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Inhibition of pattern recognition receptor-mediated inflammation by bioactive phytochemicals: a review of recent research*

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Abstract

Emerging evidence reveals that pattern-recognition receptors (PRR), Toll-like receptors (TLR) and Nucleotide-binding oligomerization domain proteins (NOD) mediate both infection-induced and sterile inflammation by recognizing pathogen-associated molecular patterns and endogenous molecules, respectively. PRR-mediated chronic inflammation is a determinant for the development and progression of chronic diseases including cancer, atherosclerosis and insulin resistance. Recent studies demonstrated that certain phytochemicals inhibit PRR-mediated pro-inflammation. Curcumin, helenalin, cinnamaldehyde and sulforaphane, containing α, β-unsaturated carbonyl or isothiocyanate group, respectively, that are known to interact with free SH groups in cysteine residues, but not resveratrol (with no unsaturated carbonyl group), inhibit TLR4 activation by interfering with TLR4 receptor dimerization. Similarly, curcumin, as well as parthenolide and helenalin, but not resveratrol and EGCG, also inhibits NOD2 activation by interfering with NOD2 dimerization. In contrast, resveratrol, EGCG, luteolin and structural analogs of luteolin, specifically inhibit TLR3 and TLR4 signaling by targeting TBK1 and RIP1 in TRIF complex. Together, these results suggest that PRRs and downstream signaling components are molecular targets for dietary strategies to reduce PRR-mediated chronic inflammation and consequent risks of chronic diseases.

Introduction

Pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and Nucleotide-binding oligomerization domain proteins (NODs) detect invading pathogens by recognizing pathogen-associated molecular patterns (PAMPs), and activate innate immune responses for host defense. However, it is now well documented that these PRRs can also be activated by a variety of endogenous molecules derived from tissue injury and elicit sterile inflammation to initiate wound-healing processes. Host defense and wound healing are the key biological processes for the survival of all multicellular organisms. Emerging evidence suggests that PRRs can detect metabolic disturbance and bridge immune responses to metabolic

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homeostasis [1]. Such functional diversity of PRRs may be achieved by the ability of PRRs to recognize a wide variety of agonists [2]. However, such promiscuous nature of agonist specificity can make PRRs vulnerable to dysregulation leading to chronic inflammation, which in turn can promote the development and progression of chronic diseases.

Many phytochemicals have been known for their anti-inflammatory, chemopreventive, and cardioprotective properties [3-5]. An increasing number of studies also indicates that diet rich in anti-inflammatory phytochemicals may have beneficial effects in ameliorating metabolic syndrome, a constellation of abnormal cardiometabolic factors that increase risk of cardiovascular disease and type 2 diabetes [6]. However, the mechanisms by which such beneficial effects are mediated is not well understood. Recent evidence revealed that certain phytochemicals inhibit PRR activation by targeting the receptor itself or their specific downstream signaling molecules [7-13]. These results suggest the possibility that PRR-mediated inflammation and consequent risk of development of chronic disease can be suppressed by diets we consume every day. This review summarizes and discusses the significance and implication of recent findings that certain phytochemicals inhibit the activation of PRRs.

Pattern recognition receptors (PRRs) and downstream signaling pathway

TLRs and NODs are two key PRRs involved in host defense. TLRs are type I transmembrane receptors composed of extracellular leucine-rich repeat (LRR) motifs, and a cytoplasmic Toll/interleukin-1 receptor (TIR) homology domain [14-15]. Thirteen TLRs have been identified in human and mouse, which are ubiquitously expressed in various tissues [16]. TLRs detect invading pathogens by recognizing PAMPs, and are responsible for the induction of innate immune responses for elimination of the pathogens.

NODs as cytoplasmic PRRs recognize conserved moieties of bacterial peptidoglycan and activate proinflammatory signaling pathways [17]. NODs are composed of a C-terminal leucine-rich repeat (LRR) domain for ligand recognition, a nucleotide-binding oligomerization domain (NOD), and an N-terminal Caspase-recruitment domain (CARD) for the initiation of downstream signaling. NOD1 recognizes a dipeptide, γ-D-glutamyl-*meso*diaminopimelic acid (iE-DAP), from most Gram-negative bacteria and specific Gram-positive bacteria. NOD2 recognizes a muramyl dipeptide, MurNAc-L-Ala-D-isoGln (MDP) from both Gram-positive and Gram-negative bacteria [18-19].

PRRs can be also activated by endogenous molecules with non-microbial origin [20-29]. These molecules are generated during tissue injuries and cell death as well as from nutrient metabolism. The downstream signaling pathways of TLRs and NODs have been extensively reviewed [14, 17, 30] and are not the focus of this review. Downstream signaling pathways of PRRs and known targets of phytochemicals are depicted in Fig. 1.

PRR-mediated inflammation and chronic diseases

TLRs and NODs recognize PAMPs and activate innate immune responses that are required for host defense against invading pathogens. In addition to infection-induced inflammation, these PRRs can also be activated by endogenous molecules of non-microbial origins, leading to sterile inflammation. Dysregulation of infection-induced or sterile inflammation can increase the risks of development and progression of many chronic diseases, including atherosclerosis, cancer and insulin resistance, as depicted in Fig. 2.

Atheroslerosis is considered to be an inflammatory disease, in that inflammation drives the formation, progression and rupture of the atherosclerotic plaques [31]. Recent studies using atherosclerosis prone LDLr-/- or apoE-/- mice have shown that deficiency of TLR2, TLR4

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or its immediate adaptor protein myeloid differentiation primary response gene 88 (MyD88) has resulted in reduction in plaque lesions [32-34]. On the other hand, recurrent administrations of TLR4 agonist lipopolysaccharide (LPS) [35] or TLR2 agonist, Pam3CSK4 (the synthetic bacterial lipoprotein mimetic) were shown to accelerate atherogenesis [32]. These results suggest that TLR4 and/or TLR2-mediated inflammation is involved in the development of atherosclerosis. Various endogenous molecules that activate TLR4 and might contribute to the development of atherosclerosis have been identified. For example, recent studies have shown that minimally oxidized LDL (mmLDL) and its active components, polyoxygenated cholesteryl ester hydroperoxides, are involved in activation of TLR4, which leads to cytoskeletal rearrangement and intracellular lipid accumulation through activation of spleen tyrosine kinase and its downstream signaling targets [36-39]. Extensive reviews on the activation of TLRs by their endogenous agonists, leading to atherosclerosis can be found elsewhere [2, 40-42]) and are not the focus of this review.

The continuous activation of TLRs by chronic infection, such as *Helicobater pylori*, can cause chronic inflammation leading to increased risk of gastric cancer [43-44]. TLRs are also responsible for recognizing microbial pathogens, such as Epstein-Barr virus [45], hepatitis B and C virus [46-47] and *Helicobater pylori* [48], which are important etiological agents of human cancer. In supporting of the roles in cancer, the association of many polymorphisms in TLRs with human cancer has been reported [49-50]. Moreover, dysregulation of sterile inflammation during wound healing can cause chronic inflammation providing the microenvironment that can promotes pathogenic processes including tumorigenesis. Certain types of cancer are considered as wounds that do not heal [51]. The activation of TLRs and development of cancer have also been extensively reviewed [52-54] and therefore are not the focus of this review.

Even in absence of infection or overt tissue injury, low grade chronic inflammation is known to be associated with obesity and insulin resistance. Many inflammatory signals are known to impair insulin receptor signaling leading to insulin resistance. Serine kinases Interleukin-1 receptor-associated kinase (IRAK-1), IkappaB kinase β (IKKβ) and c-Jun N-terminal kinase (JNK) inhibit insulin signaling by phosphorylating Insulin Receptor Substrate 1 (IRS-1) on serine residues, which in turn impedes the normal association of IRS-1 with insulin receptor, thereby impairing downstream propagation of insulin signaling [55-57]. Since IRAK-1, IKKβ and JNK are the downstream components of TLR signaling pathways, it is conceivable that the activation of TLRs would lead to impairment of insulin signaling. In supporting this notion, mice lacking TLR4 were protected from lipid infusion-induced suppression of whole body glucose metabolism and insulin signaling in muscle [58]. Mice lacking TLR2 were protected from high fat feeding-induced adiposity, insulin resistance and other metabolic abnormalities [59]. Saturated fatty acids (SFAs) have been suggested to be the endogenous agonists for TLR4 and TLR2 in vivo. Previous work have shown that SFAs activate TLR4 or TLR2 (dimerized with TLR6 or TLR1) in murine macrophage RAW264.7 cells [26-27], myotubes C2C12 cells [60] and 3T3-L1 adipocytes [58]. Together, these results support the role of TLRs in the development of obesity and insulin resistance. Therefore, the dietary factors which inhibit TLR activation may improve insulin sensitivity by alleviating TLR-mediated impairment of insulin signaling.

The significance of NOD1 and NOD2 in immune responses is evident from the linkage of their mutations with inflammatory diseases in humans and the increased susceptibility of NOD1-/- and NOD2-/- mice to gastrointestinal bacterial infections. While mutations in NOD1 have been associated with increased susceptibility to asthma and inflammatory bowel disease [61-62], Mutations in NOD2 have been linked to susceptibility to inflammatory granulomatous disorders, such as Crohn's disease [63-64], Blau syndrome [65] and early on-set sarcoidoisis [66]. NOD1-/-mice are more susceptible to *H. pylori* oral infections with

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higher bacterial burden and mortality rates than wild-type [67]. On the other hand, NOD2-/- mice were shown to display increased bacterial loads when infected with *L. monocytogenes* via the oral route, compared to their wild-type counterparts [68]. Therefore, NODs play a critical role in host defense against bacterial infection. Dysregulation of NOD signaling pathways may lead to the development of certain chronic diseases. Recent study revealed that bioactive phytochemicals inhibit the activation of PRRs by targeting the receptors or their downstream signaling molecules [11] . How such inhibition of NODs by phytochemicals is related to their anti-inflammatory, chemopreventive, and cardioprotective properties is a very intriguing question that needs further investigation.

Inhibition of PRR activation by bioactive phytochemicals

Inhibition of PRR dimerization by phytochemicals

The conceptual clue for the phytochemicals to inhibit PRR activation was derived from the results demonstrating that the relative potency of different sesquiterpene lactones in inhibiting LPS-induced expression of cyclooxygenase-2 (COX-2; a TLR4 target gene) was directly related to the presence of α-methylene-gamma-lactone moiety that can confer a Michael addition to sulfhydryl group [69]. Furthermore, sulfhydryl compounds abolish the inhibitory effects of a sesquiterpene lactone on LPS-induced expression of COX-2 [69]. Subsequent studies showed that many phytochemicals with inhibitory effects on NF-κB activation or chemopreventive efficacy contain the structural motif conferring a Michael addition [70-71]. Recent studies have showed that the phytochemicals with the structural motif conferring a Michael addition inhibit PRR activation by interfering with the receptor dimerization [7, 9, 11, 13]. These have provided a new paradigm for understanding of the beneficial effects of phytochemicals on chronic diseases.

Curcumin, a polyphenol found in the plant *Curcuma longa* (Fig. 3), has been shown to suppress the activation of NF-κB induced by various pro-inflammatory stimuli, presumably through inhibition of IKKβ kinase activity or DNA binding of p65 [72-73]. A recent study revealed that curcumin inhibits MyD88-induced NF-κB activation. However, curcumin did not inhibit interferon regulatory factor 3 (IRF3) activation induced by another immediate TLR4 downstream component TIR-domain-containing adaptor inducing interferon-beta (TRIF), suggesting that the target of curcumin is the receptor itself, but not the downstream components of TRIF pathway [7]. Further studies indicate that curcumin and helenalin, which contain α, β-unsaturated carbonyl group, but not resveratrol (with no unsaturated carbonyl group, Fig. 3), inhibit TLR4 activation by interfering with receptor dimerization [7] (Fig. 4A). Such conclusion was further supported by the result that curcumin inhibits ligand-independent dimerization of constitutively active TLR4. The receptor dimerization is known to be the initial step of TLR4 activation [74-75]. Inhibition of receptor dimerization of TLR4 by phytochemicals with the structural motif conferring a Michael addition suggests that these phytochemicals might modify the free sulfhydryl groups of cysteine residues in TLR4, leading to interference of disulfide formation. Moreover, the activation loop of IKKβ containing cysteine residues has also been reported to be modified by Michael addition [76]. Similarly, cinnamaldehyde (Fig. 3), which also contains α, β-unsaturated carbonyl group, inhibited agonist-induced TLR4 dimerization [9]. The suppression of TLR4 dimerization by cinnamaldehyde was reversed by thiol donors, dithiothreitol and N-acetyl-L-cysteine, suggesting the involvement of cysteine modification in the inhibitory effects of anti-inflammatory phytochemicals [9]. Indeed, sulforaphane (SFN, Fig. 3), an isothiocyanate, formed adducts with cysteine residues in the extracellular domain of TLR4 as determined by micro LC-MS/MS analysis after incubation of recombinant TLR4 extracellular domain with SFN (Fig. 4C-D) [13]. This modification by SFN resulted in the blockade of TLR4 dimerization (Fig. 4B), which was reversed by thiol supplementation. Together, these data suggest that the inhibition of TLR activation by certain anti-inflammatory phytochemicals

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may be attributed to the modification of cysteines in TLR4 extracellular domain and the subsequent blockade of receptor dimerization through their electrophilic α, β-unsaturated carbonyl groups or isothiocyanate group (Fig. 3). Moreover, these results also suggest that one of targets of anti-inflammatory phytochemicals is TLR4 receptor complex and that the receptor dimerization can be an effective target for dietary and pharmacological agents to ameliorate chronic inflammatory diseases resulting from dysregulated TLR activation.

Similar to TLR4, NODs also contain LRR domain in their structure which may be involved in receptor dimerization and the dimerization of NODs is also the initial step of ligand-induced receptor activation [17]. Curcumin, parthenolide and helenalin (Fig. 3) were shown to inhibit NOD2 ligand MDP-induced NF-κB activation and target gene (IL-8) expression in a dose-dependent manner (Fig. 5A). In contrast, (-)-epigallocatechin-3-galate (EGCG, Fig. 3) and resveratrol, which do not contain the structural motif conferring Michael addition, exhibited no inhibitory effects (Fig. 5A) [11]. Moreover, curcumin inhibited NOD2 over-expression-induced NF-κB activation in 293T cells in a dose-dependent manner (Fig. 5B). However, curcumin did not inhibit NF-κB in 293T cells that over-express the immediate downstream signaling molecule Rip-like interactive clarp kinase (RICK), suggesting that the target of curcumin in inhibiting NOD2 signaling pathway must be located upstream of NOD2-RICK interaction, instead of downstream of RICK (Fig. 5B). Indeed, curcumin inhibited MDP-induced NOD2 dimerization in a dose-dependent manner. Curcumin also inhibited NOD2 dimerization induced by another NOD2 agonist, lauric acid (Fig. 5C) [11]. Similar inhibition of NOD2 dimerization was also observed with parthenolide with α, β-unsaturated carbonyl group [11]. It remains to be determined whether these phytochemicals prevent or alleviate NOD-mediated chronic inflammation and consequent risk of chronic diseases.

The studies with curcumin, cinnamaldehyde, and sulforaphane, which have structural motifs that can interfere with receptor dimerization, have provided a paradigm that PRR dimer assembly could be a novel target for the anti-inflammatory strategies to ameliorate chronic inflammation resulting from dysregulated PRR activation.

Inhibition of MyD88-independent signaling pathways downstream of TLR3 and TLR4 by phytochemicals

Phytochemicals without the structural motif conferring Michael addition(e.g., resveratrol, EGCG) did not inhibit TLR4 dimerization; however, they specifically inhibited the MyD88-independent TRIF complex containing serine kinases TANK binding kinase 1 (TBK1) and Receptor –interacting protein 1 (RIP1), the downstream signaling components of TLR3 and TLR4 [8, 10, 12]. Despite numerous reports demonstrating the inhibitory effects of resveratrol on NF-κB activation and target gene expression induced by various proinflammatory stimuli [77-78], the direct molecular target has not been fully identified. Although NF-κB can be stimulated by multiple signaling pathways derived from the activation of different types of receptors including TLRs and NODs [79], the inhibition by resveratrol of NF-κB activated by different agonists suggests that the targets of resveratrol are likely to be downstream signaling components responsible for the activation of the transcription factor, rather than the receptors themselves. Resveratrol suppressed NF-κB activation and COX-2 expression following TLR3 and TLR4 stimulation, but not TLR2 or TLR9 in RAW264.7 cells (Fig. 6A) [8]. Moreover, resveratrol inhibited NF-κB activation induced by stimulation of TRIF, but not by MyD88. Furthermore, the suppressive effect of resveratrol on LPS-induced NF-κB activation was abolished in TRIF-deficient mouse embryonic fibroblasts, but not in MyD88-deficient macrophages (Fig. 6B-C), suggesting that resveratrol specifically inhibits MyD88-independent signaling pathways downstream of TLR3 and TLR4. In further delineating the target of resveratrol, it was found that resveratrol inhibited the kinase activity of TBK1 and the NF-κB activation induced by RIP1 [8] (Fig.

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6D). Together, these results demonstrate that resveratrol specifically inhibits TLR3 and TLR4 signaling pathway by targeting TBK1 and RIP1 in TRIF complex. Additional studies showed that EGCG, luteolin and structural analogs of luteolin such as quercetin, chrysin, and eriodictyol, also inhibited TRIF signaling pathway by targeting TBK1 kinase [10, 12]. Together, the studies suggest that TBK1 in TRIF pathway, can be targeted by phytochemicals to suppress the activation of TLR3 and TLR4 and their functional consequences. Future studies will identify additional phytochemicals that can target TBK1

Future directions

Excessive generation of stimulatory endogenous molecules, or the presence of insufficient amounts of endogenous molecules that inhibit the activation of PRRs, can increase the propensity to PRR activation. Although PRR-mediated innate immune responses are a part of the essential elements for host defense and wound healing, the termination of PRR-mediated innate immune responses is required to prevent tissue damage and chronic inflammation. Currently, the mechanisms by which PRR-mediated inflammatory responses are resolved are not well understood. It has been reported that negative regulation of TLRs can be achieved at multiple levels [80-81]. MicroRNA-mediated RNA interference is also emerging as an important regulatory mechanism that operates at the translation level to modulate TLR-mediated immune responses [82-85]. For example, the production of miR-146 was up-regulated by TLR4 activation. The expression of IRAK and Tumor necrosis factor receptor associated factor 6 (TRAF6) proteins, the downstream signaling components of TLR4, was suppressed by the miR-146 family [82]. On the other hand, NOD2 agonists activated PI3K pathways, which negatively regulate NOD-induced NF-κB activation [86]. These results suggest that the activation of PRRs stimulates both pro-inflammatory and intrinsic resolution pathways, probably with different time course. Therefore, the duration and intensity of PRR-induced inflammation can be modulated not only by the strength of initial activation but also by timely resolution of the inflammation. The findings that the activation of PRRs is inhibited by certain phytochemicals suggest that these dietary components can act as extrinsic resolution factors for PRR-mediated inflammation. Future studies will reveal whether the intrinsic resolution pathways of PRR-induced inflammation can also be modulated by dietary factors, including bioactive phytochemicals.

The fact that the activation of these PRRs is dynamically modulated by dietary factors [2] suggests that PRRs can act as the sensor for disturbance in metabolic homeostasis. Thus, PRRs act as the fulcrum for Yin and Yang of inflammation modulated by dietary factors (Fig. 7). Identifying molecular targets by which dietary factors modulate PRR-mediated signaling pathways and target gene expression would provide new opportunities to reduce the risk and to manage chronic inflammatory diseases resulting from the dysregulation of PRR-mediated inflammatory responses.

Abbreviations

CARD	caspase-recruitment domain
COX	cyclooxygenase
DAMPs	danger-associated molecular patterns
DTT	dithiothreitol
IKK	IkappaB kinase
EGCG	(-)-epigallocatechin-3-gallate

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IRAK	Interleukin-1 receptor-associated kinase
IRF3	Interferon regulatory factor 3
IRS-1	Insulin receptor substrate-1
JNK	c-Jun N-terminal kinase
LDL	low density lipoprotein
LPS	lipopolysaccharide
LRR	leucine-rich repeat
MDP	muramyl dipeptide, MurNAc-l-Ala-disoGln
MyD88	myeloid differentiation primary response gene 88
NAC	N-acetyl-L-cysteine
NF-κB	nuclear factor kappa B
NODs	nucleotide-binding oligomerization domain proteins
PAMPs	pathogen- associated molecular patterns
PI3K	phosphoinositide-3-kinase
PRRs	pattern recognition receptors
RICK	Rip-like interactive clarp kinase
SFA	Saturated fatty acid
TBK1	TANK binding kinase 1
TIR	Toll/interleukin-1 receptor
TLR	Toll-like receptor
TRAF	Tumor necrosis factor receptor associated factor
TRIF	TIR-domain-containing adaptor inducing interferon-beta

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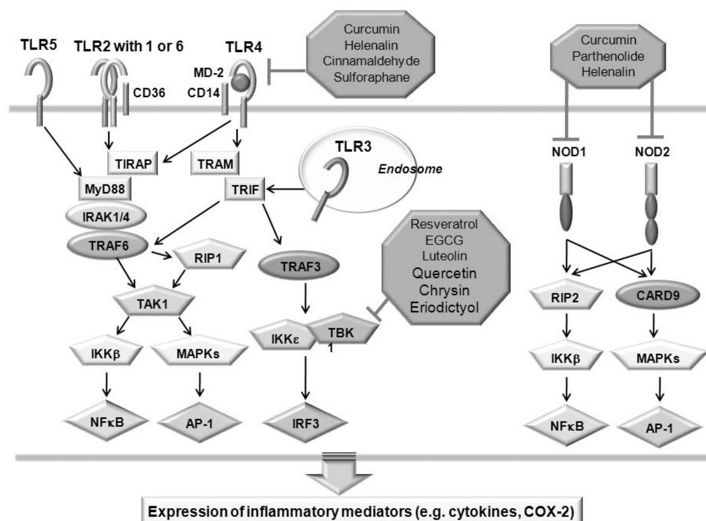

Fig.1. Identified molecular targets of phytochemicals on PRR-mediated inflammatory signaling pathway

TLRs and NODs are pathogen recognition receptors (PRRs) that detect conserved molecules of pathogens. Stimulation of TLRs by ligands leads to the recruitment of adaptor molecules such as MyD88 and TRIF through the interaction of TIR domains, leading to MyD88 dependent and MyD88-independent (TRIF dependent) signaling pathway. The activation of NODs leads to the recruitment of RICK/RIP2 through CARD–CARD interactions, leading to activation of inflammatory signaling pathway. Curcumin, helenalin, cinnamaldehyde, and sulforaphane inhibit TLR4-mediated NF-κB activation by inhibiting TLR4 dimerization. Resveratrol, EGCG, luteolin, quercetin, chrysin, and eriodictyol directly inhibit TBK1 kinase activity. Similarly, curcumin, helenalin and parthenolide also inhibit NOD2 mediated NF-κB activation, presumably through inhibition of NOD2 dimerization.

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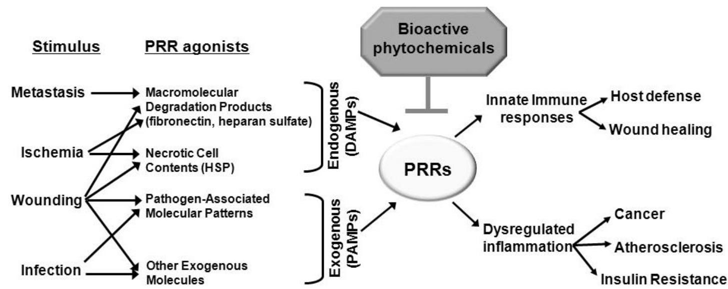

Fig. 2. Bioactive phytochemicals suppress PRR-mediated inflammation, leading to decreased risks of development and progression of chronic diseases

Both pathogen associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) from endogenous molecules from tissue injury and degradation can activate PRRs to culminate in innate immune responses, leading to host defense against invading pathogens, and promote tissue remodeling and wound healing. Dysregulated septic or sterile inflammation may increase the risks of chronic diseases. Bioactive phytochemicals may be beneficial in treating or preventing these chronic diseases through suppression of PRR activation.

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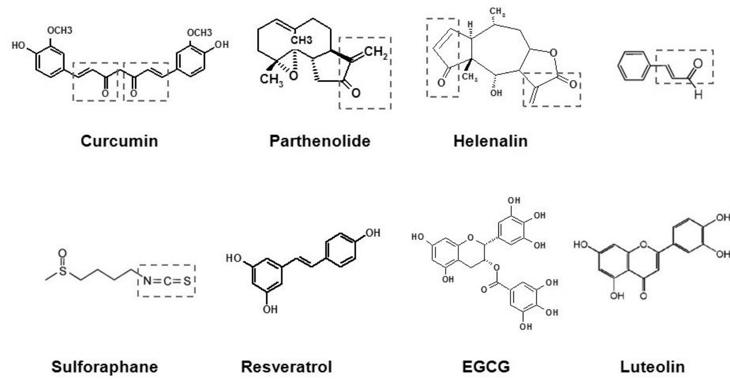
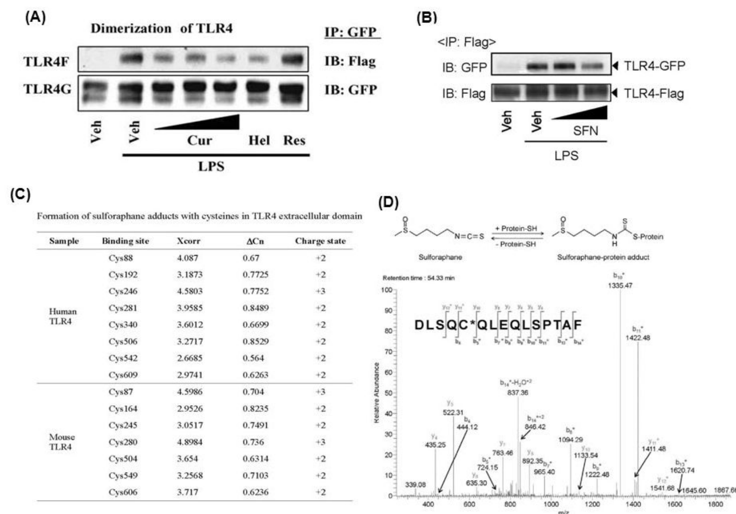


Fig.3. The chemical structures of phytochemicals curcumin, parthenolide, helenalin, cinnamaldehyde, sulforaphane, resveratrol, EGCG, and luteolin

The α,β-unsaturated carbonyl groups in curcumin, parthenolide, helenalin, and cinnamaldehyde are boxed in red. An isothiocyanate group in sulforaphane is boxed in blue.



Formation of sulforaphane adducts with cysteines in TLR4 extracellular domain

Sample	Binding site	Xcorr	ΔCn	Charge state
Human TLR4	Cys88	4.087	0.67	+2
	Cys192	3.1873	0.7725	+2
	Cys246	4.5803	0.7752	+3
	Cys281	3.9585	0.8489	+2
	Cys340	3.6012	0.6699	+2
	Cys506	3.2717	0.8529	+2
	Cys542	2.6685	0.564	+2
	Cys609	2.9741	0.6263	+2
Mouse TLR4	Cys87	4.5986	0.704	+3
	Cys164	2.9526	0.8235	+2
	Cys245	3.0517	0.7491	+2
	Cys280	4.8984	0.736	+3
	Cys504	3.654	0.6314	+2
	Cys549	3.2568	0.7103	+2
	Cys606	3.717	0.6236	+2

Fig. 4. Curcumin and sulforaphane inhibits TLR4 dimerization induced by LPS and sulforaphane binds to cysteines in TLR4 extracellular domain

(A, B). Ba/F3 cells expressing TLR4-Flag (TLR4F), TLR4-GFP (TLR4G), MD2-Flag (MD2F), and CD14 were pre-treated with curcumin (10, 20, 50 μM), helenalin (5 μM), resveratrol (50 μM), or sulforaphane (10, 20 μM) for 1 h and then treated with LPS (50 ng/ml) for 20 min. Cell lysates were subjected to immunoprecipitation (IP) and immunoblotted (IB) with antibody as indicated. (C) Summary of cysteines in human and mouse TLR4, which bind to sulforaphane. Extracellular domain of human TLR4 (a.a. 27-631) or mouse TLR4 (a.a. 26-629) was incubated with sulforaphane and micro LC-MS/MS analysis was performed. (D) (Upper panel) Schematic diagram of reaction of sulforaphane with protein cysteine residue. (Lower panel) MS/MS spectrum of the SFN-cysteine adducts at human TLR4 (Asp502-Phe516; DLSQC^{506}QLEQLSPTAF). * denotes fragment ions with one SFN. Cur, curcumin; Hel, helenalin; and Res, resveratrol; SFN, sulforaphane. Reproduced with permission from *Biochemical Pharmacology* and *Journal of Immunology*.

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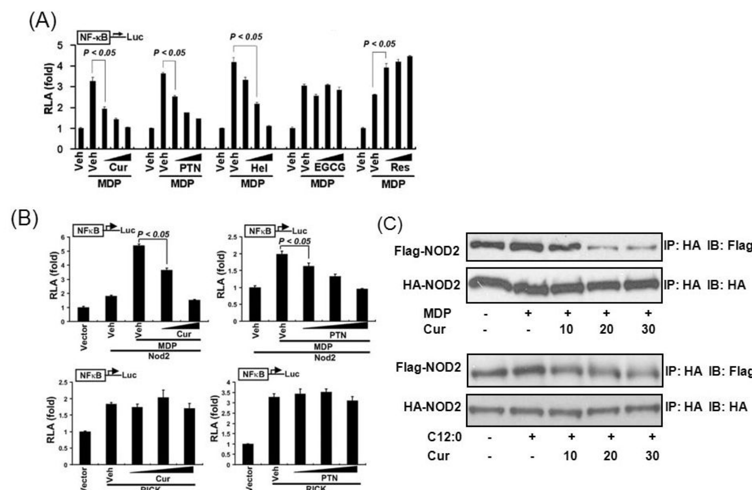

Fig. 5. Curcumin inhibits NOD2 dimerization induced by MDP and lauric acid

(A) HCT116 cells were transfected with NF-κB-luciferase and β-galactosidase reporters. The cells were pretreated with curcumin (10, 20, and 30 μM), parthenolide (5, 10, and 15 μM), helenalin (1, 3, and 5 μM), EGCG (10, 30, and 50 μM), or resveratrol (10, 30, and 50 μM) for 1 h and then coincubated with MDP (50 μM) for additional 6 h. (B) HEK293T cells were cotransfected with NF-κB-luciferase and β-galactosidase reporter and either Nod2 expression vector (top panels) or RICK expression vector (bottom panels). The cells were pretreated with curcumin (10, 20 μM) or parthenolide (5, 10, and 15 μM) for 1 h and then co-incubated with MDP (200 ng/ml) for an additional 6 h (top panels) or were treated with curcumin (10, 20, and 30 μM) or parthenolide (5, 10, and 15 μM) for 6 h (bottom panels). Relative luciferase activity (RLA) was normalized with β-galactosidase activity. Values are mean ± S.E.M (n = 3). Statistically significant difference ($p < 0.05$) is indicated. (C) HEK293T cells were co-transfected with HA-Nod2 and Flag-Nod2 cDNA expression vectors. The cells were pretreated with curcumin (10, 20, and 30 μM) for 1 h and then co-incubated with MDP (200ng/ml) (upper) or lauric acid (C12:0; 100 μM) (lower) for 15 min. Nod2 proteins from cell lysates were immunoprecipitated (IP) with anti-HA affinity matrix, and HA-Nod2 and Flag-Nod2 proteins were detected by Western blotting (IB) using anti-HA and anti-Flag antibodies. Cur, curcumin; PTN, parthenolide; Hel, helenalin; and Res, resveratrol. Reproduced with permission from *Molecular Pharmacology*.

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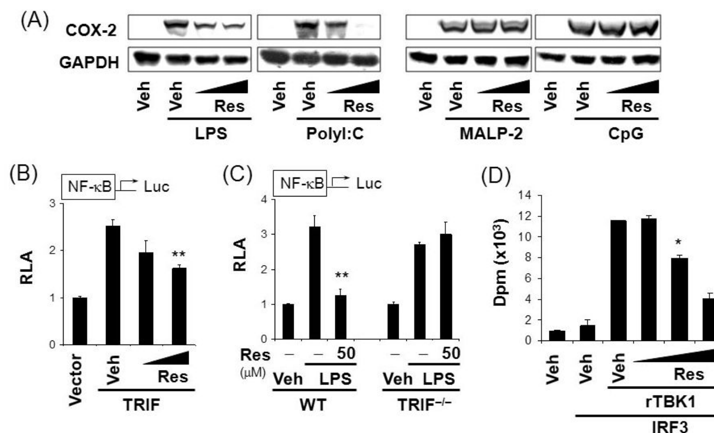

Fig.6. Resveratrol suppresses the functional activity of TBK1

(A) RAW264.7 cells were treated with resveratrol (30, 50 μM) for 1 hr and further stimulated with LPS (5 ng/ml), polyI:C (10 μg/ml), MALP-2 (2 ng/ml), or CpG DNA (ODN1668, 0.2 μM) for 18 hrs. Cell lysates were analyzed for COX-2 and GAPDH immunoblots. (B) RAW264.7 cells were transfected with NF-κB-luciferase reporter plasmid and the TRIF expression plasmid. Cells were further treated with resveratrol (30, 50 μM) for 18 hrs. (C) TRIF-deficient (TRIF-/-) or wild-type mouse embryonic fibroblasts were transfected with NF-κB-luciferase reporter plasmid. Cells were treated with resveratrol for 1 hr and further stimulated with LPS (100 ng/ml) for 18 hrs. Relative luciferase activity (RLA) was determined. Values are mean±S.E.M. (n=3). (D) *In vitro* TBK1 kinase assay was performed using recombinant active TBK1 (rTBK1) with IRF3 in the presence of resveratrol (20, 50, 100 μM). Values are mean ± S.E. ($n = 2$). **, Significantly different from (B) TRIF plus vehicle or (C) LPS alone, $p<0.01$. *,, Significantly different from (D) vehicle plus rTBK1 plus IRF3, $p < 0.05$. Veh, vehicle. Res, resveratrol. WT, wild-type. Reproduced with permission from *Journal of Immunology*.

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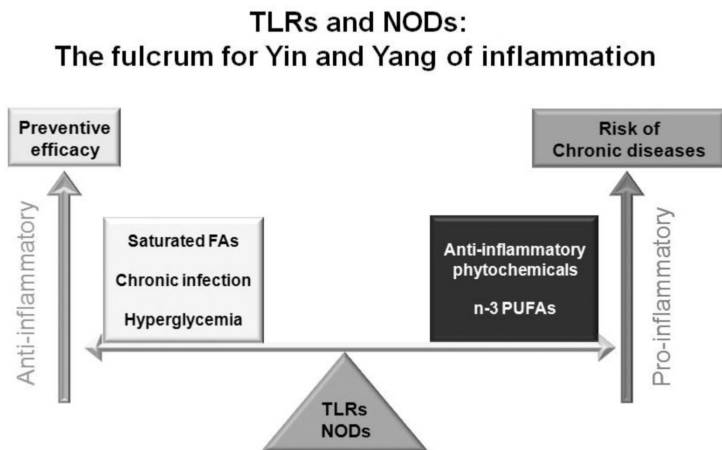

Fig. 7. TLRs and NODs are the fulcrum in the Yin-Yang of inflammation

Activation of these PRRs is dynamically modulated by dietary factors suggesting that PRRs can act as a sensor for disturbance in metabolic homeostasis. Identifying molecular targets by which dietary factors modulate PRR-mediated signaling pathways and target gene expression would provide new opportunity to reduce the risks and to manage chronic inflammatory diseases resulting from the dysregulation of PRR-mediated inflammatory responses.