A narrative review of the role of the Notch signaling pathway in rheumatoid arthritis

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Background and Objective: Rheumatoid arthritis (RA) is a chronic autoimmune disease affected by genetics and the environment factors. Its early diagnosis and treatment are difficult, and the infection risk is serious. The treatment effects for most patients were not significant, which has become a difficult challenge to overcome. Cell signals play an important role in regulating basic cellular activities such as immunity. Notch signaling is a near secretory signal that can affects many processes of cell normal morphogenesis, including the differentiation of pluripotent progenitor cells, apoptosis, cell proliferation and the formation of cell boundary. In addition, the expression and activation of Notch signaling are increased in the synovial cells and vascular endothelial cells of RA patients. The purpose of this review was to elucidate the related mechanisms of Notch signaling in RA progression, as well as the potential therapeutic value of Notch signaling in a variety of autoimmune diseases.

Methods: Literatures about Notch signaling and RA were extensively reviewed to analyze and discuss.

Key Content and Findings: This article briefly reviews the role of Notch signaling in RA. It also summarizes the functional role of Notch signaling in the treatment of RA, with the goal to provide a new treatment option for RA patients.

Conclusions: In this review, the approach we discussed focuses on Notch signaling as a potential therapeutic target against RA, enriching therapeutic strategies for inflammatory diseases including RA.

Keywords: Rheumatoid arthritis (RA); Notch signaling; cytokines; T helper cells (Th cells); macrophages

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease affected by heredity and environmental factors and is mainly chronic (1). The main clinical features of RA are synovial hyperplasia and pannus formation, resulting in irreversible joint injury and severe disability. Patients usually present with symmetrical polyarthritis of small joints in the limbs, accompanied by morning stiffness and occasionally systemic symptoms (2,3). The main pathological features of RA are macrophage and lymphocyte infiltration, synovial

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Table 1 The search strategy summary

Items	Specification
Date of search (specified to date, month and year)	25, October 2021
Databases and other sources searched	PubMed, Web of Science, and EMBASE databases
Search terms used (including MeSH and free text search terms and filters) (note: please use an independent supplement table to present detailed search strategy of one database as an example)	"Rheumatoid arthritis", "Notch signaling pathway", "macrophages", and "Inflammation/infection"
Timeframe	Up to December 2021
Inclusion and exclusion criteria (study type, language restrictions, etc.)	None
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Yue Zhuang and Wenke Lu conducted the selection independently; consensus was obtained by all researchers' discussion
Any additional considerations, if applicable	None

fibroblast proliferation and joint destruction (4). The incidence rate of RA is approximately 0.5–1% (5), while the incidence rate in mainland China is 0.42%, and the total number of patients living with RA is approximately 5 million (6). The peak age of occurrence for RA is 20–45 years old and is mostly in women, with the prevalence rate of men to women of approximately 1:4 (7). Thus far, the pathogenesis of RA is unclear. At present, it is generally accepted that genetic and environmental factors play a key role in the prevalence of RA, and the risk of RA in patients with a family history increases 3–5 times (8). The influence of the environment on RA is divided into internal and external factors. Similar to other autoimmune diseases, the impact of intestinal microbiota on disease risk and progression in RA also plays a very important role (9). External factors such as eating habits and lifestyle are also associated with RA. A higher incidence of RA occurs on a low-vitamin, high-carbohydrate, meat, high-protein, and high-speed rail diet. Smoking is considered to be one of the most serious external risk factors for RA development. The current study highlights the pathophysiology of RA patients associated with smoking, such as the occurrence of oxidative stress, the release of inflammatory cytokines and the altered epigenetics (10). Epidemiological and animal model studies confirmed the association between smoking and RA (11). If the treatment is not sufficient, it will bring irreversible lifelong disability to the patient. Unfortunately, the diagnosis and treatment effect of some RA patients is not ideal, and biotherapy also increases the risk of infection. Therefore, it is very important to understand the pathogenesis of RA and find new treatment strategies.

Notch signaling, a near secretory signal that mediates intercellular signal transmission through receptor-ligand interactions between adjacent cells (12), is a highly conserved regulatory signaling pathway present in all mammalian cells (13). Notch signaling is involved in proliferation, survival and differentiation during embryonic development and survival (14,15). Previous studies have focused on the activation of Notch signaling in synovial cells (16), blood vessel endothelial cells (17) and peripheral blood lymphocytes (18) of RA patients. In addition, the Notch-1 intracellular domain (N1ICD) and Notch-3 intracellular domain (N3ICD) were upregulated in the synovium of RA patients and collagen-induced arthritis (CIA) rats, and the Notch-4 signaling pathway is involved in RA synovial fibroblasts (19). In this review, we focus on the different conditions and interactions of Notch signaling within RA pathogenesis, and we also discuss using Notch signaling as a targeting challenge against RA. We present the following article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-142/rc)

Methods

The information used to write this paper was collected from the sources listed in *Table 1*.

Discussion

Notch signal pathway

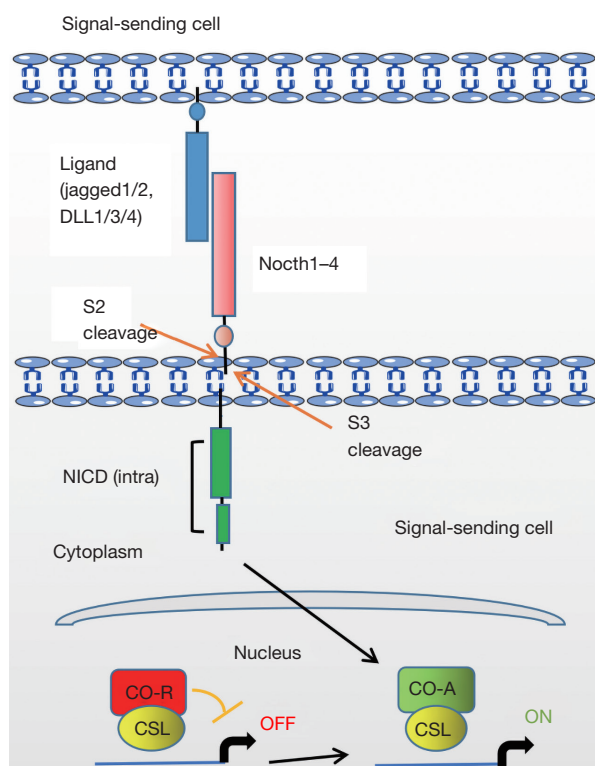The Notch family contains four transmembrane receptors

Figure 1 Basic cascade of intracellular Notch signaling. DLL, Delta-like; NICD, Notch intracellular domain; CSL, CBF1/Su(H)/LAG1; CO-R, co-repressor; CO-A, co-activator.

(Notch1–4), which interact with Notch ligands. Classical or typical Notch ligands are single pass membrane proteins Jagged1 and jagged2 and Delta-like (DLL)1, DLL3, and DLL4 (20,21). Once the Notch ligand binds to the Notch receptor on adjacent cells, triggering intracellular Notch signaling cascade (*Figure 1*), including: (I) the cleavage of extracellular a disintegrin and metalloprotease (ADAM) family protease; (II) the cytoplasmic cleavage of Notch intracellular domain (NICD) by the γ-secretase; (III) NICD translocates into the nucleus, which interacts with the CBF1/Su(H)/LAG1 (CSL) family of transcription factors to activate its downstream targets, such as Hes family (22). Many Notch target genes have recently been identified in different cell types, which has been considered to be the main way of Notch activation.

Mechanisms of Notch signaling in the RA progression

Effect of Notch signaling on cytokine secretion in RA
Cytokines are signaling molecules that coordinate the systemic or local immune response of the body.

Unfortunately, dysregulation of cytokine release and signaling mediates pathogenic effects (23). Notch signaling regulates embryonic development, differentiation processes and tissue homeostasis in multiple organ systems (14). Thus, dysregulation of Notch signaling is associated with inflammatory disease processes, including RA (24), atherosclerosis (25), systemic lupus erythematosus (26), systemic sclerosis (27), experimental autoimmune encephalomyelitis (28), and infectious diseases (29,30). Recent studies have shown that Notch signal expression and activation can stimulate synovial cells, thereby accelerating the production of proinflammatory cytokines in RA (16,19,24,31,32). Also, overexpression of N1ICD in mouse macrophages increases the content of the proinflammatory cytokines tumor necrosis factor α (TNF-α) and interleukin (IL)-6 (33). Notch signaling has also been shown to mediate TNF-α-induced IL-6 production in fibroblast-like synovial cells in RA, exacerbating RA (34). On the other hand, in primary human and mouse macrophages, interferon γ (IFN-γ, the most potent macrophage activator) strongly enhances jagged-1 expression and reduces DLL1 and DLL4 expression (35). These results indicate that Notch signal activation promotes cytokine secretion and aggravates RA.

The relationship between Notch signal and oxygen in RA
Changes in oxygen content and inflammogenesis in the synovium of arthritis have been fully shown to play critical roles in the progression of RA (36). In addition, it has been reported that the synovial tissues of RA patients are relatively anoxic compared to the noninflammatory synovial tissues of non-RA patients (37). In inflamed joints, oxygen levels were negatively correlated with vascular distribution, oxidative damage, and synovial inflammation levels (38,39). Hypoxia regulates hypoxia-inducible factors (HIFs) and vascular endothelial growth factor (VEGF). VEGF and other key genes induce angiogenesis and promote cell growth (40). For instance, hypoxia promotes cell proliferation of RA-fibroblast-like synoviocytes (FLSs) and RA angiogenesis by increasing glucose-6-phosphate isomerase (G6PI) (41). It has also been reported that hypoxia can induce autophagy (42). Hypoxia stress is mediated by HIF-1α and is a strong signal for the initiation of autophagy (43). Low PO_2 levels in synovial tissues were inversely correlated with NICD expression *in vivo*, and Notch-1/HIF-1α interactions mediated hypoxia-induced angiogenesis and invasion of RA (44). Meanwhile, Notch-1 and Notch-3 are involved in hypoxia-induced activation of RA synovial fibroblasts (45). Furthermore, the contents

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of HIF-1α, N1ICD and N3ICD are highly expressed in synovial tissues of RA patients. *In vitro*, loss of Notch-1 and Notch-3 inhibited hypoxia-induced invasion and angiogenesis in rheumatoid arthritis synovial fibroblasts (RASFs). The overexpression of N1ICD and N3ICD promotes this process. In addition, Notch-1 mediates RASF migration and epithelial-mesenchymal transformation (EMT) under hypoxia conditions, while Notch-3 mediates antiapoptotic and autophagy processes. Furthermore, animal models of CIA have shown that N1ICD and LY411575 (N3ICD inhibitor) have therapeutic effects in improving symptoms and disease severity (46). The above studies indicate that hypoxia promotes the incidence of RA, while hypoxia also activates Notch signaling, so Notch signaling plays an important role in the pathogenesis of RA.

The relationship between Notch signaling and T cell differentiation

Notch signaling not only regulates cell fate during development but also affects the growth and survival of progenitor cells. In the immune system, Notch plays a crucial role in maintaining hematopoietic stem cells, guiding lymphocyte development and regulating T cell differentiation and function (14,47-49). In addition to regulating lymphocyte development, Notch receptor and ligand interactions can also serve as antigen-presenting cells to promote T cell differentiation. Peripheral T cell activation mediated by T cell receptor (TCR) is a routine process of the adaptive immune system. Ulteriorly, the activation of CD4+ T cells is achieved by binding antigen to TCR delivered by major histocompatibility complex (MHC) class II molecules (50). Costimulatory signals between B7 (CD80/CD86) and CD28 leads to the occurrence of several downstream signaling molecular events, such as T cell activation and proliferation (51). Notch stimulates T cell effector function by kinase-dependent signaling of phosphatidylinositol 3 downstream of TCR and CD28 by promoting Akt kinase and rapamycin activation to enhancing function and survival in response to lower antigen doses (52,53). Notch signaling is mediated by enhancing protein kinase PKCθ, which is involved in TCR and CD28 signaling, as well as the regulation of actin cytoskeleton (54). In addition, Notch signaling mediates effector T cell differentiation, such as CD4+ T helper cells (Th1, 2, 9 and 17) and CD8+ T cells (55). In terms of function, Th1 cells play a crucial role in clearing intracellular pathogens and viruses and mediating autoimmune diseases. Th1 cells regulate the genealogy-specific transcription factor T-bet

and secrete the IFN-γ. Th2 cells play a crucial role in mediating the immunity of helminth parasites and allergic reactions. Differentiation of Th2 cells is induced by the cytokine IL-4 and requires the GATA3 to release IL-4, 5 and 13. Th17 cells play an important role in fighting extracellular bacterial and fungal infections and controlling body autoimmunity. IL-6 and TGF-β produce Th17 cells, which secrete IL-17 and IL-23 and regulate retinoic acid-related orphan receptor γt (ROR-γt) (56) (*Figure 2*).

Effect of Notch signaling on Th1 cell differentiation in RA
The characteristic Th1 genes, *TBX21* and *IFNG*, were confirmed as the direct targets of Notch (55,57). Depletion of IFN-γ secreted by Th1 cells in *in vivo* Leishmania mice infected with Notch-1/Notch-2 dual gene defects has been shown, but Th1 cell function has not been reported in DN-MAML transgenic or conditional RBPJκ knockout in mice (58-60). Thus, signaling that regulates Th1 differentiation is involved in Notch RBPJκ independence. Deletion of the *Jagged1* or *Mindbomb1* genes was found to be essential for the expression of functional Notch ligands without affecting Th1 cell differentiation *in vitro* (61,62). The ability of DLL1/DLL4 to promote Th1 cell differentiation has been demonstrated in several experiments, such as the reduction of anti-DLL4 antibodies *in vivo* affecting T cell secretion of IFN-γ and TNF-α (63,64). DLL1 blockade also reduced the number of Th1 cells in allograft models (65). Th cells in RA patients exhibit altered Notch receptor expression profiles and enhanced Notch signal activation compared with healthy controls (18). In addition, Notch signaling is involved in specific Th1 and Th17 types of amplification, involving Notch-3 and DLL1, so selective inhibition of Notch-3 or DLL1-mediated Notch signaling provides novel strategies for the treatment of RA (66).

Effect of Notch signaling on Th17 cell differentiation in RA
In recent years, many researchers have conducted in-depth studies on the regulation of Th17 differentiation in naive CD4+ T cells. Studies have shown that Th17 cells and proinflammatory cytokines produced by Th17 cells, such as IL-17A, IL-17F, IL-21, and IL-22, are associated with various autoimmune and inflammatory diseases (67,68). The pathogenic role of the cytokines IL-17 and Th17 cells has been found in several autoimmune diseases, including RA (69), primary sclerosis (70), psoriasis (71), Crohn's disease (72), and systemic lupus erythematosus (73). IL-17 expression is increased in both the serum and articular fluid of patients with active RA, which in turn promotes the secretion of various cytokines by synovial cells, enhances osteoclast activity, inhibits chondrocyte synthesis, and
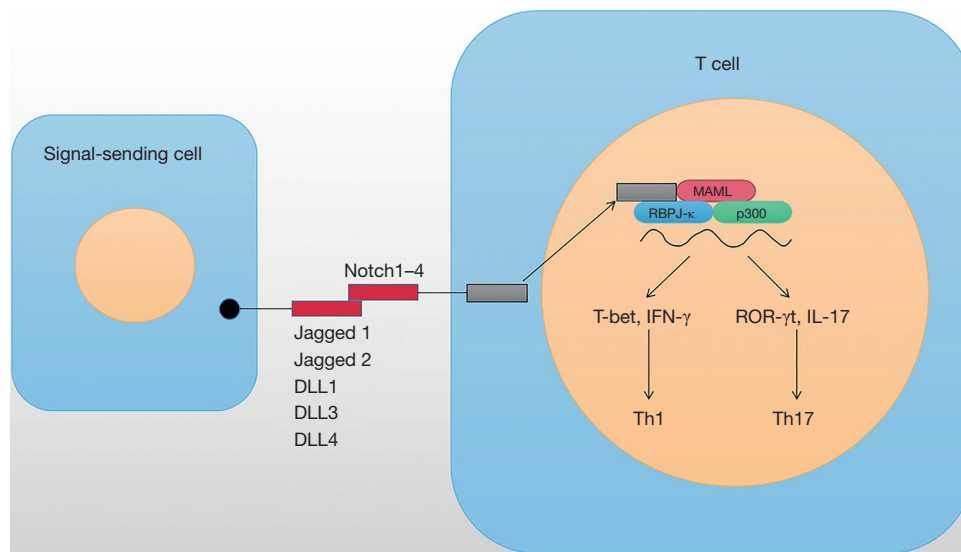
Figure 2 The role of Notch signaling in T cell differentiation. DLL, Delta-like; IL, interleukin; IFN-γ, interferon γ; ROR-γt, retinoic acid-related orphan receptor γt.

ultimately leads to bone erosion (74). Notch signaling is enhanced in RA patients, with altered expression patterns in helper T cells (18). Notch signaling regulates Th17 cell function by integrating signals supplied by cytokine such as IL-6 or TGF-β. In addition, TGF-β is a cytokine active in the gut that binds with IL-6 to promote Th17 cells (75). Studies have shown that the *ROR-γT*, *IL-17*, and *IL-23R* gene promoters are direct targets of Notch; thus, when Notch signaling is blocked, Th17 cell differentiation is impaired (68,76,77). Notch-1 binds directly to the *ROR-γT* and *IL-17* promoters to regulate Th17 differentiation (78). Notch-3 presents a critical role in antigen-specific T cell differentiation, and Notch-3 blocking inhibits the activation of Th1 and Th17 cell in CIA mice (66).

The effect of Notch signaling on macrophage in RA

Macrophages are key immune cells in the presence of host tissues that play a critical role in organismal defense and inflammation to endogenous and exogenous stimuli (79-81). Macrophages present a key role in the pathophysiology of RA. It has been reported that the number of macrophages in synovial tissue is evidently associated with disease severity when activated (82). Meanwhile, macrophages not only produce a variety of proinflammatory cytokines and chemokines but also promote cartilage and bone destruction in RA through a variety of mechanisms, showing a wide range of proinflammatory, destructive and remodeling properties, which have a significant impact

on the acute and chronic stages of RA pathogenesis (4,83,84). In addition, macrophages are influenced by RA or intercellular contact produced by innate immune cells, T cells, and fibroblasts (85). It is worth noting that the activation of aromatic hydrocarbon receptor (AHR) is related to the pathogenesis of RA. AHR agonists inhibit the expression of proinflammatory cytokines in macrophages, which are key cells in the pathogenesis of RA, suggesting specific circuits that regulate the AHR pathway in RA macrophages. However, the high expression of miR-223 in bone marrow cells prevented the inhibition of IL-β, IL-6, and TNF-α by the AHR. This connection of the miR-223/AHR pathway provides conceptual evidence for miR-223 antagonist-based RA therapies. What's more, notch-regulated miR-223 has been shown to target AHR pathways and increase cytokine production in macrophages of patients with RA (86,87). In addition, our study has shown that the imbalance between M1 and M2 macrophages is considered to be one of the main causes of RA (*Figure 3*). M1 macrophages were detected mainly in patients with severe RA, while M2 macrophages were detected in patients with mild or clinical remission of RA (88,89). M1 macrophages produce proinflammatory cytokines such as TNF-α and IL-1β, which are highly expressed in RA patients, whereas M2 macrophages produce anti-inflammatory cytokines such as IL-4 and IL-10, which are under expressed in RA patients (90). Interestingly, the interrelationship between cytokines in macrophages and
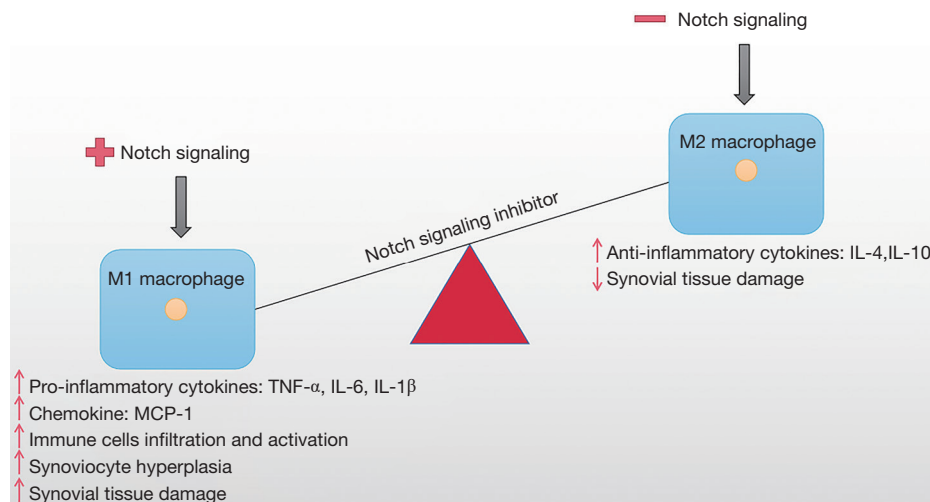
Figure 3 Notch signaling coordination of macrophage polarization in the RA synovial junctions. IL, interleukin; TNF-α, tumor necrosis factor α; MCP-1, monocyte chemoattractant protein-1; RA, rheumatoid arthritis.

Notch signaling further highlighting the key role of Notch signaling in modulating macrophage immune responses (23). Notch signaling guides the proinflammatory response of macrophages during inflammation and infection, DLL1 and Notch target genes were upregulated following Gram-positive/negative bacterial infection in human monocytes (91,92), seems to indicate that Notch signaling appears to negatively regulate immune defense mechanisms in macrophages. In RAW264.7 macrophages, miR-146 promoted the polarization of M2 macrophages and reduced the polarization of M1 macrophages by targeting Notch-1 signaling (93). These evidences clearly indicates an important role for Notch signaling in the pathogenesis of RA. Together, targeted Notch signals in the myeloid system may be a novel potential target in the pathogenesis of RA by controlling the polarized balance of M1 and M2 macrophages and reconstructing the homeostasis immune system.

The therapeutic role of Notch signaling in RA

Targeting Notch signaling was found to reduce inflammation and minimize associated tissue damage (24,94). Some enzymatic reactions mediate the regulation and activation of Notch signaling, providing potential adversarial targets for disease treatment. For example, γ-secretase inhibitors prevent Notch receptor cleavage, and specific antibodies block either Notch receptors or ligands. It is important that crosstalk between Notch and other signaling provides an opportunity for a combination therapy that can target many pathways simultaneously, which may increase therapeutic effectiveness (95). Notch-activated M1 macrophages reduce joint tissue injury in mice with inflammatory arthritis (96). The Notch signaling inhibitor LY411575, which inhibits both Notch-1 and Notch-3, was used to treat CIA in rats (97). In patients with RA, lncRNA MALAT1 is reduced and hsa-miR155-3p is elevated, coregulating changes in the Notch signaling pathway (98). Survivin alleviates RA by activating the Notch signaling pathway to promote RA fibroblast-like synovial cell proliferation and the expression of angiogenic-related proteins and inhibit apoptosis (99). Unfortunately, no single clinical product to date can safely and efficiently target Notch patients. Therefore, further studies are needed to elucidate the biological significance of Notch signaling. We need to discover new strategies (e.g., new targets, new antagonists) to provide a promising treatment for immune diseases such as RA.

Conclusions

Notch signaling is an evolutionarily highly conserved intercellular signaling pathway, and an increasing number of studies have confirmed that Notch signaling is a promising target for pathological therapy by regulating the development and function of immune cells. Therefore, it is important to understand the dynamic balance of beneficial and harmful activation of Notch signals, especially for the

study of the mechanism. A large body of evidence supports the role of Notch signaling in the inflammatory response of macrophages and helper T cells to various stimuli. Due to its ease of pharmacological application, treatment targeting Notch signaling in multiple pathological conditions may provide a promising strategy to improve inflammation. Evidence shows that targeting Notch signaling can reduce tissue inflammation and minimize tissue injury under many pathological conditions, including RA. Unfortunately, the use of Notch signal targeting remains many challenges against immune diseases. More in-depth research is needed to clarify the mechanism of various Notch receptors and ligands in RA, which will make it easier to understand how Notch signal drives the body's immune response and contribute novel therapeutic options for inflammatory diseases. In conclusion, Notch signaling in RA and other autoimmune diseases is a promising therapeutic target, which will help us to better develop effective therapeutic strategies for related inflammatory diseases in the future.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-142/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-142/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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