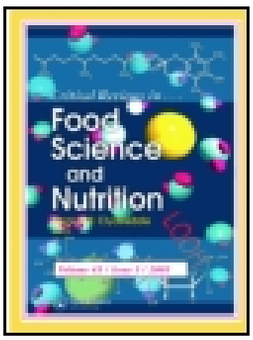

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# Regulation of the intestinal tight junction by natural polyphenols: a mechanistic perspective

Guan Yang[1], Shima Bibi[1], Min Du[2], Takuya Suzuki[3], Mei-Jun Zhu[1]*

[1]*School of Food Science, Washington State University, Pullman, WA 99164, USA;* [2]*Department of Animal Science, Washington State University, Pullman, WA 99164, USA;* [3]*Department of Biofunctional Science and Technology, Hiroshima University, Higashi-Hiroshima, 739-8528, Japan*

(*): Corresponding author: Meijun Zhu, Ph.D., Associate Professor, School of Food Science, Washington State University, Pullman, WA 99163; Phone: (509) 335-4016; Fax: (509) 335-4815; E-mail: meijun.zhu@wsu.edu

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**Abbreviations**

**AMPK**, AMP-activated protein kinase; **AOM**, azoxymethane; **BTK**, Bruton's tyrosine kinase; **DAG**, diacylglycerol; **DSS**, dextran sodium sulfate; **ERK**, extracellular signal-regulated kinase; **GSE**, grape seed extract; **GSH-px**, glutathione peroxides; **IBD**, inflammatory bowel disease; **IFN-γ**, interferon gamma; **IKK**, IκB kinase; **JAM**, junctional adhesion molecule; **IL,** interleukin; **JNK**, c-Jun N-terminal kinases; **MAPK**, mitogen-activated protein kinase; **MAPKAP**, MAPK activated protein; **MDCK**, Madin-Darby canine kidney; **MLCK**, Myosin light chain kinase; **MNK**, MAPK-interacting kinases; **NF-κB**, nuclear factor kappa light-chain-enhancer of activated B cells; **PI3K**, phosphoinositide-3-kinase; PKB, protein kinase B; **PKC**, protein kinase C; **PLC**, phospholipase C; **PP2A**, protein phosphatase 2A; **PPAR-γ**, peroxisome proliferator activated receptor -γ ; **ROCK**, Rho-associated kinase; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **STAT**, signal transducer and activator of transcription; **TEER**, the trans-epithelial electrical resistance; **TJ**, tight junction; **TK**, tyrosine kinase; **TLR**, Toll-like receptor; **TNF-α**, tumor necrosis factor-α; **ZO**, zona occluden

**Abstract**

Impairment of the epithelial barrier function is closely linked to the pathogenesis of various gastrointestinal diseases, food allergies, type I diabetes and other systematic diseases. Plant-derived polyphenols are natural secondary metabolites and exert various physiological benefits including anti-inflammatory, anti-oxidative, anti-carcinogenic, and anti-aging effects. Recent studies also show the role of plant polyphenols in regulation of the intestinal barrier and prevention of intestinal inflammatory diseases. Here, we summarize the regulatory pathways and mediators linking polyphenols to their beneficial effects on tight junction and gut epithelial barrier functions, and provide useful information about using polyphenols as nutraceuticals for intestinal diseases.

**Keywords**: polyphenols; intestinal epithelium; barrier function; tight junction; signaling pathway

## 1. Introduction

Gut epithelium constitutes a single layer of tightly linked cells (Heath 2010). It is permeable to nutrients, minerals, water and selected antigens, while it fends off bacteria, viruses and antigenic materials. Impairment in the intestinal barrier integrity permits passage of normally excluded luminal substances, which elicits excessive immune response and hence inflammation (Suenaert *et al.* 2002; Knight *et al.* 2007; Zeissig *et al.* 2007; Lee *et al.* 2010; Camilleri *et al.* 2012). Therefore, epithelial integrity and barrier function are critical for health (Hilsden *et al.* 1999; Groschwitz & Hogan 2009; Yu 2009; Camilleri *et al.* 2012; Vaarala 2012). To maintain integrity between epithelial cells, extensive tight junctions are formed, these regulate epithelial permeability (Groschwitz & Hogan 2009). A large number of tight junction proteins are involved in the formation of tight junction complexes, including intracellular proteins: zona occludens (ZO-1, ZO-2, and ZO-3), cingulin, 7H6 and ZA-1; and transmembrane proteins: occludin, claudin, junctional adhesion molecules and tricellulin (Laukoetter *et al.* 2006; Ulluwishewa *et al.* 2011; Suzuki 2013). Disturbances in production and formation of tight junction (TJ) complex negatively impact gut epithelial barrier function, which is involved in the pathogenesis of many diseases (Scaldaferri *et al.* 2012; Vaarala 2012).

The intestinal barrier integrity is dynamically regulated by a diverse array of environmental factors such as pathogens, luminal pro-inflammatory molecules, stresses and dietary factors (Suzuki 2013). For example, Enterohemorrhagic *Escherichia coli* (Philpott *et al.* 1998) and *Clostridium perfringens* (Singh *et al.* 2000) interact with the intestinal epithelial cells to disrupt

the TJ barrier. Environmental stresses, such as alcohol and acetaldehyde, might also disrupt the barrier integrity (Rao 1998; Keshavarzian *et al.* 2009). On the other hand, certain dietary factors exert protective effects on the intestinal barrier. Non-essential amino acids such as arginine and glutamine improve intestinal immunity (Yao *et al.* 2011; Ren *et al.* 2014a; Ren *et al.* 2014b; Ren *et al.* 2014c; Yin *et al.* 2014). Butyrate promotes the intestinal barrier function (Peng *et al.* 2009), and vitamin D enhances TJ formation and prevents dextran sodium sulfate (DSS)-induced epithelial disruption (Kong *et al.* 2008).

Polyphenols are bioactive compounds present abundantly in plant-derived foods. They are grossly classified into five groups based on their structures: phenolic acids, flavonoids, anthocyanins, stilbenes, and lignans (Tsao 2010). They are known for their anti-inflammatory and anti-oxidative activities (Gupta *et al.* 2014), as well as anti-diabetic, anti-carcinogenic, anti-muscle wasting and even anti-aging activities (Cao *et al.* 2008; Chis *et al.* 2009; Wang *et al.* 2009; Kiyici *et al.* 2010; Velmurugan *et al.* 2010b; Cuevas *et al.* 2011; Narita *et al.* 2011; Su & D'Souza 2011; Wang *et al.* 2014; Yang *et al.* 2015). Recent studies also show the beneficial effects of polyphenols on epithelial barrier function. Naringenin, a major polyphenol in citrus fruits, enhances TJ formation and barrier integrity in cultured intestinal Caco-2 cell monolayers (Noda *et al.* 2012). Naringenin also alleviates the DSS-induced colitis in BALB/c mice partially through improvement of the TJ barrier (Azuma *et al.* 2013). Quercetin (found in apples, onions, leafy green vegetables and tea) and kaempferol (found in tea, broccoli, cabbage, kale, beans, endive, leak, tomato, strawberries and grapes) regulate TJ protein expression and enhance intestinal Caco-2 cell monolayer barrier integrity (Suzuki & Hara 2009; Suzuki *et al.* 2011a; Noda *et al.* 2014). In Caco-2 cells, curcumin, which is the major anti-inflammatory polyphenol

in turmeric, ameliorates barrier disruption induced by hydrogen peroxide ($H_2O_2$) (Wang *et al.* 2012b) or inflammatory cytokines such as tumor necrosis factor α (TNF-α) (Ye *et al.* 2006) and interleukin (IL)-1β production (Al-Sadi & Ma 2007). Similarly, the green tea flavonoid, epigallocatechin gallate, prevents interferon gamma (IFN-γ) (Watson *et al.* 2004), and *Staphylococcus* enterotoxin B (Watson *et al.* 2005) provoked intestinal T84 cell barrier dysfunction. Grape seed extract (GSE) that contains a mixture of polyphenols reduces the severity of selected disease markers in the proximal colon of DSS-induced colitis in rats (Cheah *et al.* 2013), and alleviates inflammatory response in TNBS-induced colitis in rats (Wang *et al.* 2011b) and IL-10-deficient mice (Wang *et al.* 2013; Bibi *et al.* 2016). GSE also increases occludin and ZO-1 expression in the gut epithelium of rats (Song *et al.* 2011a; Goodrich *et al.* 2012). Recently, our group reported that GSE supplementation ameliorates IBD indices in IL-10-deficient mice, which are associated with decreased inflammatory responses and reduced expression of claudin-2, a pore forming TJ protein, in colonic tissue (Wang *et al.* 2013). In addition, resveratrol, a polyphenol extracted from grape seed and skin, promotes the mRNA expression of tight junction proteins (Etxeberria *et al.* 2015). Currently, the exact mechanisms linking dietary polyphenols to gut epithelial health has not been established. Based on available literature, we briefly summarize possible regulatory pathways and mediators linking polyphenols to their beneficial effects on TJ protein expression and complex formation, and intestinal barrier integrity.

## 2. Polyphenols and signaling pathways regulating TJ formation

### 2.1 Polyphenols inhibit nuclear factor- κB (NF-κB) signaling

One important target of polyphenols' action *in vivo* is the inflammatory NF-κB proteins, including p65 (Rel A), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2). In un-stimulated cells, NF-κB is sequestered in the cytoplasm as an inactive non-DNA-binding complex by partnering with inhibitor κB proteins (IκBs), comprising IκBα, IκBβ, IκBγ, IκBε, Bcl-3, and precursors p100 and p105 (Caamano & Hunter 2002; Senftleben & Karin 2002; Santangelo *et al.* 2007). IκB proteins undergo phosphorylation by the IκB kinase (IKK). IKK contains two catalytic subunits, IKKα and IKKβ, and the regulatory protein termed NF-κB essential modifier (NEMO, also known as IKKγ) (Caamano & Hunter 2002; Senftleben & Karin 2002; Santangelo *et al.* 2007). Upon stimulation, IKK phosphorylates IκB resulting in its degradation from the NF-κB complex. The free NF-κB can enter the nucleus to function as transcription factors that initiate the expression of specific genes, such as pro-inflammatory cytokines.

Polyphenols exert their anti-inflammatory activity by targeting different NF-κB members (Velmurugan *et al.* 2010a; Biasi *et al.* 2011; Song *et al.* 2011b; Izzi *et al.* 2012; Wang *et al.* 2013). For example, dietary administration of 0.25 or 0.5% grape seed extract (GSE) at pre- and post-azoxymethane (AOM) treatment reduced ($P<0.001$) the AOM-induced NF-κB (p65)-positive cells (Velmurugan *et al.* 2010a). Polyphenols also affect the NF-κB pathway by inhibiting the IKK phosphorylation and/or preventing proteasomal degradation of IκB. For instance, curcumin prevents phosphorylation of IκB by inhibiting the activity of IKK (Joe *et al.* 2004). Quercetin also decreases the NF-κB activity through inhibition of IKK phosphorylation and degradation of IκB (Dias *et al.* 2005). It is also known that anthocyanins from blueberry,

blackberry, and blackcurrant attenuated lipopolysaccharide (LPS)-induced NF-κB p65 translocation to the nucleus in macrophages (Lee *et al.* 2014)

Inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), activate NF-κB signaling and impair epithelial barrier function by TJ disassembly (Kisseleva *et al.* 2006; Amasheh *et al.* 2010; Petecchia *et al.* 2012; Rotches-Ribalta *et al.* 2012; Wang *et al.* 2012a; Zhang & Li 2012). Polyphenols improve barrier function partially by antagonizing the NF-κB pathway induced by inflammatory cytokines. For example, berberine (found in European barberry, goldseal, goldthread, Oregon Grape, phellodendron and tree turmeric) supplementation ameliorated TNF-α induced claudin-1 disassembly and up-regulated claudin-2 expression in HT-29/B6 human colon monolayers. Furthermore, this effect is mimicked by BAY11-7082, a specific blocker of NF-κB pathway, indicating the beneficial effects of berberine are mediated through the NF-κB pathway (Amasheh *et al.* 2010). The role of NF-κB in the impairment of the intestinal barrier was further supported by a recent study showing treatment with pyrrolidine dithiocarbamate (PDTC), which is an inhibitor of NF-κB, upregulated the occludin and ZO-1 expression in DSS-induced colitis model (Yin *et al.* 2015b). Our previous study found that GSE decreases the protein expression of claudin-2 and increases the expression of claudin-1 in the colon of IL-10-deficient mice through down-regulation of NF-κB signaling (Wang *et al.* 2013). More recently, Piegholdt *et al* reported that the isoflavones, biochanin A (found in red clover, alfalfa sprouts, soy, peanuts, chick peas and other legumes) and prunetin (found in Prunus yedoensis (Yoshino cherry), improve transepithelial electricial resistance (TEER) and prevent TNF-α induced disruption of barrier integrity in Caco-2 cells (Piegholdt *et al.* 2014). These changes were associated with inhibition of the NF-κB pathway (Piegholdt *et al.* 2014). Anthocyanin rich extract of raspberries

significantly attenuates inflammation via inhibition of the NF-kB signaling, demonstrated by improved colonic histological architecture in a DDS-induced colitis mouse model (Li *et al.* 2014).

### 2.2 Polyphenols modulate the activity of multiple protein kinases

#### 2.2.1 Mitogen-activated protein kinases (MAPK)

MAPK are a family of Ser/Thr specific protein kinases, which play fundamental roles in a wide range of cellular processes and are often deregulated in disease states. Mammals express at least four distinctly regulated MAPKs: extracellular signal-regulated kinases (ERKs 1/2), p38MAPKs (α, β, δ, and γ), c-Jun NH2-terminal kinases (JNKs-1/2/3), and extracellular signal-regulated kinase-5 (ERK5) or BMK (Santangelo *et al.* 2007). Generally, ERK1/2 is activated by mitogens and growth factors, and does not affect the inflammatory responses directly, while JNK cascades are usually triggered by stress and inflammation. The p38 plays an active signaling role in cellular responses to stress and inflammation (Kyriakis & Avruch 2001; Boncoeur *et al.* 2008). In addition, p38 directly binds and phosphorylates transcription factors, including NF-κB and CDX-2, to induce inflammatory gene transcription (Hollenbach *et al.* 2004; Schindler *et al.* 2007; Broom *et al.* 2009). Members of the JNK family also play crucial roles in inflammation (Himes *et al.* 2006). JNK activates c-Jun, which forms hetero- or homodimers with activator protein-1 (AP-1) transcription factor, inducing the expression of inflammatory cytokines (Schmeck *et al.* 2006).

Polyphenols improve the barrier function through modulating MAPK signaling pathways (Morin 2005; Chuenkitiyanon *et al.* 2010; Shin *et al.* 2011). Anthocyanins are widely distributed polyphenolic compounds that can be found abundantly in fruits and vegetables. Anthocyanin rich

extract of raspberries prevents DSS-induced histological damage of the colon architecture by inhibiting MAPKs (Li *et al.* 2014). Quercetin, at 10 μmol/L, prevents the loss of ZO-1 and occludin induced by $H_2O_2$ through reducing p38 MAPK phosphorylation (Chuenkitiyanon *et al.* 2010). On the other hand, ERK 1/2 interacts directly with the C-terminal region of occludin and plays a crucial role in preventing EGF protection of TJs disrupted by $H_2O_2$ (Basuroy *et al.* 2006), acetaldehyde (Samak *et al.* 2011), and TNF-α (Piegholdt *et al.* 2014). Biochanin A and pruenetin were identified as potent inhibitors of ERK activation, which accounts for the barrier-improving ability of these isoflavones (Piegholdt *et al.* 2014). In HCT-116 human colon carcinoma cells, anthocyanin (45 μg/ml) treatment increases TEER, and decreases the expression of claudin-1, claudin-3 and claudin-4 (Shin *et al.* 2011), which is associated with p38 activation. Inhibition of the p38 MAPK attenuates the effects of anthocyanins on the expression of claudins, showing the importance of p38 signaling in mediating the beneficial effects of anthocyanins on epithelial barrier function (Shin *et al.* 2011). In short, these data suggest the mediatory roles of MAPK pathways in linking polyphenol compounds to the improvement of barrier function.

### 2.2.2 Phosphoinositide-3-Kinases (PI3K)/Akt

PI3K participates in numerous biological processes, including immune cell growth, differentiation, survival, proliferation, migration, and metabolism. In the immune system, impaired PI3K signaling leads to immunodeficiency, whereas unrestrained PI3K signaling contributes to autoimmunity and inflammation (Okkenhaug & Vanhaesebroeck 2003). By direct phosphorylation of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2), PI3K generates phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P3), which in turn contributes to the

activation of various downstream targets, including Ser/Thr-specific protein kinase Akt (also known as protein kinase B).

PI3K signaling pathway regulates epithelial barrier function, primarily through regulating TJ expression (Li & Neu 2009; Mankertz *et al.* 2009; Suzuki *et al.* 2011b; Rosenthal *et al.* 2012). In addition, PI3K also regulates TJ assembly. A previous study showed that the PI3K is activated by oxidative stress, which results in increased intestinal permeability, while inhibition of PI3K reduces permeability (Sheth *et al.* 2003). Glutamine deprivation decreases the TEER level and increases the intestinal permeability (Wang *et al.* 2015), which was reversed by inhibition of PI3K (Li & Neu 2009). Moreover, the PI3K pathway is responsible for the up-regulation of claudin-2 expression induced by TNF-α (Mankertz *et al.* 2009; Amasheh *et al.* 2010), and IL-6 (Suzuki *et al.* 2011b). Limited evidence points to the PI3K/Akt signaling in linking polyphenols to the epithelial barrier function. The polyphenols, naringenin and quercetin have similarities in structure to the synthetic PI3K inhibitors and hence inhibit PI3K activity (Walker *et al.* 2000; Harmon & Patel 2003). In another *in vitro* study in HT-29/B6 human colon monolayers, berberine inhibits TNF-α induced activation of the PI3K/Akt pathway and reduces the claudin-2 protein expression (Amasheh *et al.* 2010). These studies indicate that polyphenols improve the barrier integrity partly through inhibition of PI3K/Akt pathway.

### 2.2.3. Protein kinase C (PKC)

The PKC family of Ser/Thr kinases regulates a number of fundamental biological processes in epithelial cells including barrier function (Suzuki *et al.* 2008; Suzuki *et al.* 2009; Suzuki & Hara 2009). Intestinal epithelial cells express a range of PKC isoforms involved in various

pathways, i.e. conventional (cPKC) isozymes (α, βI, βII, and γ), novel (nPKC) isozymes (δ, ε, η, μ, and θ), and atypical (aPKC) isozymes (ζ and ι/λ) (Song *et al.* 2001; Farhadi *et al.* 2006). These isoforms vary in their sensitivity to activators and cofactors, thus playing different roles in the regulation of intestinal barrier function (Farhadi *et al.* 2006). The role played by the PKC family on TJs is complex. Previously, it was reported that pretreatment with PKC selective antagonists, prevents the activation of down-stream signaling induced by Toll-like receptor 2 (TLR2), which greatly enhances TEER in Caco-2 and HT-29 monolayers through recruiting ZO-1 (Cario *et al.* 2004). The activation of PKC also induces the activation of MAP kinases, which results in the tight junction disruption in the corneal epithelial cells (Wang *et al.* 2004). Moreover, over-expression of nPKCδ on renal LLC-PK1 cells has a negative effect on the TJ barrier (Mullin *et al.* 1998), and inhibition of nPKCδ by quercetin promotes actin cytoskeletal association and expression of TJ proteins in Caco-2 cells (Suzuki & Hara 2009). Besides the intestinal barrier integrity, polyphenols also play a role in improving the blood-barrier function. Green tea polyphenols prevent blood-brain barrier damage, exerting neuroprotective effects by up-regulating the expression of claudin-5, occludin and ZO-1 as well as down-regulating PKCα expression (Liu *et al.* 2013). All these indicate that polyphenols inhibit PKC to improve epithelial barrier function.

### 2.2.4. Tyrosine kinases

Tyrosine kinases are key mediators of important cellular signaling pathways controlling cell proliferation and growth via activation of tyrosine kinase receptors (Lemmon & Schlessinger 2010; Segaliny *et al.* 2015). This activation is considered the starting point for many cellular

signaling pathways by recruiting enzymatic effectors (PLCγ, PI3K, etc.) and serving as the upstream of the signaling pathways including MAPK, PI3K, PLCγ, etc. (Lemmon & Schlessinger 2010; Segaliny *et al.* 2015). Accumulating evidences suggest the significant contribution of tyrosine kinase to TJ integrity (Rao *et al.* 1997; Atkinson & Rao 2001; Seth *et al.* 2004; Elias *et al.* 2009; Amasheh *et al.* 2010; Spalinger *et al.* 2013). Specifically, $H_2O_2$ administration induces tyrosine phosphorylation of several proteins (Rao *et al.* 1997) and acetaldehyde disrupts TJs through inducing phosphorylation of several proteins including ZO-1, E-cadherin and β-catenin at their tyrosine residues, which increases the paracellular permeability in Caco-2 and T84 cell monolayers (Atkinson & Rao 2001). In patients with active Crohn's disease, mRNA and protein levels of protein tyrosine phosphatase, nonreceptor type 22, were lower compared with the controls, suggesting the importance of the tyrosine phosphorylation in maintaining TJ integrity (Spalinger *et al.* 2013). Polyphenols have been reported to regulate the activity of tyrosine kinases in various studies. The administration of genistein, a soybean polyphenol, prevents acetaldehyde-induced permeability and TJ disruption by blocking tyrosine phosphorylation (Atkinson & Rao 2001). Moreover, berberine prevents TNF-α induced claudin-1 disassembly and claudin-2 up-regulation partially via down-regulation of tyrosine kinase pathway (Amasheh *et al.* 2010). In addition, biochanin A, and pruenetin improved the barrier integrity partly through the down-regulation of ZO-1 tyrosine phosphorylation further indicating that polyphenols could exert beneficial effects on TJ integrity via inhibition of tyrosine kinase activity and tyrosine phosphorylation (Piegholdt *et al.* 2014).

### 2.2.5. Myosin light chain kinase (MLCK)

Myosin light chain kinases (MLCKs) are a family of soluble protein kinases that function principally to phosphorylate the regulatory myosin light chain (MLC-2) and thereby induce actomyosin contraction (Rigor *et al.* 2013). A number of studies have been conducted to understand the role of MLCK activity under disease conditions. It has been shown that increased MLCK activity decreases colonic epithelial barrier function (Ferrier *et al.* 2003). Inflammatory cytokines TNF-α and IFN-γ induced barrier loss, and increased MLCK phosphorylation. The TJ barrier was restored by the inhibition of MLCK *in vitro* (Zolotarevsky *et al.* 2002). MLCK inhibition also resulted in reduced ZO-1 exchange between tight junction and cytosolic pools, which suggests MLCK alters barrier regulation via a ZO-1 dependent process (Yu *et al.* 2010). Pharmacological inhibition of MLCK alleviates TJ barrier dysfunction (Scott *et al.* 2002; Ma *et al.* 2005; Gu *et al.* 2011). It is proposed that polyphenols could improve TJ integrity through down-regulation of MLCK activity (Gu *et al.* 2011; Cao *et al.* 2013). In a mouse model of endotoxinemia, pretreatment with berberine for 7 days reduces small intestine permeability and prevents TJ disruption caused by LPS, concomitant with the decreased MLCK activity (Gu *et al.* 2011). Furthermore, berberine ameliorates IFN-γ and TNF-α induced intestinal epithelial barrier dysfunction in Caco-2 cell monolayers through suppression of MLCK-MLC2 signaling pathway (Cao *et al.* 2013). These studies show that, polyphenols inhibit MLCK to improve epithelial barrier function.

### 2.2.6 AMP-activated protein kinase (AMPK)

AMP-activated protein kinase (AMPK) is a Ser/Thr protein kinase that plays an important role in maintaining cellular energy balance (Gruzman *et al.* 2009). AMPK is activated by a

number of dietary factors including polyphenol compounds (Peng *et al.* 2009; Wang *et al.* 2011a; Castillo-Pichardo & Dharmawardhane 2012; Yang *et al.* 2012; Mukai *et al.* 2013; Zhao *et al.* 2015). For example, butyrate (Peng *et al.* 2009) and forskolin (Egawa *et al.* 2008) enhance the intestinal barrier via activation of AMPK *in vitro*. AMPK is activated during calcium-induced TJ assembly in Madin-Darby canine kidney (MDCK) cells, and up-regulation of AMPK activity facilitates TJ assembly (Zhang *et al.* 2006). Attenuation of AMPK is responsible for the impaired epithelial barrier function induced by IFN-γ (Scharl *et al.* 2009). All of the above evidences indicate the critical roles of AMPK in TJ integrity. Polyphenols also affect intestinal barrier function through AMPK modulation. It has recently been shown that theaflavins-3'-O-gallate significantly increased the TJ protein (occludin, claudin-1, and ZO-1) expression as well as the phosphorylation of AMPK (Park *et al.* 2015). Inhibition of AMPK completely abolished the beneficial effects of theaflavins-3'-O-gallate on the intestinal barrier, suggesting these effects were AMPK dependent (Park *et al.* 2015). 6-Gingerol, a major pungent phenolic component in ginger, has also been demonstrated to protect the DSS-induced intestinal inflammatory disorder through the activation of AMPK (Chang & Kuo 2015). However, our recent study showed that AMPK activity was heightened in IL-10-deficient mice, where severe inflammation occurs due to the absence of a key anti-inflammatory cytokine. GSE supplementation restored AMPK to levels that are associated with the improved barrier integrity (Yang *et al.* 2014). AMPK, as an intracellular energy sensor and regulator, contributes significantly to the maintenance of the epithelial barrier, which is energy-dependent (Novak & Mollen 2015). Hallmarks of mitochondrial dysfunction, including oxidative stress and impaired ATP production have been characterized in the IBD patients (Novak & Mollen 2015). Thus, this abnormal activation of

AMPK, in IL-10-deficient mice, could be due to the impairment in energetic metabolism elicited by acute inflammation, which warrants further research.

## 2.3 Polyphenols regulate the activity of key enzymes

### 2.3.1. Protein phosphatase 2A (PP2A)

PP2A comprises a family of Ser/Thr phosphatases. It has a broad range of cellular functions such as regulating cell survival (Strack *et al.* 2004), facilitating DNA repair (Chowdhury *et al.* 2005) and others. PP2A is composed of three subunits, scaffold subunit A, regulatory subunit B and catalytic subunit C. The functional diversity of PP2A is mediated by scaffold and regulatory subunits, which interact with a wide range of proteins (Janssens & Goris 2001). These interactions facilitate the cross-talk of PP2A with several cell signaling pathways including IκB kinases, MAPK, Akt and PKC (Sontag *et al.* 1997; Van Kanegan *et al.* 2005; Grethe & Porn-Ares 2006).Interestingly, studies show that PP2A interacts with TJ proteins thus negatively regulating TJ assembly and function (Nunbhakdi-Craig *et al.* 2002; Seth *et al.* 2007; Sheth *et al.* 2009; Dunagan *et al.* 2012). Enhanced PP2A activity induces dephosphorylation of membrane TJ proteins, ZO-1, occludin, and claudin-1 at their serine residues, with an increasing paracellular permeability in MDCK cells (Nunbhakdi-Craig *et al.* 2002). This dephosphorylation is important in promoting TJ opening (Farshori & Kachar 1999; Clarke *et al.* 2000). Inhibition of PP2A and knockdown of PP2A-Cα (the catalytic subunit of PP2A) attenuate the impairment of barrier function caused by $H_2O_2$ (Sheth *et al.* 2009). Polyphenols inhibit PP2A activity (Chen *et al.* 2013; Kiss *et al.* 2013), which improves TJ formation.

### 2.3.2. Rho family of small GTPases

The Rho family of small GTPases, consisting of Rho, Rac, and Cdc42, play an important role in the regulation of epithelial structure and function (Wittchen *et al.* 2003). Its downstream effector, Rho-associated kinase (ROCK) is a Ser/Thr kinase, which is important in regulating TJ permeability (Walsh *et al.* 2001). In cultured T84 cell monolayers, ROCK inhibition prevents proper localization of TJ proteins during TJ assembly, enhancing paracellular permeability (Walsh *et al.* 2001). ROCKs could also alter the cell-cell adhesion and actin cytoskeleton organization by phosphorylating MLC (Grassie *et al.* 2012). Moreover, a previous study has demonstrated that Rho GTP exchange factor ARHGEF11, mediates RhoA – MLC2 signaling pathways at cell-cell junctions, functioning in cooperation with ZO-1, therefore regulating the paracellular barrier and the organization of the apical junctional complex and peri-junctional actomyosin ring of epithelial cells (Itoh *et al.* 2012). Because polyphenols suppress Rho activity (Higashi *et al.* 2005; McLaughlin *et al.* 2006), polyphenols may decrease the TJ permeability through attenuating the Rho activity.

**2.4 Polyphenols reduce reactive oxygen species (ROS)**

The maintenance of redox homeostasis is of critical importance for normal cellular processes and organ function. This homeostasis is tightly regulated by the pro-oxidative and anti-oxidative systems. Oxidative stress is considered to be an imbalance between the production of ROS and their elimination through anti-oxidative mechanisms (Pi *et al.* 2010; Reuter *et al.* 2010; Kanninen *et al.* 2011). ROS production correlates with inflammation and alterations in several signal pathways including MAPK and NF-κB (Morgan & Liu 2011; Liu *et al.* 2012; Park *et al.* 2012). $H_2O_2$ induced oxidative stress impairs TJs in the intestinal epithelium (Sheth *et al.* 2003;

Chuenkitiyanon *et al.* 2010; Duan *et al.* 2016). Dietary glutamate and aspartate are beneficial in alleviating the oxidative stress induced by diquat (Yin *et al.* 2015a) and $H_2O_2$ (Duan *et al.* 2016). Plant-derived polyphenols also exhibit anti-inflammatory and anti-oxidative effects on various disease models (Edgecombe *et al.* 2000; Cao *et al.* 2008; Chis *et al.* 2009; Cuevas *et al.* 2011; Nadour *et al.* 2012; Rajamurugan *et al.* 2012; Periasamy & Alshatwi 2013). Oregano essential oil, which is enriched with phenolic compounds, reduces the activation of superoxide dismutase (SOD) and glutathione peroxide (GSH-Px), and improves dysfunction of intestinal epithelial barrier in rats (Wei *et al.* 2015). These data suggest that polyphenols may improve the barrier function through the down-regulation of ROS.

## 3. Implications and Conclusions

Basic and clinical studies have shown that increased intestinal permeability is associated with various diseases, such as IBD, celiac disease, irritable bowel syndrome, and type I diabetes (Suzuki 2013). Dietary polyphenol supplementation might provide a potential therapy for these diseases. Interest in studying the mechanisms connecting polyphenol compounds and barrier integrity has grown in recent years (Table 1). However, in most cases, available studies were performed in cultured cells. Moreover, it is still unclear whether the polyphenols directly exert effects on epithelial development or mediates the epithelial formation through the regulation of TJs. Thus, further *in vivo* and mechanistic studies are needed, which will likely lead to the development of polyphenolic compounds as therapeutic and preventive approaches against the barrier-defect related diseases.

Additionally, existing data suggests that polyphenols exert their beneficial effects in TJ regulation via diversified pathways, which crosstalk with each other. For example, in intestinal cells, PKC activation is accompanied by progressively decreasing MLC2 phosphorylation. Meanwhile, an acute increase in MLCK phosphorylation concurrent with PKC activation leads to the decrease in MLC2 phosphorylation and improvement of barrier integrity (Turner *et al.* 1999). Furthermore, other signaling pathways and mediators may also interact with pathways, discussed in this review, which regulate TJ integrity and their regulatory roles in polyphenols' mediated TJ barrier function. Future investigations are required to elucidate the precise mechanisms involving these signaling pathways and their interactions which underlying the polyphenol-mediated protective effects on intestinal TJ formation and barrier function. Knowing these pathways and their cross-talks may also help to further understand the clinical use of the polyphenolic compounds in the prevention and treatment of diseases associated with intestinal barrier defects.

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**Table 1.** Summary of direct evidence of the mechanisms connecting the polyphenol compounds and barrier integrity.

| Experimental setting | Polyphenol source | Signaling pathways | Response | Reference |
|---|---|---|---|---|
| TNF-α induced HT29/B6 cells | Berberine | NF-κB↓ PI3K/Akt↓ tyrosine kinases↓ | Ameliorated claudin-1 disassembly and claudin 2↓ | (Amasheh *et al.* 2010) |
| IL-10-deficient mice | Grape seed extract | NF-κB↓ | Claudin-1↑ and claudin 2↓ | (Wang *et al.* 2013; Yang *et al.* 2014) |
| TNF-α induced CaCO-2 cells | Biochanin A, prunetin | NF-κB↓ ERK↓ tyrosine kinases↓ | Improved TEER, Claudin 1→, occludin→, ZO-1→, E-cadherin → | (Piegholdt *et al.* 2014) |
| DDS-induced colitis in mice | Anthocyanin rich extract of raspberries | NF-κB↓ MAPKs↓ | Improved Colonic histological architecture | (Li *et al.* 2014) |
| Hydrogen peroxide induced ECV304 | Quercetin | p38↓ | ZO-1↑and occludin↑ | (Chuenkitiyanon *et al.* 2010) |

| | | | | |
|---|---|---|---|---|
| monolayers | | | | |
| HCT-116 human colon carcinoma cells | Anthocyanin | p38↑ | Improved TEER, claudin-1↓, claudin-3↓ and claudin-4↓ | (Shin *et al.* 2011) |
| Caco-2 cells | Quercetin | PKCδ↓ | ZO-2↑, occludin↑, claudin-1↑, claudin-4↑ | (Suzuki & Hara 2009) |
| Blood-brain barrier damage | Green tea polyphenols | PKCα↓ | Claudin-5↑, occludin↑ and ZO-1↑ | (Liu *et al.* 2013) |
| Acetaldehyde-induced Caco-2 cells | Genistein | Tyrosine kinases↓ | Improved TEER, distribution of ZO-1 and occludin↑ | (Atkinson & Rao 2001) |
| LPS challenged mouse | Berberine | MLCK↓ | Distribution of claudin-1, claudin-4, ZO-1 and occludin↑ Intestinal permeability↓ | (Gu *et al.* 2011) |
| IFN-γ and TNF-α Induced CaCO-2 cells | Berberine | MLCK↓ | Distribution of claudin-1, ZO-1 and occludin↑ Intestinal permeability↓ | (Cao *et al.* 2013) |
| | Theaflavins- | AMPK↑ | Occludin↑, claudin-1↑, | (Park *et al.* |

| | | | | |
|---|---|---|---|---|
| Caco-2 cells | 3'-O-gallate | | and ZO-1↑ | 2015) |
| DSS-treated Caco-2 monolayers | 6-gingerol | AMPK↑ | Improved TEER | (Chang & Kuo 2015) |
| IL-10-deficient mice | Grape seed extract | AMPK↓ | Claudin-1↑ and claudin 2↓ | (Yang *et al.* 2014) |
| Diquat-induced rats | Oregano essential oils | SOD and GSH-Px↓ | ZO-1↑and occludin↑ | (Wei *et al.* 2015) |

↑, increase or improve; ↓, decrease; →, not change.