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PERSPECTIVE

Romance of the three kingdoms: RORgammat allies with HIF1alpha against FoxP3 in regulating T cell metabolism and differentiation

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Regulatory T (Treg) cells play an essential role in immune homeostasis by controlling the function of various immune effector cells, including RAR-related orphan receptor gammat$^+$ (RORγt$^+$) T helper 17 (Th17) cells. Foekhead box P_3 ($FoxP_3$) is the master regulator of Treg cell function, while RORγt is the key transcription factor for the induction of the interleukin (IL)-17 family of cytokines during Th17 cell differentiation. FoxP3 can directly interact with and negatively regulate the function of RORγt, to determine the balance between induced Treg (iTreg) and Th17 cell polarization. Two recent independent studies from the Pan and Chi Labs have shown how hypoxia-inducible factor 1 alpha (HIF1α) is able to tip the balance of T cell differentiation toward the Th17 lineage by responding to the local changes in metabolic shift or an increase in proinflammatory mediators in the microenvironment. By allying with HIF1α, RORγt wins the fight against FoxP3 and Treg cell commitment.

The immune system is tightly controlled by the balance of multiple innate and adaptive immune cells to distinguish between self and non-self and maintain immune homeostasis. The fight between pro- and anti-inflammatory responses is often compared to the Yin-Yang philosophy, where these opposites are naturally bound but interconnect in order to maintain a healthy balance. Transcription factor networks play a central role in regulating the differentiation and function of immune cells, including T cells. In the past decade, the forkhead family transcription factor Foekhead box P_3 ($FoxP_3$) has drawn much attention due to its essential role in controlling the fate of the immunosuppressive regulatory T (Treg) cell. Another dominant player is RAR-related orphan receptor gammat (RORγt), a short isoform of the orphan nuclear receptor RORγ, which is instrumental in controlling the development and function of interleukin (IL)-17 producing inflammatory effector T (Teff) cells known as helper T (Th) 17 cells. These transcription factors could be described as the Yin and Yang of the T cell immune system since they are intimately connected and share common differential cues; however, RORγt and FoxP3 also act reciprocally to keep one another in check.

Previously studies have shown how naïve T cells can be differentiated into induced FoxP3$^+$ Treg (iTreg) cells via their co-stimulation with anti-CD3/CD28 antibodies plus transforming growth factor β (TGFβ) treatment, or into RORγt$^+$ Th17 cells by the extra addition of the proinflammatory cytokine IL-6 (Park et al., 2005; Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006). How the balance between Th17 and iTreg cell differentiation is precisely controlled remains rather unclear, although it has been reported that FoxP3 can directly bind to RORγt to then inhibit RORγt-dependent transcriptional activation of its key target genes such as IL-17 (Zhou et al., 2008; Chen et al., 2011).

The interplay between RORγt and FOXP3 in T cell differentiation has been made clearer since the role of hypoxia and the key transcription factor, hypoxia-inducible factor 1α (HIF1α), has now been identified as being essential for promoting T cell differentiation toward the Th17-lineage away from iTreg cell polarization (Dang et al., 2011; Shi et al., 2011). By allying with HIF1α, RORγt wins the battle against FoxP3 in favor of Th17 differentiation. HIF1α, as the third party, complicates this Yin-Yang relationship between RORγt

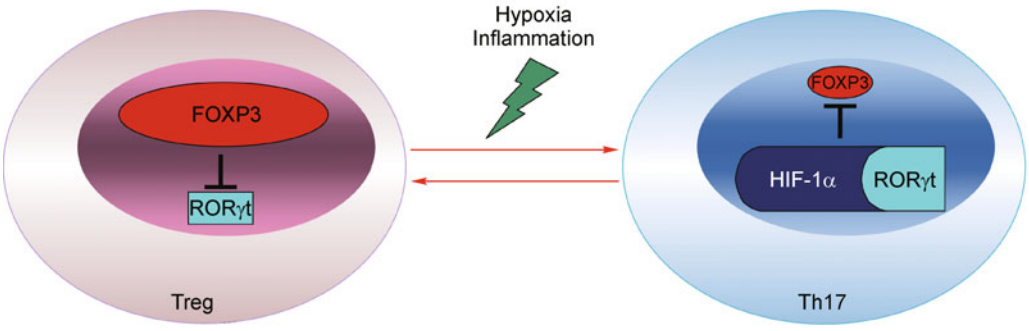

Figure 1. Regulatory T cells are controlled by its master transcription factor FOXP3, whereas Th17 cells utilize the combination of HIF1α and RORγt to induce its differentiation. At the protein level, FOXP3 can inhibit the function of RORγt to favor Treg differentiation, but RORγt requires the recruitment of HIF1α to fight back against FOXP3 by targeting it for degradation. HIF1α can also be activated by hypoxic and non-hypoxic signals to favor HIF1α/RORγt-dependent Th17 differentiation.

and FoxP3. The emergence and dynamics of the HIF1α story is more akin to the interplay between the generals and armies of the Three Kingdoms era. Here, we witness the war of these transcription factors in the Th17/Treg axis (Fig. 1).

Chi and colleagues investigated the role of HIF1α in regulating T cell metabolism, focusing on glucose metabolism and its involvement in T cell differentiation. By performing *in vitro* T cell differentiation assays, the authors found that HIF1α, which has been extensively studied in the tumor biology field as a critical regulator of cell metabolism (Finley et al., 2011), was moderately expressed in Th1 cells, highly expressed in Th17 cells, but was undetectable in Th2 or Treg cells (Shi et al., 2011). The high expression of HIF1α was found to be essential for driving the glycolytic gene expression program in Th17 cells since the HIF1α deficient T cells had a much lower expression of the key transporters and enzymes including Glut1, HK2, GPI, TPI, Eno, PKM, LDHα, MCT4, and also failed to differentiate into functional IL-17 expressing Th17 cells. When naïve CD4 T cells were grown under Th17 skewing conditions, the HIF1α deficient T cells neither significantly changed the induction and expression of RORγt and RORα, nor the phosphorylation status of signal transducer and activator of transcription 3 (STAT3), but were downregulated in IL-23R expression. This resulted in the suppression of Th17 polarization, the upregulation of FoxP3 expression to further antagonize RORγt function, and the induction of the expression of Treg cell phenotypic markers such as CTLA4 and Gpr83 through TGFβ signal. By the addition of 2-Deoxy-D-glucose (2-DG), which has the 2-hydroxyl group replaced by hydrogen to prevent its further glycolysis, or Rapamycin to promote the differentiation of Treg cells but not other Th subsets (Battaglia et al., 2006; Kopf et al., 2007; Haxhinasto et al., 2008; Zeiser et al., 2008; Delgoffe et al., 2011), the authors found that fewer IL-17 producing cells were generated under Th17-skewing conditions. Additionally, IL-6 promoted, but Rapamycin inhibited, the expression of HIF1α. Recently, Ramsdell and colleagues showed how FoxP3$^+$ Treg cells and other Teff cells including Th17 cells have distinguishable differences in both glycolysis and lipid oxidation (Michalek et al., 2011). It should therefore be interesting to study whether HIF1α plays any role in suppressing lipid oxidation during Th17 and iTreg cell differentiation.

Pan and colleagues have shown how HIF1α works with RORγt at multiple levels (Dang et al., 2011). Firstly, the HIF1α gene can be transcriptionally activated by the inflammatory cytokine-mediated activation of the JAK-STAT3 signal pathway that is prominent during Th17 polarization, but is independent of hypoxic stimuli. Secondly, HIF1α that is induced during T cell differentiation can actively promote RORγt gene transcription. Lastly, HIF1α binds to the RORγt protein complex including histone modification enzymes such as p300 which specifically functions at the promoter region of RORγt target genes (e.g. IL-17A) to induce their transcription and cell differentiation toward the Th17 cell lineage. On the other hand, HIF1α interacts and regulates FOXP3 at the posttranslational level by interacting with the C-terminal domain of FOXP3 to promote its degradation in a proteasome-dependent manner. The hydroxylase targeting sites of HIF1α (P402 and P564) are critical for the ability of HIF1α in deregulating FOXP3, since non-proline hydroxylatable HIF1α is unable to mediate FOXP3 degradation. The knockdown of the HIF1α ubiquitin ligase complex subunit PHD2 can also prevent FOXP3 degradation, which suggests that HIF1α may recruit its own E3 ligase complex to FOXP3. HIF1α knockout mice were found to be resistant to experimental autoimmune encephalomyelitis (EAE); here, HIF1α-deficient T cells were found to be resistant to hypoxia-driven Th17 cell generation *in vitro*, but there was an increase in the frequency of FoxP3$^+$ cells and degree of Treg cell differentiation *in vitro* and *in vivo*.

The three transcription factors, HIF1α, RORγt and FOXP3, work in conjunction with other cofactors to regulate gene transcription. HIF1α acts with its heterodimer partner ARNT, which can alternatively bind to HIF1β (Semenza and Wang, 1992; Weidemann and Johnson, 2008), while FOXP3 can form a homodimer or heterodimerize with other subfamily

members such as FOXP1 or multiple histone modification enzymes such as TIP60, p300 or HDAC7 in a large molecular complex (Li et al., 2006). Whether these heteromeric partners of HIF1α and FoxP3 play a role in regulating their interaction with RORγt is unclear and it remains an interesting topic for future study.

The stability of HIF1α itself is reversely regulated by the histone deacetylase SIRT3 (Finley et al., 2011), so the association of p300 with HIF1α and RORγt may reciprocally stabilize both of these transcription factors by preventing their negative regulation by SIRT3. Moreover, FOXP3 is an acetylated protein in Treg cells (Li et al., 2007) and FOXP3 complex stability is dependent on the balance between p300 and SIRT1 activity (van Loosdregt et al., 2010); so how HIF1α can destabilize a binding partner like FOXP3 (Dang et al., 2011), but stabilize the expression of others such as RORγt (Dang et al., 2011; Shi et al., 2011) or p53 (An et al., 1998) *in vivo* remains an interesting issue to be solved. It is also important to understand how and whether HIF1α allies with other transcription factors which are known to be essential for the differentiation and function of Th17 cells, including Batf (Schraml et al., 2009), IκBζ (Okamoto et al., 2010), and IRF4 (Brustle et al., 2007). IRF4 also interacts with FoxP3 and its expression is essential for $FoxP3^+$ Treg cell-mediated suppression of the Th2 response (Zheng et al., 2009). It would therefore be interesting to determine whether HIF1α can regulate the function of IRF4, and how HIF1α may regulate other major metabolic pathways including lipid (Michalek et al., 2011), amino acid (Cobbold et al., 2009) and nucleic acid metabolism (Tong et al., 2009) in Th17 and Treg cells.

In summary, the intriguing studies by Dang et al. and Shi et al. have highlighted the functional importance of the dynamic, temporal, and chromatin site-specific transcriptional complexes in regulating gene transcription, which leads to the precise control of crucial metabolic pathways involved in immune cell differentiation and function. The inherent differences in the metabolic pathways found in different T cell types have been utilized to augment the divide between opposing T cell differentiation programs. HIF1α has been used by RORγt as an ally to strengthen its own game but in turn acts to ambush the FOXP3/Treg differentiation pathway. How hypoxia and inflammation work in conjunction to fine tune the balance of the Th17/Treg axis remains to be elucidated—but nonetheless, there seems to be a potential for targeting these key metabolic pathways and dynamic ensembles of transcription factor complexes for the treatment of diseases such as autoimmune disease, where Th17 and Treg cell functions are sought to be suppressed or augmented, respectively.

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