



Sulforaphane Regulates Macrophage M1/M2 Polarization to Attenuate Macrophage-induced Caco-2 Cell Injury in an Inflammatory Environment

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ABSTRACT

Background: The imbalance between M1 and M2 macrophage activation is closely associated with the pathogenesis of inflammatory bowel diseases (IBDs). Sulforaphane (SFN) plays an important role in the treatment of inflammatory diseases.

Objective: To investigate the effect of SFN on macrophage polarization and its underlying regulatory mechanism.

Methods: Mouse bone marrow-derived macrophages (BMDMs) were treated with SFN and an Nrf2 inhibitor, Brusatol. M1 macrophages were induced by LPS and IFN- γ stimulation, whereas M2 macrophages were induced by stimulation with IL-4 and IL-13. LPS-stimulated BMDMs were co-cultured with Caco-2 cells. Flow cytometry, qRT-PCR, and Western blot were performed to assess macrophage polarization. Cell function was assessed using CCK8 assay, transepithelial electrical resistance (TEER) assay, and biochemical analysis.

Results: Higher concentrations of SFN resulted in better intervention effects, with an optimal concentration of 10 μ M. SFN decreased the levels of IL-12, IL-6, and TNF- α , as well as the percentages of CD16/32 in M1 BMDMs. At the same time, SFN increased the levels of YM1, Fizz1, and Arg1 as well as the percentages of CD206⁺ cells in M2 BMDMs. In addition, SFN enhanced the accumulation of Nrf2, NQO1, and HO-1 in M1 BMDMs, and the downregulation of Nrf2 reversed the regulatory effect of SFN on M1/M2 macrophages. LPS-stimulated BMDMs induced Caco-2 cell damage, which was partially alleviated by SFN.

Conclusion: Our findings indicate that SFN may act as an Nrf2 agonist to regulate macrophage polarization from M1 to M2. Furthermore, SFN may represent a potential protective ingredient against IBD.

Keywords: Inflammatory Bowel Disease; Macrophage; Nrf2/ARE; Sulforaphane

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INTRODUCTION

Macrophages are fundamental cellular constituents of the body's innate and adaptive immunity and play a central role in stimulating the inflammatory cascades and facilitating tissue repair in response to injury (1, 2). Accumulating evidence points to the essential role of macrophages in the treatment of inflammatory diseases and cancer (3, 4). In addition, macrophages can be classified into two major categories at the extreme, M1 and M2, depending on their microenvironment, with M1 being pro-inflammatory and M2 being anti-inflammatory (5). M1 macrophages, induced by lipopolysaccharide (LPS) and interferon- γ (IFN- γ), are responsible for producing pro-inflammatory cytokines such as interleukin (IL)-6, IL-12, and tumor necrosis factor- α (TNF- α) to protect the body from infectious agents and maintain homeostasis (6). In contrast, IL-4 and IL-13 stimulate M2 macrophages to secrete anti-inflammatory cytokines and express allosteric heat shock protein 70 (Hsp70) modulator (Ym1), found in inflammatory zone 1 (Fizz1), and arginase-1 (Arg-1) to participate in inflammation resolution and tissue repair (6). Therefore, managing macrophage polarization is crucial for the treatment of inflammatory diseases and tumors.

In recent years, the therapeutic potential of phytochemicals in the treatment of inflammatory diseases has received considerable attention due to their efficacy, specificity, and minimal side effects (7). Research suggests that phytochemicals may regulate inflammation and tumor progression through the management of macrophage polarization (8, 9). One such phytochemical is sulforaphane (SFN, 1-isothiocyanato-4-methylsulfinyl butane), which occurs naturally in cruciferous vegetables (10). The antioxidant, anti-inflammatory, and anti-apoptotic properties of SFN make it a promising treatment for several diseases (11-13). Studies have shown that SFN inhibits

the activation of the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing-3 (NLRP3) inflammasome to limit the secretion of inflammatory factors and reduce retinal ganglion cell death (14). Studies have also reported that SFN attenuates oxidative stress, limits the expression of inflammatory cytokines, and reduces apoptosis, thereby protecting intestinal epithelial cells from LPS-induced damage (15). SFN has been shown to activate numerous downstream target genes *via* the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) signaling pathway with potent antioxidant activity (16, 17). When exposed to bacterial or viral stimuli, SFN is known to interfere with inflammatory responses by activating Nrf2, suppressing pro-inflammatory cytokines while stimulating heme oxygenase-1 (HO-1) (18). SFN was found to inhibit the expression of pro-inflammatory mediators and enhance the expression of Nrf2, HO-1, and anti-inflammatory cytokines, thereby protecting microglial cells from LPS-induced damage (19).

SFN may also potentially act as a modulator of M1/M2 macrophage activity (20). Studies have found that SFN helps to reduce the expression of M1 macrophage markers induced by LPS (21). SFN reverses the upregulation of nitric oxide (NO) concentration and diverse inflammatory mediators caused by LPS in macrophages and activates the expression of Nrf2 and HO-1 (22). Regulation of macrophage function by SFN has been suggested as a potential target for treating hemorrhagic shock/resuscitation (23, 24) and renal injury (25). However, whether SFN mediates macrophage polarization through the Nrf2/ARE pathway remains to be investigated.

Inflammatory bowel diseases (IBDs) refer to chronic conditions that result in inflammation and mucosal damage in the gastrointestinal tract. They include Crohn's disease and ulcerative colitis (26). Studies have shown that IBDs impose a significant

burden on the global economy (27). While the exact cause of IBD remains unclear, recent evidence suggests that the state of macrophages plays a critical role in initiating and reducing inflammation in IBD (28). Research has shown that promoting the polarization of M2 macrophages can aid in tissue repair, reducing the symptoms of IBD (29). However, the effects of SFN on M2 macrophage polarization and IBD pathogenesis remain uncertain.

Therefore, our study was designed to examine the effects of SFN on M1 and M2 macrophage activity and the expression of related inflammatory cytokines. In addition, we investigated potential mechanisms by which suppression of the Nrf2/ARE signaling pathway may counteract the effects of SFN. Furthermore, we wondered how SFN affects the viability and barrier integrity of Caco-2 cells in the presence of macrophage-induced stimulation by LPS.

MATERIALS AND METHODS

Cell Culture and Treatment

Mouse bone marrow-derived macrophages (BMDMs, AW-YCM003, Abiowell) were maintained in a complete macrophage medium consisting of 50 ng/mL macrophage-stimulating factor (M-CSF) and Roswell Park Memorial Institute (RPMI)-1640. BMDMs were isolated from bone marrow and differentiated from bone marrow monocytes. To investigate the effects of SFN on BMDMs and screen for the optimal concentration, BMDMs were stimulated with SFN (C4733, APExBIO) at concentrations of 1, 10, 20, and 50 μ M for 6 h. (22). To evaluate the regulation of Nrf2 on SFN function, BMDMs were pretreated with the Nrf2 inhibitor Brusatol (100 nM, 172924, Selleckchem) for 18 h. (30). BMDMs were then induced to differentiate. In addition, human colorectal adenocarcinoma Caco-2 cells (AW-CCH056, Abiowell) were cultured in minimal essential medium supplemented with a non-essential amino acid solution, 20% fetal bovine serum,

and 1% penicillin/streptomycin. Cells were maintained at 37°C in a 5% CO₂ incubator. Mycoplasma test certificates indicated a negative result.

Establishment of a Co-culture System

To simulate an inflammatory environment in the intestine, we performed a co-culture procedure involving macrophages and Caco-2 cells. Specifically, Caco-2 cells were seeded in Transwell chambers at a density of 4×10^5 cells per well and cultured until a fully differentiated Caco-2 monolayer was formed. BMDMs (4×10^6 cells/wells) were seeded on the basal side and left treated with LPS (+/-) (100 ng/mL for 3 h) (31), followed by exposure to SFN (+/-) (10 μ M for 6 h). In the co-culture system, LPS was chosen to stimulate BMDMs to simulate an *in vitro* inflammatory environment in the intestine (31-33). Stimulation with LPS alone can induce M1 (pro-inflammatory) phenotype in BMDMs, resulting in increased secretion of pro-inflammatory factors and decreased secretion of anti-inflammatory factors (34, 35). After replacing the medium with complete DMEM, the transwell chambers containing the Caco-2 cells were inserted into the plate containing the BMDMs. The medium replacement was performed to minimize any interference of LPS on Caco-2 cells (36, 37). The cells were specifically divided into three groups: Caco-2/BMDM group (the co-culture of Caco-2 cells with untreated BMDMs), Caco-2/BMDM (LPS) group (the co-culture of LPS-treated BMDMs, after medium replacement, with Caco-2 cells), and Caco-2/BMDM (LPS+SFN) group (the co-culture of LPS- and SFN-treated BMDMs, after medium replacement, with Caco-2 cells).

Macrophage Polarization

BMDMs are supplemented with inflammatory cytokines to induce differentiation into M1 or M2 macrophages (38). Briefly, BMDMs were stimulated with LPS (1 μ g/mL, L2880, Sigma) and IFN- γ (10 ng/mL, 315-05-20, Peprotech) for 72 h to

differentiate into the M1 phenotype, defined as the M1 group. BMDMs were stimulated with IL-4 (20 ng/mL, 200-04-51, Peprotech) and IL-13 (10 ng/mL, 200-13-2, Peprotech) for 72 h to differentiate into M2 phenotype to establish the M2 group. Simultaneously, 10 μ M SFN was added to define the M1+SFN or M2+SFN groups. Pretreatment of BMDMs with 100 nM Brusatol for 18 h was served as M1+SFN+Brusatol or M2+SFN+Brusatol groups.

Measurement of Transepithelial Electrical Resistance (TEER)

To assess the fusion and integrity of the cell monolayer, TEER was measured (39). Caco-2 cells (4×10^5 cells/well) were seeded into 24-well transwell plates with a 0.4 μ m pore size (Corning). The cells were monitored daily until the TEER value exceeded 150 Ω -cm², indicating a tight monolayer. The transwell plates containing Caco-2 cells were then placed into wells containing BMDMs treated with LPS (+/-) and SFN (+/-). After 48 h, TEER values were measured using a Millicell-ERS instrument (Millipore).

Cell Counting Kit-8 (CCK8) Assay

Cells were seeded at a density of 5×10^3 cells/well/100 μ L in a 96-well plate with three replicates. CCK8 (10 μ L/well, AWC0114a, Abiowell) was then added. The cell plates were transferred to a 37°C incubator and incubated for 4 h, after which we recorded

the optical density (OD) values of each group at 450 nm using a microplate reader (MB-530, HEALES).

Quantitative Real-time PCR (qRT-PCR)

Total RNA was extracted from the cells using Trizol (15596026, Thermo). The resulting RNA was assayed for absorbance values using an ultraviolet (UV) spectrophotometer, with OD260/280 and OD260/230 values greater than 2. RNA was then converted to cDNA using the HiFiScript cDNA Synthesis Kit (CW2569, ConWin). Amplification was performed using the UltraSYBR Mixture Kit (CW2601, ConWin) on the QuantStudio™ 1 platform. The amplification program consisted of 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 15 s. The relative mRNA expression of the target gene was calculated using the $2^{-\Delta\Delta C_t}$ method, with β -actin as the reference gene. Primer sequences for the target gene were designed using Primer5 (PREMIER Biosoft, Vancouver, Canada), listed in Table 1.

Flow Cytometry

Cells (1×10^5 cells/100 μ L) were washed with PBS and then resuspended in 100 μ L of culture medium. The cells were incubated in the dark for 30 min with the corresponding antibodies CD16/32 (11-0161-82, eBioscience) or CD206 (MA5-16870, Invitrogen). After another washing with PBS, the cells were

Table 1. Primer sequences

Target	F (5'-3')	R (5'-3')
IL-12	TGAAGACATCACACGGGACCA	CAGCTCCCTCTTGTGTGGAA
IL-6	GACTTCCATCCAGTTGCCTT	ATGTGTAATTAAGCCTCCGACT
TNF- α	AGCACAGAAAGCATGATCCG	CACCCCGAAGTTCAGTAGACA
YM1	AGGGCCCTTATTGAGAGGAG	AGCTGGTACAGCAGACAAGAC
Fizz1	GGGATGACTGCTACTGGGTG	TCAACGAGTAAGCACAGGCA
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Nrf2	GCTCCTATGCGTGAATCCCAA	TTTGCCCTAAGCTCATCTCGT
NQO1	GGGGTGGCTCACTTACATCAG	TAGGTGCTTGGCTTGGTGAC
HO-1	TCCATGTTGACTGACCACGACT	CCCACCCCTCAAAAGATAGCC
β -actin	ACATCCGTAAAGACCTCTATGCC	TACTCCTGCTTGCTGATCCAC

IL-6: interleukin-6; TNF- α : tumor necrosis factor-alpha; Arg-1: arginase-1; Nrf2: nuclear factor erythroid 2-related factor 2; HO-1: heme oxygenase-1; NQO1: NADPH quinone dehydrogenase 1

resuspended in a culture medium. Analysis was then performed on a Beckman Coulter CytoFLEX flow cytometer (A00-1-1102).

Detection of Lactate Dehydrogenase (LDH)

LDH levels in Caco-2 cells were detected according to the instruction manual of the LDH assay kit (A020-2, Nanjing Jiancheng Bioengineering Institute). Blank, standard, test, and control wells were set up for measurement at a wavelength of 450 nm to determine the OD values.

Western Blot

Total protein was obtained from BMDMs using radioimmunoprecipitation assay buffer (RIPA, AWB0136, Abiowell). After quantification using a bicinchoninic acid (BCA) assay kit (AWB0104, Abiowell), the total protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The membranes were then blocked with 5% skimmed milk powder and incubated overnight at 4°C with Nrf2 (1:2500, 80593-1-RR), NQO1 (1:4000, 11451-1-AP), HO-1 (1:3000, 10701-1-AP) and β -actin (1:5000, 66009-1-Ig). The membranes were then incubated with HRP-conjugated rabbit antibody (1:6000, SA00001-2) or mouse antibody (1:5000, SA00001-1) for 90 min at room temperature. All antibodies were purchased from Proteintech. Finally, the target proteins were visualized using ChemiScope6100 (CLiNX) before exposure to ECL Plus (AWB0005, Aiwowell). Band densities were counted using Quantity One 4.6 software (Bio-Rad).

Statistical Analysis

All values are expressed as mean $\pm$ standard deviation and statistical analysis was performed using GraphPad Prism 9. The Kolmogorov-Smirnov and exploratory descriptive statistics tests were used to analyze normal distribution and homogeneity of variance. Comparisons between the two

groups were carried out by unpaired t-test, while comparisons between multiple groups were performed by ANOVA (Analysis of Variance) followed by Tukey's post hoc test. $P < 0.05$ was considered a significant difference.

RESULTS

SFN Inhibited Polarization of M1 Macrophages

To determine the optimal concentration of SFN for our interventions and to assess the potential cytotoxicity of SFN, we examined the effect of different concentrations of SFN (1 μ M, 10 μ M, 20 μ M, and 50 μ M) on the viability of BMDMs. Our results indicated that SFN at 1 and 10 μ M had no cytotoxicity on BMDMs, but it decreased BMDM viability at 20 and 50 μ M (Fig. 1A). Therefore, we used 10 μ M SFN for subsequent experiments. Next, to investigate the effect of SFN on M1 polarization, we stimulated BMDMs with LPS and IFN- γ to differentiate into the M1 phenotype. Compared with the control group, M1 BMDMs exhibited elevated levels of IL-12, IL-6, and TNF- α , as well as increased expression of CD16/32. Supplementation with SFN reversed these elevated trends (Figs. 1B, 1C). Our observations indicated that SFN impeded the M1 polarization of macrophages.

SFN Promoted M2 Macrophage Polarization

To further evaluate the regulation of SFN on M2 polarization, we stimulated BMDMs with IL-4 and IL-13 to facilitate differentiation into the M2 phenotype. Our results demonstrated that compared with the control group, M2 BMDMs exhibited increased levels of YM1, Fizz1, and Arg-1 as well as enhanced expression of CD206. SFN supplementation further amplified these increased trends (Figs. 2A, 2B). These observations indicated that SFN was beneficial in enhancing the M2 phenotype of M2 BMDMs.

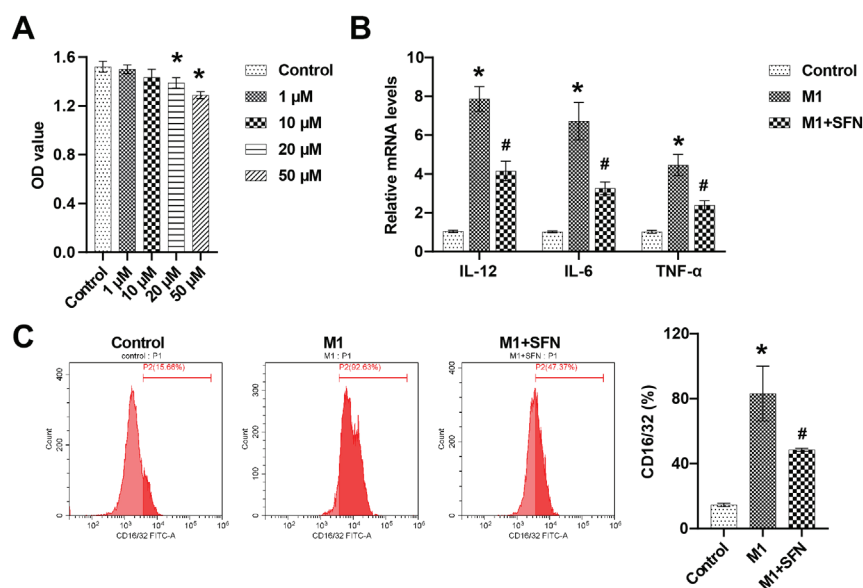


Fig. 1. SFN inhibits M1 macrophage polarization. (A) CCK8 assay was utilized to determine the effect of different concentrations of SFN (1 μ M, 10 μ M, 20 μ M, and 50 μ M) on the viability of M1 BMDMs. * p <0.05 vs. control. (B) The effect of 10 μ M SFN on the mRNA levels of IL-12, IL-6, and TNF- α in M1 BMDMs was investigated by qRT-PCR. (C) Flow cytometry was applied to examine the effect of 10 μ M SFN on the CD16/32 ratio in M1 BMDMs. * p <0.05 vs. control, # p <0.05 vs. M1. BMDM: bone marrow-derived macrophage; SFN: sulforaphane

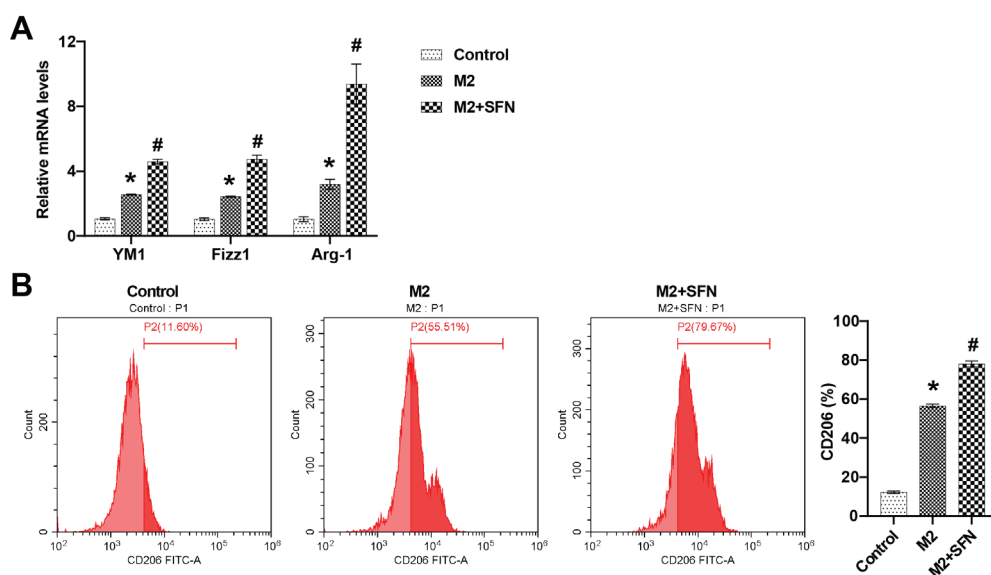


Fig. 2. SFN promotes M2 macrophage polarization. (A) qRT-PCR was employed to investigate the effect of 10 μ M SFN on the mRNA level changes of YM1, Fizz1, and Arg1 in M2 BMDMs. (B) The effect of 10 μ M SFN on the ratio of CD206 in M2 BMDMs was examined by flow cytometry. * p <0.05 vs. control, # p <0.05 vs. M2. BMDM: bone marrow-derived macrophage; SFN: sulforaphane; Arg-1: arginase-1

SFN Inhibited M1 Macrophage Polarization Through Nrf2/ARE Signaling

To investigate the regulation of Nrf2-ARE by SFN, we examined the mRNA and protein expression of Nrf2, NADPH quinone dehydrogenase 1 (NQO1), and HO-1 in the

M1+SFN group. Our data indicated that compared with the control group, inducing BMDMs toward M1 polarization resulted in increased expression of Nrf2, NQO1, and HO-1, which was further exacerbated by SFN supplementation (Figs. 3A, 3B).

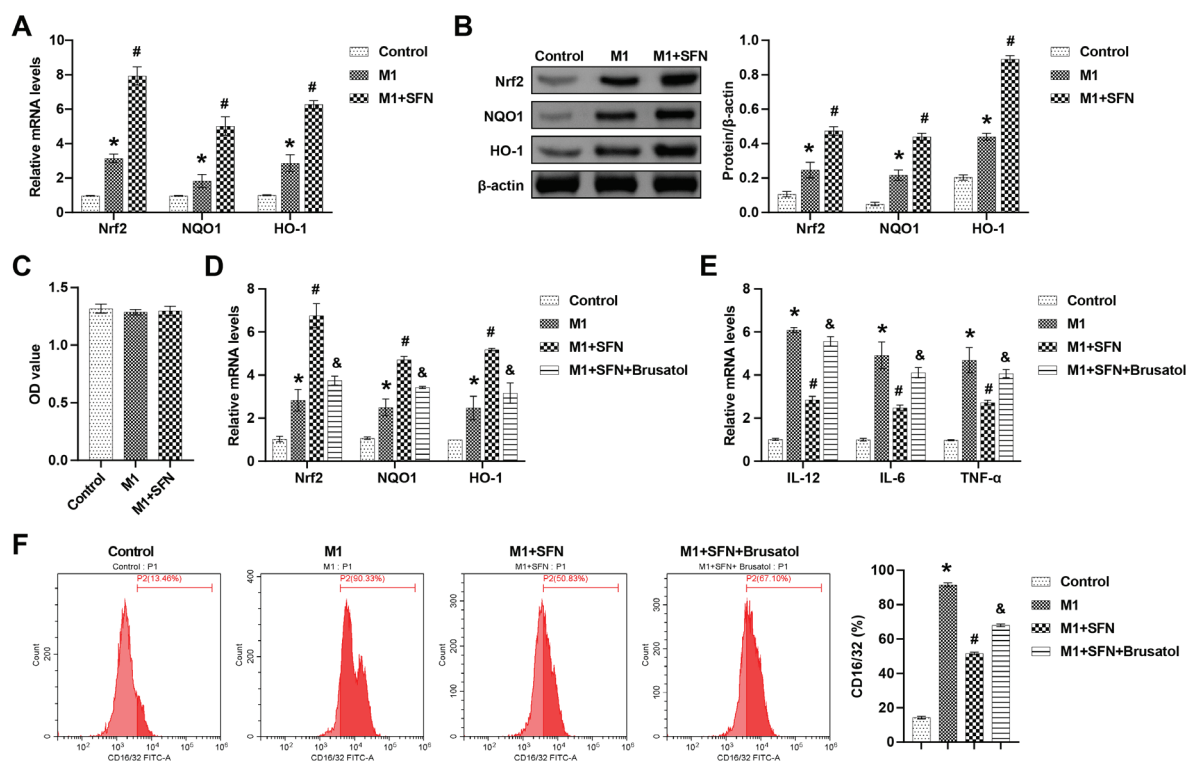


Fig. 3. SFN stimulates the Nrf2/ARE pathway to hinder M1 macrophage polarization. (A) The effect of 10 μ M SFN on the mRNA expression of Nrf2, NQO1, and HO-1 in M1 BMDMs was determined. (B) The effect of 10 μ M SFN on the protein abundance of Nrf2, NQO1, and HO-1 in M1 BMDMs was investigated by Western blot. * p <0.05 vs. control, # p <0.05 vs. M1. (C) The effect of 10 μ M SFN on the viability of M1 BMDMs was examined by CCK8 assay. (D) The effect of Brusatol on the mRNA levels of SFN-regulated Nrf2, NQO1, and HO-1 was measured. (E) The effect of Brusatol on the mRNA levels of SFN-regulated IL-12, IL-6, and TNF- α was detected. (F) The effect of Brusatol on the percentage of SFN-regulated CD16/32 was analyzed by flow cytometry. * p <0.05 vs. control, # p <0.05 vs. M1, & p <0.05 vs. M1+SFN. BMDM: bone marrow-derived macrophage; SFN: sulforaphane; IL-6: interleukin-6; TNF- α : tumor necrosis factor-alpha; HO-1: heme oxygenase-1; ARE: antioxidant response element; Nrf2: nuclear factor erythroid 2-related factor 2; NQO1: NADPH quinone dehydrogenase 1

These data suggest that SFN can induce the Nrf2/ARE signaling pathway. Furthermore, there was no significant difference in the viability of M1 BMDMs among the three groups (Fig. 3C). Next, we selected the Nrf2 inhibitor Brusatol to investigate whether the Nrf2-ARE pathway plays a regulatory role in SFN-mediated macrophage polarization. Our data revealed that compared with the M1+SFN group, the M1+SFN+Brusatol group exhibited decreased mRNA levels of Nrf2, NQO1, and HO-1 (Fig. 3D), thereby reversing the beneficial effect of SFN on the Nrf2/ARE signaling pathway. Compared with the control group, M1 BMDMs exhibited increased levels of IL-12, IL-6, TNF- α , and the expression of CD16/32. In contrast, the M1+SFN group

showed a decreasing trend, which was reversed by the application of Brusatol (Figs. 3E, 3F). These findings suggested that SFN promoted Nrf2/ARE signaling to inhibit M1 macrophage polarization.

SFN Promoted M2 Macrophage Polarization via the Nrf2/ARE Pathway

To evaluate the regulation of Brusatol on SFN-promoted M2 macrophage polarization, we examined the levels of YM1, Fizz1, and Arg1 in the M2+SFN+Brusatol group. Our data indicated that compared with the control group, M2 BMDMs exhibited increased levels of YM1, Fizz1, Arg-1, and the expression of CD206. Compared with the M1 group, the M2+SFN group showed

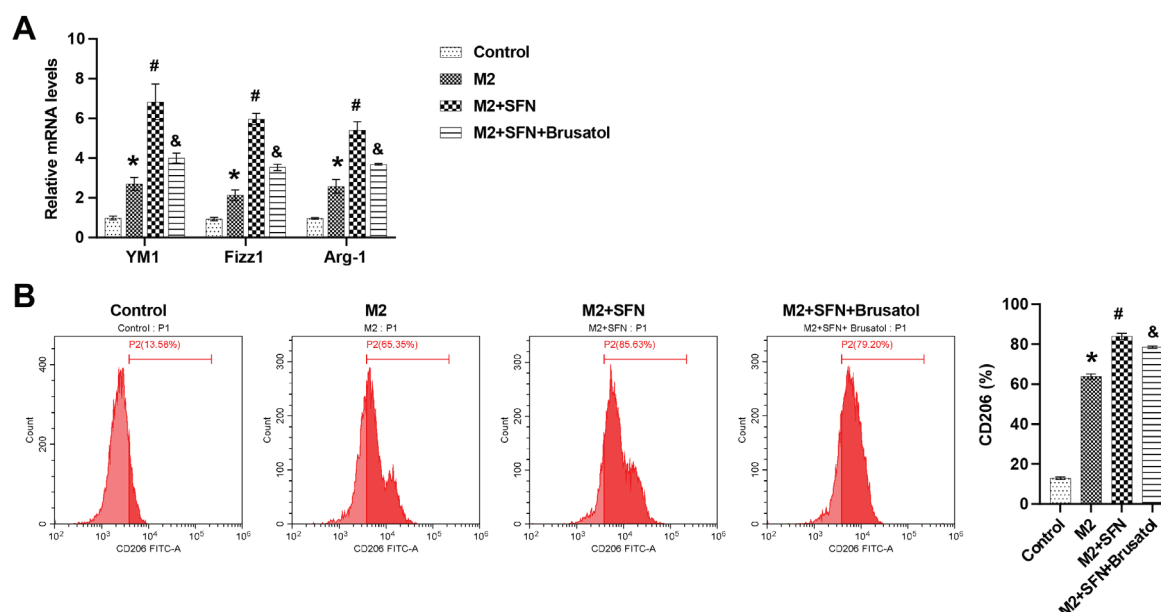


Fig. 4. SFN induces the Nrf2/ARE pathway to promote M2 macrophage polarization. (A) The effect of Brusatol on the mRNA levels of SFN-regulated YM1, Fizz1, and Arg1 was examined. (B) The effect of Brusatol on the percentage of SFN-regulated CD206 was investigated by flow cytometry. * $p < 0.05$ vs. control, # $p < 0.05$ vs. M2, & $p < 0.05$ vs. M2+SFN. SFN: sulforaphane; ARE: antioxidant response element; Nrf2: nuclear factor erythroid 2-related factor 2; Arg-1: arginase-1

an upward trend, which was reversed by the application of Brusatol (Figs. 4A, 4B). Our findings suggested that SFN promoted Nrf2/ARE signaling to induce M2 macrophage polarization.

SFN Attenuated Caco-2 Cell Injury Induced by LPS-stimulated Macrophages

LPS has been reported to promote macrophage polarization toward the M1 phenotype and the secretion of pro-inflammatory cytokines (34, 40). Therefore, to further investigate the effect of SFN on macrophage function in an inflammatory environment, we co-cultured LPS-exposed BMDMs with Caco-2 cells. The results showed that LPS-stimulated BMDMs led to a decrease in Caco-2 cell viability, whereas SFN partially restored Caco-2 cell viability (Fig. 5A). LPS-stimulated BMDMs caused an increase in LDH levels in Caco-2 cells, whereas SFN inhibited the increase (Fig. 5B). Additionally, the TEER values of Caco-2 cell monolayers decreased following exposure to LPS-stimulated BMDMs, whereas SFN reversed this trend (Fig. 5C). These results

indicated that SFN reduced the damage caused by LPS-stimulated macrophages to Caco-2 cells.

Subsequently, we examined whether SFN affected the polarization status of LPS-stimulated macrophages. The results showed that LPS induced an increase in the CD16/32 ratio and a decrease in the frequency of CD206⁺ cells in BMDMs, and SFN treatment further reversed these trends (Fig. 5D). Additionally, LPS resulted in an increase in the levels of IL-10 and TNF- α , as well as a decrease in the levels of IL-10 and TGF- β in BMDMs. SFN treatment further reversed these trends (Figs. 5E, 5F). These results demonstrated that SFN inhibited the LPS-induced M1 macrophage polarization and the secretion of inflammatory factors.

DISCUSSION

Due to their excellent safety and pharmacological activity, phytochemicals in diseases have attracted extensive attention for their potential application in various diseases.

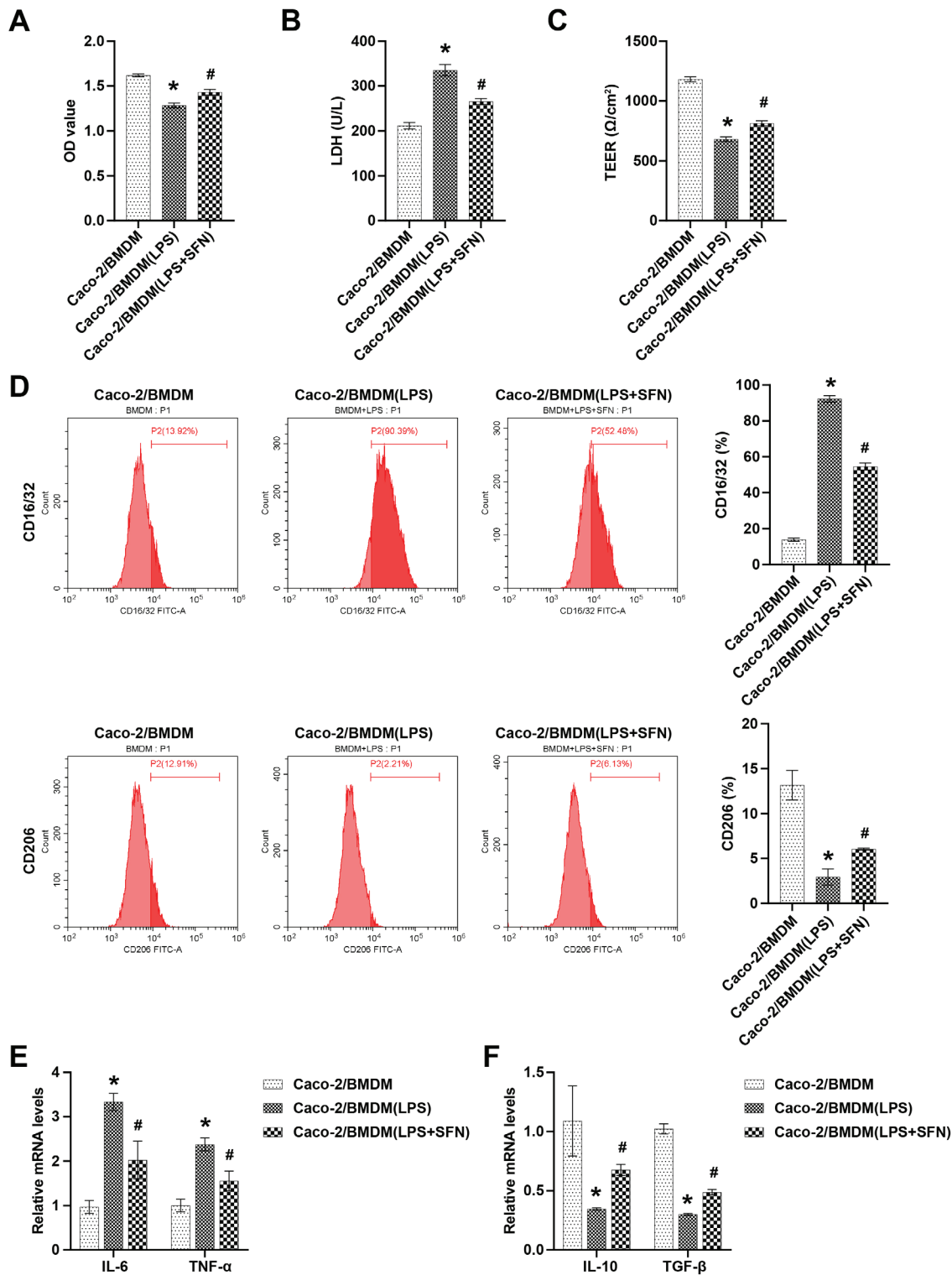


Fig. 5. SFN regulates Caco-2 cell function and macrophage polarization induced by LPS-stimulated BMDMs. Caco-2 cells grown on transwell inserts were co-cultured with LPS-stimulated BMDMs. (A) Cell viability of Caco-2 cells after co-incubation with BMDMs as detected by CCK8 assay. (B) The level of LDH was examined in Caco-2 cells after co-incubation with BMDMs. (C) TER across cell monolayers were measured. (D) The ratios of CD16/32- or CD206-positive cells were assessed in BMDMs after co-incubation with Caco-2 cells. (E) The mRNA levels of IL-6 and TNF- α were detected in BMDMs after co-incubation with Caco-2 cells. (F) The mRNA levels of IL-10 and TGF- β were detected in BMDMs after co-incubation with Caco-2 cells. * $p < 0.05$ vs. Caco-2/BMDM, # $p < 0.05$ vs. Caco-2/BMDM(LPS). BMDM: bone marrow-derived macrophage; SFN: sulforaphane

The antagonistic effects of phytochemicals have been reported in areas such as cancers (41), nerve damage (42), and prevalent inflammatory diseases (7). Among these, SFN is considered to be a phytochemical with broad therapeutic activities derived from cruciferous vegetables (43). In this study, we stimulated BMDMs to differentiate into the M1 or M2 phenotype with inflammatory factors and evaluated the effects of SFN on macrophage activity. We also examined the effect of LPS-stimulated macrophages on Caco-2 cell viability and cell monolayer barrier integrity and evaluated the role of SFN. The results showed that SFN suppressed M1 macrophage activity and induced M2 macrophage polarization. In addition, the anti-inflammatory activity of SFN may be related to the Nrf2/ARE-mediated antioxidant response. LPS stimulated macrophage M1 polarization, resulting in decreased Caco-2 cell viability and the loss of Caco-2 cell barrier integrity. This phenomenon was reversed by SFN.

Maintaining the balance of M1/M2 macrophages is critical for the management of several diseases, including cancer and inflammatory disorders (44). Phytochemicals have been found to provide a new paradigm for the treatment of inflammatory diseases by modulating macrophage polarization (45). For example, *Nelumbo nucifera* flower extracts can effectively inhibit TNF- α production in human macrophages (46), while ethanol extracts from mulberry fruit have been shown to inhibit inducible nitric oxide synthase (iNOS) expression and nitric oxide production in mouse macrophages (47). In the present study, we screened for the optimal intervention concentration of 10 μ M SFN and then used LPS and IFN- γ to stimulate the polarization of BMDM toward M1. The results showed that SFN decreased the production of IL-12, IL-6, and TNF- α and the expression of CD16/32 compared with the M1 group. SFN also increased the production of YM1, Fizz1, and Arg1 and the expression of CD206 compared with the

M2 group. Thus, our results suggested that SFN induced M2 polarization and inhibited M1 polarization of macrophages. However, it is important to note that macrophages are defined as a continuum of M1 and M2 macrophages, with their expression and transcriptional profiles changing along a spatial and temporal continuum (48). Macrophages are capable of adopting an intermediate phenotype with mixed M1 and M2 characteristics in response to various cues from the tissue microenvironment (4). In addition, the pro-inflammatory phenotype of M1 macrophages is also characterized by increased production of reactive oxygen species (ROS), whereas M2 macrophages exhibit low ROS production (49, 50). Defining the phenotype of macrophages is considered a major challenge, but it is crucial to reflect the heterogeneity of activated macrophages and to assess the complexity of their functions in disease.

Phytochemicals have been found to affect the progression of IBD. Astragaloside IV and loganin promote M1 to M2 macrophage polarization, resulting in attenuation of DSS-induced intestinal inflammation (51, 52). Triptolide activates the Nrf2/HO-1 signaling pathway to reduce ROS generation and promote anti-inflammatory macrophage infiltration (53). Previous research has demonstrated the protective effects of SFN against IBD. Inhibition of pro-inflammatory pathways and levels of pro-inflammatory factors are currently available and are approved therapies for IBD (54). Dextran sulfate sodium (DSS)-induced IBD mice exhibit weight loss, colon shortening, and decreased serum IL-6 levels (55). In IBD patients and the DSS-induced IBD mouse model, the intestinal barrier is compromised, and tight junctions are disrupted (56, 57). SFN has been shown to increase body weight, colon length, and reverse intestinal dysbiosis in mice with DSS-induced IBD (58). SFN has also been found to attenuate LPS-induced secretion of pro-inflammatory factors, reduce permeability, and alleviate oxidative stress in

the intestinal epithelium (15). In our study, we observed that LPS-stimulated BMDMs resulted in decreased viability, increased LDH levels, and decreased TEER levels in Caco-2 cells, indicating compromised intestinal mucosal barrier integrity (59). SFN treatment prevented the damage to the epithelial cells caused by LPS-stimulated BMDMs. Furthermore, sequencing analysis has revealed the presence of inflammatory fibroblasts, neutrophils, or M1 macrophages in the colon of IBD patients that are absent in healthy individuals (60). The IBD model exhibited macrophage M1 polarization and pro-inflammatory factor production. G protein-coupled 84 (GPR84) regulates the composition of the macrophage pool in the intestine and thus influences the pathological features of IBD. In colonic tissue from IBD patients, the majority of macrophages co-express GPR84 and CD86 (39). Downregulation of M2 macrophage markers and upregulation of M1 macrophage markers were observed in the colonic tissue of IBD mice (61). Previous studies have shown that deletion of extracellular matrix protein 1 (ECM1) in macrophages induced M2 macrophage activation and increased Arg-1 expression in colonic tissues, which suppressed the pathological response in IBD (62). Additionally, miR-497 has been found to inhibit the nuclear factor-kappaB (NF- κ B) signaling pathway, thereby attenuating DSS-induced IBD symptoms and LPS-induced inflammation (63). These findings suggest that macrophage targeting is involved in mediating the progression of IBD. In the co-culture system created in our study, LPS induced upregulation of CD16/32 expression and elevated levels of IL-6 and TNF- α , while it inhibited CD206 expression and decreased levels of IL-10 and TGF- β . Notably, SFN reversed these trends. Compared with the study by Tang et al. (53), our study did not comprehensively discuss the effects of SFN on macrophage composition and intestinal inflammation in an IBD mouse model due to time and budget limitations. In addition, the

lack of investigation of the efficacy and safety of SFN in more diverse IBD *in vitro* and *in vivo* models and clinical samples is also a major limitation of our study. Taken together, these results indicate that SFN promotes M2 macrophage activation and restores Caco-2 monolayer barrier integrity.

Nrf2 is widely recognized as an antioxidant regulator (64), and there is evidence that its upregulation can impede the production of nitric oxide, prostaglandin E2, and ROS in macrophages, thereby suppressing M1 macrophage polarization and levels of pro-inflammatory markers (65). Therefore, we speculated that the regulation of SFN on M1/M2 macrophages might be mediated by Nrf2 signaling. Our findings were consistent with previous studies suggesting that SFN may act as an Nrf2 inducer (66). Specifically, we observed increased accumulation of Nrf2 and its downstream molecules HO-1 and NQO1 in response to SFN treatment compared with the M1 group. Of note, HO-1 and NQO1 have ARE sites (67). Furthermore, the Nrf2 inhibitor brusatol reversed the SFN-induced accumulation of Nrf2, NQO1, and HO-1. Brusatol administration also upregulated the levels of IL-12, IL-6, and TNF- α , as well as the ratio of CD16/32 compared with the M1+SFN group. Compared with the M2+SFN group, Brusatol downregulated the levels of YM1, Fizz1, and Arg1, as well as the proportions of CD206. These results indicated that SFN could interfere with macrophage M1 polarization and contribute to M2 polarization, in part at least by activating the Nrf2/ARE signaling pathway. It has been found that SFN can induce the generation of ROS in bladder cancer cells to induce apoptosis, which may be related to the Nrf2 pathway (68). Although we were unable to explore the potential mechanism by which SFN regulates ROS generation in macrophages due to funding constraints, we have reason to believe that the regulation of SFN on M1/M2 macrophages may be related to Nrf2/ARE-mediated antioxidant responses, which deserves further

investigation and discussion. Whether SFN affects macrophage activity in IBD through the Nrf2/ARE signaling pathway also remains to be explored. In addition to the Nrf2 pathway, SFN may mediate different signaling pathways to influence the immune environment in IBD. Beta-hydroxybutyrate has been shown to promote M2 macrophage polarization in a signal transducer and activator of transcription (STAT) 6-dependent manner, thereby aiding in the resolution of intestinal inflammation and mucosal repair (69). Lactic acid has been reported to limit the NLRP3 inflammasome and M1 macrophage polarization, thereby alleviating DSS-induced colitis (70). Furthermore, blocking the NF- κ B pathway in macrophages has been suggested as a potential strategy for IBD therapy (71). It remains to be determined whether SFN mediates the progression of IBD by modulating the STAT, NLRP3, and NF- κ B pathways.

Furthermore, due to time and funding constraints, our study only established a Caco-2/BMDM(LPS+/-) co-culture system to simulate intestinal inflammation and investigate the regulation of intestinal inflammation by SFN. Previous studies have established a Caco-2(LPS+/-)/THP-1 co-culture model to investigate the regulation of intestinal inflammation by (10Z)-Debromohymenialdisine. LPS-stimulated Caco-2 cells induced increased release of iNOS, NO, IL-1 β , IL-6, and TNF- α in THP-1 cells, and the drug reversed the levels of these factors, indicating the potential therapeutic effects of the drug on aberrant intestinal inflammation in IBD (72). It is necessary to establish a co-culture system of LPS-treated Caco-2 cells with stimulated BMDMs to further define the crosstalk between macrophages and intestinal inflammation. This will help answer how inflamed epithelial cells in IBD attract M0 macrophages (untreated BMDMs) and polarize them toward M1 macrophages, as well as how treatment with SFN alleviates the pro-inflammatory environment. Therefore,

more evidence is needed to support SFN targeting macrophage activity to inhibit IBD progression. In addition to the composition of macrophages, further research is needed to explore the regulation of SFN on the state of other immune cells.

Tumor-associated macrophages, which exhibit pro-tumor physiological properties, play a critical role in tumorigenesis (73). Interestingly, our study found that SFN induced the polarization of M2 macrophages. However, SFN has been shown to have potential tumor suppressor properties (74). Inflammation is closely associated with cancer development, and chronic inflammation is known to promote tumorigenesis (75). Conversely, inhibition of inflammation may serve to halt tumor growth and differentiation (76). The tumor suppressor p53 is known to play a pivotal role in this process (77, 78), as evidenced by the fact that p53 deficiency enhances chronic inflammatory responses and promotes tumor progression (79). Previous research has indicated that SFN may exert an anti-cancer response in a p53-dependent manner. Specifically, SFN stimulates p53 expression, thereby limiting tumor progression (80, 81). Interestingly, iron overload has been shown to increase ROS levels and induce p53 acetylation, thereby promoting M1 macrophage polarization (82). It has also been shown that increased ROS levels induce p53 expression in macrophages (82). Given this information, we hypothesized that SFN may negatively affect the function of M2-like macrophages by stimulating ROS production and activating the p53 signaling pathway. This would ultimately serve to hinder tumor progression. However, further evidence is needed to substantiate this speculation.

In conclusion, our study suggests a potential mechanism by which SFN induces the Nrf2/ARE pathway to facilitate macrophage M1-to-M2 polarization. These findings have important implications for the application of SFN in pharmacology and may open up new avenues for “green chemoprevention”.

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AUTHORS' CONTRIBUTION

TY, ZL, HJ, QL and JP designed the study, performed the research, analyzed data, and wrote the paper. All authors contributed to the article and approved the submitted version.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Locati M, Curtale G, Mantovani A. Diversity, Mechanisms, and Significance of Macrophage Plasticity. *Annu Rev Pathol*. 2020;15:123-47.
- Austermann J, Roth J, Barczyk-Kahlert K. The Good and the Bad: Monocytes' and Macrophages' Diverse Functions in Inflammation. *Cells*. 2022;11(12).
- Kadomoto S, Izumi K, Mizokami A. Macrophage Polarity and Disease Control. *Int J Mol Sci*. 2021;23(1).
- DeNardo DG, Ruffell B. Macrophages as regulators of tumour immunity and immunotherapy. *Nat Rev Immunol*. 2019;19(6):369-82.
- Skytthe MK, Graversen JH, Moestrup SK. Targeting of CD163(+) Macrophages in Inflammatory and Malignant Diseases. *Int J Mol Sci*. 2020;21(15).
- Kolliniati O, Ieronymaki E, Vergadi E, Tsatsanis C. Metabolic Regulation of Macrophage Activation. *J Innate Immun*. 2022;14(1):51-68.
- Shin SA, Joo BJ, Lee JS, Ryu G, Han M, Kim WY, et al. Phytochemicals as Anti-Inflammatory Agents in Animal Models of Prevalent Inflammatory Diseases. *Molecules*. 2020;25(24).
- Liao H, Ye J, Gao L, Liu Y. The main bioactive compounds of *Scutellaria baicalensis* Georgi. for alleviation of inflammatory cytokines: A comprehensive review. *Biomed Pharmacother*. 2021;133:110917.
- Li LG, Peng XC, Yu TT, Xu HZ, Han N, Yang XX, et al. Dihydroartemisinin remodels macrophage into an M1 phenotype via ferroptosis-mediated DNA damage. *Front Pharmacol*. 2022;13:949835.
- Panjwani AA, Liu H, Fahey JW. Crucifers and related vegetables and supplements for neurologic disorders: what is the evidence? *Curr Opin Clin Nutr Metab Care*. 2018;21(6):451-7.
- Liebman SE, Le TH. Eat Your Broccoli: Oxidative Stress, NRF2, and Sulforaphane in Chronic Kidney Disease. *Nutrients*. 2021;13(1):266.
- Schepici G, Bramanti P, Mazzon E. Efficacy of Sulforaphane in Neurodegenerative Diseases. *International Journal of Molecular Sciences*. 2020;21(22):8637.
- Coutinho LdL, Junior TCT, Rangel MC. Sulforaphane: An emergent anti-cancer stem cell agent. *Frontiers in Oncology*. 2023;13.
- Gong Y, Cao X, Gong L, Li W. Sulforaphane alleviates retinal ganglion cell death and inflammation by suppressing NLRP3 inflammasome activation in a rat model of retinal ischemia/reperfusion injury. *International Journal of Immunopathology and Pharmacology*. 2019;33.
- Zhang YJ, Wu Q. Sulforaphane protects intestinal epithelial cells against lipopolysaccharide-induced injury by activating the AMPK/SIRT1/PGC-1 α pathway. *Bioengineered*. 2021;12(1):4349-60.
- Ruhe RT, Suzuki K. The Integrative Role of Sulforaphane in Preventing Inflammation, Oxidative Stress and Fatigue: A Review of a Potential Protective Phytochemical. *Antioxidants (Basel)*. 2020;9(6).
- Thiruvengadam M, Venkidasamy B, Subramanian U, Samynathan R, Ali Shariati M, Rebezov M, et al. Bioactive Compounds in Oxidative Stress-Mediated Diseases: Targeting the NRF2/ARE Signaling Pathway and Epigenetic Regulation. *Antioxidants (Basel)*. 2021;10(12).
- Mahn A, Castillo A. Potential of Sulforaphane as a Natural Immune System Enhancer: A Review. *Molecules*. 2021;26(3).
- Subedi L, Lee J, Yumnam S, Ji E, Kim S. Anti-Inflammatory Effect of Sulforaphane on LPS-Activated Microglia Potentially through JNK/AP-1/NF- κ B Inhibition and Nrf2/HO-1 Activation. *Cells*. 2019;8(2).
- Ramirez-Pavez T, Garcia-Penaranda A, Garcia-Ibanez P, Yepes-Molina L, Carvajal M, Ruiz-Alcaraz AJ, et al. Potential of Sulforaphane and Broccoli Membrane Vesicles as Regulators of M1/M2 Human Macrophage Activity. *Int J Mol Sci*. 2022;23(19).
- Bahiraii S, Brenner M, Yan F, Weckwerth W, Heiss EH. Sulforaphane diminishes moonlighting of pyruvate kinase M2 and interleukin 1 β expression in M1 (LPS) macrophages. *Frontiers in Immunology*. 2022;13.
- Ruhe RT, Ma S, Suzuki K. Sulforaphane Protects

- Cells against Lipopolysaccharide-Stimulated Inflammation in Murine Macrophages. *Antioxidants* (Basel). 2019;8(12).
23. K Q, Y L, W L, P L, X Z, Q Z, et al. SULFORAPHANE ADMINISTRATION AFTER HEMORRHAGIC SHOCK/ RESUSCITATION IN MICE REDUCES THE SECRETION OF INFLAMMATORY CYTOKINES AND INCREASES THE IMMUNOCOMPETENCE OF SPLENIC MACROPHAGES. *Shock*. 2023;59(- 1540-0514 (Electronic)):486-92.
 24. W L, J G, A F, K H, F B, C W, et al. Sulforaphane-Dependent Up-Regulation of NRF2 Activity Alleviates Both Systemic Inflammatory Response and Lung Injury After Hemorrhagic Shock/ Resuscitation in Mice. *Shock*. 2022;57(- 1540-0514 (Electronic)):221-9.
 25. Liu H, Yang X, Tang K, Ye T, Duan C, Lv P, et al. Sulforaphane elicits dual therapeutic effects on Renal Inflammatory Injury and crystal deposition in Calcium Oxalate Nephrocalcinosis. *Theranostics*. 2020;10(16):7319-34.
 26. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. *Mayo Clin Proc*. 2019;94(1):155-65.
 27. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2017;390(10114):2769-78.
 28. Saez A, Herrero-Fernandez B, Gomez-Bris R, Sanchez-Martinez H, Gonzalez-Granado JM. Pathophysiology of Inflammatory Bowel Disease: Innate Immune System. *Int J Mol Sci*. 2023;24(2).
 29. Zhou X, Li W, Wang S, Zhang P, Wang Q, Xiao J, et al. YAP Aggravates Inflammatory Bowel Disease by Regulating M1/M2 Macrophage Polarization and Gut Microbial Homeostasis. *Cell Rep*. 2019;27(4):1176-89 e5.
 30. Yang L, Shen C, Estrada-Bernal A, Robb R, Chatterjee M, Sebastian N, et al. Oncogenic KRAS drives radioresistance through upregulation of NRF2-53BP1-mediated non-homologous end-joining repair. *Nucleic Acids Res*. 2021;49(19):11067-82.
 31. Tanoue T, Nishitani Y, Kanazawa K, Hashimoto T, Mizuno M. In vitro model to estimate gut inflammation using co-cultured Caco-2 and RAW264.7 cells. *Biochem Biophys Res Commun*. 2008;374(3):565-9.
 32. Ren M-T, Gu M-L, Zhou X-X, Yu M-S, Pan H-H, Ji F, et al. Sirtuin 1 alleviates endoplasmic reticulum stress-mediated apoptosis of intestinal epithelial cells in ulcerative colitis. *World Journal of Gastroenterology*. 2019;25(38):5800-13.
 33. De Gregorio A, Serafino A, Krasnowska EK, Superti F, Di Fazio MR, Fuggetta MP, et al. Protective Effect of *Limosilactobacillus fermentum* ME-3 against the Increase in Paracellular Permeability Induced by Chemotherapy or Inflammatory Conditions in Caco-2 Cell Models. *International Journal of Molecular Sciences*. 2023;24(7).
 34. Islam SU, Lee JH, Shehzad A, Ahn EM, Lee YM, Lee YS. Decursinol Angelate Inhibits LPS-Induced Macrophage Polarization through Modulation of the NFkappaB and MAPK Signaling Pathways. *Molecules*. 2018;23(8).
 35. Yuan S-N, Wang M-x, Han J-L, Feng C-Y, Wang M, Wang M, et al. Improved colonic inflammation by nervonic acid via inhibition of NF-κB signaling pathway of DSS-induced colitis mice. *Phytomedicine*. 2023;112.
 36. Xu Y-W, Xing R-X, Zhang W-H, Li L, Wu Y, Hu J, et al. Toxoplasma ROP16I/III ameliorated inflammatory bowel diseases via inducing M2 phenotype of macrophages. *World Journal of Gastroenterology*. 2019;25(45):6634-52.
 37. Kordulewska NK, Topa J, Tańska M, Cieślińska A, Fiedorowicz E, Savelkoul HFJ, et al. Modulatory Effects of Osthole on Lipopolysaccharides-Induced Inflammation in Caco-2 Cell Monolayer and Co-Cultures with THP-1 and THP-1-Derived Macrophages. *Nutrients*. 2020;13(1).
 38. Ortiz-Robles CD, Cortés-Montoya V, Reyes-Aguilar SS, Albores A, Calderón-Aranda ES. Low concentrations of lead disturb phenotypical markers of the inflammatory and the anti-inflammatory profile of bone marrow-derived macrophages from BALB/c mice. *Toxicology*. 2021;460.
 39. Zhang Q, Chen LH, Yang H, Fang YC, Wang SW, Wang M, et al. GPR84 signaling promotes intestinal mucosal inflammation via enhancing NLRP3 inflammasome activation in macrophages. *Acta Pharmacol Sin*. 2022;43(8):2042-54.
 40. Yang K, Xu J, Fan M, Tu F, Wang X, Ha T, et al. Lactate Suppresses Macrophage Pro-Inflammatory Response to LPS Stimulation by Inhibition of YAP and NF-kappaB Activation via GPR81-Mediated Signaling. *Front Immunol*. 2020;11:587913.
 41. Ranjan A, Ramachandran S, Gupta N, Kaushik I, Wright S, Srivastava S, et al. Role of Phytochemicals in Cancer Prevention. *Int J Mol Sci*. 2019;20(20).
 42. Fakhri S, Piri S, Moradi SZ, Khan H. Phytochemicals Targeting Oxidative Stress, Interconnected Neuroinflammatory, and Neuroapoptotic Pathways Following Radiation. *Curr Neuropharmacol*. 2022;20(5):836-56.
 43. Yagishita Y, Fahey JW, Dinkova-Kostova AT, Kensler TW. Broccoli or Sulforaphane: Is It

- the Source or Dose That Matters? *Molecules*. 2019;24(19).
44. Geiss C, Salas E, Guevara-Coto J, Regnier-Vigouroux A, Mora-Rodriguez RA. Multistability in Macrophage Activation Pathways and Metabolic Implications. *Cells*. 2022;11(3).
 45. Koh YC, Yang G, Lai CS, Weerawatanakorn M, Pan MH. Chemopreventive Effects of Phytochemicals and Medicines on M1/M2 Polarized Macrophage Role in Inflammation-Related Diseases. *Int J Mol Sci*. 2018;19(8).
 46. Sranujit RP, Noysang C, Tippiyawat P, Kooltheat N, Luetragoon T, Usuwanthim K. Phytochemicals and Immunomodulatory Effect of *Nelumbo nucifera* Flower Extracts on Human Macrophages. *Plants (Basel)*. 2021;10(10).
 47. Lee D, Lee SR, Kang KS, Kim KH. Bioactive Phytochemicals from Mulberry: Potential Anti-Inflammatory Effects in Lipopolysaccharide-Stimulated RAW 264.7 Macrophages. *Int J Mol Sci*. 2021;22(15).
 48. Milich LM, Ryan CB, Lee JK. The origin, fate, and contribution of macrophages to spinal cord injury pathology. *Acta Neuropathol*. 2019;137(5):785-97.
 49. Yang Y, Wang Y, Guo L, Gao W, Tang TL, Yan M. Interaction between macrophages and ferroptosis. *Cell Death Dis*. 2022;13(4):355.
 50. Griess B, Mir S, Datta K, Teoh-Fitzgerald M. Scavenging reactive oxygen species selectively inhibits M2 macrophage polarization and their pro-tumorigenic function in part, via Stat3 suppression. *Free Radic Biol Med*. 2020;147:48-60.
 51. Tian L, Zhao J-L, Kang J-Q, Guo S-b, Zhang N, Shang L, et al. Astragaloside IV Alleviates the Experimental DSS-Induced Colitis by Remodeling Macrophage Polarization Through STAT Signaling. *Frontiers in Immunology*. 2021;12.
 52. Liu S, Shen H, Li J, Gong Y, Bao H, Zhang J, et al. Loganin inhibits macrophage M1 polarization and modulates sirt1/NF- κ B signaling pathway to attenuate ulcerative colitis. *Bioengineered*. 2020;11(1):628-39.
 53. Tang B, Zhu J, Zhang B, Wu F, Wang Y, Weng Q, et al. Therapeutic Potential of Triptolide as an Anti-Inflammatory Agent in Dextran Sulfate Sodium-Induced Murine Experimental Colitis. *Frontiers in Immunology*. 2020;11.
 54. Giuffrida P, Cococcia S, Delliponti M, Lenti MV, Di Sabatino A. Controlling Gut Inflammation by Restoring Anti-Inflammatory Pathways in Inflammatory Bowel Disease. *Cells*. 2019;8(5).
 55. Zhang S, Xu W, Wang H, Cao M, Li M, Zhao J, et al. Inhibition of CREB-mediated ZO-1 and activation of NF- κ B-induced IL-6 by colonic epithelial MCT4 destroys intestinal barrier function. *Cell Proliferation*. 2019;52(6).
 56. Dong L, Xie J, Wang Y, Jiang H, Chen K, Li D, et al. Mannose ameliorates experimental colitis by protecting intestinal barrier integrity. *Nature Communications*. 2022;13(1).
 57. Ibrahim S, Zhu X, Luo X, Feng Y, Wang J. PIK3R3 regulates ZO-1 expression through the NF- κ B pathway in inflammatory bowel disease. *International Immunopharmacology*. 2020;85.
 58. Zhang Y, Tan L, Li C, Wu H, Ran D, Zhang Z. Sulforaphane alter the microbiota and mitigate colitis severity on mice ulcerative colitis induced by DSS. *AMB Express*. 2020;10(1):119.
 59. Tu J, Xu Y, Xu J, Ling Y, Cai Y. Chitosan nanoparticles reduce LPS-induced inflammatory reaction via inhibition of NF- κ B pathway in Caco-2 cells. *Int J Biol Macromol*. 2016;86:848-56.
 60. Garrido-Trigo A, Corraliza AM, Veny M, Dotti I, Melón-Ardanaz E, Rill A, et al. Macrophage and neutrophil heterogeneity at single-cell spatial resolution in human inflammatory bowel disease. *Nature Communications*. 2023;14(1).
 61. Zhu W, Chen Q, Li Y, Wan J, Li J, Tang S. HIF-1 α -Overexpressing Mesenchymal Stem Cells Attenuate Colitis by Regulating M1-like Macrophages Polarization toward M2-like Macrophages. *Biomedicine*. 2023;11(3).
 62. Zhang Y, Li X, Luo Z, Ma L, Zhu S, Wang Z, et al. ECM1 is an essential factor for the determination of M1 macrophage polarization in IBD in response to LPS stimulation. *Proc Natl Acad Sci U S A*. 2020;117(6):3083-92.
 63. Zhang M, Yang D, Yu H, Li Q. MicroRNA-497 inhibits inflammation in DSS-induced IBD model mice and lipopolysaccharide-induced RAW264.7 cells via Wnt/ β -catenin pathway. *Int Immunopharmacol*. 2021;101(Pt B):108318.
 64. Morris G, Gevezova M, Sarafian V, Maes M. Redox regulation of the immune response. *Cell Mol Immunol*. 2022;19(10):1079-101.
 65. Luo JF, Shen XY, Lio CK, Dai Y, Cheng CS, Liu JX, et al. Activation of Nrf2/HO-1 Pathway by Nardochinoid C Inhibits Inflammation and Oxidative Stress in Lipopolysaccharide-Stimulated Macrophages. *Front Pharmacol*. 2018;9:911.
 66. Wei J, Zhao Q, Zhang Y, Shi W, Wang H, Zheng Z, et al. Sulforaphane-Mediated Nrf2 Activation Prevents Radiation-Induced Skin Injury through Inhibiting the Oxidative-Stress-Activated DNA Damage and NLRP3 Inflammasome. *Antioxidants (Basel)*. 2021;10(11).
 67. Xiang Q, Zhao Y, Lin J, Jiang S, Li W. The Nrf2 antioxidant defense system in intervertebral disc degeneration: Molecular insights. *Exp Mol Med*. 2022;54(8):1067-75.
 68. Xie H, Chun FK, Rutz J, Blaheta RA. Sulforaphane Impact on Reactive Oxygen Species (ROS) in

- Bladder Carcinoma. *Int J Mol Sci.* 2021;22(11).
69. Huang C, Wang J, Liu H, Huang R, Yan X, Song M, et al. Ketone body β -hydroxybutyrate ameliorates colitis by promoting M2 macrophage polarization through the STAT6-dependent signaling pathway. *BMC Medicine.* 2022;20(1).
70. Sun S, Xu X, Liang L, Wang X, Bai X, Zhu L, et al. Lactic Acid-Producing Probiotic *Saccharomyces cerevisiae* Attenuates Ulcerative Colitis via Suppressing Macrophage Pyroptosis and Modulating Gut Microbiota. *Frontiers in Immunology.* 2021;12.
71. Zhang Y, Tao M, Chen C, Zhao X, Feng Q, Chen G, et al. BAFF Blockade Attenuates DSS-Induced Chronic Colitis via Inhibiting NLRP3 Inflammasome and NF- κ B Activation. *Frontiers in Immunology.* 2022;13.
72. Lee SM, Kim N-H, Lee S, Kim YN, Heo J-D, Rho J-R, et al. (10Z)-Debromohymenialdisine from Marine Sponge *Stylissa* sp. Regulates Intestinal Inflammatory Responses in Co-Culture Model of Epithelial Caco-2 Cells and THP-1 Macrophage Cells. *Molecules.* 2019;24(18).
73. Pan Y, Yu Y, Wang X, Zhang T. Tumor-Associated Macrophages in Tumor Immunity. *Front Immunol.* 2020;11:583084.
74. Kaiser AE, Baniasadi M, Giansiracusa D, Giansiracusa M, Garcia M, Fryda Z, et al. Sulforaphane: A Broccoli Bioactive Phytocompound with Cancer Preventive Potential. *Cancers (Basel).* 2021;13(19).
75. Barabutis N, Schally AV, Siejka A. P53, GHRH, inflammation and cancer. *EBioMedicine.* 2018;37:557-62.
76. Greten FR, Grivnenkov SI. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity.* 2019;51(1):27-41.
77. Kubra KT, Akhter MS, Uddin MA, Barabutis N. P53 versus inflammation: an update. *Cell Cycle.* 2020;19(2):160-2.
78. Hernandez Borrero LJ, El-Deiry WS. Tumor suppressor p53: Biology, signaling pathways, and therapeutic targeting. *Biochim Biophys Acta Rev Cancer.* 2021;1876(1):188556.
79. Shi D, Jiang P. A Different Facet of p53 Function: Regulation of Immunity and Inflammation During Tumor Development. *Front Cell Dev Biol.* 2021;9:762651.
80. Kan S, Wang J, Sun G. Sulforaphane regulates apoptosis- and proliferation-related signaling pathways and synergizes with cisplatin to suppress human ovarian cancer. *Int J Mol Med.* 2018;42:2447-58.
81. Wang Y, Wu H, Dong N, Su X, Duan M, Wei Y, et al. Sulforaphane induces S-phase arrest and apoptosis via p53-dependent manner in gastric cancer cells. *Sci Rep.* 2021;11(1):2504.
82. Zhou Y, Que KT, Zhang Z, Yi ZJ, Zhao PX, You Y, et al. Iron overloaded polarizes macrophage to proinflammation phenotype through ROS/acetyl-p53 pathway. *Cancer Med.* 2018;7(8):4012-22.