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RESEARCH HIGHLIGHT

Impaired regulatory T cell function in autoimmune diseases: are microRNAs the culprits?

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MicroRNAs (miRNAs) are evolutionarily conserved small non-coding RNA sequences, approximately 22 nucleotides in length, that act as negative regulators of target genes via binding to the 3′-untranslated region of mRNAs, leading to mRNA degradation or the inhibition of mRNA translation. In animal cells, the biogenesis of miRNAs begins in the nucleus with the transcription of double-stranded pri-miRNA (1–3 kb) and ends as a single-stranded 'mature miRNA' in the cytoplasm. Drosha and Dicer are the key enzymes that play critical roles in miRNA biogenesis.[1,2] miRNA regulates various cellular and physiological processes, and the dysregulated expression of miRNA has been linked to several pathologies, including autoimmune diseases.[2,3]

$CD4^+$ T helper (T_H) cells act as key regulators of adaptive immunity. The activation and differentiation of naive $CD4^+$ T cells into distinct subsets, such as T_H1, T_H2, T_H17, and $CD4^+$ $CD25^+FOXP3^+$ regulatory T cells (Tregs), are accomplished by various signals obtained from antigen-loaded major histocompatibility complex class II molecules, co-stimulatory molecules, and polarizing cytokines.[4–6] Tregs that serve as immunosuppressors could be thymic in origin (natural Tregs) or derived (induced) at the periphery from naive $CD4^+$ T cells. Tregs maintain immune tolerance by modulating the functions of innate immune cells, effector T cells, and B cells.[6–8]

Defects in the Tregs paralyze their suppressive capabilities.[9–12] Evidence suggests that autoimmune diseases in humans, such as systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis (RA), are linked with impaired Tregs, which has been further verified in different animal models. In RA, although Tregs are enriched at the site of inflammation (synovium), the suppressive ability of Tregs is reduced. The attenuated Treg function might be linked to pro-inflammatory cytokines (IL-6, IL-21 or tumor-necrosis factor (TNF-α)) and to functional mutation or polymorphism of FOXP3.[9–12] A recent report by Alla Skapenko and colleagues suggests that dysregulated miRNA expression in Tregs contributes to impaired Treg functions in RA (Figure 1).[13]

MiRNAs (such as miR-146a, miR-155, miR-21, miR-142-3p, and miR-31) and miRNA-processing enzymes (Dicer and Drosha) are directly or indirectly linked to lymphocyte differentiation and function, including Tregs.[6,13–15] The data obtained by Zhang *et al.* suggest that miR-31 negatively regulates the generation of peripherally derived Tregs by inhibiting retinoic acid-inducible protein 3 (Gprc5a; Figure 1). In an experimental autoimmune encephalomyelitis (EAE) model, miR-31 expression was upregulated in splenocytes and pathogenic T-cells. The conditional deletion of miR-31 led to the enhanced induction of peripherally derived Tregs and decreased the severity of EAE in mice.[15] In addition, the expression of miR-155 and miR-146a provides favorable conditions for Tregs. MiR-155 controls Treg homeostasis by suppressing the expression of suppressor of cytokine signaling 1 (SOCS1). MiR-146a regulates the expression of signal transducer and activator of transcription-1 (STAT1) and plays a critical role in Treg-mediated control of T_H1 response.[6] The dysregulated expression of miR-146a and miR-155 is frequently reported in RA, and hence Alla Skapenko and colleagues aimed to investigate the regulation of miR-146a and miR-155 in the Tregs of patients with RA and their regulation of Treg functions and disease activity.[13]

By using freshly isolated peripheral blood mononuclear cells from RA patients and healthy donors, the authors first explored the basal levels of miR-146a and miR-155 in $CD25^+$ Tregs and $CD25^-$ non-Tregs. Further, both cell populations were stimulated for 24 and 48 h using anti-CD3/CD28 monoclonal antibodies. The authors observed that compared with healthy controls, miR-146a expression was significantly

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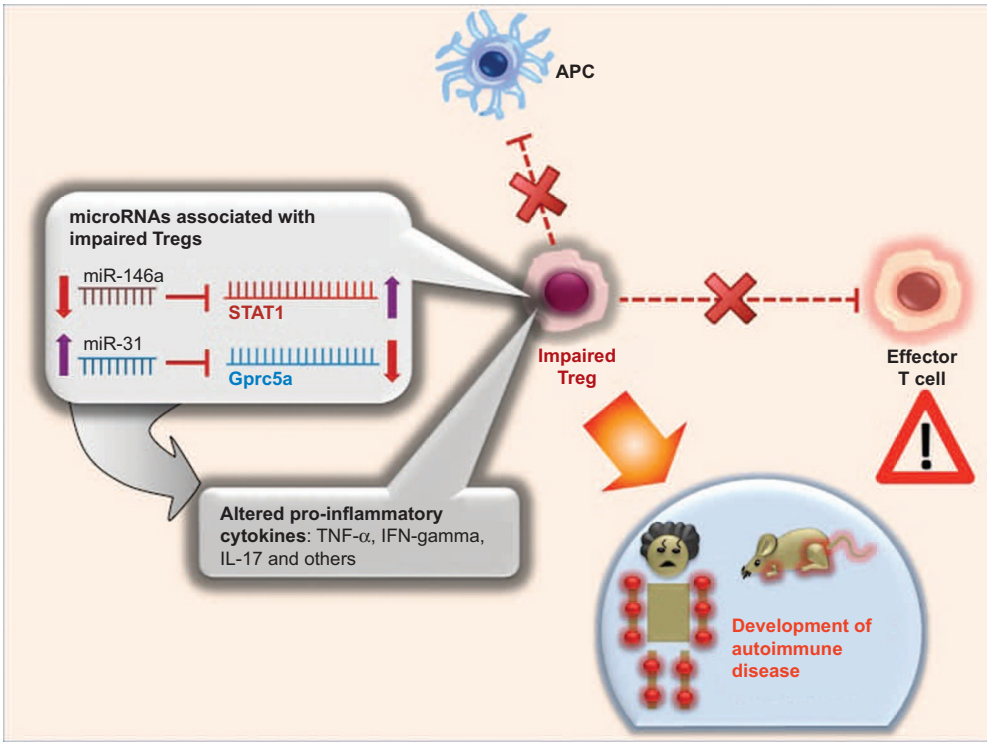

Figure 1 MicroRNAs contribute to impaired regulatory T cell function in autoimmune diseases. Tregs are critical for maintaining immune tolerance. However, Tregs are reported to be defective in their functions in several autoimmune diseases, which could be attributed to changes in the expression of miRNAs, such as miR-146a and miR-31, that modulate the expression levels of STAT1 and Gprc5a, respectively, leading to inflammatory cytokine responses.

suppressed in Tregs isolated from RA patients but not in $CD25^{-}$ T cells. In contrast, the expression of miR-155 was upregulated in both populations. Further investigations suggested that the clinical parameters of RA disease activity were negatively correlated with the expression levels of miR-146a but not with miR-155. For instance, RA patients with lower disease activity expressed higher miR-146a in Tregs, and vice versa.[13]

The expression levels of miR-146a and miR-155 are positively regulated by the nuclear factor kappa B (NF-κB) pathway. However, these miRNAs control the NF-κB pathway in a negative feedback loop. While miR-146a suppresses the expression of IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6), miR-155 targets IκB kinase epsilon (IKKε). Therefore, the authors aimed to investigate the effect of the above miRNAs on the regulation of the NF-κB pathway. Using gene expression-based analysis of IRAK1, TRAF6, and IKKε in the Tregs of RA patients, they observed that there was no correlation between the miR-146a and miR-155 levels and the expression of NF-κB pathway molecules.[13] Furthermore, the authors also analyzed the expression of two additional genes, STAT1 and SOCS1, that are known targets of miR-146a and miR-155, respectively. They determined that upon T-cell antigen receptor stimulation, STAT1, and SOCS1 expression levels are upregulated. However, STAT1 was directly correlated with the expression level of miR-146a in RA patients with active disease but not in patients with low disease activity (Figure 1). In contrast, SOCS1 expression was not correlated with the expression of miR-155, suggesting the involvement of another mechanism that regulates SOSC1 expression in RA.

Considering the alterations in the expression of selective genes and miRNAs in RA patients, the authors investigated the modulation of the secretion of pro-inflammatory cytokines such as interferon- gamma, TNF-α, and IL-17. They observed that the expression levels of these cytokines were upregulated in the Tregs of RA patients with active disease but not in patients with low disease activity or in healthy controls. The authors further verified the expression of pro-inflammatory cytokines in Tregs via modulating the miRNA profile using mimic and/or antagomir methods.[13] They determined that the miR-146a mimics suppressed the expression of pro-inflammatory cytokines and STAT1 and also observed the reverse effect with antagomirs. However, miR-155 mimics induced cytokine production. The combination of mimics or antagomirs of miR-146a and miR-155 showed similar results to the ones observed with miR-146a alone.

In conclusion, this study determined that the suppression of miR-146a in RA facilitates the activation of STAT1, which contributes to the pro-inflammatory phenotype of Tregs. These observations also suggest that miRNAs are the master regulators of RA pathogenesis and are functionally involved in the regulation of the Treg phenotype by modulating the target gene expression. It is not known whether the reduced expression

of miR-146a in the Tregs of RA patients with active disease was a repercussion of signaling by inflammatory cytokines. As neutralization of TNF-α has been shown to rescue the defective functions of Tregs in RA,[9–11] it would be worth exploring whether such effects are associated with the modulation of miR-146a and other miRNAs.

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