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Using flavonoids as a therapeutic intervention against rheumatoid arthritis: The known and unknown

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ABSTRACT

Rheumatoid arthritis (RA) is an intricate autoimmune disease involved in numerous factors. Aberrant immune responses at joint sites are considered primary in the pathogenesis of RA. The complex interactions may occur between distinct immune cells, aiming at amplifying and accelerating inflammatory responses in inflamed joints. At present, gut-joint axis hypothesis holds the idea that RA originates in the gut as a result of coactions between the intestinal immune cells and dysbiotic microbiota. Dysbiosis causes intestinal inflammation and alterations in intestinal permeability, which provides a pathological basis for the transfer of activated intestinal immune cells and their products to the joints through systemic circulation or other ways. Some therapeutic options widely utilized for the treatment of RA are associated with gut-joint axis, suggesting modulation of gut-joint axis may be a promising strategy in preventing and treating RA. Flavonoids are a type of polyphenol widely existed in herbs and foods showing anti-RA potentials. However, the mechanisms by which flavonoids mitigate RA have not been well organized. In this review, we outline and discuss current understanding of the underlying mechanisms of anti-RA flavonoids through immunoregulation, gut-joint axis, and inflammatory responses, providing a reference for developing novel strategies for the treatment and prevention of RA.

1. Introduction

Rheumatoid arthritis (RA) refers to a chronic autoimmune disorder of unknown etiology. The capital features of RA are persistent synovitis accompanied by extra-articular organ involvement and autoantibodies including rheumatoid factor (RF) and anti-citrullinated peptide protein antibodies (ACPAs) production. Genetic background and environmental exposure are the risk factors that contribute to the development of RA [1]. Epidemiological data have shown that RA affects 0.5-1.0% of adults, and women and elderly people are susceptible to the disorder [2]. Clinically, the primary therapeutic agents of RA, including disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and glucocorticoid, mitigate synovitis and systemic inflammation and relieve the pain of RA patients. However, these drugs can't produce adequate effects on the improvement of the disorder and their serious side effects including hepatorenal toxicity also restrict their clinical application [3]. Lately, biological agents are able to apply as arthritis is out-of-control or toxic effects arise with DMARDs. Owing to nasty infections and high costs, their use remains limited [3]. Fundamentally, the application of anti-RA drugs is restricted as a consequence of the pathogenesis of RA that is not well understood.

Perturbations in immune homeostasis in joints is considered dominate in RA. It has been well reported that a variety of immune cells contribute significantly to maintaining the immune function homeostasis of host and to the pathogenesis of RA [4]. In individuals with established RA, substantial data have reported that infiltration and aberrant activation/inhibition of immune cells, such as T cells, B cells, and macrophage s, are abundant in synovial tissues, which contributes to initiating and perpetuating joints inflammatory milieu [5]. The complex interplays that may occur among different immune cells, intending to expand and facilitate the inflammatory response involving joints [6]. At present, extraordinary progress has been made in the development of drugs targeting immune cells. Data came from experimental arthritis models and clinical diagnosis in individuals with RA prove that these target-specific drugs can significantly reduce pro-inflammatory cytokines, RF, ACPAs, and C-reactive protein in peripheral blood and synovial fluid [7,8]. Nevertheless, the relevance between their pharmacological effects and the reduced biomarkers of RA warrants further investigation.

The gut is generally considered to be the largest immune organ of the host owing to the maximum innate and adaptive immune cells inhab-

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itation [9]. The intestinal microbiota that resides within the host gut mucous membranes comprises over 100 trillion bacteria. Compelling evidence supports that the normal gut microbe is essential for maintaining enteral and parenteral immune responses [10–12]. However, dysbiosis occurs frequently as gut microbiota is susceptible to environmental and host-related factors, thereby resulting in gut inflammation and leakiness [13], triggering bacteria penetration into the extra-intestine. Subsequently, with dysfunctional microbiota stimulation, the intestine-derived immune cells are activated and secrete pro-inflammatory cytokines, which can alter local and follow-up systemic immune responses, eventually boosting the pathogenesis of RA [14]. Mounting data conducted in different mouse models of RA and individuals with RA prove that the intestinal dysbiosis, together with intestinal inflammation and leakiness can potentially bring about arthritis [15–20]. The gut-joint axis hypothesis which holds the idea that RA begins in the intestine has recently been put forward [21]. Data reinforcing the hypothesis are that a number of intestinal innate and adaptative immune cells are amplified and activated in synovium and systemic circulation of RA patients [22]. However, few therapeutic methods have focused on gut-joint axis so far.

Flavonoids refer to a series of compounds formed by the interconnection of two phenyl rings with phenolic hydroxyl groups through the central three carbon atoms [23]. They can mainly be classified into flavone, flavonol, flavonone, flavanonol, anthocyanidin, and chalcone [24] (Fig. 1). Flavonoids with wide distribution and relatively low toxicity can be consumed safely in diet and show potent anti-inflammatory and anti-oxidant effects. Moreover, the activity of flavonoids antioxidants is in strong correlation with their chemical structures [25,26]. Flavonoids intervention with low cost is widely used in clinical treatment of various diseases, such as neurodegenerative diseases, cancers, and eye diseases [27–30]. Emerging evidence has revealed that flavonoids elicit the anti-RA potentials. Flavonoids improve symptoms of RA via multiple targets involved in immunoregulation, modulation of gut-joint axis, and inhibition of inflammatory responses.

In this review, we seek to outline the current understanding of the underlying mechanisms of anti-RA flavonoids via immune responses and gut-joint axis, providing a reference for developing novel strategies for treating and preventing RA. The literatures selected is based on a search of Pubmed between 2011 and 2021 and all doses of flavonoids presented in literatures are in accordance with the range given in the consensus document [31].

2. The roles of flavonoids in immune responses

It is well known that the main symptoms of RA are chronic inflammation in the joints, which leads to joint destruction [32]. Defining critical cellular subtypes and their activation/inhibition states in the inflamed joints is a key step in searching therapeutic targets for RA [4]. T cells, B cells, and macrophages have been established relevance to RA pathogenesis and are found in abundance in inflamed synovial membranes of RA patients [33–36] (Fig. 2). The mechanisms of flavonoids targeting these immune cells to ease the symptoms of RA are summarized in Table 1.

2.1. The roles of flavonoids in T lymphocytes

$CD4^+$ helper T (Th) cells engages in the development of RA and mainly differentiate into three subtypes with distinct immunological function. Th1 cells, originated from naïve Th cells that are mainly driven and induced by interleukin (IL)-12 produces interferon (IFN)-γ and tumor necrosis factor (TNF)-α [37] and plays an important role of pro-inflammatory. IL-4 produced by basophils, eosinophils, and mature Th2 cells is the primary signal for Th2 cells lineage differentiation. Th2 cells produce anti-inflammatory cytokines like IL-4, IL-5, IL-10, and IL-13 [38]. These cytokines can induce proliferation of Th2 cells while suppressing that of Th2 cells as we as maintaining the balance of Th1/Th2. Differentiation of Th17 cells is promoted by IL-6, IL-23, and IL-1β. Th17 cells are mainly secreting IL-17, which contributes to cartilage and bone destruction [39]. Regulator T (Treg) cells also contribute importantly to adaptive immune responses. Induced Treg cells are the result of peripheral T cell activation in the presence of transforming growth factor (TGF)-β costimulation and lack of pro-inflammatory cytokines [33,40]. TGF-β and IL-10 produced by Treg cells have potent anti-inflammatory effects through blocking of T cells division and differentiation into Th17 [41]. In RA patients, the drifts of Th1/Th2 and Th17/Treg are widely existed [42,43]. Re-maintaining the homostasis of them is the crucial idea to develop anti-RA drugs.

Flavonoids derived from herbs and foods can effectively improve RA by modulating T cells in collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AIA) experimental models. Acacetin is a natural flavonoid extracted from *Saussurea involucrate* (Kar. et Kir.) Sch. -Bip and can significantly repress the incidence of CIA and preventing the pathological alteration by expanding Treg cells and narrowing Th1 and Th17 cells in spleen and inguinal lymph nodes [44]. Cinnamtannin D1, a flavonoid derived from the food spice *Cinnamomum tamala*, elicits potent anti-RA property by regulating Th17/Treg balance in mice with CIA [45]. Quercetin, a flavonoid widely existed in fruits and vegetables, shows robust anti-arthritic effect in pre-clinical and clinical studies with unknow underlying mechanisms. Yang et al. report that quercetin can substantially yield an obvious mitigation of arthritic manifestations by decreasing the percentage of Th17 cells and increasing that of Treg cells [46]. Naringin, a well-known flavanone glycoside found in citrus fruits, can dramatically regulate Th1/Th2 balance to improve autoimmune arthritis in AIA mice [47]. Taken together, flavonoids can effectively ameliorate the symptoms of RA by regulating the homostasis of T cells.

2.2. The roles of flavonoids in B lymphocytes

The production of RF and ACPAs is one of the characteristics of RA, and can even occur earlier than clinical symptoms. Stimulated by antigen in synovial tissues, B lymphocytes proliferate and differentiate into a large number of plasma cells, which can synthesize and secrete antibodies as well as pro-inflammatory mediators and circulate in the peripheral blood [48,49]. Recently, B cells depletion therapy in RA has led to some surprises, however, the potential side effects can't be neglected [50–52].

Epigallocatechin gallate, a bioactive flavonoid in green tea, can effectively inhibit the migration of B cells to extravascular space, including joints and may be a promising drug for RA treatment [53]. Epigallocatechin-3-gallate is also a bioactive flavonoid derived from green tea and have a therapeutic effect in CIA rats by depleting B cells [54]. In brief, using flavonoids intervention targeting B cells is an effective approach to ameliorate the symptoms of RA.

2.3. The roles of flavonoids in macrophages

Macrophages that are differentiated from peripheral blood monocytes get involved in the initiation and perpetuation of inflammation, adhesion and migration of leukocyte, and degradation of matrix. It has been revealed that the inflamed synovial membrane and the cartilage-pannus junction are populated by macrophages [36], suggesting these cells may intimately associated with the pathogenetic cascade of RA. Interestingly, Macrophages are highly plastic and can switch from one phenotype to another. Stimulated by lipopolysaccharide (LPS) or IFN-γ, macrophages can differentiate into M1 subtypes, which can produce reactive oxygen species (ROS), nitric oxide (NO), and a large number of pro-inflammatory cytokines, showing pro-inflammatory effects and subsequently causing articular cartilage injury. In the presence of IL-4 or IL-13 stimulation, macrophages can differentiate into M2 subtypes. M2 secrete anti-inflammatory cytokines and promote synovial tissues repair [36,55]. Although treatment of RA with anti-macrophage approaches is

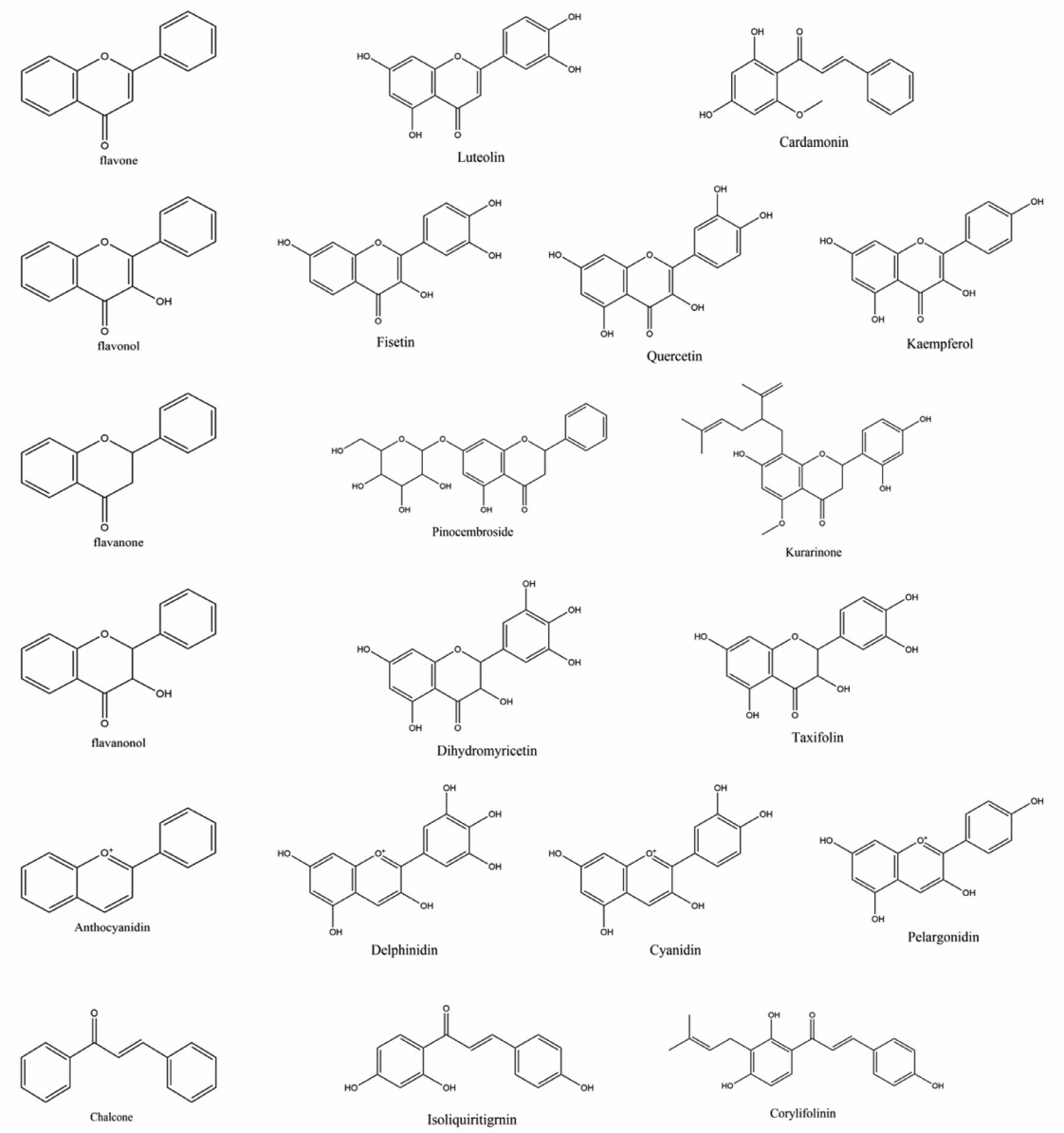

Fig. 1. The structures of flavonoids and their representative compounds.

theoretically therapeutic, regulation of macrophages polarization seems to be more promising for the treatment of RA.

Flavonoids also possess anti-RA potentials by regulating macrophages polarization. Hesperidin, a natural flavonoid widely presented in citrus fruits, can significantly relieve the symptoms of RA by inhibiting the polarization of macrophages to M1 [56]. Similarly, malvidin-3-O-β glucoside derived from fruits can also ease RA symptoms by inhibiting the polarization of macrophages to M1 [57]. Silibinin, a natural polyphenolic flavonoid extracted from the herb *Silybum marianum*, can induce the macrophages M2 polarization to mitigate the symptoms of RA [58]. Thus, flavonoids can effectively ameliorate the symptoms of RA by modulating macrophages polarization.

3. The roles of flavonoids in gut-joint axis

The gut-joint axis consists of two consecutive steps. First, the occurrence of dysbiosis accompanied by intestinal inflammation and barrier dysfunction provides the opportunity for the interactions between intestinal immune cells and dysbiotic microbiota. Second, the activated intestinal immune cells with the abilities of pro-inflammatory cytokines production enter the joints through systemic circulation or other ways, thus leading to the pathogenesis of RA (Fig. 3). Therefore, there are two therapeutic targets for RA through gut-joint axis: (1) improve intestinal dysbiosis; (2) inhibit intestinal immune cells proliferation or trafficking.

3.1. The roles of flavonoids in intestinal dysbiosis

Intestinal dysbiosis is an aberrant microbial ecological state characterized by pathobionts bloom, commensals reduction, and diversity loss. Enteric inflammation, diet, and xenobiotics are the main factors that contributes to dysbiosis [65]. Substantial data have demonstrated that dysbiosis occurs in different experimental mouse models of RA and individuals with RA. For example, Rogier et al. have demonstrated that gut microbiota undergoes significant changes in the preclinical phase of CIA, as characterized by reduced abundance of *Bacteroidetes* and raised that of *Firmicutes* [66]. Some cohort studies have also proved that great alterations in the intestinal microbial community are presented in individuals with new-onset and established RA [67–70]. Data from fecal

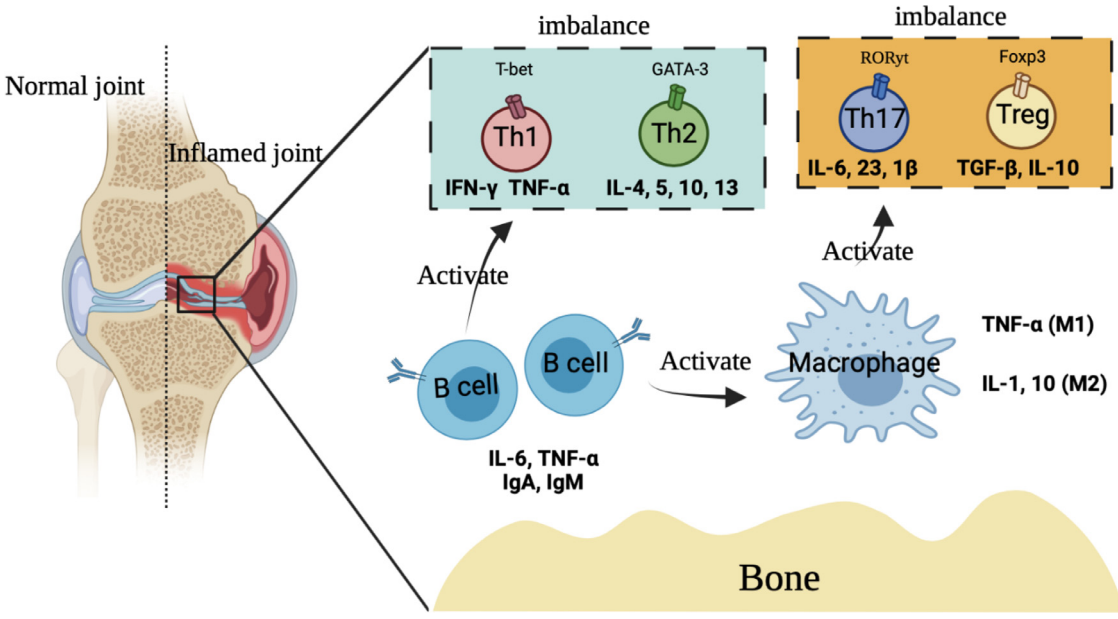

Fig. 2. The interactions among immune cells in inflamed joints. T cells, B cells, and macrophages contribute essentially to rheumatoid arthritis (RA). T cells differentiate into several subtypes that produce different cytokines, primarily including Th 1, Th2, Th17 and Treg cells. B cells with pro-inflammatory cytokines secretion and autoantibodies production can activate Th1 and macrophages. Macrophages encompass two subtypes, M1 and M2, and can activate Th17 cells.

Table 1
The roles of flavonoids in T cells, B cells, and macrophages.

Flavonoid name	Experimental models	Biological findings	Reference
Acacetin	Male DBA/1J mice with CIA	Treg cells ↑, Th17 cells↓, Th1 cells↓, regulate Th17/Treg	[44]
Cinnamtannin D1	Male DBA/1J mice with CIA; Naïve CD4 cells with TGF-β, IL-6, anti-IL-2/4, anti-IFN-γ stimulation or TGF-β, IL-2, anti-IL-4, anti-IFN-γ stimulation	Treg cells ↑, Th17 cells↓, regulate Th17/Treg; Treg cells differentiation ↑, Th17 cells differentiation ↓	[45]
Quercetin	Peripheral blood mononuclear cells with CD3, anti-CD28, IL-23, IL-6, IL-1β, anti-IL-4, anti-IFN-γ stimulation; Female Wista rats with CIA	Th17 cells differentiation ↓; Treg cells ↑, Th17 cells↓, regulate Th17/Treg	[46,59]
Epigallocatechin-3-gallate	Male DBA/1J mice with CIA; IFN-γ KO mice with CIA; Male SD rats with AIA	Treg cells ↑, Th17 cells ↓, regulate Th17/Treg; B cells ↓	[53,60]
Icariin	Male C57BL/6 mice with CIA	Th17 cells ↓	[61]
Anthocyanin	Male DBA/1J mice with CIA; Human $CD4^+$ T cells with anti-CD3, anti-CD28, anti-IFN-c, anti-IL-4, IL-1β, IL-6 stimulation	Th17 cells ↓, Th17 cells differentiation ↓	[62]
Silibinin	Female Wista rats with CIA; Naïve CD4+CD62L+ T cells with anti-CD3, anti-CD28, TGF-β, IL-6, anti-IFN-γ, anti-IL-4 stimulation; RAW264.7 cells with LPS, IFN-γ, or IL-4 stimulation	Th17 cells ↓, Th17 cells differentiation ↓; Macrophage M2 polarization ↑	[58]
Oroxylin	Male DBA/1 mice with CIA	Treg cells ↑, Th17 cells↓, regulate Th17/Treg radio	[63]
Naringenin	Male DBA/1J mice with CIA	Th1 cells ↓, Th17 cells ↓	[64]
Naringin	Female Balb/c mice with AIA	Th1 cells ↑, Treg cells ↑, Th2 cells↓	[47]
Kurarinone	Male DBA/1 mice with CIA	Th1 cells ↓, Th17 cells ↓	[64]
Hesperidin	RAW264.7 cells with LPS stimulation	Macrophage M1 polarization ↓	[56]
Malvidin-3-O-β glucoside	RAW264.7 cells with LPS stimulation	Macrophage M1 polarization ↓	[57]

CIA, collagen-induced arthritis; AIA, adjuvant-induced arthritis

transplantation and antibiotics treatment experiments in mice prove the potential cause relationship between gut microbiota and RA. Maeda et al. have shown that colonization of SKG mice with faecal microbiota from patients with RA shows an elevated susceptibility to arthritis [71]. Jubair et al. have reported that depleting the microbiota of the mice with a cocktail of antibiotics before the induction of CIA leads to a significant reduction in the severity of disorder [72].

In light of the gut-joint axis, intestinal inflammation and gut barrier dysfunction are also major contributors in the development of RA. The occurrence of dysbiosis can trigger and perpetuate the aberrant activation and expansion of intestinal innate immune cells, therefore leading to intestinal inflammation [73]. The link between dysbiosis and gut barrier is well conducted. The presence of normal gut microbiota can induce the differentiation of $ROR\gamma t^+NKp46^+$ natural killer-like cells with IL-22 production, which can promote gut integrity and inhibit bacterial infiltration [74]. However, perturbations in gut microbiota disturb the process, causing the gut barrier dysfunction [75]. Moreover, elevated serum zonulin concentrations is associated with intestinal dysbiosis [76]. Zonulin, a biomarker of gut permeability, can initiate proteinase-activated receptor 2 (PAR2)-dependent transactivation of epidermal growth factor receptor (EGFR), therefore causing tight junction disassembly [77]. Although no direct evidence shows the correlations among intestinal inflammation, gut barrier and RA, a cohort study have reported a considerable association between RA and inflam-

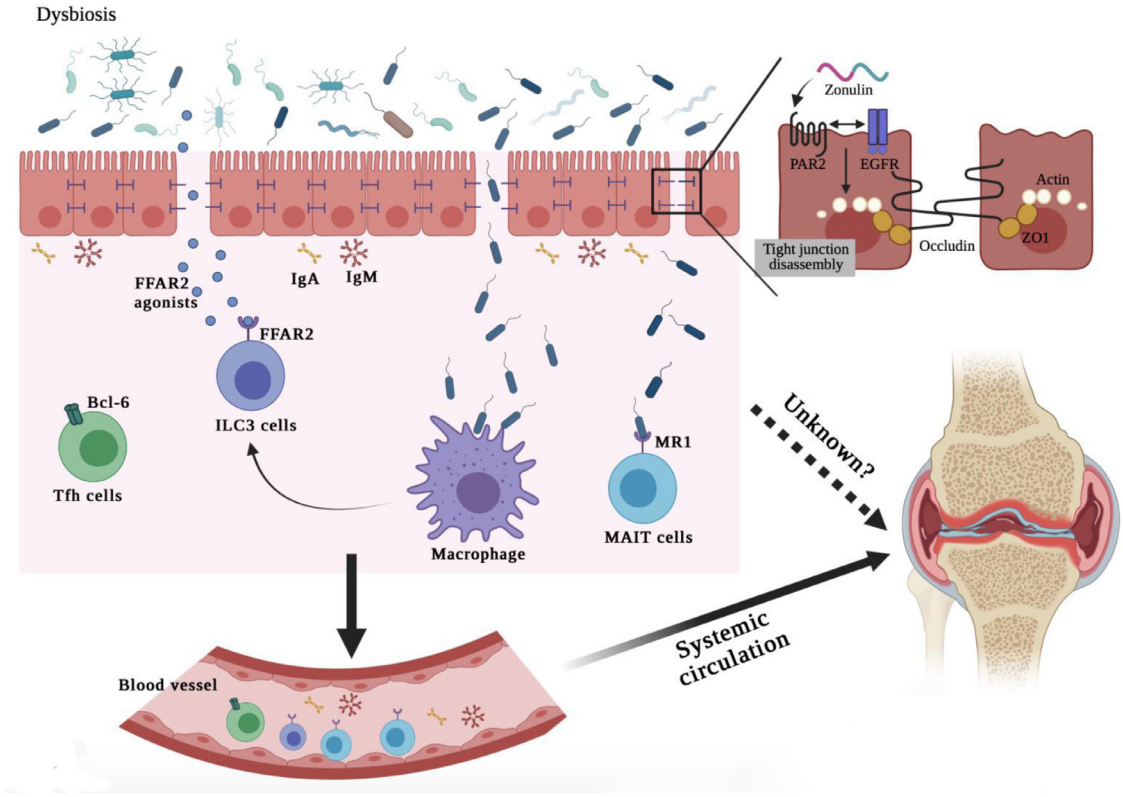

Fig. 3. The pathogenesis of rheumatoid arthritis (RA) through gut-joint axis. Intestinal dysbiosis accompanied with intestinal inflammation and gut barrier dysfunction leads to the interactions between intestinal immune cells and aberrant microbes. Subsequently, the activated intestinal immune cells such as group 3 innate lymphoid (ILC3) cells, mucosa-associated invariant T (MAIT) cells, and T follicular helper (Tfh) cells traffic to the joints via systemic circulation, therefore promoting the pathogenesis of RA.

matory bowel disease (IBD) [78]. IBD is also an autoimmune disease with similar pathogenic mechanisms of RA and characterized by chronic intestinal inflammation and gut leakiness, suggesting that intestinal inflammation and gut barrier dysfunction have a potential contributor to the development RA. Taken together, improving intestinal dysbiosis is a possible therapeutic method to improve the development of RA.

At present, Aa et al. reported that Kaempferol, a natural flavonol found in many edible herbs, elicit anti-arthritis activities by re-balancing dysbiosis in CIA mice [79]. Due to similar pathogenic mechanisms between RA and IBD, some studies have revealed that some flavonoids can effectively alleviate the symptoms of IBD, which can provide some hints for RA therapies using flavonoids intervention. Ren et al. have reported that acacetin can significantly improve the clinical symptoms of dextran sulfate sodium (DSS)-induced colitis in mice by modulating gut microbiota and inhibiting intestinal inflammation [80]. Zhu et al. have revealed that baicalin, a flavonoid extracted from the root of herb *Astmgali Radix*, can protect the rats against trinitrobenzene sulphonic acid (TNBS)-induced colitis by regulating gut microbiota, especially increasing the *Butyricimonas* abundance [81]. Phloretin, a flavonoid found in pears of fruits, shows anti-IBD effects by remodeling gut microbiota, especially enriching *Lactobacillus* [82]. Pinocembrin, a plant-derived flavonoid with anti-inflammation effects, can improve the severity of DSS-induced colitis in mice by regulating gut microbiota and repairing gut barrier [83]. Taken together, data presented above suggest that flavonoids may be a type of promising drugs for RA treatment, which warrants further development [84].

3.2. The roles of flavonoids in intestinal immune cells proliferation and trafficking

Intestinal immune cells mainly include Group 3 innate lymphoid (ILC3) cells, mucosa-associated invariant T (MAIT) cells and intestinal T follicular helper (Tfh) cells. ILC3 cells are primarily situated at epithelial barrier surfaces and involved in response to the extra-intestinal pathogens and in maintaining intestinal homeostasis. With commensal microbes and their products (such as free fatty acid receptor 2 agonism) stimulation, ILC3 cells are activated and expanded in the intestine and produce pro-inflammatory mediators including IL-17 and IL-22 [85,86]. In a CIA mouse model, ILC3 cells are significantly amplified in flamed joints [87]. In individuals with early RA, the number of ILC3 cells are significantly elevated in inguinal lymph node biopsy [88]. MAIT cells, mainly situated at mucosal and epithelial barrier, are innate-like T cells that bridge gut microbiota and intestinal immune responses [89]. They are activated by certain bacteria and subsequently produce pro-inflammation mediators. Blunted MAIT cells frequency in systemic circulation and elevated of that in inflamed tissues are common features in various autoimmune diseases [89]. In the context of RA, MAIT cells are abundant in the synovial fluid and high levels of pro-inflammation mediators can promote the migration of MAIT cells from blood to the joints [90]. Tfh cells are a subset of activated $CD4^+$ T cells which can assist the formation and maintenance of germinal centers (GC). Tfh promote B lymphocytes differentiation and autoantibodies production by secreting IL-21 [91]. The migration of Tfh cells from the intestine to the joints has been well conducted [92]. A cohort study has demonstrated that a higher percentage of circulating Tfh cells and higher serum level of IL-21 in RA patients [93]. At present, it is well understood that these cells can enter the joints through systemic circulation. Nevertheless, there may have other ways promote the communication between gut and joints, which needs further investigation. Taken together, inhibiting the proliferation and migration of intestinal immune cells is also a therapeutic way for the treatment of RA.

Although no research targeting the intestinal immune cells to improve RA using flavonoids intervention has been reported, some investigators have demonstrated that flavonoids can have a therapeutic role

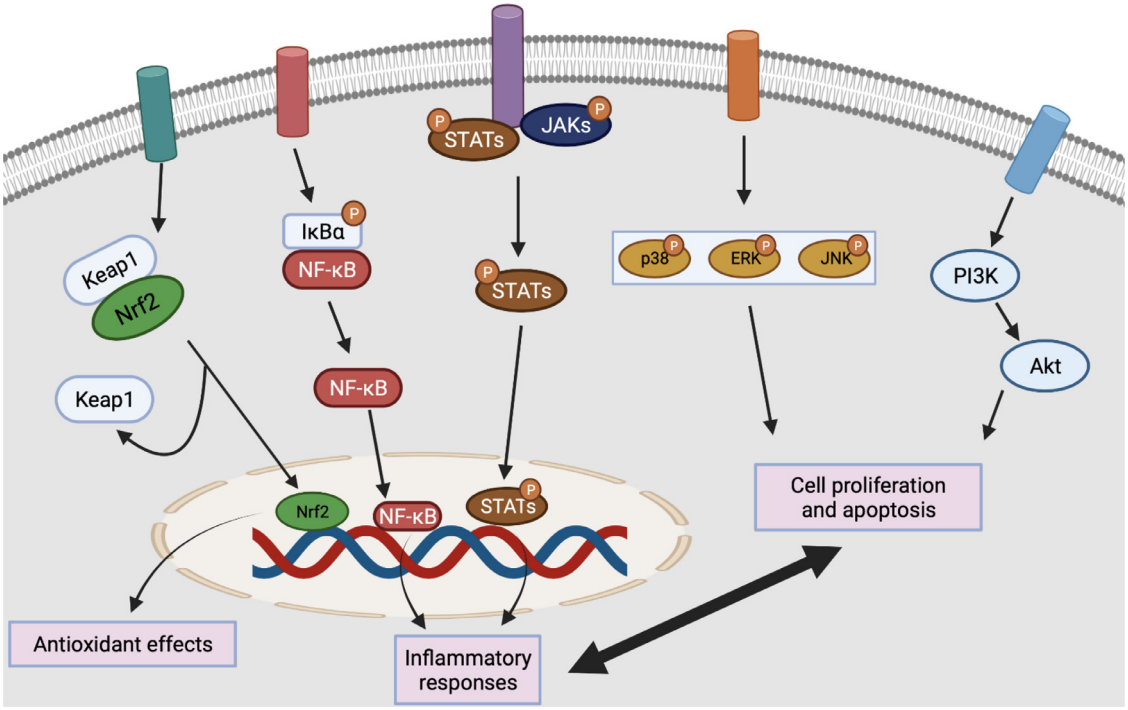

Fig. 4. Therapeutic signaling pathways of flavonoids

in some autoimmune diseases through intestinal immune cells, which is also beneficial for the anti-RA flavonoid drugs development. Yang et al. have revealed that baicalin attenuates lupus autoimmunity by inhibiting Tfh cells differentiation and pro-inflammation cytokines production [94].

4. The roles of flavonoids in inflammatory mediators

Inflammatory mediators including histamine, cytokines, chemokines, integrins, and proteases that are generated by immune cells secretion and genetic expression are responsible for bone erosion and inflammation at synovial sites and for the progress of RA. Nowadays, balancing and resolving inflammation are regarded as promising strategies for RA. Flavonoids are well-known for their robust anti-inflammatory effects and overwhelming evidence shows that flavonoids lighten the symptoms of RA through inhibiting local and systemic inflammation responses. Consumption of cinnamtannin D1 can significantly increase TGF-β and IL-10 levels while decrease those of IL-6, IL-17 and IL-1β in serum of CIA mice [45]. Hesperidin treatment can effectively suppress the expression of matrix metalloproteinases (MMP) in LPS-induced FLS cells [54]. Malvidin-3-O-β glucoside treatment can substantially inhibit the secretion of IL-1, IL-6, and NO in LPS-induced macrophages [57]. Icariin addition can dramatically inhibit the expression of β3 integrin and MMP9 in bone marrow-derived macrophage cells [61]. Administration of oroxylin A can memorably decrease the serum levels of IL-1β, IL-6, IL-17, and TNF-α [63]. Kurarinone can also markedly decrease the serum and paw tissues levels of IFN-γ, TNF-α, IL-6, and IL-17A. Taken together, flavonoids targeting inflammatory mediators can effectively improve the development of RA.

5. Therapeutic signaling pathways of flavonoids

The development of RA is an intricate process involving many signaling pathways, primarily including PI3K/Akt, MAPK, NF-κB, STAT, and Nrf2 signaling pathways (Fig. 4).

5.1. The roles of flavonoids in PI3K/Akt signaling pathway

Synovial hyperplasia and inflammation are considered to be the pathological features of RA. In the context of RA, fibroblast-like synovial (FLS) cells can secrete a plenty of inflammatory cytokines and continuously stimulate FLS cells, resulting in uncontrolled proliferation. PI3K/Akt signaling pathway is deemed to be a bridge between proliferation and apoptosis of FLS cells. In RA-FLS cells, PI3K and Akt are highly expressed, and have an effect on the excessive migration of FLS cells. Moreover, lots of inflammatory cytokines such as IL-17 and IL-21 can promote the inflammatory proliferation of FLS cells by inducing and triggering PI3K [95]. Previous studies have demonstrated that LY294002, an inhibitor of PI3K, can dramatically improve synovial hyperplasia and inflammation in mice with CIA and AIA [56], suggesting targeting PI3K/Akt signaling pathway is an effective mean to inhibit the progress of RA. Nowadays, increasing literatures have reported that flavonoids can significantly improve the development of RA in experimental mouse models through PI3K/Akt signaling pathway. Qi et al. have reported that hesperidin can significantly inhibit the expression of PI3K and p-Akt in M1 macrophages and FLS cells. Moreover, upon LY294002 intervention, the anti-arthritis effects of hesperidin are dampened in AIA mice [56]. Liu et al. have revealed that epigallo-catechin-3-gallate can suppress the expression of p-Akt in B cells of CIA rats, therefore repressing the development of RA [54].

5.2. The roles of flavonoids in MAPK signaling pathway

MAPK signaling pathway including extracellular signal-related kinase (ERK-1/2), p38 (p38$\alpha/\beta/\gamma/\delta$), c-Jun N-terminal kinase (JNK-1/2/3), and ERK5 that regulates a series of cytokines, chemokines, and enzymes. MAPK signaling pathway is hyperactive in synovial tissues and leads to persistent inflammation and abnormal hyperplasia [96,97]. The selective inhibitors targeting MAPK signaling pathway have been proposed to be an effective approach for the treatment of RA [98]. Similarly, the MAPK signaling pathway is inhibited accompanied with reduced symptoms of RA upon flavonoids treatment. Sun et al. have demonstrated that the flavonoids, extracted from the herb *Flemingia philippinen-*

sis, can improve the symptoms of RA by inhibiting the expression of p-ERK1, p-p38, and p-JNK in paw tissues of CIA mice [99]. Zhai et al. have reported that liquiritin, a flavonoid extracted from the herb *Glycyrrhiza uralensis*, can significantly inhibit the IL-1β-induced RA-FLS proliferation by down-regulating p-p38, and p-JNK [100].

5.3. The roles of flavonoids in NF-κB and STAT signaling pathways

NF-κB and STAT also contribute significantly to RA. When NF-κB and STAT are activated, they then shift to the nucleus and gets involved in the regulation of inflammatory response, cell proliferation and apoptosis [101,102]. NF-κB and STAT are profoundly and sustainably activated in CIA and AIA mice, which are improved by flavonoids. Bai et al. have reported that baicalin can alleviate CIA in rats and repress RA-FLS proliferation by down-regulating nuclear p65 expression [103]. Bao et al. have reported that Genkwanin, a flavonoid isolated from the herb *Daphne genkwa*, exerts anti-RA effects by down-regulating the expression of p-STAT3 and p-NF-κB in paw tissues of AIA mice [101].

5.4. The roles of flavonoids in NRF2 signaling pathways

Oxidative stress is involved in the pathogenesis and pathological process of RA. Upon activating Nrf2 signaling pathway, various antioxidant enzymes (such as heme oxygenase-1, HO-1) are released to regulate the oxidative stress state of RA [104]. It has been well conducted that Nrf2-deficiency increases susceptibility to RA [105] and the mice with Nrf2-knockout background shows higher levels of pro-inflammatory mediators than their wild-type littermates [106]. The Nrf2 signaling pathway is activated accompanied with reduced symptoms of RA upon flavonoids treatment. Karatas et al. have reported that epigallocatechin 3-gallate had anti-arthritic effects by up-regulating the expression of Nrf2 and HO1 in joint tissues of CIA rats [107]. Su et al. have reported that calycosin, a flavonoid isolated from the herb *Astragali Radix*, can suppress the expression of pro-inflammatory mediators in RA-FLS by up-regulating the expression of Nrf2 and HO1 [108].

6. Concluding remarks

Nowadays, gut microbiota has been a hot target in a variety of diseases. Alterations in the composition of gut microbiota have been observed in mice with CIA and individuals with established RA [21], suggesting gut microbiota may also get involved in the occurrence and progression of RA. With the advent of metagenome sequencing and the development of germ-free and humanized mouse models, the association between gut microbiota and RA has been well understood. In light of these existing data, the concepts of gut-joint axis are described, replenishing the pathogenesis of RA.

The main drawback of flavonoids is that they take effect slowly and need to be taken for a long time, which is also related to their poor absorption. Most of flavonoids are taken orally and can encounter commensal bacteria in the small and large intestine. These microbes collectively encode 150-fold-more genes than human genome [109], showing a rich enzyme repository with drug-metabolizing potentials. In fact, there are quite a number of flavonoids metabolized by gut microbiota, and the metabolites possess better absorption and more active pharmacological activities than their parent drugs. Take scutellarin, a flavonoid with high effectiveness in clinic, for example. The oral bioavailability of scutellarin, is exceptionally low. In healthy volunteers and rats, the oral bioavailability of scutellarin was found to be merely 2.2% and 0.67% respectively [110]. The physiological effects of scutellarin are in notable contrast to its poor bioavailability. Although scutellarin seems to be absorbed in the form of scutellarein, no aglycone but isoscutellarin is detected in the portal vein plasma and the plasma concentrations of isoscutellarin exceed that of scutellarin by about 30-fold. Furthermore, gut microbiota also gets involved in the process of isoscutellarin transformation and isoscutellarin shows more excellent pharmacological effects than scutellarin [110]. Taken together, focus on flavonoids gut microbiota-derived metabolites seems to be a promising method to search for novel drugs.

Although using flavonoids as a therapeutic intervention against RA is at a very initial stage and still needs a lot of pre-clinical and clinical data, researches are shedding light on flavonoids in potential clinical treatment and prevention of RA. Further studies are required specifically to define the exact step of gut-joint axis (especially the intestinal mucosal-derived immune cells trafficking step) during the pathogenesis of RA and to develop flavonoids for RA treatment with the focus on the interplays with flavonoids and gut microbiota.

Declaration of competing interest

No conflicts of interest in this article

Acknowledgements

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