation of RORγt [127]. TGF-β has been shown to mediate USP4 nuclear-to-cytoplasmic transport [128]. It is not clear at this point what the relative contributions of USP4 and USP17 are to the overall stabilization of RORγt *in vivo*.

While RORγt seems to be a direct substrate for USP4 and USP17, another deubiquitinylating enzyme, DUBA (OTUD5), also affects RORγt protein stability without any detectable direct interaction. Deficiency in DUBA results in drastically increased IL-17A production and accumulation of RORγt in IL-17 producing cells both *in vitro* and *in vivo* [120]. Protein stabilization in the absence of a deubiquitinase suggests an indirect effect, and indeed DUBA interacts with and stabilizes UBR5, which in turn promotes the degradation of RORγt [120]. This is a common theme in the ubiquitin field, where in many cases deubiquitinases regulate the stability of E3 ligases and in turn, indirectly, regulate the activity and/or stability of downstream substrates. Another well-studied example is USP7 regulating the stability of MDM2 which in turn affects p53 levels [129]. DUBA and UBR5 form a stable complex in T cells [120], and presumably co-regulate several substrates besides RORγt. It is currently not known if DUBA can also associate with ITCH.

A large body of literature suggests that the proteasome is directly involved in regulating the transcriptional activity of NRs, including RORs [130–132]. For instance, two proteasome subunits, PSMB6 and PSMC5, have been shown to interact with ROR receptors [72,133]. The role of the proteasome in this context includes both proteolytic and non-proteolytic activities [130]. Interestingly, the proteasome inhibitor MG-132 was found to inhibit transcriptional activity of NRs, including ERα and RORα [134,135]. Mechanistically this observation is still incompletely understood, likely complex and to some extent target gene-specific. In part, gene activation may require proteolytic removal of NR:co-repressor complexes [130–132]. Co-activators recruit E3 ligases and subsequently ubiquitinylated co-repressors are targeted for degradation. However, proteasomal turnover of chromatin-bound NRs themselves seems to be required for transcriptional activity. Consistent with this notion, Hairless (Hr) functions as an effective repressor of ROR-induced transcriptional activation, in part by stabilizing RORs and protecting them from degradation [135,136]. This apparent paradox is explained by a model in which degradation is required for the disruption of the transcription initiation complex, thus facilitating the transition to a productive elongation complex and elongation of transcription. NR removal enables the reassembly of transcriptional complexes (promoter recharging) to ensure multiple rounds of transcription [130–132].

Decoding of the myriad forms and sites of post-translational modification governing RORγt function is emerging as an area of intense investigation. Layered atop those individual modification is the implication of cross talk and contingency existing between different PTM types. As already discussed, acetylation can protect lysine residues from ubiquitinylation. Phosphorylation, on the other hand, is a common trigger of ubiquitinylation. Interruption of any of these processes can impinge on the likelihood and consequence of others.

4.4. Regulation of RORγt expression and function by other NRs

Several other NRs modulate RORγt function, although mostly by regulating its expression. The fact that NR ligands, including

retinoic acid, vitamin D, and several PPARγ agonists, are protective in pre-clinical T cell-mediated autoimmunity models, such as EAE, colitis, or collagen-induced arthritis provides circumstantial evidence for this concept [137–140]. Activation of RARα, a receptor closely related to RORγt, by all-trans-retinoic-acid (ATRA) for example, strongly interferes with Th17 differentiation by suppressing RORγt expression and inducing FOXP3, thus promoting the development of regulatory T cells [141,142]. Peroxisome proliferator-activated receptor γ (PPARγ) is a NR that forms heterodimers with retinoid X receptors (RXR) [143]. PPARγ also suppresses RORγt expression and hence functions as a suppressor for Th17 differentiation. Ligand-activated PPARγ blocks RORγt expression by stabilizing binding of the co-repressor SMRT to the *Rorc* promoter [144].

REV-ERBα and REV-ERBβ are NRs with an atypical LBD that lacks the AF2 region, and thus cannot interact with co-activators [145]. Instead, they interact constitutively with NCORs and function as repressors of transcription [34,146]. Interestingly, REV-ERBs recognize similar RORE motifs as RORs and have been shown to antagonize ROR signaling in various settings [34,147]. However, the interplay and co-regulation of gene expression between REV-ERBs and RORs has mostly been studied in metabolism and circadian rhythm [34]. While surprisingly a direct co-regulation of immune-relevant RORγt target genes by REV-ERBs has not been demonstrated to date, REV-ERBα-deficient mice have alterations in Th17 differentiation [148]. However, this effect relies, at least in part, on transcriptional regulation of RORγt expression. REV-ERBα suppresses the expression of the transcription factor NFIL3, which in turn inhibits RORγt expression. The report by Yu et al. was also the first to link Th17 cell development to the circadian clock network through the transcription factor REV-ERBα [148]. Providing a very similar mechanism, a more recent report further links seasonal changes in MS disease activity to differences in melatonin levels. Melatonin induces the expression of the repressor NFIL3 by inhibiting REV-ERBα· Treatment with melatonin ameliorates disease in an experimental model of MS and directly interferes with the differentiation of human and mouse Th17 cells [149].

4.5. Regulation of RORγt by other transcription factors

Since the identification of RORγt as the master transcription factor of Th17 cells, a number of other components have been identified that together form a transcriptional network that regulates Th17 cell differentiation (Fig. 7A). BATF and IRF4 function as pioneering factors that open chromatin early upon T cell activation. In fact, most if not all RORγt binding sites in T cells are co-bound by BATF/IRF4 [150]. IκBζ belongs to the Bcl3 family of nuclear proteins. It interacts with NF-κB and regulates downstream biological functions. IκBζ is indispensable for Th17 cell development by cooperating with RORγt to activate the *Il17a* promoter and inducing Th17 cell differentiation [151]. Runx1 is another transcription factor that can interact with RORγt and binds cooperatively to the *Il17a* promoter to augment Th17 differentiation. Runx1 is required for IL-17 production in Th17 cells. Runx1 also controls the balance of Th17 and Treg cell development [152]. As already discussed, environmental factors play important roles in regulating Th17 cell differentiation. Hypoxia-inducible factor 1 (HIF-1α) is an essential transcription factor under hypoxia condition to control the metabolic switch from oxidative phosphorylation to aerobic glycolysis. HIF-1α is up-regulated in Th17 cells in a STAT3-dependent manner. Importantly, HIF-1α is indispensable for Th17 cell development. T cells deficient in HIF-1α expression fail to differentiate into Th17 cells *in vitro*. HIF-1α directly binds to the *Rorc* promoter and induces transcription. In addition, HIF-1α also directly interacts with RORγt and recruits

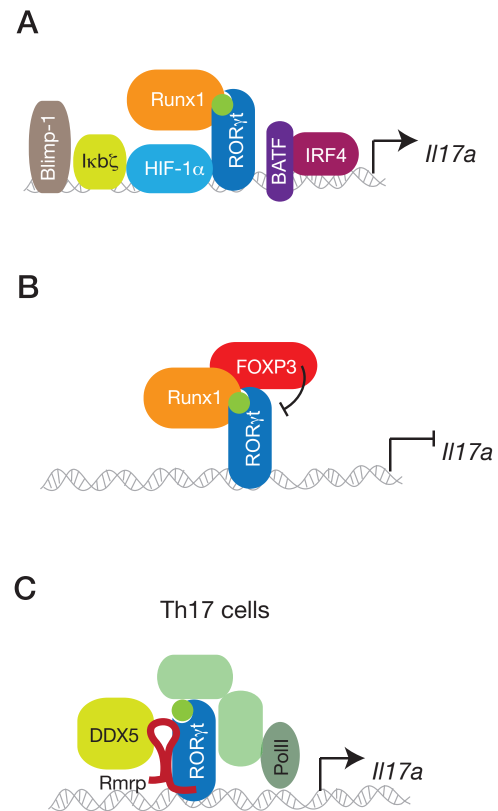

Fig. 7. IL-17-transcriptional network.
(A) Transcription factors cooperating with RORγt. RORγt functions within a transcriptional network to regulate target gene expression. BATF and IRF4 open chromatin to allow transcription of *Il17a* (left panel). IκBζ activates the *Il17a* promoter and Runx1 cooperatively binds the *Il17a* promoter with RORγt. HIF-1α directly interacts with RORγt and recruits p300. Blimp-1 co-binds many promoter regions with RORγt and co-regulates target gene expression.
(B) RORγt interacts with and is inhibited by FOXP3. During Th17 and T regulatory cell differentiation *in vitro* as well as in certain regulatory T cell subsets *in vivo*, RORγt and FOXP3 are co-expressed and interact with each other. FOXP3 functions as a repressor of RORγt by binding to its LBD.
(C) RORγt regulation by Rmrp and DDX5 in Th17 cells. The DEAD-box protein 5 (DDX5) interacts with RORγt and activates its transcriptional activity. The lncRNA Rmrp facilitates the DDX5/RORγt interaction selectively in Th17 cells.

p300 to regulate *Il17a* gene expression in Th17 cells [153]. Recently, Blimp-1 was described to be induced by IL-23 in pathogenic Th17 cells and to promote pathogenicity in inflammatory disease models, such as EAE. Genome-wide occupancy studies revealed that Blimp-1 binds in proximity to RORγt binding sites in the regulatory regions of many Th17 genes including *Il23r*, *Il17a*, *Il17f*, and *Csf2*, and regulates their expression. However, it is unclear whether there is a direct interaction between RORγt and Blimp-1 [154].

It had been noticed early on that the cell fates of Th17 cells and regulatory T cells are closely linked during *de novo* differentiation *in vitro*. TGF-β induces the expression of both RORγt and FOXP3, the forkhead family transcriptional repressor important for the development and function of regulatory T cells [155,156]. Depending on the culture conditions, a range of phenotypes from pro-inflammatory Th17 cells (generated with IL-6, IL-1β and IL-23 in the absence of TGF-β) over suppressive Th17 cells (generated with IL-6 and low amounts of TGF-β) to regulatory T cells (generated with IL-2 and high amounts of TGF-β) can be obtained [155–160]. Indeed, FOXP3 and RORγt are transiently co-expressed in this process (Fig. 7B). Both factors interact with one another [160,161], and in fact FOXP3 has been found to co-localize with RORγt binding to DNA in cultured T cells [93]. FOXP3 also interacts with

RORα and this interaction, most likely analogous to the one with RORγt, has been characterized in much detail. FOXP3 inhibits its transcriptional activity [162]. This interaction was mapped to the second exon for FOXP3 which is missing in a second shorter isoform expressed in human. The shorter FOXP3 isoform also does not interact with RORγt [161]. Interestingly, this region of FOXP3 contains a "co-activator like" LXXLL motif, which was shown to interact with AF2 of RORα. Mutation of the LXXLL motif in FOXP3 abolishes the interaction with and repression by FOXP3. Additionally, the inhibition of RORα (or RORγt) by FOXP3 does not require an intact forkhead domain, demonstrating that FOXP3 functions independently of DNA-binding [161,162].

Functionally, FOXP3 and RORγt antagonize one another. *In vitro,* the expression of one or the other is extinguished eventually by signals provided by IL-6 or IL-2, respectively. Interestingly, more recently a stable subset of RORγt + FOXP3 + regulatory T cells has been identified in the intestinal lamina propria [163–165]. This subset is critically import to regulate gut-specific immune responses [163,164]. RORγt and FOXP3 appear to co-regulate a number of genes, presumably through FOXP3-mediated suppression of certain RORγt target genes, resulting in signatures of both Tregs and Th17 cells being expressed in these cells [165]. How the co-expression of FOXP3 and RORγt is stably maintained is currently not known.

4.6. Rmrp – a long non-coding RNA as co-regulator of RORγt

In recent years, RNASeq approaches have revealed that mammalian cells transcribe a large proportion (about two-thirds) of their genomic DNA in a highly regulated, cell type-specific manner, most of it into non-coding RNA (ncRNA) [166,167]. Arbitrarily, ncRNAs exceeding 200nt in length have been designated long non-coding RNAs (lncRNA). Although we are only beginning to appreciate the various crucial roles played by this highly abundant class of transcripts, several functions for lncRNAs in regulating high-order chromosomal dynamics, telomere biology, subcellular structural organization or transcription factor activity have already been established [168–171].

Work by Gangqing et al. analyzing a large number of T cell subsets, ranging from thymic precursors to various Th cell subsets, in mice revealed a highly dynamic and cell-specific expression of more than 1500 genomic regions that generate lncRNAs. These regions are adjacent to genes encoding proteins critically involved in regulating immunological function, and many of them are bound and regulated by key T cell transcription factors such as T-bet, GATA-3, STAT4 and STAT6 [172]. Similarly, Spurlock et al. identified more than 2000 lncRNAs expressed in human T cell subsets. Recapitulating some of the findings in mice, the authors identified clusters of lncRNAs that are Th lineage-specific in their expression, and are intragenic or adjacent to Th lineage-specific genes encoding proteins with immunologic functions [173]. The role of the vast majority of these lncRNA is still unknown.

Huang et al. recently demonstrated that the transcriptional activity of RORγt in Th17 cells is critically dependent on one particular lncRNA [174]. Leading up to this realization, a new co-activator of RORγt in Th17 cells, the DEAD-box protein 5 (DDX5), was identified in a liquid-chromatography-tandem mass spectroscopy (LC–MS/MS)-based approach. DDX5 belongs to a large family of RNA helicases that hydrolyze adenosine-5-triphosphate to unwind RNA [175], and has previously been described as a transcriptional co-activator for other NRs [176,177]. DDX5 co-regulates the transcription of nearly 40% of RORγt target genes, including *Il17a* and *Il17f* [174]. Indeed, DDX5 is required for Th17-mediated inflammatory pathologies, including colitis and EAE [174]. The observation that the RNA helicase activity of DDX5 is essential for its function in Th17 cells ultimately led to the identification and characterization of an lncRNA component of the DDX5/RORγt complex (Fig. 7C). The RNA component of mitochondrial ribonuclease protein complex (Rmrp) is a lncRNA that is known for its role in mitochondrial RNA processing (MRP) and maturation of 5.8 S ribosomal RNA [178]. Interestingly, mutations in Rmrp in human result in a rare autosomal recessive disorder named cartilage-hair hypoplasia (CHH) characterized by skeletal dysplasia, hypoplastic hair, neuronal dysplasia of the intestine, predisposition to lymphoma and defective immunity [179]. However, the role of Rmrp in immune cells was poorly understood. Elegant studies using CRISPR to generate mutants of Rmrp in mice demonstrate a critical role in facilitating the DDX5/RORγt interaction and RORγt target gene transcription [174]. Curiously, both DDX5 and Rmrp, despite being highly expressed in thymocytes, are dispensable for RORγt function in thymocyte development [174]. In these cells, Rmrp does not co-precipitate with DDX5. Similarly, the development of lymph nodes is normal in mice with either mutant Rmrp or a lymphoid cell-specific deficiency in DDX5, suggesting that LTi are intact in these mice [174,180]. These findings do not only raise important questions as to what are the signals and molecular triggers of DDX5/Rmrp/RORγt complex formation, but also potentially provide a very attractive new approach for targeting the RORγt/Th17 pathway pharmacologically for the treatment of inflammatory and autoimmune disorders, by eliminating unintended effects on thymocytes and LTi cells. Most certainly the functional characterization of Rmrp in Th17 cells will only be the first in a series of discoveries in this exciting new area of immune regulation through lncRNAs.

5. Concluding remarks

As detailed before, RORγt plays an essential role in establishing and maintaining adaptive immune responses by enabling the formation of secondary and tertiary lymphoid organs and by regulating thymic T cell development and Th17 cell differentiation. However, the requirement of RORγt for the development of several innate lymphoid cells underscores its importance in innate immunity, as well. Lastly, the strong association with autoimmunity makes RORγt an important drug target. Partly driven by this realization, we have come a long way from "orphan" NR RORγt to a much more granular understanding of its complex biology and the various regulatory circuits that govern RORγt expression and activity. Presumably best studied to date are the transcriptional networks that function in concert with RORγt itself to regulate IL-17 expression. More relevant from a drug development aspect, we have also made enormous progress in developing highly selective and potent inverse agonists targeting the transcriptional activity of RORγt. Still lagging behind but rapidly evolving is our understanding of the endogenous ligands that drive RORγt function within the cell. It seems now clear that these ligands are intermediates or products of the cholesterol synthesis pathway, and we are beginning to uncover other cellular pathways that determine the availability of these ligands. It will be interesting to further investigate the crosstalk and feedback loops between cellular (lipid) metabolism and RORγt activity, to better understand their dynamics and how they relate to and shape the pro-inflammatory nature of the immune cell through modulating RORγt target gene expression. Another part of RORγt biology that is still largely unexplored is the co-factor landscape, and its influence on various RORγt functions. Uncovering and understanding these selective co-factor interactions will be an important area of future research and holds the potential to develop highly selective therapies that avoid detrimental on-target side effects. Another piece in the puzzle is the rapidly evolving appreciation of post-translational modifications as important modulators of RORγt function. In some cases, PTMs might be the cause of selective co-factor interactions,

or they might regulate DNA-binding (acetylation) or stability (ubiquitinylation). It is surprising that although several RORγt phosphorylation sites have been detected, none of them has been studied functionally. While kinases have been classic drug targets for a long time, recent progress greatly expands our ability to target E3 ubiquitin ligases and deubiquitinases. Future research in these areas will allow us to further deepen our understanding of RORγt biology and to fully realize the potential of targeting this pathway in the clinic.

Conflicts of interest

Sascha Rutz, Celine Eidenschenk and James R. Kiefer are employees of Genentech. Wenjun Ouyang is an employee of Amgen.

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References

[1] M. Robinson-Rechavi, H. Escriva Garcia, V. Laudet, The nuclear receptor superfamily, J. Cell Sci. 116 (2003) 585–586.
[2] M. Becker-André, E. André, J.F. DeLamarter, Identification of nuclear receptor mRNAs by RT-PCR amplification of conserved zinc-finger motif sequences, Biochem. Biophys. Res. Commun. 194 (1993) 1371–1379.
[3] C. Carlberg, R. Hooft van Huijsduijnen, J.K. Staple, J.F. DeLamarter, M. Becker-André, RZRs, a new family of retinoid-related orphan receptors that function as both monomers and homodimers, Mol. Endocrinol. 8 (1994) 757–770, doi: http://dx.doi.org/10.1210/mend.8.6.7935491.
[4] T. Hirose, R.J. Smith, A.M. Jetten, ROR gamma: the third member of ROR/RZR orphan receptor subfamily that is highly expressed in skeletal muscle, Biochem. Biophys. Res. Commun. 205 (1994) 1976–1983, doi:http://dx.doi.org/10.1006/bbrc.1994.2902.
[5] F.R. Santori, Nuclear hormone receptors put immunity on sterols, Eur. J. Immunol. 45 (2015) 2730–2741, doi:http://dx.doi.org/10.1002/eji.201545712.
[6] J.A. Kallen, J.-M. Schlaeppi, F. Bitsch, S. Geisse, M. Geiser, I. Delhon, et al., X-ray structure of the hRORalpha LBD at 1.63 A: structural and functional data that cholesterol or a cholesterol derivative is the natural ligand of RORalpha, Structure 10 (2002) 1697–1707.
[7] C. Stehlin-Gaon, D. Willmann, D. Zeyer, S. Sanglier, A. Van Dorsselaer, J.-P. Renaud, et al., All-trans retinoic acid is a ligand for the orphan nuclear receptor ROR beta, Nat. Struct. Biol. 10 (2003) 820–825, doi:http://dx.doi.org/10.1038/nsb979.
[8] Y. Wang, N. Kumar, L.A. Solt, T.I. Richardson, L.M. Helvering, C. Crumbley, et al., Modulation of retinoic acid receptor-related orphan receptor alpha and gamma activity by 7-oxygenated sterol ligands, J. Biol. Chem. 285 (2010) 5013–5025, doi:http://dx.doi.org/10.1074/jbc.M109.080614.
[9] Y. Wang, N. Kumar, C. Crumbley, P.R. Griffin, T.P. Burris, A second class of nuclear receptors for oxysterols: regulation of RORalpha and RORgamma activity by 24S-hydroxycholesterol (cerebrosterol), Biochim. Biophys. Acta 1801 (2010) 917–923, doi:http://dx.doi.org/10.1016/j.bbalip.2010.02.012.
[10] L. Jin, D. Martynowski, S. Zheng, T. Wada, W. Xie, Y. Li, Structural basis for hydroxycholesterols as natural ligands of orphan nuclear receptor RORgamma, Mol. Endocrinol. 24 (2010) 923–929, doi:http://dx.doi.org/10.1210/me.2009-0507.
[11] X. Hu, Y. Wang, L.-Y. Hao, X. Liu, C.A. Lesch, B.M. Sanchez, et al., Sterol metabolism controls T(H)17 differentiation by generating endogenous RORγ agonists, Nat. Chem. Biol. 11 (2015) 141–147, doi:http://dx.doi.org/10.1038/nchembio.1714.
[12] F.R. Santori, P. Huang, S.A. van de Pavert, E.F. Douglass, D.J. Leaver, B.A. Haubrich, et al., Identification of natural RORγ ligands that regulate the development of lymphoid cells, Cell Metab. 21 (2015) 286–297, doi:http://dx.doi.org/10.1016/j.cmet.2015.01.004.
[13] M.A. Ortiz, F.J. Piedrafita, M. Pfahl, R. Maki, TOR: a new orphan receptor expressed in the thymus that can modulate retinoid and thyroid hormone signals, Mol. Endocrinol. 9 (1995) 1679–1691, doi:http://dx.doi.org/10.1210/mend.9.12.8614404.
[14] I.I. Ivanov, B.S. McKenzie, L. Zhou, C.E. Tadokoro, A. Lepelley, J.J. Lafaille, et al., The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells, Cell 126 (2006) 1121–1133, doi: http://dx.doi.org/10.1016/j.cell.2006.07.035.
[15] G. Eberl, D.R. Littman, The role of the nuclear hormone receptor RORgammat in the development of lymph nodes and Peyer's patches, Immunol. Rev. 195 (2003) 81–90.
[16] A.M. Jetten, Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism, Nucl. Recept Signal. 7 (2009) e003, doi:http://dx.doi.org/10.1621/nrs.07003.
[17] T. Korn, E. Bettelli, M. Oukka, V.K. Kuchroo, IL-17 and Th17Cells, Annu. Rev. Immunol. 27 (2009) 485–517, doi:http://dx.doi.org/10.1146/annurev.immunol.021908.132710.
[18] M. Cherrier, C. Ohnmacht, S. Cording, G. Eberl, Development and function of intestinal innate lymphoid cells, Curr. Opin. Immunol. (2012), doi:http://dx.doi.org/10.1016/j.coi.2012.03.011.
[19] K. Ghoreschi, A. Laurence, X.-P. Yang, K. Hirahara, J.J. O'Shea, T helper 17 cell heterogeneity and pathogenicity in autoimmune disease, Trends Immunol. 32 (2011) 395–401, doi:http://dx.doi.org/10.1016/j.it.2011.06.007.
[20] L.A. Tesmer, S.K. Lundy, S. Sarkar, D.A. Fox, Th17 cells in human disease, Immunol. Rev. 223 (2008) 87–113, doi:http://dx.doi.org/10.1111/j.1600-065X.2008.00628.x.
[21] M.R. Chang, H. Rosen, P.R. Griffin, RORs in autoimmune disease, Curr. Top. Microbiol. Immunol. 378 (2014) 171–182, doi:http://dx.doi.org/10.1007/978-3-319-05879-5_8.
[22] J.R. Huh, D.R. Littman, Small molecule inhibitors of RORγt: targeting Th17 cells and other applications, Eur. J. Immunol. 42 (2012) 2232–2237, doi: http://dx.doi.org/10.1002/eji.201242740.
[23] B.P. Fauber, S. Magnuson, Modulators of the nuclear receptor retinoic acid receptor-related orphan receptor-((ROR (or RORc), J. Med. Chem. 57 (2014) 5871–5892, doi:http://dx.doi.org/10.1021/jm401901d.
[24] F. Isono, S. Fujita-Sato, S. Ito, Inhibiting RORγt/Th17 axis for autoimmune disorders, Drug Discov. Today 19 (2014) 1205–1211, doi:http://dx.doi.org/10.1016/j.drudis.2014.04.012.
[25] A. Medvedev, Z.H. Yan, T. Hirose, V. Giguère, A.M. Jetten, Cloning of a cDNA encoding the murine orphan receptor RZR/ROR gamma and characterization of its response element, Gene 181 (1996) 199–206.
[26] Y.W. He, M.L. Deftos, E.W. Ojala, M.J. Bevan, RORgamma t a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells, Immunity 9 (1998) 797–806.
[27] E. André, K. Gawlas, M. Becker-André, A novel isoform of the orphan nuclear receptor RORbeta is specifically expressed in pineal gland and retina, Gene 216 (1998) 277–283.
[28] I. Villey, R. de Chasseval, J.P. de Villartay, RORgammaT, a thymus-specific isoform of the orphan nuclear receptor RORgamma/TOR, is up-regulated by signaling through the pre-T cell receptor and binds to the TEA promoter, Eur. J. Immunol. 29 (1999) 4072–4080, doi:http://dx.doi.org/10.1002/(SICI)1521-4141(199912)29:12<4072:AID-IMMU4072>3.0.CO;2-E.
[29] A.M. Jetten, J.H. Joo, Retinoid-related orphan receptors (RORs): roles in cellular differentiation and development, Adv. Dev. Biol. 16 (2006) 313–355, doi:http://dx.doi.org/10.1016/S1574-3349(06)16010-X.
[30] S. Jolly, N. Journiac, B. Vernet-der Garabedian, J. Mariani, RORalpha, a key to the development and functioning of the brain, Cerebellum 11 (2012) 451–452, doi:http://dx.doi.org/10.1007/s12311-011-0339-1.
[31] R.L. Fitzsimmons, P. Lau, G.E.O. Muscat, Retinoid-related orphan receptor alpha and the regulation of lipid homeostasis, J. Steroid Biochem. Mol. Biol. 130 (2012) 159–168, doi:http://dx.doi.org/10.1016/j.jsbmb.2011.06.009.
[32] A. Swaroop, D. Kim, D. Forrest, Transcriptional regulation of photoreceptor development and homeostasis in the mammalian retina, Nat. Rev. Neurosci. 11 (2010) 563–576, doi:http://dx.doi.org/10.1038/nrn2880.
[33] H. Duez, B. Staels, The nuclear receptors Rev-erbs and RORs integrate circadian rhythms and metabolism, Diab. Vasc. Dis. Res. 5 (2008) 82–88, doi: http://dx.doi.org/10.3132/dvdr.2008.0014.
[34] D.J. Kojetin, T.P. Burris, REV-ERB and ROR nuclear receptors as drug targets, Nat. Rev. Drug Discov. 13 (2014) 197–216, doi:http://dx.doi.org/10.1038/nrd4100.
[35] G. Eberl, S. Marmon, M.-J. Sunshine, P.D. Rennert, Y. Choi, D.R. Littman, An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells, Nat. Immunol. 5 (2004) 64–73, doi:http://dx.doi.org/10.1038/ni1022.
[36] G. Eberl, D.R. Littman, The role of the nuclear hormone receptor RORγt in the development of lymph nodes and Peyer's patches, Immunol. Rev. 195 (2003) 81–90, doi:http://dx.doi.org/10.1034/j.1600-065X.2003.00074.x.
[37] S.A. van de Pavert, R.E. Mebius, New insights into the development of lymphoid tissues, Nat. Rev. Immunol. 10 (2010) 664–674, doi:http://dx.doi.org/10.1038/nri2832.
[38] Z. Sun, D. Unutmaz, Y.R. Zou, M.J. Sunshine, A. Pierani, S. Brenner-Morton, et al., Requirement for RORgamma in thymocyte survival and lymphoid organ development, Science 288 (2000) 2369–2373.
[39] S. Kurebayashi, E. Ueda, M. Sakaue, D.D. Patel, A. Medvedev, F. Zhang, et al., Retinoid-related orphan receptor gamma (RORgamma) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 10132–10137.
[40] H. Xie, M.S. Sadim, Z. Sun, RORgammat recruits steroid receptor coactivators to ensure thymocyte survival, J. Immunol. 175 (2005) 3800–3809.
[41] E. Ueda, S. Kurebayashi, M. Sakaue, M. Backlund, B. Koller, A.M. Jetten, High incidence of T-cell lymphomas in mice deficient in the retinoid-related orphan receptor RORgamma, Cancer Res. 62 (2002) 901–909.
[42] S. Okada, J.G. Markle, E.K. Deenick, F. Mele, D. Averbuch, M. Lagos, et al., IMMUNODEFICIENCIES. Impairment of immunity to Candida and Mycobacterium in humans with bi-allelic RORC mutations, Science 349 (2015) 606–613, doi:http://dx.doi.org/10.1126/science.aaa4282.

[43] I.I. Ivanov, L. Zhou, D.R. Littman, Transcriptional regulation of Th17 cell differentiation, Semin. Immunol. 19 (2007) 409–417, doi:http://dx.doi.org/10.1016/j.smim.2007.10.011.
[44] X.O. Yang, B.P. Pappu, R. Nurieva, A. Akimzhanov, H.S. Kang, Y. Chung, et al., T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma, Immunity 28 (2008) 29–39, doi:http://dx.doi.org/10.1016/j.immuni.2007.11.016.
[45] J. Yang, M.S. Sundrud, J. Skepner, T. Yamagata, Targeting Th17 cells in autoimmune diseases, Trends Pharmacol. Sci. 35 (2014) 493–500, doi:http://dx.doi.org/10.1016/j.tips.2014.07.006.
[46] P. Miossec, J.K. Kolls, Targeting IL-17 and TH17 cells in chronic inflammation, Nat. Rev. Drug Discov. 11 (2012) 763–776, doi:http://dx.doi.org/10.1038/nrd3794.
[47] I.J. McEwan, Nuclear receptors: one big family, Methods Mol. Biol. 505 (2009) 3–18, doi:http://dx.doi.org/10.1007/978-1-60327-575-0_1.
[48] L.A. Solt, P.R. Griffin, T.P. Burris, Ligand regulation of retinoic acid receptor-related orphan receptors: implications for development of novel therapeutics, Curr. Opin. Lipidol. 21 (2010) 204–211, doi:http://dx.doi.org/10.1097/MOL.0b013e328338ca18.
[49] V. Giguère, M. Tini, G. Flock, E. Ong, R.M. Evans, G. Otulakowski, Isoform-specific amino-terminal domains dictate DNA-binding properties of ROR alpha, a novel family of orphan hormone nuclear receptors, Genes Dev. 8 (1994) 538–553.
[50] V. Giguère, B. Beatty, J. Squire, N.G. Copeland, N.A. Jenkins, The orphan nuclear receptor ROR alpha (RORA) maps to a conserved region of homology on human chromosome 15q21-q22 and mouse chromosome 9, Genomics 28 (1995) 596–598, doi:http://dx.doi.org/10.1006/geno.1995.1197.
[51] L.D. McBroom, G. Flock, V. Giguère, The nonconserved hinge region and distinct amino-terminal domains of the ROR alpha orphan nuclear receptor isoforms are required for proper DNA bending and ROR alpha-DNA interactions, Mol. Cell. Biol. 15 (1995) 796–808.
[52] H. Sundvold, S. Lien, Identification of a novel peroxisome proliferator-activated receptor (PPAR) gamma promoter in man and transactivation by the nuclear receptor RORalpha1, Biochem. Biophys. Res. Commun. 287 (2001) 383–390, doi:http://dx.doi.org/10.1006/bbrc.2001.5602.
[53] V. Giguère, L.D. McBroom, G. Flock, Determinants of target gene specificity for ROR alpha 1: monomeric DNA binding by an orphan nuclear receptor, Mol. Cell. Biol. 15 (1995) 2517–2526.
[54] M. Schräder, C. Danielsson, I. Wiesenberg, C. Carlberg, Identification of natural monomeric response elements of the nuclear receptor RZR/ROR. They also bind COUP-TF homodimers, J. Biol. Chem. 271 (1996) 19732–19736.
[55] J. Kallen, J.-M. Schlaeppi, F. Bitsch, I. Delhon, B. Fournier, Crystal structure of the human RORalpha Ligand binding domain in complex with cholesterol sulfate at 2.2 A, J. Biol. Chem. 279 (2004) 14033–14038, doi:http://dx.doi.org/10.1074/jbc.M400302200.
[56] Y. Li, M.H. Lambert, H.E. Xu, Activation of nuclear receptors: a perspective from structural genomics, Structure 11 (2003) 741–746.
[57] B.D. Darimont, R.L. Wagner, J.W. Apriletti, M.R. Stallcup, P.J. Kushner, J.D. Baxter, et al., Structure and specificity of nuclear receptor-coactivator interactions, Genes Dev. 12 (1998) 3343–3356.
[58] C.K. Glass, M.G. Rosenfeld, The coregulator exchange in transcriptional functions of nuclear receptors, Genes Dev. 14 (2000) 121–141.
[59] D.M. Heery, E. Kalkhoven, S. Hoare, M.G. Parker, A signature motif in transcriptional co-activators mediates binding to nuclear receptors, Nature 387 (1997) 733–736, doi:http://dx.doi.org/10.1038/42750.
[60] E.M. McInerney, D.W. Rose, S.E. Flynn, S. Westin, T.M. Mullen, A. Krones, et al., Determinants of coactivator LXXLL motif specificity in nuclear receptor transcriptional activation, Genes Dev. 12 (1998) 3357–3368.
[61] D.M. Heery, S. Hoare, S. Hussain, M.G. Parker, H. Sheppard, Core LXXLL motif sequences in CREB-binding protein, SRC1, and RIP140 define affinity and selectivity for steroid and retinoid receptors, J. Biol. Chem. 276 (2001) 6695–6702, doi:http://dx.doi.org/10.1074/jbc.M009404200.
[62] L. Nagy, H.Y. Kao, J.D. Love, C. Li, E. Banayo, J.T. Gooch, et al., Mechanism of corepressor binding and release from nuclear hormone receptors, Genes Dev. 13 (1999) 3209–3216.
[63] D. Moras, H. Gronemeyer, The nuclear receptor ligand-binding domain: structure and function, Curr. Opin. Cell Biol. 10 (1998) 384–391.
[64] L. Nagy, H.Y. Kao, D. Chakravarti, R.J. Lin, C.A. Hassig, D.E. Ayer, et al., Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase, Cell 89 (1997) 373–380.
[65] X. Hu, M.A. Lazar, The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors, Nature 402 (1999) 93–96, doi:http://dx.doi.org/10.1038/47069.
[66] H.E. Xu, T.B. Stanley, V.G. Montana, M.H. Lambert, B.G. Shearer, J.E. Cobb, et al., Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARalpha, Nature 415 (2002) 813–817, doi:http://dx.doi.org/10.1038/415813a.
[67] Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor, EMBO J. 14 (1995) 3741–3751.
[68] M.G. Rosenfeld, V.V. Lunyak, C.K. Glass, Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response, Genes Dev. 20 (2006) 1405–1428, doi:http://dx.doi.org/10.1101/gad.1424806.
[69] V. Perissi, M.G. Rosenfeld, Controlling nuclear receptors: the circular logic of cofactor cycles, Nat. Rev. Mol. Cell Biol. 6 (2005) 542–554, doi:http://dx.doi.org/10.1038/nrm1682.
[70] N.J. McKenna, B.W. O'Malley, Minireview: nuclear receptor coactivators-an update, Endocrinology 143 (2002) 2461–2465, doi:http://dx.doi.org/10.1210/endo.143.7.8892.
[71] W. Xu, Nuclear receptor coactivators: the key to unlock chromatin, Biochem. Cell Biol. 83 (2005) 418–428, doi:http://dx.doi.org/10.1139/o05-057.
[72] G.B. Atkins, X. Hu, M.G. Guenther, C. Rachez, L.P. Freedman, M.A. Lazar, Coactivators for the orphan nuclear receptor RORalpha, Mol. Endocrinol. 13 (1999) 1550–1557, doi:http://dx.doi.org/10.1210/mend.13.9.0343.
[73] D.A. Gold, S.H. Baek, N.J. Schork, D.W. Rose, D.D. Larsen, B.D. Sachs, et al., RORalpha coordinates reciprocal signaling in cerebellar development through sonic hedgehog and calcium-dependent pathways, Neuron 40 (2003) 1119–1131.
[74] H.P. Harding, G.B. Atkins, A.B. Jaffe, W.J. Seo, M.A. Lazar, Transcriptional activation and repression by RORalpha, an orphan nuclear receptor required for cerebellar development, Mol. Endocrinol. 11 (1997) 1737–1746.
[75] J.M. Harris, P. Lau, S.L. Chen, G.E.O. Muscat, Characterization of the retinoid orphan-related receptor-alpha coactivator binding interface: a structural basis for ligand-independent transcription, Mol. Endocrinol. 16 (2002) 998–1012, doi:http://dx.doi.org/10.1210/mend.16.5.0837.
[76] S. Kurebayashi, T. Nakajima, S.-C. Kim, C.-Y. Chang, D.P. McDonnell, J.-P. Renaud, et al., Selective LXXLL peptides antagonize transcriptional activation by the retinoid-related orphan receptor RORgamma, Biochem. Biophys. Res. Commun. 315 (2004) 919–927, doi:http://dx.doi.org/10.1016/j.bbrc.2004.01.131.
[77] P. Lau, P. Bailey, D.H. Dowhan, G.E. Muscat, Exogenous expression of a dominant negative RORalpha1 vector in muscle cells impairs differentiation: rORalpha1 directly interacts with p300 and myoD, Nucleic Acids Res. 27 (1999) 411–420.
[78] D.R. Johnson, J.M. Lovett, M. Hirsch, F. Xia, J.D. Chen, NuRD complex component Mi-2beta binds to and represses RORgamma-mediated transcriptional activation, Biochem. Biophys. Res. Commun. 318 (2004) 714–718, doi:http://dx.doi.org/10.1016/j.bbrc.2004.04.087.
[79] A.M. Jetten, Recent advances in the mechanisms of action and physiological functions of the retinoid-related orphan receptors (RORs), Curr. Drug Targets Inflamm. Allergy 3 (2004) 395–412.
[80] D.R. Littman, Z. Sun, D. Unutmaz, M.J. Sunshine, H.T. Petrie, Y.R. Zou, Role of the nuclear hormone receptor ROR gamma in transcriptional regulation thymocyte survival, and lymphoid organogenesis, Cold Spring Harb. Symp. Quant. Biol. 64 (1999) 373–381.
[81] E.F. Greiner, J. Kirfel, H. Greschik, D. Huang, P. Becker, J.P. Kapfhammer, et al., Differential ligand-dependent protein–protein interactions between nuclear receptors and a neuronal-specific cofactor, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 7160–7165.
[82] P. Lau, S.J. Nixon, R.G. Parton, G.E.O. Muscat, RORalpha regulates the expression of genes involved in lipid homeostasis in skeletal muscle cells: caveolin-3 and CPT-1 are direct targets of ROR, J. Biol. Chem. 279 (2004) 36828–36840, doi:http://dx.doi.org/10.1074/jbc.M404927200.
[83] J.R. Huh, M.W.L. Leung, P. Huang, D.A. Ryan, M.R. Krout, R.R.V. Malapaka, et al., Digoxin and its derivatives suppress T(H)17 cell differentiation by antagonizing RORγt activity, Nature (2011), doi:http://dx.doi.org/10.1038/nature09978.
[84] P. Soroosh, J. Wu, X. Xue, J. Song, S.W. Sutton, M. Sablad, et al., Oxysterols are agonist ligands of RORγt and drive Th17 cell differentiation, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12163–12168, doi:http://dx.doi.org/10.1073/pnas.1322807111.
[85] C. Wang, N. Yosef, J. Gaublomme, C. Wu, Y. Lee, C.B. Clish, et al., CD5L/AIM regulates lipid biosynthesis and restrains th17 cell pathogenicity, Cell 163 (2015) 1413–1427, doi:http://dx.doi.org/10.1016/j.cell.2015.10.068.
[86] J. Kurokawa, S. Arai, K. Nakashima, H. Nagano, A. Nishijima, K. Miyata, et al., Macrophage-derived AIM is endocytosed into adipocytes and decreases lipid droplets via inhibition of fatty acid synthase activity, Cell Metab. 11 (2010) 479–492, doi:http://dx.doi.org/10.1016/j.cmet.2010.04.013.
[87] H. Gronemeyer, J.-A. Gustafsson, V. Laudet, Principles for modulation of the nuclear receptor superfamily, Nat. Rev. Drug Discov. 3 (2004) 950–964, doi:http://dx.doi.org/10.1038/nrd1551.
[88] O. René, B.P. Fauber, G. de, L. Boenig, B. Burton, C. Eidenschenk, C. Everett, et al., Minor structural change to tertiary sulfonamide RORc ligands led to opposite mechanisms of action, ACS Med. Chem. Lett. 6 (2015) 276–281, doi:http://dx.doi.org/10.1021/ml500420y.
[89] B.P. Fauber, A. Gobbi, P. Savy, B. Burton, Y. Deng, C. Everett, et al., Identification of N-sulfonyl-tetrahydroquinolines as RORc inverse agonists, Bioorg. Med. Chem. Lett. 25 (2015) 4109–4113, doi:http://dx.doi.org/10.1016/j.bmcl.2015.08.028.
[90] B.P. Fauber, A. Gobbi, K. Robarge, A. Zhou, A. Barnard, J. Cao, et al., Discovery of imidazo[1,5-a]pyridines and -pyrimidines as potent and selective RORc inverse agonists, Bioorg. Med. Chem. Lett. 25 (2015) 2907–2912, doi:http://dx.doi.org/10.1016/j.bmcl.2015.05.055.
[91] L.A. Solt, N. Kumar, P. Nuhant, Y. Wang, J.L. Lauer, J. Liu, et al., Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand, Nature 472 (2011) 491–494, doi:http://dx.doi.org/10.1038/nature10075.
[92] M. Scheepstra, S. Leysen, G.C. van Almen, J.R. Miller, J. Piesvaux, V. Kutilek, et al., Identification of an allosteric binding site for RORγt inhibition, Nat. Commun. 6 (2015) 8833, doi:http://dx.doi.org/10.1038/ncomms9833.
[93] S. Xiao, N. Yosef, J. Yang, Y. Wang, L. Zhou, C. Zhu, et al., Small-molecule RORγt antagonists inhibit T helper 17 cell transcriptional network by divergent

mechanisms, Immunity 40 (2014) 477–489, doi:http://dx.doi.org/10.1016/j.immuni.2014.04.004.
[94] N. Kumar, B. Lyda, M.R. Chang, J.L. Lauer, L.A. Solt, T.P. Burris, et al., Identification of SR 2211: a potent synthetic RORγ-selective modulator, ACS Chem. Biol. 7 (2012) 672–677, doi:http://dx.doi.org/10.1021/cb200496y.
[95] L.A. Solt, N. Kumar, Y. He, T.M. Kamenecka, P.R. Griffin, T.P. Burris, Identification of a selective RORγ ligand that suppresses T(H)17 cells and stimulates T regulatory cells, ACS Chem. Biol. 7 (2012) 1515–1519, doi:http://dx.doi.org/10.1021/cb3002649.
[96] J.R. Huh, E.E. Englund, H. Wang, R. Huang, P. Huang, F. Rastinejad, et al., Identification of potent and selective diphenylpropanamide RORγ inhibitors, ACS Med. Chem. Lett. 4 (2013) 79–84, doi:http://dx.doi.org/10.1021/ml300286h.
[97] Ursolic acid suppresses interleukin-17 (IL-17) production by selectively antagonizing the function of RORgamma t protein, J. Biol. Chem. 286 (2011) 22707–22710, doi:http://dx.doi.org/10.1074/jbc.C111.250407.
[98] J. Skepner, R. Ramesh, M. Trocha, D. Schmidt, E. Baloglu, M. Lobera, et al., Pharmacologic inhibition of RORγt regulates Th17 signature gene expression and suppresses cutaneous inflammation in vivo, J. Immunol. 192 (2014) 2564–2575, doi:http://dx.doi.org/10.4049/jimmunol.1302190.
[99] M.R. Chang, B. Lyda, T.M. Kamenecka, P.R. Griffin, Pharmacologic repression of retinoic acid receptor-related orphan nuclear receptor γ is therapeutic in the collagen-induced arthritis experimental model, Arthritis Rheumatol. 66 (2014) 579–588, doi:http://dx.doi.org/10.1002/art.38272.
[100] Y. Wang, N. Kumar, P. Nuhant, M.D. Cameron, M.A. Istrate, W.R. Roush, et al., Identification of SR 1078, a synthetic agonist for the orphan nuclear receptors RORα and RORγ, ACS Chem. Biol. 5 (2010) 1029–1034, doi:http://dx.doi.org/10.1021/cb100223d.
[101] W. Zhang, J. Zhang, L. Fang, L. Zhou, S. Wang, Z. Xiang, et al., Increasing human Th17 differentiation through activation of orphan nuclear receptor retinoid acid-related orphan receptor γ (RORγ) by a class of aryl amide compounds, Mol. Pharmacol. 82 (2012) 583–590, doi:http://dx.doi.org/10.1124/mol.112.078667.
[102] M.R. Chang, V. Dharmarajan, C. Doebelin, R.D. Garcia-Ordonez, S.J. Novick, D. S. Kuruvilla, et al., Synthetic RORγt agonists enhance protective immunity, ACS Chem. Biol. 11 (2016) 1012–1018, doi:http://dx.doi.org/10.1021/acschembio.5b00899.
[103] N.R. West, S. McCuaig, F. Franchini, F. Powrie, Emerging cytokine networks in colorectal cancer, Nat. Rev. Immunol. 15 (2015) 615–629, doi:http://dx.doi.org/10.1038/nri3896.
[104] W. Zou, N.P. Restifo, T(H)17 cells in tumour immunity and immunotherapy, Nat. Rev. Immunol. 10 (2010) 248–256, doi:http://dx.doi.org/10.1038/nri2742.
[105] M. Anbalagan, B. Huderson, L. Murphy, B.G. Rowan, Post-translational modifications of nuclear receptors and human disease, Nucl. Recept Signal. 10 (2012) e001, doi:http://dx.doi.org/10.1621/nrs.10001.
[106] H. Faus, B. Haendler, Post-translational modifications of steroid receptors, Biomed. Pharmacother. 60 (2006) 520–528, doi:http://dx.doi.org/10.1016/j.biopha.2006.07.082.
[107] C. Rochette-Egly, Nuclear receptors: integration of multiple signalling pathways through phosphorylation, Cell. Signal. 15 (2003) 355–366.
[108] H.W. Lim, S.G. Kang, J.K. Ryu, B. Schilling, M. Fei, I.S. Lee, et al., SIRT1 deacetylates RORγt and enhances Th17 cell generation, J. Exp. Med. 212 (2015) 607–617, doi:http://dx.doi.org/10.1084/jem.20132378.
[109] Q. Wu, J. Nie, Y. Gao, P. Xu, Q. Sun, J. Yang, et al., Reciprocal regulation of RORγt acetylation and function by p300 and HDAC1, Sci. Rep. 5 (2015) 16355, doi:http://dx.doi.org/10.1038/srep16355.
[110] W. Gu, R.G. Roeder, Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain, Cell 90 (1997) 595–606.
[111] J.E. Hoberg, A.E. Popko, C.S. Ramsey, M.W. Mayo, IkappaB kinase alpha-mediated derepression of SMRT potentiates acetylation of RelA/p65 by p300, Mol. Cell. Biol. 26 (2006) 457–471, doi:http://dx.doi.org/10.1128/MCB.26.2.457-471.2006.
[112] A.K. Ghosh, J. Varga, The transcriptional coactivator and acetyltransferase p300 in fibroblast biology and fibrosis, J. Cell Physiol. 213 (2007) 663–671, doi:http://dx.doi.org/10.1002/jcp.21162.
[113] H.-S. Kwon, M. Ott, The ups and downs of SIRT1, Trends Biochem. Sci. 33 (2008) 517–525, doi:http://dx.doi.org/10.1016/j.tibs.2008.08.001.
[114] J. Zhang, S.-M. Lee, S. Shannon, B. Gao, W. Chen, A. Chen, et al., The type III histone deacetylase Sirt1 is essential for maintenance of T cell tolerance in mice, J. Clin. Invest. 119 (2009) 3048–3058, doi:http://dx.doi.org/10.1172/JCI38902.
[115] R. Zhang, H.-Z. Chen, J.-J. Liu, Y.-Y. Jia, Z.-Q. Zhang, R.-F. Yang, et al., SIRT1 suppresses activator protein-1 transcriptional activity and cyclooxygenase-2 expression in macrophages, J. Biol. Chem. 285 (2010) 7097–7110, doi:http://dx.doi.org/10.1074/jbc.M109.038604.
[116] U.H. Beier, T. Akimova, Y. Liu, L. Wang, W.W. Hancock, Histone/protein deacetylases control Foxp3 expression and the heat shock response of T-regulatory cells, Curr. Opin. Immunol. 23 (2011) 670–678, doi:http://dx.doi.org/10.1016/j.coi.2011.07.002.
[117] H.-S. Kwon, H.W. Lim, J. Wu, M. Schnölzer, E. Verdin, M. Ott, Three novel acetylation sites in the Foxp3 transcription factor regulate the suppressive activity of regulatory T cells, J. Immunol. 188 (2012) 2712–2721, doi:http://dx.doi.org/10.4049/jimmunol.1100903.
[118] A.M. Weissman, Themes and variations on ubiquitylation, Nat. Rev. Mol. Cell Biol. 2 (2001) 169–178, doi:http://dx.doi.org/10.1038/35056563.
[119] Y. Kulathu, D. Komander, Atypical ubiquitylation – the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages, Nat. Rev. Mol. Cell Biol. 13 (2012) 508–523, doi:http://dx.doi.org/10.1038/nrm3394.
[120] S. Rutz, N. Kayagaki, Q.T. Phung, C. Eidenschenk, R. Noubade, X. Wang, et al., Deubiquitinase DUBA is a post-translational brake on interleukin-17 production in T cells, Nature (2014), doi:http://dx.doi.org/10.1038/nature13979.
[121] M. Kathania, P. Khare, M. Zeng, B. Cantarel, H. Zhang, H. Ueno, et al., Itch inhibits IL-17-mediated colon inflammation and tumorigenesis by ROR-γt ubiquitination, Nat. Immunol. (2016), doi:http://dx.doi.org/10.1038/ni.3488.
[122] X. Wang, J. Yang, L. Han, K. Zhao, Q. Wu, L. Bao, et al., TRAF5-mediated K63-linked polyubiquitination play essential role in positive regulation of RORγt on promoting IL-17A expression, J. Biol. Chem. (2015), doi:http://dx.doi.org/10.1074/jbc.M115.664573 (jbc.M115.664573).
[123] P. Xie, TRAF molecules in cell signaling and in human diseases, J Mol Signal 8 (2013) 7, doi:http://dx.doi.org/10.1186/1750-2187-8-7.
[124] X. Liu, H. Li, B. Zhong, M. Blonska, S. Gorjestani, M. Yan, et al., USP18 inhibits NF-γB and NFAT activation during Th17 differentiation by deubiquitinating the TAK1-TAB1 complex, J. Exp. Med. 210 (2013) 1575–1590, doi:http://dx.doi.org/10.1084/jem.20122327.
[125] B. Zhong, X. Liu, X. Wang, S.H. Chang, X. Liu, A. Wang, et al., Negative regulation of IL-17-mediated signaling and inflammation by the ubiquitin-specific protease USP25, Nature (2012), doi:http://dx.doi.org/10.1038/ni.2427.
[126] L. Han, J. Yang, X. Wang, Q. Wu, S. Yin, Z. Li, et al., The E3 deubiquitinase USP17 is a positive regulator of retinoic acid-related orphan nuclear receptor γt (RORγt) in Th17 cells, J. Biol. Chem. 289 (2014) 25546–25555, doi:http://dx.doi.org/10.1074/jbc.M114.565291.
[127] J. Yang, P. Xu, L. Han, Z. Guo, X. Wang, Z. Chen, et al., Cutting edge: ubiquitin-specific protease 4 promotes Th17 cell function under inflammation by deubiquitinating and stabilizing RORγt, J. Immunol. 194 (2015) 4094–4097, doi:http://dx.doi.org/10.4049/jimmunol.1401451.
[128] L. Zhang, F. Zhou, Y. Drabsch, R. Gao, B.E. Snaar-Jagalska, C. Mickanin, et al., USP4 is regulated by AKT phosphorylation and directly deubiquitylates TGF-β type I receptor, Nat. Cell Biol. 14 (2012) 717–726, doi:http://dx.doi.org/10.1038/ncb2522.
[129] D. Vucic, V.M. Dixit, I.E. Wertz, Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death, Nat. Rev. Mol. Cell Biol. 12 (2011) 439–452, doi:http://dx.doi.org/10.1038/nrm3143.
[130] B.R. Keppler, T.K. Archer, H.K. Kinyamu, Emerging roles of the 26 S proteasome in nuclear hormone receptor-regulated transcription, Biochim. Biophys. Acta 1809 (2011) 109–118, doi:http://dx.doi.org/10.1016/j.bbagrm.2010.08.005.
[131] W. Zhou, J.M. Slingerland, Links between oestrogen receptor activation and proteolysis: relevance to hormone-regulated cancer therapy, Nat. Rev. Cancer. 14 (2014) 26–38.
[132] J.H. Lee, M.J. Lee, Emerging roles of the ubiquitin-proteasome system in the steroid receptor signaling, Arch. Pharm. Res. 35 (2012) 397–407, doi:http://dx.doi.org/10.1007/s12272-012-0301-x.
[133] A.M. Jetten, J.H. Joo, Retinoid-related orphan receptors (RORs): roles in cellular differentiation and development, Adv. Dev. Biol. 16 (2006) 313–355, doi:http://dx.doi.org/10.1016/S1574-3349(06)16010-X.
[134] D.M. Lonard, Z. Nawaz, C.L. Smith, B.W. O'Malley, The 26 S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation, Mol. Cell 5 (2000) 939–948.
[135] A.N. Moraitis, V. Giguère, The co-repressor hairless protects RORalpha orphan nuclear receptor from proteasome-mediated degradation, J. Biol. Chem. 278 (2003) 52511–52518, doi:http://dx.doi.org/10.1074/jbc.M308152200.
[136] A.N. Moraitis, V. Giguère, C.C. Thompson, Novel mechanism of nuclear receptor corepressor interaction dictated by activation function 2 helix determinants, Mol. Cell. Biol. 22 (2002) 6831–6841, doi:http://dx.doi.org/10.1128/MCB.22.19.6831-6841.2002.
[137] D.S. Straus, C.K. Glass, Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms, Trends Immunol. 28 (2007) 551–558, doi:http://dx.doi.org/10.1016/j.it.2007.09.003.
[138] C. Klemann, B.J.E. Raveney, A.K. Klemann, T. Ozawa, S. von Hörsten, K. Shudo, et al., Synthetic retinoid AM80 inhibits Th17 cells and ameliorates experimental autoimmune encephalomyelitis, Am. J. Pathol. 174 (2009) 2234–2245, doi:http://dx.doi.org/10.2353/ajpath.2009.081084.
[139] C.G. Mayne, J.A. Spanier, L.M. Relland, C.B. Williams, C.E. Hayes, 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis, Eur. J. Immunol. 41 (2011) 822–832, doi:http://dx.doi.org/10.1002/eji.201040632.
[140] H. Wen, J.F. Baker, Vitamin D, immunoregulation, and rheumatoid arthritis, J. Clin. Rheumatol. 17 (2011) 102–107, doi:http://dx.doi.org/10.1097/RHU.0b013e31820edd18.
[141] D. Mucida, Y. Park, G. Kim, O. Turovskaya, I. Scott, M. Kronenberg, et al., Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid, Science 317 (2007) 256–260, doi:http://dx.doi.org/10.1126/science.1145697.
[142] K.M. Elias, A. Laurence, T.S. Davidson, G. Stephens, Y. Kanno, E.M. Shevach, et al., Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway, Blood 111 (2008) 1013–1020, doi:http://dx.doi.org/10.1182/blood-2007-06-096438.

[143] R.T. Gampe, V.G. Montana, M.H. Lambert, A.B. Miller, R.K. Bledsoe, M.V. Milburn, et al., Asymmetry in the PPARgamma/RXRalpha crystal structure reveals the molecular basis of heterodimerization among nuclear receptors, Mol. Cell 5 (2000) 545–555.
[144] L. Klotz, S. Burgdorf, I. Dani, K. Saijo, J. Flossdorf, S. Hucke, et al., The nuclear receptor PPAR gamma selectively inhibits Th17 differentiation in a T cell-intrinsic fashion and suppresses CNS autoimmunity, J. Exp. Med. 206 (2009) 2079–2089, doi:http://dx.doi.org/10.1084/jem.20082771.
[145] B. Dumas, H.P. Harding, H.S. Choi, K.A. Lehmann, M. Chung, M.A. Lazar, et al., A new orphan member of the nuclear hormone receptor superfamily closely related to Rev-Erb, Mol. Endocrinol. 8 (1994) 996–1005, doi:http://dx.doi.org/10.1210/mend.8.8.7997240.
[146] L. Yin, M.A. Lazar, The orphan nuclear receptor Rev-erbalpha recruits the N-CoR/histone deacetylase 3 corepressor to regulate the circadian Bmal1 gene, Mol. Endocrinol. 19 (2005) 1452–1459, doi:http://dx.doi.org/10.1210/me.2005-0057.
[147] S. Raghuram, K.R. Stayrook, P. Huang, P.M. Rogers, A.K. Nosie, D.B. McClure, et al., Identification of heme as the ligand for the orphan nuclear receptors REV-ERBalpha and REV-ERBbeta, Nat. Struct. Mol. Biol. 14 (2007) 1207–1213, doi:http://dx.doi.org/10.1038/nsmb1344.
[148] X. Yu, D. Rollins, K.A. Ruhn, J.J. Stubblefield, C.B. Green, M. Kashiwada, et al., TH17 cell differentiation is regulated by the circadian clock, Science 342 (2013) 727–730, doi:http://dx.doi.org/10.1126/science.1243884.
[149] M.F. Farez, I.D. Mascanfroni, S.P. Méndez-Huergo, A. Yeste, G. Murugaiyan, L.P. Garo, et al., Melatonin contributes to the seasonality of multiple sclerosis relapses, Cell 162 (2015) 1338–1352, doi:http://dx.doi.org/10.1016/j.cell.2015.08.025.
[150] M. Ciofani, A. Madar, C. Galan, M. Sellars, K. Mace, F. Pauli, et al., A validated regulatory network for th17 cell specification, Cell 151 (2012) 289–303, doi:http://dx.doi.org/10.1016/j.cell.2012.09.016.
[151] K. Okamoto, Y. Iwai, M. Oh-Hora, M. Yamamoto, T. Morio, K. Aoki, et al., IkappaBzeta regulates T(H)17 development by cooperating with ROR nuclear receptors, Nature 464 (2010) 1381–1385, doi:http://dx.doi.org/10.1038/nature08922.
[152] F. Zhang, G. Meng, W. Strober, Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells, Nat. Immunol. 9 (2008) 1297–1306, doi:http://dx.doi.org/10.1038/ni.1663.
[153] E.V. Dang, J. Barbi, H.-Y. Yang, D. Jinasena, H. Yu, Y. Zheng, et al., Control of TH17/Treg balance by hypoxia-Inducible factor 1, Cell (2011), doi:http://dx.doi.org/10.1016/j.cell.2011.07.033.
[154] R. Jain, Y. Chen, Y. Kanno, B. Joyce-Shaikh, G. Vahedi, K. Hirahara, et al., Interleukin-23-Induced transcription factor blimp-1 promotes pathogenicity of t helper 17Cells, Immunity 44 (2016) 131–142, doi:http://dx.doi.org/10.1016/j.immuni.2015.11.009.
[155] L. Zhou, I.I. Ivanov, R. Spolski, R. Min, K. Shenderov, T. Egawa, et al., IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways, Nat. Immunol. 8 (2007) 967–974, doi:http://dx.doi.org/10.1038/ni1488.
[156] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, et al., Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells, Nature 441 (2006) 235–238, doi:http://dx.doi.org/10.1038/nature04753.
[157] K. Ghoreschi, A. Laurence, X.-P. Yang, C.M. Tato, M.J. McGeachy, J.E. Konkel, et al., Generation of pathogenic T(H)17 cells in the absence of TGF-β signalling, Nature 467 (2010) 967–971, doi:http://dx.doi.org/10.1038/nature09447.
[158] Y. Zheng, D. Danilenko, P. Valdez, I. Kasman, J. Eastham-Anderson, J. Wu, et al., Interleukin-22 a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis, Nature 445 (2007) 648–651.
[159] S. Rutz, R. Noubade, C. Eidenschenk, N. Ota, W. Zeng, Y. Zheng, et al., Transcription factor c-Maf mediates the TGF-β-dependent suppression of IL-22 production in T(H)17 cells, Nat. Immunol. 12 (2011) 1238–1245, doi:http://dx.doi.org/10.1038/ni.2134.
[160] L. Zhou, J.E. Lopes, M.M.W. Chong, I.I. Ivanov, R. Min, G.D. Victora, et al., TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function, Nature 453 (2008) 236–240, doi:http://dx.doi.org/10.1038/nature06878.
[161] K. Ichiyama, H. Yoshida, Y. Wakabayashi, T. Chinen, K. Saeki, M. Nakaya, et al., Foxp3 inhibits RORgammat-mediated IL-17A mRNA transcription through direct interaction with RORgammat, J. Biol. Chem. 283 (2008) 17003–17008, doi:http://dx.doi.org/10.1074/jbc.M801286200.
[162] J. Du, C. Huang, B. Zhou, S.F. Ziegler, Isoform-specific inhibition of ROR alpha-mediated transcriptional activation by human FOXP3, J. Immunol. 180 (2008) 4785–4792.
[163] E. Sefik, N. Geva-Zatorsky, S. Oh, L. Konnikova, D. Zemmour, A.M. McGuire, et al., Individual intestinal symbionts induce a distinct population of ROR γ+ regulatory T cells, Science (2015), doi:http://dx.doi.org/10.1126/science.aaa9420 (aaa9420).
[164] C. Ohnmacht, J.-H. Park, S. Cording, J.B. Wing, K. Atarashi, Y. Obata, et al., The microbiota regulates type 2 immunity through RORγt+ T cells, Science (2015), doi:http://dx.doi.org/10.1126/science.aac4263 (aac4263).
[165] B.-H. Yang, S. Hagemann, P. Mamareli, U. Lauer, U. Hoffmann, M. Beckstette, et al., Foxp3(+) T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation, Mucosal Immunology. (2015), doi:http://dx.doi.org/10.1038/mi.2015.74.
[166] N. Maeda, T. Kasukawa, R. Oyama, J. Gough, M. Frith, P.G. Engström, et al., Transcript annotation in FANTOM3: mouse gene catalog based on physical cDNAs, PLoS Genet. 2 (2006) e62, doi:http://dx.doi.org/10.1371/journal.pgen.0020062.
[167] S. Djebali, C.A. Davis, A. Merkel, A. Dobin, T. Lassmann, A. Mortazavi, et al., Landscape of transcription in human cells, Nature 489 (2012) 101–108, doi:http://dx.doi.org/10.1038/nature11233.
[168] T.R. Mercer, M.E. Dinger, J.S. Mattick, Long non-coding RNAs: insights into functions, Nat. Rev. Genet. 10 (2009) 155–159, doi:http://dx.doi.org/10.1038/nrg2521.
[169] A. Fatica, I. Bozzoni, Long non-coding RNAs: new players in cell differentiation and development, Nat. Rev. Genet. 15 (2014) 7–21, doi:http://dx.doi.org/10.1038/nrg3606.
[170] J.T.Y. Kung, D. Colognori, J.T. Lee, Long noncoding RNAs: past, present, and future, Genetics 193 (2013) 651–669, doi:http://dx.doi.org/10.1534/genetics.112.146704.
[171] J.L. Rinn, H.Y. Chang, Genome regulation by long noncoding RNAs, Annu. Rev. Biochem. 81 (2012) 145–166, doi:http://dx.doi.org/10.1146/annurev-biochem-051410-092902.
[172] G. Hu, Q. Tang, S. Sharma, F. Yu, T.M. Escobar, S.A. Muljo, et al., Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation, Nat. Immunol. 14 (2013) 1190–1198, doi:http://dx.doi.org/10.1038/ni.2712.
[173] C.F. Spurlock, J.T. Tossberg, Y. Guo, S.P. Collier, P.S. Crooke, T.M. Aune, Expression and functions of long noncoding RNAs during human T helper cell differentiation, Nat. Commun. 6 (2015) 6932, doi:http://dx.doi.org/10.1038/ncomms7932.
[174] W. Huang, B. Thomas, R.A. Flynn, S.J. Gavzy, L. Wu, S.V. Kim, et al., DDX5 and its associated lncRNA Rmrp modulate TH17 cell effector functions, Nature 528 (2015) 517–522, doi:http://dx.doi.org/10.1038/nature16193.
[175] P. Linder, E. Jankowsky, From unwinding to clamping – the DEAD box RNA helicase family, Nat. Rev. Mol. Cell Biol. 12 (2011) 505–516, doi:http://dx.doi.org/10.1038/nrm3154.
[176] E.L. Clark, A. Coulson, C. Dalgliesh, P. Rajan, S.M. Nicol, S. Fleming, et al., The RNA helicase p68 is a novel androgen receptor coactivator involved in splicing and is overexpressed in prostate cancer, Cancer Res. 68 (2008) 7938–7946, doi:http://dx.doi.org/10.1158/0008-5472.CAN-08-0932.
[177] N.C. Wortham, E. Ahamed, S.M. Nicol, R.S. Thomas, M. Periyasamy, J. Jiang, et al., The DEAD-box protein p72 regulates ERalpha-/oestrogen-dependent transcription and cell growth, and is associated with improved survival in ERalpha-positive breast cancer, Oncogene 28 (2009) 4053–4064, doi:http://dx.doi.org/10.1038/onc.2009.261.
[178] C.L. Hsieh, T.A. Donlon, B.T. Darras, D.D. Chang, J.N. Topper, D.A. Clayton, et al., The gene for the RNA component of the mitochondrial RNA-processing endoribonuclease is located on human chromosome 9p and on mouse chromosome 4, Genomics 6 (1990) 540–544.
[179] A.N. Martin, Y. Li, RNase MRP RNA and human genetic diseases, Cell Res. 17 (2007) 219–226, doi:http://dx.doi.org/10.1038/sj.cr.7310120.
[180] W. Huang, D.R. Littman, Regulation of RORγt in inflammatory lymphoid cell differentiation, Cold Spring Harb. Symp. Quant. Biol. 027615 (2016), doi:http://dx.doi.org/10.1101/sqb.2015.80.027615.
[181] J. Chao, I. Enyedy, K. Van Vloten, D. Marcotte, K. Guertin, R. Hutchings, et al., Discovery of biaryl carboxylamides as potent RORγ inverse agonists, Bioorg. Med. Chem. Lett. 25 (2015) 2991–2997, doi:http://dx.doi.org/10.1016/j.bmcl.2015.05.026.
[182] B.P. Fauber, O. René, Y. Deng, J. Devoss, C. Eidenschenk, C. Everett, et al., Discovery of 1-{4-[3-fluoro-4-((3S,6R)-3-methyl-1,1-dioxo-6-phenyl-[1,2]thiazinan-2-ylmethyl)-phenyl]-piperazin-1-yl}-ethanone (GNE-3500): a potent, selective, and orally bioavailable retinoic acid receptor-related orphan receptor C (RORc or RORγ) inverse agonist, J. Med. Chem. 58 (2015) 5308–5322, doi:http://dx.doi.org/10.1021/acs.jmedchem.5b00597.
[183] N. Kumar, L.A. Solt, J.J. Conkright, Y. Wang, M.A. Istrate, S.A. Busby, et al., The benzenesulfoamide T0901317 [N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-benzenesulfonamide] is a novel retinoic acid receptor-related orphan receptor-alpha/gamma inverse agonist, Mol. Pharmacol. 77 (2010) 228–236, doi:http://dx.doi.org/10.1124/mol.109.060905.

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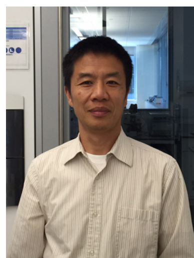

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