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The Emerging Landscape of RORγt Biology

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The transcription factor retinoid-related orphan receptor gamma t (RORγt) has emerged as an exciting target for inflammatory diseases. Xiao et al. (2014) show that a new class of RORγt antagonists can inhibit the inflammatory function of T helper 17 cells without altering RORγt occupancy on its target genes.

The discovery of pathogenic T helper 17 (Th17) cells nearly a decade ago (Ivanov et al., 2006) has led to the development of targeting strategies to inhibit inflammatory T cell activities for the treatment of autoimmune disorders. At the core of these therapeutic approaches is antibody-mediated neutralization of interleukin-23 (IL-23) and IL-17, which is showing encouraging results for treatment of psoriasis, multiple sclerosis, Crohn's disease, and ankylosing spondylitis (Patel et al., 2013, Sandborn et al., 2012). These clinical proof-of-concept studies underscore the advantage and effectiveness of suppressing retinoid-related orphan receptor gamma t (RORγt)$^{+}$IL-23R^{+} Th17 cells. Additional approaches include (1) blockade of IL-23R signaling through the suppression of JAK2- and TYK2-dependent STAT3 activation and (2) direct inhibition of RORγt—the nuclear hormone receptor (NHR) transcription factor that controls Th17 cell development and function. In the current issue of *Immunity*, Xiao et al. (2014) describe the screening and validation of small-molecule inhibitors of RORγ. What distinguishes this study from others is the combined use of a large-scale pharmaceutical discovery platform with innovative genome-wide analysis of RORγt target genes in Th17 cells. Aside from its potential clinical value, the advantage of small-molecule inhibitors of a NHR transcription factor is the opportunity to dissect the molecular mechanisms of RORγt regulation and the RORγt-dependent transcriptional landscape not only in Th17 cells but also in other RORγt-expressing cell types.

Screening through a library of (~2 million) small-molecule compounds, Xiao et al. (2014) identified TMP778, TMP920, and GSK805, which bound to and inhibited the RORγ ligand-binding domain (LBD) from interacting with cofactor peptide steroid receptor coactivator 1 (SRC-1) in a fluorescence resonance energy transfer assay. These compounds were highly selective for RORγ, and importantly, the inhibitory effect of the compounds on RORγt was confirmed through the suppression of effector cytokines from Th17 cells. The compounds also inhibited two different RORγt-dependent models of autoimmunity in vivo: experimental autoimmune encephalomyelitis and imiquimod-induced skin inflammation (Skepner et al., 2014). Treatment not only delayed the onset of disease but also reduced the severity of disease progression in both models. These results support the identification of compounds that not only bind to RORγt but also effectively inhibit RORγt function both in vitro and in vivo.

RORγt belongs to a family of NHR transcriptional factors that are composed of protein structures with a separate DNA-binding domain (DBD) and LBD. The DBD imparts gene-targeting specificity, whereas the LBD serves as control switches for transcription regulatory functions (Huh and Littman, 2012). Over the past few years, the search for RORγt inhibitors has yielded molecules that engage the LBD, induce conformational changes, and prevent recruitment of coactivators (e.g., SRCs). Examples include diverse chemical structural classes of compounds, including digoxin, SR1001, and ursolic acid (Huh et al., 2011, Solt et al., 2011, Xu et al., 2011). Digoxin and SR1001 bind the LBD of RORγ and inhibit the recruitment of coactivator SRCs by disrupting helix H12 of the RORγt ligand-binding pocket. Ursolic acid also interrupts RORγ LBD interaction with SRC-1, and all of the compounds effectively inhibit expression of genes that are preferentially expressed in Th17 cells. However, until now, the direct effects of RORγt inhibitors on RORγt-dependent transcription programs have not been analyzed in detail.

By taking the innovative approach of pairing chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq) data, Xiao et al. (2014) were able to gain broad insights into the direct transcriptional effects of the RORγt inhibitors on the basis of changes in RORγt occupancy on its target genes, as well as global transcriptional modulations. As expected, many of the genes that were suppressed after treatment with the inhibitors were Th17-cell-specific genes, such as *Il17a* and *Il23r*, confirming the strong inhibition of Th17 cell differentiation and IL-17 production. However, similar to *Rorc*$^{-/-}$ cells, RORγt-inhibited cells also led to the induction of signature genes from other CD4^{+} T helper cell lineages, such as *Il4* and *Tbx21*. As observed for many other transcriptional regulators, RORγt functions as both an activator and a repressor of a wide range of target genes, and the effect of the inhibitors reveals an important function of RORγt in positively regulating the transcriptional signature of Th17 cells while suppressing those of other T helper cell lineages.

How do the inhibitors suppress the activity of RORγt? Is it simply by interrupting RORγt from binding its target genes, or is it by disrupting RORγt interaction with other transcription factors or coactivators? The answer proved to be both, but interestingly, it was specific to the compound itself. Xiao et al. (2014) compared compound-induced changes in RORγt gene occupancy by using genome-wide ChIP-seq, and surprisingly, the data revealed remarkable insights for the mechanism of action between the different RORγt inhibitors (Figure 1). Whereas compound TMP920 clearly abrogated

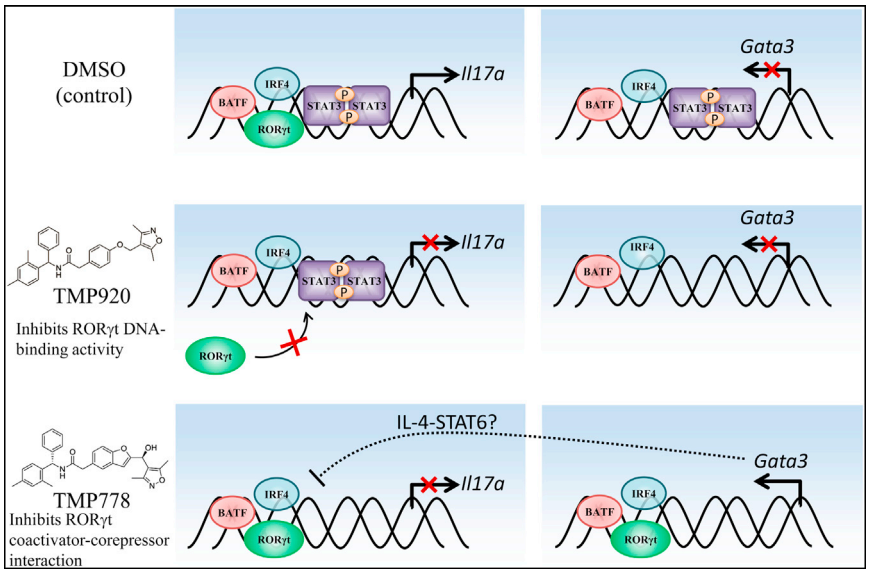

Figure 1. Mechanisms of Functional RORγt Inhibition by Small-Molecule Inhibitors
(Top) RORγt, IRF4, BATF, and STAT3 occupancy on *Il17a* and *Gata3* in Th17 cells. RORγt and coregulatory transcription factors induce *IL17a* transcription, whereas *Gata3* expression is inhibited.
(Center) Treatment with TMP920 during Th17 cell polarization inhibits RORγt occupancy on *Il17a*, and *IL17a* transcription is inhibited. RORγt occupancy on *Gata3* is not affected by treatment with TMP920.
(Bottom) In contrast to treatment with TMP920, treatment with TMP778 preserves RORγt occupancy on *IL17a*; however, *IL17a* transcription is inhibited. TMP778 bound to RORγt allows occupancy at additional target genes, including *Gata3*. Whether the suppression of *Il17a* is through the inhibition of RORγt interaction with coregulatory proteins or through indirect suppression from *Gata3* transactivation and GATA3-induced proteins such as IL-4 and downstream STAT6 signaling remains to be determined.

RORγ occupancy of target sequences, RORγ binding activities were preserved when cells were treated with TMP778 or GSK805. An unanticipated effect of TMP778 or GSK805 was that these compounds appeared to stabilize RORγt occupancy in certain genomic loci, and interestingly, treatment with either compound led to RORγt occupancy in new binding sites normally not seen in Th17 cells. A region of particular interest was the binding of RORγt to *Gata3* when cells were treated with TMP778 or GSK805. These compounds induced an increase in both *Gata3* mRNA and protein expression. In light of this interesting observation, additional unanswered questions arise. How do the compounds alter RORγt DNA binding, and do the additional RORγt occupancy sites contribute to the suppressed Th17 cell phenotype? It is possible that transactivation of *Gata3* by RORγt participates in the inhibition of Th17 cell signature genes in cells treated with TMP778 or GSK805, perhaps through the induction of GATA3 target genes (Figure 1). Additional studies analyzing the effect of the compounds on RORγt interaction with coactivators and corepressors through a proteomics approach might further shed valuable insights into the molecular mechanisms of RORγt inhibition.

The strategic value of analyzing the transcriptional profile in conjunction with RORγt target-gene occupancy is the unprecedented glimpse into the potential diversity in the inhibitory mechanisms of RORγ small-molecule antagonists. Although the global downstream effects after treatment with a range of compounds might appear similar, the data presented clearly show that the actual molecular mechanism can be quite diverse. The data highlight two important points: (1) combining an unbiased genome-wide transcription factor binding assay via ChIP-seq with global transcriptional modulation analysis demonstrates a profound alteration of the Th17 cell transcriptional signature after treatment with RORγ antagonists and (2) RORγt might lead to "fine tuning" of $CD4^+$ T helper cell fate by supporting the Th17 cell circuitry while repressing the "default" Th2 cell fate. Treatment with the compounds effectively demonstrates the dual nature of RORγt as a transcriptional activator and repressor of genes that destabilize Th17 cells. Studies understanding the interaction between RORγt and coactivators or corepressors and how this association regulates Th17 cell fate will be an exciting future research avenue.

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