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Andrographolide and its derivatives are effective compounds for gastrointestinal protection: a review

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Abstract. – OBJECTIVE: Andrographolide and its derivatives have many functions, such as anti-infection, anti-tumor, neuroprotection, and immune regulation. However, the gastrointestinal protective effects, especially gastrointestinal tumors, and inflammation-related diseases of andrographolide and its derivatives have not been well summarized and discussed. In this review, we aimed to summarize and discuss the pharmacological effects and underlying mechanisms of andrographolide and its derivatives in gastrointestinal protection, with a view to revealing more possibilities of andrographolide and its derivatives in gastrointestinal diseases prevention therapy.

MATERIALS AND METHODS: The data in this review are searched and selected from PubMed with the keywords: Andrographolide and Andrographolide derivatives, and relevant data with gastrointestinal protection are extracted and discussed.

RESULTS: Andrographolide and its derivatives have prophylactic and therapeutic effects in gastrointestinal disorders such as GU, gastric cancer, colorectal cancer, and inflammatory bowel disease.

CONCLUSIONS: Andrographolide and its derivatives are effective compounds for gastrointestinal protection.

Key Words:
Andrographolide, Gastrointestinal system, Cancer, Inflammation, Apoptosis.

Introduction

The acanthaceous plant *Andrographis paniculata* (*A. paniculata*) was widely used in ancient Asia to treat febrile diseases as it purportedly "clears away heat" and detoxifies. In India, *A. paniculata* is used to treat skin infections, dysentery, and diabetes mellitus, whereas in ancient China, it was used to treat diseases such as fever and laryngitis. More than 120 kinds of chemical constituents have been reported for *A. paniculata*, mainly diterpene lactones and flavonoids, as well as phenylpropanoids and other compounds. Andrographolide is a natural diterpene lactone of *A. paniculata* (Figure 1) and is one of its main active ingredients. Due to its antibacterial and anti-inflammatory properties, and its therapeutic effects on upper respiratory tract infections, andrographolide has attracted considerable attention as a natural antibiotic[1-3].

Modern pharmacological studies[4-10] have described antioxidant, anti-inflammatory, anti-viral, and immune-modulating effects of andrographolide and its derivatives, and they are widely used to treat hepatitis, organ fibrosis, metabolic diseases, cancers, and autoimmune diseases. Recently, basic and clinical studies have reported the protective effects of andrographolide and its derivatives on the gastrointestinal tract. This review will discuss the beneficial and emerging roles of andrographolide and its derivatives on the gastrointestinal tract, focusing on the molecular mechanisms of its local and systemic actions in the stomach and intestinal tract.

Gastric Ulcers (GUs)

A GU is an ulcer located between the cardia and pylorus and is the most common disease of the upper digestive tract. It is characterized by destruction of the gastric mucosa and secondary damage to gastric tissue by pepsin and gastric acid. Under normal circumstances, the integrity of the gastric mucosa is maintained by an intact

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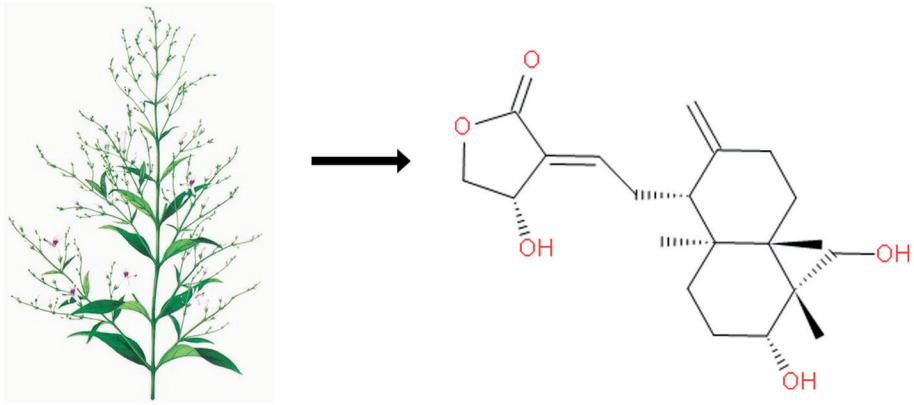

Figure 1. *Andrographis paniculata* and the molecular formula of andrographolide.

barrier structure and enhanced defense mechanisms, including a mucus bicarbonate barrier, an epithelial barrier, continuous epithelial renewal, blood flow through mucosal microcirculation, and a mucosal microvascular endothelial barrier. Gastric mucosa also maintains mucosal integrity through defense mechanisms, such as a mucus barrier, an epithelial barrier, gastric mucosal blood flow, and protective cytokines. Although the complex pathogenesis of GUs has been studied for decades, the mechanisms underlying their etiology remain unclear. Certainly, a variety of factors, such as alcohol consumption, bacterial infection, non-steroidal anti-inflammatory drug (NSAID) use, or those that lead to mucosal layer damage play pivotal roles in GU pathogenesis. *In vivo* studies[11-13] with animal models have demonstrated that andrographolide or its derivatives alleviates morphological and pathological damage associated with ethanol- and NSAID-induced GUs; more specifically, they reduced the ulcer area, increased the thickness of the mucus layer, and attenuated mucosal congestion and swelling. These gastroprotective effects of andrographolide were confirmed by morphological and histological analyses and indicate that andrographolide is a potential natural compound for the treatment of GUs. In the following sections, some aspects of the gastroprotective effects of andrographolide and its derivatives in GU will be discussed.

Antioxidant Activity

The antioxidant activity of andrographolide and its mechanisms have been summarized in previous studies[14]. For instance, andrographolide was shown to block, both *in vitro* and *in vivo*, hyperglycemia-triggered reactive oxygen species (ROS) generation by suppressing NADPH oxidase activation and augmenting nuclear factor erythroid 2-related factor 2 (Nrf2) expression[15]. Andrographolide-induced Nrf2 expression modulates enzymes in the glutathione and thioredoxin antioxidant systems, leading to enhanced redox status of liver cells[6]. Andrographolide has also been reported to exert strong antioxidant effects in cardiovascular, respiratory, and respiratory diseases, as well as in arthritis[2,8,16-19].

Evidence[11,13] supporting the antioxidant activity of andrographolide and its derivatives has been described in animal models of GUs, which include increasing gastric levels of glutathione, catalase, superoxide dismutase, prostaglandin E2, cyclooxygenase-1, and cyclooxygenase-2 while dramatically decreasing the levels of malonaldehyde. Thus, the gastroprotective effects of andrographolide in GUs may result from its ability to ameliorate damage from oxidative stress. However, the underlying mechanism(s) by which andrographolide suppresses oxidative stress and reduces ROS need investigation. Of note, whether andrographolide exerts its antioxidant effects for gastric protection via the Nrf2 signaling pathway is unknown.

Antibacterial Activity

Helicobacter pylori (H. pylori) is a recognized cause of gastritis and gastric and duodenal ulcers. *Helicobacter pylori* produces the highly active urease, which can catalyze urea hydrolysis to generate ammonia and carbon dioxide, thereby lowering the pH environment of the gastric mu-

cosa and allowing bacteria to colonize the stomach. Thus, an effective strategy in GU treatment would be to inhibit urease activity to reduce bacterial reproduction. Andrographolide has not been shown to have inhibitory effects on *H. pylori*, but a recent study has demonstrated that labdane diterpenoids isolated from *A. paniculata* effectively inhibits *H. pylori*[20]. Moreover, Mo et al[21] reported that andrographolide sodium bisulfite is a competitive inhibitor of the thiol groups in urease in a slow-binding manner that is both reversible and concentration dependent, indicating that andrographolide may be a promising urease inhibitor in treating urease-related diseases.

The above data imply that andrographolide may confer gastric protection by inhibiting *H. pylori* activity, but more evidence is required to prove this hypothesis.

Other Activity

Saranya et al[11] demonstrated that rats pretreated with andrographolide (1, 3, or 5 mg/kg) for 30 days exhibited less severe ethanol-induced gastric lesions, likely resulting from andrographolide-induced suppression of gastric acid secretion and maintenance of mucus layer integrity, as reflected by reduced H^+/K^+-ATPase activity and sustained mucin levels, respectively. Furthermore, this study also demonstrated that andrographolide decreased myeloperoxidase activity; myeloperoxidase is an inflammatory marker that allows the infiltration of neutrophils into gastric mucosa. In a study by Yao et al[12], andrographolide-pretreated mice exhibited greatly attenuated intragastric-ethanol-administration-induced imbalance of gastric mucosal vascular homeostasis via the inhibition of the 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3-mediated glycolysis pathway in gastric mucosal vascular endothelial cells. An *in vivo* study reported that andrographolide sodium bisulfite may have anti-apoptotic properties as it significantly increased the expression of heat shock protein 70 and B-cell lymphoma-2 (Bcl-2) protein while suppressing the Bax protein in the gastric tissue[13]. Together, these findings demonstrate that andrographolide confers gastric protection likely by regulating inflammation and vascular hemostasis with its anti-apoptotic properties, these mechanisms provide novel strategies for gastric protection.

Gastrointestinal Cancers

Cancer is essentially a polygenic disease characterized by the activation of one or more proto-oncogenes or by mutations and/or deletions of tumor suppressor genes, both resulting in cells that escape normal growth control mechanisms. Consequently, immortalized cancer cells become aggressive and display malignant phenotypes. The malignant proliferation of tumor cells prominently manifests as a disorder of the cell cycle, for example, with the imbalance of cyclin and cyclin-dependent kinases, two key regulatory molecule families[22]. It also manifests as the activation or inhibition of certain key signaling pathways regulating the growth, differentiation, and invasion of tumor cells. Thus, the promotion of cell apoptosis is essential in restricting further cancer development.

Andrographolide exhibits tremendous potential in the treatment of gastrointestinal cancers, as it has been discovered to have multiple targets and signaling pathways in the treatment of colon cancer. Moreover, some preliminary *in vitro* studies have shown that andrographolide has a chemo-preventive effect in gastrointestinal cancers. For example, andrographolide reduces the viability of HT-29 cells in a dose- and time-dependent manner; at lower doses, the cell cycle is halted in the G_2/M phases, but it is held in G_0/G_1 phases at higher doses[23]. In this section, the underlying mechanisms of andrographolide and its derivatives against gastrointestinal cancers, especially its pro-apoptotic activity, will be discussed.

Regulation of the Wnt/β-Catenin Signaling Pathway

The Wnt signaling pathways are a set of signal transduction pathways with multiple downstream channels that are activated when Wnt-protein ligands bind to membrane G protein-coupled receptors. Wnt signal transduction pathways play crucial roles in biological development, cell transport, and apoptosis. Accordingly, aberrations in Wnt pathways cause various growth-related pathologies and cancers. The most well-studied Wnt downstream pathway is the canonical Wnt/β-catenin pathway, in which Wnt regulates gene expression via β-catenin/T-cell factors (TCFs); the Wnt/β-catenin pathway thus has implications for gastrointestinal cancer.

Previous studies have reported that andrographolide can activate the Wnt/β-catenin signaling pathway in different diseases. Jiang et al[24] demonstrated that andrographolide exerts pro-osteogenic effects via activation of the Wnt/β-catenin signaling pathway. Furthermore, andrographolide was reported to promote neural differentiation of

rat adipose tissue-derived stromal cells through Wnt/β-catenin signaling, indicated by increased expression of nuclear β-catenin and inhibition of glycogen synthase kinase-3β (GSK-3β), a key enzyme in the Wnt/β-catenin signaling cascade[25]. Andrographolide was also shown to activate the canonical Wnt signaling pathway via non-ATP-competitive inhibition of GSK-3β[26]. Altogether, these studies indicate that andrographolide can activate the Wnt/β-catenin signaling pathway and provide additional evidence that andrographolide is a competitive inhibitor of GSK-3β[27].

In contrast to the researches reported above, andrographolide and its derivatives exert anti-cancer effects by inhibiting the Wnt pathway. For example, 19-O-triphenylmethyl andrographolide (RS-PP-050) inhibited β-catenin transcription by repressing TCF/lymphocyte enhancer factor (LEF) activity in colorectal cells overexpressing β-catenin and decreasing endogenous expression of Wnt target genes[28]. Moreover, RS-PP-050 reduced protein expression of the active form of β-catenin by blocking phosphorylation at Ser675 of β-catenin, which is critical for β-catenin nuclear translocation. In HT29 colon cancer cells, the andrographolide analogue 3A.1 (19-tert-butyldiphenylsilyl-8, 17-epoxy andrographolide) was found to significantly reduce TCF/LEF promoter activity in the Wnt/β-catenin signaling pathway[29]. Accordingly, analogue 3A.1 suppressed the expression of Wnt target genes and β-catenin protein and increased the activity of GSK-3β kinase[29]. These findings suggest the therapeutic potential of andrographolide derivatives in the treatment of gastrointestinal cancer.

The contradictory effects of andrographolide in different studies are still a mystery. One answer may be that andrographolide has a bidirectional regulatory effect on the Wnt signaling pathway. On the one hand, it inhibits pathway activation caused by cancer gene mutations, whereas on the other hand, it activates the Wnt pathway in certain diseases to regulate abnormal pathologies. However, the currently available data are not enough to support this hypothesis, and further investigations need to be done before any conclusions can be drawn.

Regulation of The Intrinsic Apoptosis Pathway

A well-known, crucial anti-cancer strategy is to trigger tumor cell apoptosis, which includes two signaling pathways, the intrinsic pathway and the extrinsic pathway[30,31]. The intrinsic apoptosis pathway, also called the mitochondrion-mediated procaspase-activation pathway, is regulated by members of the Bcl-2 family and, in response to DNA destruction from chemotherapy or radiotherapy, can be initiated by activating the tumor suppressor protein p53[30].

Andrographolide and its derivatives are thought to suppress cancers by regulating the intrinsic apoptosis pathway (Figure 2). In particular, andrographolide treatment increased the expression of apoptosis-related proteins, such as caspase-3, Bax, and poly ADP-ribose polymerase (PARP), and attenuated the expression of the antiapoptotic protein Bcl-2 in Uppsala 87 Malignant Glioma cells [32]. Similarly, in andrographolide-treated B16F-10 melanoma cells, the expression of the proapoptotic proteins p53, Bax, caspase-9, and caspase-3 was upregulated, whereas protein expression of the antiapoptotic Bcl-2 was downregulated[33]. These studies[34-38] confirm data from earlier studies demonstrating that andrographolide and its derivatives induce apoptosis via Bcl-2-mediated survival signaling and modulating p53-induced caspase-3-mediated proapoptotic signaling in certain cancer cells.

Mechanistic studies have reported that andrographolide and its derivatives can also promote the apoptosis of gastrointestinal cancer cells through the intrinsic apoptotic pathway. For example, using the BGC-823 human gastric cancer cell line, Li et al[39] found that andrographolide suppressed cell growth and induced cell apoptosis by upregulating Bax and caspase-3 expression and downregulating Bcl-2 expression. Similarly, the treatment of SGC7901 gastric cancer cells with media containing different concentrations of andrographolide (5, 20, and 40 μg/mL) promoted cell apoptosis (16.5%, 19.9%, and 28.4%, respectively) and was likely related to the upregulation of Bax expression and the downregulation of Bcl-2 expression[40]. Andrographolide has also been shown to suppress colon cancer via the inherent apoptosis pathway. It induced apoptosis in human HT-29 colon cancer cells, which seems to be linked to augmented intracellular ROS levels and disruption of the mitochondrial membrane potential via the regulation of caspase-3 activity[23]. Moreover, Khan et al[41] demonstrated that andrographolide enhanced early and late apoptosis in SW-480 cells in a dose- and time-dependent manner *via* the upregulation of the proapoptotic proteins Bax and Bad and the downregulation of the antiapoptotic protein Bcl-2. Finally, treat-

ing gastric cancer (MKN45) and colon cancer (HCT116) cells with medium containing gradient concentrations of andrographolide (0, 12.5, 50, or 100 μM) has been reported to enhance the expression of the pro-apoptotic proteins p53 and Noxa, also known as phorbol-12-myristate-13-acetate-induced protein 1, a p53 downstream target gene[42]. These numerous reseraches demonstrate the pro-apoptosis activity of andrographolide in the treatment of gastrointestinal cancer; nevertheless, it is necessary to synthesize new andrographolide analogues that are more efficacious than the parent compound.

Various *in vitro* studies have illustrated the beneficial effects of using chemically modified forms of andrographolide to promote cell apoptosis in treating gastrointestinal cancer. Of note, new benzylidene derivatives of andrographolide were reported as potential anticancer agents in the treatment of breast and colon cancer; they were shown to downregulate cyclin-dependent kinase 4 to promote G_1 phase cell cycle arrest and induce apoptosis[43]. Song et al[44] found a series of novel indolo [3,2-b] andrographolide derivatives that, in a concentration-dependent manner, induced early and late cellular apoptosis in HCT116 (human colon cancer) cells. Likewise, treatment of HCT116 cells with di-spiropyrrolidino and di-spiropyrrolizidino oxindole andrographolide derivatives upregulated Bax, p53, caspase-3, caspase-9, and cleaved PARP while downregulating Bcl-2 and p65[36]. In AGS gastric cancer cells, 2 μM of the andrographolide analogue 19-triisopropyl andrographolide, or analogue-6, significantly inhibited the activity of DNA topoisomerase IIα enzyme, induced DNA damage, and activated cleaved PARP-1 and caspase3 (p53 was not activated), resulting in late cellular apoptosis[45]. Additionally, in a series of novel andrographolide derivatives that were semi-synthesized and screened, SR207

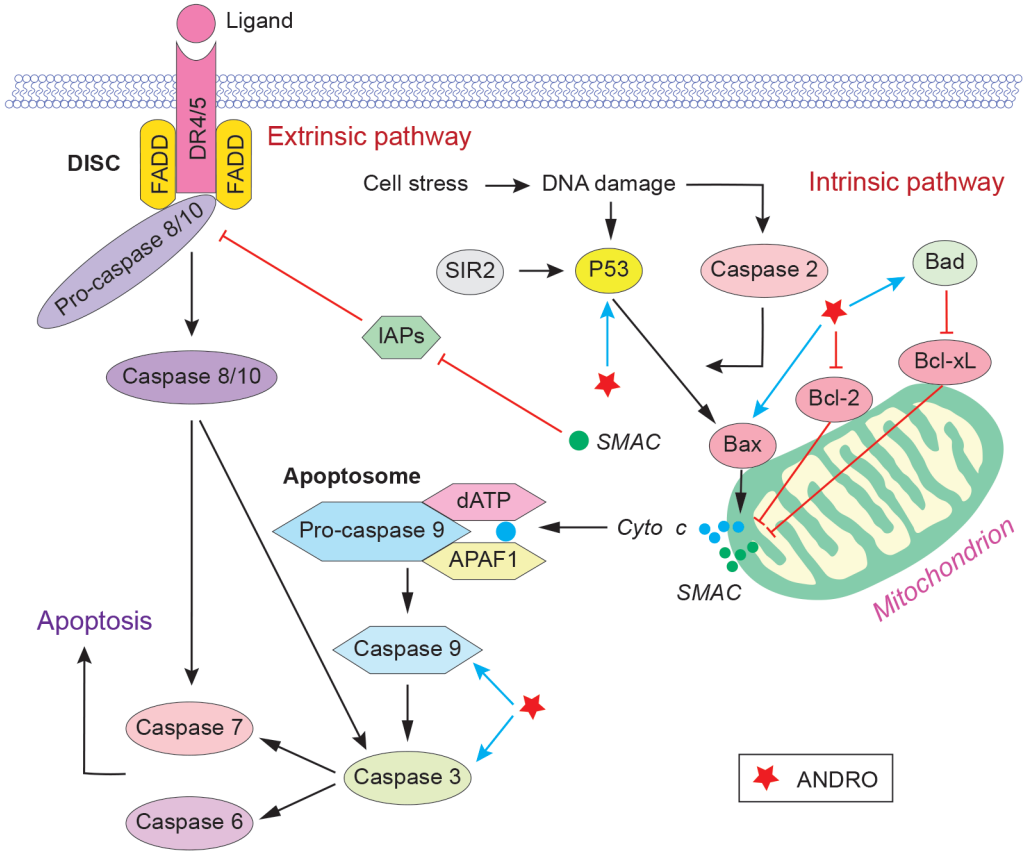

Figure 2. Regulation of the intrinsic and extrinsic apoptosis pathways by andrographolide and its derivatives. ANDRO: andrographolide and its derivatives; DR: death receptors; FADD: Fas-associating protein with a novel death domain; IAPs: inhibitor of apoptosis proteins; SIR: silent information regulator; SMAC: second mitochondria-derived activator of caspases; Cyto: cytochrome; Bax: BCL2-associated X; Bcl: B-cell lymphoma; dATP: deoxyadenosine triphosphate; APAF: apoptotic protease activating factor.

was determined to be the most effective; it has the ability to induce apoptosis in HCT116 cancer cells via DNA fragmentation[46]. SRJ09 (3,19-(2-bromobenzylidene) andrographolide) is a semisynthetic andrographolide derivative that, compared to andrographolide, exhibited improved activity *in vitro* by producing rapid cell killing effects[47]. Its anti-tumor activity was evaluated *in vivo* using athymic mice carrying HCT-116 colon tumor xenografts; SRJ09 significantly attenuated tumor growth in these mice, demonstrating for the first time *in vivo* that SRJ09 may be a potential anti-colon cancer drug.

Regulation of the Extrinsic Apoptosis Pathway

The extrinsic apoptosis pathway, or death receptor (DR)-mediated procaspase-activation pathway, is triggered by the activation of DRs by ligands, especially the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)[48]. TRAIL binds to the DRs DR4 and DR5 and following the recruitment of Fas-associated protein with death domain and procaspases-8/10 to form a death-inducing signaling complex, triggers an apoptotic cascade and resulting cell death[48,49] (Figure 2). Notably, the activation of the TRAIL signaling pathway selectively induces apoptosis of cancer but not normal cells[49]; thus, it has been recognized as a promising target for cancer therapies, which include recombinant forms of TRAIL, TRAIL receptor agonists, and other therapeutic agents, such as herbal extracts.

Recently, andrographolide has been confirmed to activate TRAIL, as well as other extrinsic apoptosis-promoting factors in cancer cells. Andrographolide at subtoxic concentrations preferentially increased the sensitivity of prostate cancer cells to TRAIL-induced apoptosis by upregulating DR4 and p53 expression[50]. Similarly, andrographolide sensitized bladder cancer cells to TRAIL-mediated apoptosis by upregulating DR4 and DR5 in a p53-dependent manner[51]. Andrographolide also has anti-gastrointestinal cancer effects via the modulation of the extrinsic apoptosis pathway. Lim et al[52] demonstrated that andrographolide inhibited cell growth and triggered apoptotic and non-apoptotic cell death in human gastric cancer cells (AGS, SNU638, and SNU601) *via* the activation of TRAIL-induced apoptosis through the induction of DR5 expression. Furthermore, andrographolide pretreatment dramatically augmented TRAIL-induced activation of caspase-8 and caspase-3, as well as cleavage of PARP (a caspase-3 substrate), likely through upregulation of DR4, a process dependent on the presence of functional p53[53]. Altogether, these studies imply that andrographolide can exert its anticancer effects by regulating the external apoptosis pathway and consequently, further support its potential use in gastrointestinal cancer therapy (Figure 2).

Regulation of Endoplasmic Reticulum (ER) Stress

Imbalances in calcium homeostasis and unfolded or misfolded proteins lead to protein accumulation in the ER, triggering ER stress, which results in cell apoptosis and is associated with cancer development. The unfolded protein response (UPR) is a highly conserved pathway that allows the cell to manage ER stress. It can activate three transcription factors: inositol-requiring enzyme 1 (IRE1), protein kinase R-like ER kinase, and activating transcription factor 6; and induce apoptosis through three main mechanisms: C/EBP homologous protein (CHOP) pathway, apoptosis signal-regulating kinase 1 and c-Jun N-terminal kinase (JNK) pathways, and caspase-12 pathway.

Compounds that stimulate cancer cells to increase ER stress and induce apoptosis may be useful for anti-cancer treatment. Andrographolide was found to significantly promote the death of T84, HCT116, and COLO 205 colon cancer cells, likely through ER stress-induced apoptosis, but not normal cells[54]. Accordingly, a decreased Bax/Bcl-2 ratio was found, along with increased expression of glucose-regulated protein 78, IRE-1, X-box binding protein 1, and CHOP. Other studies with the T84 and COLO 205 human colon cancer cell lines showed that andrographolide treatment, by increasing ROS levels, promoted ER stress and upregulated UPR signaling, leading to apoptosis [55]. Notably, in the presence of N-acetyl-L-cysteine, a ROS scavenger, andrographolide-induced cell death, activation of UPR signaling, and expression of CHOP and the apoptotic elements Bax and caspase 3 were all inhibited, indicating that the presence of ROS is crucial for andrographolide to exert its pro-apoptotic effect[55].

Given that ER stress has been well-documented in the pathogenesis of tumor development, andrographolide likely exerts its protective effects in gastrointestinal cancer by promoting apoptosis through inducing ER stress. However, this hypothesis should be evaluated carefully in future studies using different models.

Regulation of Resistance to Chemotherapy Agents and Synergistic Effects

Chemotherapy and targeted therapy remain the foremost options for the treatment of gastrointestinal tumors, as they prolong overall survival and improve the quality of life in cancer patients. However, their downsides, which remain unresolved, include various unbearable side effects and drug resistance[56,57]. One of the most well-known chemotherapeutic agents used in the treatment of colorectal cancer is 5-fluorouracil (5-Fu)[58,59]. However, a growing concern in the clinical practice of colorectal cancer is resistance to 5-Fu therapy. Thus, potential co-therapy agents to treat 5-Fu-resistant patients are urgently needed.

Several studies have shown that andrographolide may have synergistic therapeutic effects during 5-Fu treatment of colorectal cancer. For example, a recent study demonstrated that andrographolide reversed colorectal cancer resistance to 5-Fu treatment and re-sensitized HCT116/5-FuR cells (HCT116 cells that are 5-Fu resistant) to the cytotoxic effects of 5-Fu[60]. Further analysis found that andrographolide/5-Fu co-treatment increased HCT116/5-FuR cell apoptosis; Bax levels also increased significantly. As andrographolide can directly interact with Bax, this andrographolide-Bax interaction prevented Bax degradation and promoted mitochondrial-mediated apoptosis[60]. Similarly, Su et al[61] found that andrographolide, by enhancing cell apoptosis, synergistically improved the anti-proliferation effect of 5-Fu in HCT-116 cells. Notably, andrographolide has been reported to ameliorate a side effect of 5-Fu treatment – i.e., intestinal mucositis-and did not weaken the anti-H22 tumor effect of 5-Fu[62]. Although further confirmation is needed, the synergistic effect of andrographolide during 5-Fu treatment and its attenuation of 5-Fu side effects provide new ideas for gastrointestinal tumor treatments.

Regulation of Angiogenesis

Angiogenesis refers to the formation of new blood vessels from pre-existing blood vessels. In normal tissue, blood vessels distribute nutrients and oxygen for development, vascular remodeling, wound healing, pregnancy, and menstrual cycles. Vascular endothelial growth factor receptor (VEGFR) is a cytokine receptor crucial in pro-angiogenic signaling; it is activated by vascular endothelial growth factor (VEGF) and is present on the surface of endothelial cells when tumor angiogenesis is initiated. Pathological angiogenesis is driven by the overexpression of pro-angiogenic factors, and it has been recognized as a necessary process for tumor cell proliferation and metastasis in cancer development[63]. Thus, an urgent need exists for agents that block angiogenesis by depriving tumor tissues of nutrients and oxygen[64,65].

Andrographolide has been demonstrated *in vitro* to dramatically inhibit the proliferation, migration, and invasion of human umbilical vein endothelial cells (HUVECs) with no evident cytotoxicity at low concentrations[12,66,67]. Indeed, evidence supporting the anti-angiogenic effects of andrographolide *in vitro* and *in vivo* has been published (Figure 3). Andrographolide was reported to inhibited angiogenesis by inhibiting the proliferation, migration, invasion, and tube formation of HUVECs[68]. Lim et al[67] showed that andrographolide treatment (20 μM) significantly inhibited VEGF-induced (10 ng/mL) sprout formation and regressed neo-angiogenesis in chick embryos. This anti-angiogenic effect results from andrographolide binding to the ATP-binding pocket of VEGFR2 and blocking the VEGF pathway, indicating that andrographolide may exert its anti-angiogenic effects by targeting VEGFR2[67]. Accordingly, the anti-angiogenic activity of andrographolide has been shown to be mediated by the inhibition of VEGFA-induced phosphorylation and its activation of VEGFR2 and downstream targets such as mitogen-activated protein kinases (MAPKs); however, andrographolide has no effect on VEGFR2 kinase activity[69]. Andrographolide treatment markedly inhibited angiogenesis in a dose-dependent manner (5, 10, and 20 μM) compared to dimethylsulfoxide treatment in chick embryo yolk sac membrane and chick embryo chorioallantoic membrane assays[70]. Further experiments with HUVECs found that andrographolide significantly inhibited the expression of miR-21-5p, which targets tissue inhibitors of metalloproteinase 3 (TIMP3) to regulate TIMP3/matrix metallopeptidase (MMP) signaling and promote angiogenesis[70]. Blanchard et al[71] reported that five consecutive days of andrographolide (50 mg/kg, I.P. or P.O.) treatment inhibited tumor angiogenesis in athymic mice that had HCT116 cells injected into the flank to induce tumor development. Moreover, andrographolide treatment dramatically decreased the expression of VEGF165, phosphorylated protein kinase B (AKT), and fork head box protein M1 levels, while significantly elevating the expression of the endogenous angiogenesis inhibitor Tsp-2. The anti-angiogenic effect of andrographolide in colorectal cancer is further supported by

the finding that andrographolide antagonizes TNF-α-induced interleukin (IL)-8 expression in HCT116 cells[72]; IL-8 is highly expressed in colorectal cancer and is implicated in angiogenesis[73-75]. These results indicate that andrographolide has potential as a novel anti-IL-8 agent in suppressing angiogenesis in gastrointestinal cancer.

The synthesis of andrographolide derivatives has attracted considerable attention for their anti-angiogenic activity and increased cytotoxicity toward cancer cells. In particular, a newly synthesized andrographolide derivative (andrographolide-40) was found to significantly suppress blood vessel formation *in vivo* in zebrafish and inhibited proliferation, migration, and tube formation of HUVECs *in vitro*[76]. Andrographolide-9, a 15-benzylidene substituted derivative of andrographolide, by attenuating the VEGF/VEGFR2/AKT signaling pathway, was more effective than andrographolide in inhibiting the migration and formation of VEGF-induced capillary-like tubes in HUVECs[77]. Similarly, andrographolide-26b (12α-isomer) dramatically inhibited VEGF-induced proliferation, migration, invasion, and formation of capillary-like structures in HUVECs, partly by blocking VEGF/VEGFR2 signaling pathways and reducing MMP-9 expression[78]. Finally, compound A5, a novel semi-synthetic andrographolide analogue, inhibited VEGF-induced tube formation in HUVECs in a concentration-dependent manner and suppressed VEGF-induced phosphorylation of VEGFR2, extracellular signal-regulated kinase 1 and 2 (ERK1/2), and p38 kinase, indicating that compound A5 inhibits angiogenesis by blocking the VEGFR2-p38/ERK1/2 signaling pathway[79].

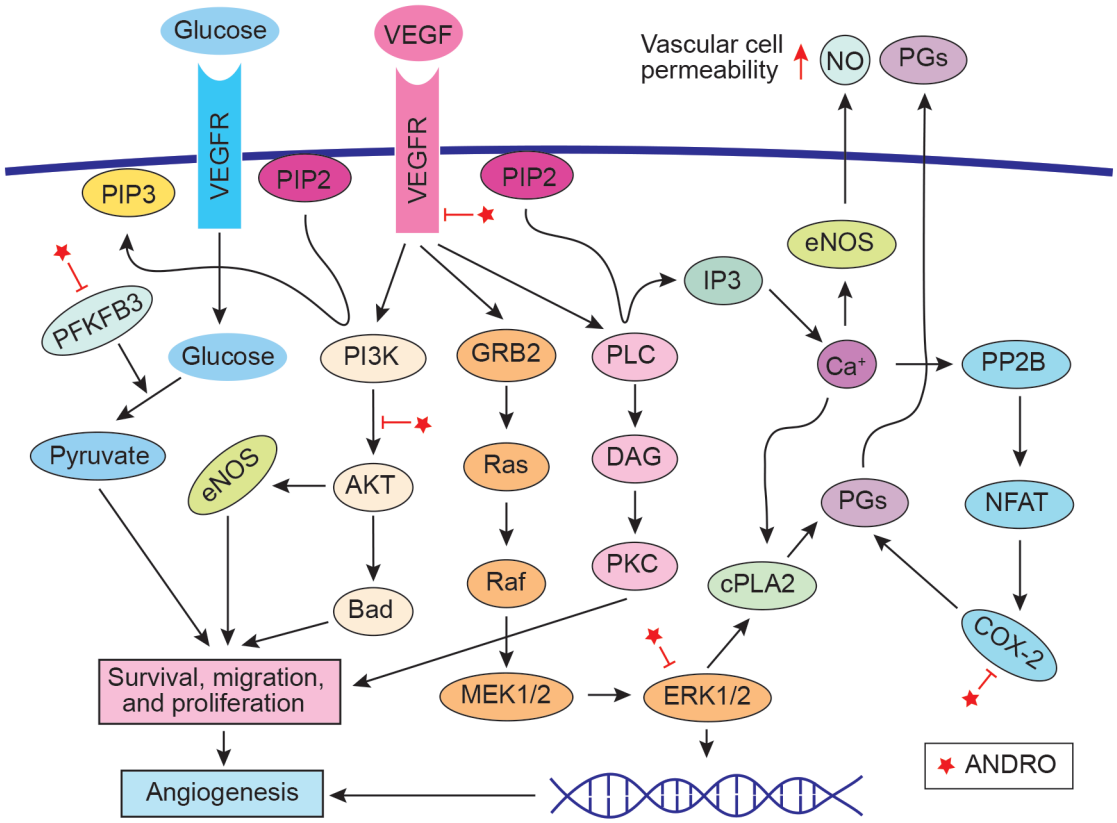

Figure 3. Regulation of angiogenesis by andrographolide and its derivatives. VEGFR: vascular endothelial growth factor receptor; PIP2: phosphatidylinositol (4,5) bisphosphate; GRB: growth factor receptor-bound protein; ERK: extracellular signal-regulated kinase; cPLA: cytoplasmic phospholipase A; PG: prostaglandin; COX: cyclooxygenase; NFAT: nuclear factor of activated T cells; PP2B/CaN: calcineurin; PIP3: phosphatidylinositol 3,4,5-trisphosphate; PLC: phospholipase C; DAG: diacylglycerol; PKC: protein kinase C; PI3K: phosphatidylinositol 3-kinase; AKT: protein kinase B; Bad: BCL2 antagonist of cell death; eNOs: endothelial nitric oxide synthase; PFKFB3: 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3.

Other Mechanisms

Other mechanisms by which andrographolide exerts its anti-cancer effects have been reported. For example, andrographolide may alter the tumor microenvironment by changing the extracellular matrix via increasing TIMP-1/2 expression and reducing MMP-2/9 expression[80]. In an earlier study, Jiang et al[81] studied the effects of andrographolide on the adhesion of gastric cancer cells to activated endothelial cells as well as the expression of certain cell adhesion molecules. They found that andrographolide suppressed the adhesion of gastric cancer cells expressing high levels of sialyl Lewis(X) to human vascular endothelial cells by suppressing E-selectin expression.

In summary, andrographolide and its derivatives exert powerful anti-gastrointestinal cancer effects by inhibiting cancer cell proliferation and metastasis as well as promoting apoptosis. Thus, they may represent putative therapeutic agents for gastrointestinal cancers. Nevertheless, additional evidence from *in vivo* and clinical studies is urgently required.

Ulcerative Colitis (UC)

UC is a chronic, non-specific inflammatory colon disease whose clinical signs include abdominal pain, bloody diarrhea, weight loss, tenesmus, and vomiting. It follows a long course and is associated with recurring attacks, which greatly impairs the quality of life of its sufferers. UC may result in higher cancerization rates. Currently, the common clinical treatments for UC include adrenocorticotropic hormones and immunosuppressive and anti-infective agents. However, some patients with refractory UC show no signs of improvement after treatment with these current drugs, and their long-term use can produce general side effects such as gastrointestinal dysfunction, joint pain, skin rashes, and especially, impaired reproductive function. Consequently, alternative therapies are urgently needed.

A recent study with an oxazolone (OXZ)-induced UC rat model found that andrographolide dramatically reduced colonic disease activity index and mortality in a dose-dependent manner while significantly improving colon length and spleen coefficient, indicating that andrographolide may be a potential UC treatment[82]. Although the selected models were different, these findings corroborate with those of previous reports using *in vivo* and *in vitro* models demonstrating that andrographolide has a protective effect in UC[83-85]. In this section, the potential mechanisms and future prospects of andrographolide's protective effects in UC will be discussed.

Regulation of Nuclear Factor Kappa B (NF-κB) and/or MAPK Pathway Activation

The Toll-like receptor 4 (TLR4) signaling pathway is initiated by lipopolysaccharides (LPS) and involves the activation of MAPK, the induction of NF-κB, and lastly, the activation of inflammation-related genes encoding pro- and anti-inflammatory cytokines and chemokines[86,87]. NF-κB plays a critical role in gene induction across diverse cellular responses, particularly with respect to inflammation and innate immunity[88,89]. The NF-κB family of transcription factors are increasingly recognized as crucial players in the many steps of UC initiation and progression[90]. Consequently, strategies that suppress NF-κB-mediated intestinal inflammation may ameliorate the pathological damage and intestinal dysfunction found in UC patients; indeed, aminosalicylic acid drugs (such as sulfasalazine) and some specific anti-inflammatory herbal extracts confer beneficial therapeutic effects[91-95]. Notably, macrophages, due to their critical role in inflammation, have been broadly employed in *in vitro* cell models of inflammatory bowel disease, especially UC, in the development of therapeutic drugs.

As a potent anti-inflammatory compound, andrographolide and its derivatives have been shown *in vitro* and *in vivo* to regulate the NF-κB pathway (Figure 3A). In an LPS-induced macrophage model, andrographolide treatment extensively inhibited the NF-κB pathway[85]. Moreover, andrographolide treatment not only suppressed upstream signaling pathways mediated by TLR4, including dephosphorylation of IκB kinase α/β, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα), and IL-1 receptor-associated kinase 4, but also inhibited the critical downstream proinflammatory transcription factor p65, resulting in decreased production of proinflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α)[85].

Andrographolide could also inhibit the MAPK pathway, as evidenced by phosphorylation levels of ERK1/2, JNK, p38, and activator protein 1[85]. These results indicate that andrographolide likely reduces LPS-induced inflammation in macrophages by attenuating NF-κB and MAPK-mediated inflammatory responses. Indeed, andrographolide and its derivative 3,14,19-triacetyl andrographolide produced anti-inflamma-

tory effects in a mouse model of dextran sulfate sodium-induced UC by inhibiting the NF-κB and MAPK pathways[85,96]. The suppression of NF-κB and MAPK by andrographolide is related to the activation of adenosine monophosphate protein kinase (AMPK)[85], a critical molecule in the regulation of bioenergy metabolism and inflammation[97,98], indicating that MAPK pathway inhibition via AMPK may be a potential mechanism underlying the effects of andrographolide treatment in UC (Figure 3A).

Multiple studies have reported the anti-inflammatory effects of andrographolide or its derivatives in UC. For example, andrographolide treatment significantly alleviated histological damage in an OXZ-induced UC rat model. The treatment improved crypt structure, reduced inflammatory cell infiltration, and inhibited p65 expression[99]. Andrographolide sulfonate has also been found to suppress the phosphorylation of p65 and P38 in damaged colon tissue in a 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced experimental colitis model[100]. Lastly, andrographolide-lipoic acid conjugate (AL-1) attenuated the expression of p-p65 and p-IκBα in TNBS-induced colitis mice[84]. Based on reports of how andrographolide and its derivatives affect the NF-κB and MAPK pathways to regulate inflammation in other models and diseases[101-104], andrographolide and its derivatives could be effective agents for the treatment of UC (Figure 3A). However, the way in which andrographolide inhibits various targets in the NF-κB and MAPK pathways and whether these inhibitory mechanisms can be observed in UC patients remain largely unknown. Therefore, additional mechanistic and clinical studies are needed.

Regulation of the T Helper (Th) 17-Involved Immune Response

A disordered immune response has been proposed as the leading cause of UC; in recent years, emerging evidence has confirmed the crucial role of IL-17 in the development UC[105,106]. Th17 cells are $CD4^+$ T cells that can secrete IL-17 under the regulation of the nuclear transcription factor retinoic acid-related orphan receptor-γt (ROR-γt) and through IL-17 secretion, can activate various immune cells to promote the development of the inflammatory response.

Prior studies have reported an association between IL-17-producing Th17 cells and autoimmune diseases, including UC[107]. Accordingly, the percentage of Th17 cells in $CD4^+$ cells collected from peripheral blood mononuclear cells (PBMCs) of UC patients has been found to exceed those of healthy donors[108], implying that Th17 regulation is a potential therapeutic avenue for UC treatment. In fact, some compounds that selectively suppress Th17 cell development and function have been identified as possible therapeutic options for treating human autoimmune diseases, including inflammatory bowel disease[109-112].

Using a TNBS-induced experimental colitis mouse model, UC PBMCs, and LPS-treated PBMCs, andrographolide treatment was observed to inhibit excessive pro-inflammatory cytokine expression, including that of TNF-α, IL-1β, IL-6, and IL-23[108]. This inhibition was connected to a reduction in Th17 cells and a suppression of IL-17A and ROR-γt protein levels, implying that andrographolide inhibits the Th17-involved immune response as well as downstream pro-inflammatory factors to suppress the inflammation response, ultimately resulting in the amelioration of UC (Figure 3B). Andrographolide sulfonate (trade name: Xi-Yan-Ping Injection), a water-soluble form of andrographolide, has been found to inhibit $CD4^+$ T-cell infiltration and the differentiation of Th1 and Th17 subsets in TNBS-induced colitis in mice and LPS-treated PBMCs, likely through signal transducer and activator of transcription (STAT) 3 signaling, indicating that andrographolide sulfonate, by regulating the Th17-involved immune response, may be a novel agent in UC treatment[113] (Figure 4B).

Others

Aberrant Th1 and Th2 responses, especially an excessive Th2 response, contribute to UC development[114]; thus, restoration of the Th1/Th2 balance may be a promising avenue for treating UC. A recent investigation using an OXZ-induced UC rat model found that andrographolide can effectively improve inflammation by regulating the balance of related inflammatory factors via blockage of the IL-4R-STAT6 pathway[82]. This pathway is essential in regulating Th2-induced cytokine production and epithelial barrier function in OXZ-induced colitis[115].

Furthermore, in TNBS-induced colonic injury, AL-1 has been reported to promote the peroxisome proliferator-activated receptor (PPAR)-γ pathway[84], an upstream target of NF-κB that is negatively correlated with NF-κB, indicating that the protective effects of AL-1 in colitis mice were likely mediated by the activation of PPAR-γ. Finally, andrographolide or its derivatives were

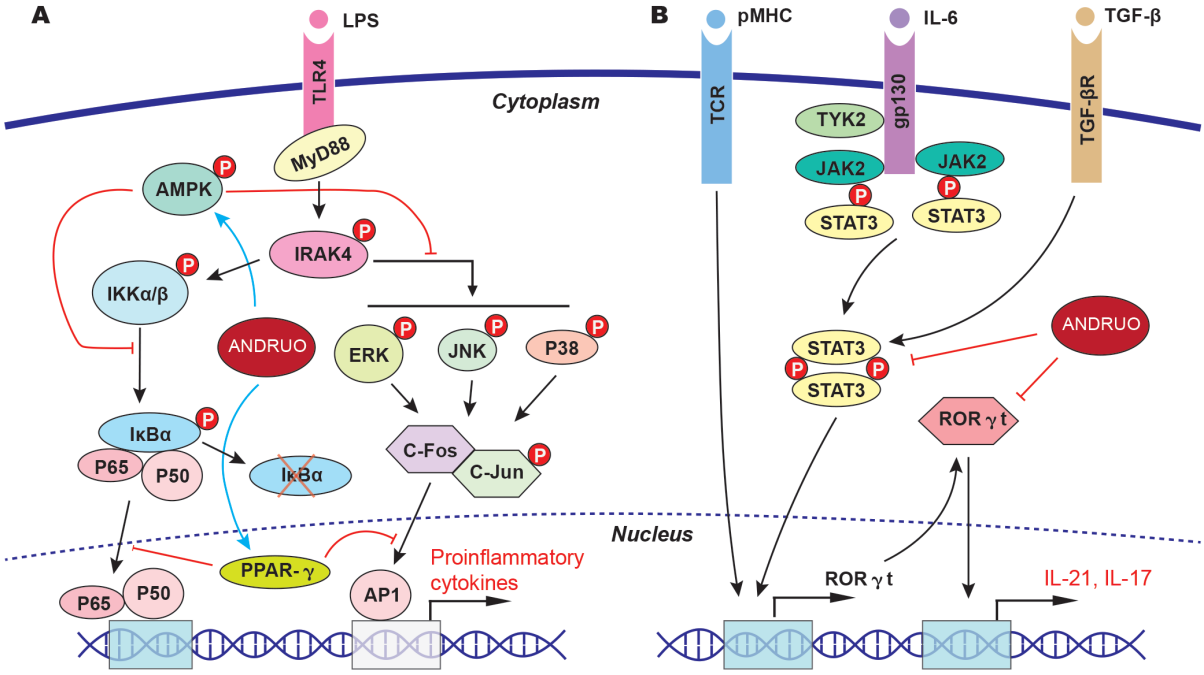

Figure 4. The underlying mechanisms of andrographolide and its derivatives in treating UC. A. Andrographolide and its derivatives regulate NF-κB- and MAPK-pathway activation. B. Andrographolide and its derivatives regulate Th17-involved immune response. NF-κB: nuclear factor kappa B; MAPK: mitogen-activated protein kinase; Th17: T helper 17; LPS: lipopolysaccharide; TLR4: toll-like receptor 4; MyD88: myeloid differentiation factor 88; IRAK4: interleukin-1 receptor-associated kinase 4; IKK: inhibitor of nuclear factor kappa-B kinase; AMPK: adenosine 5'-monophosphate (AMP)-activated protein kinase; PPAR-γ: peroxisome proliferators-activated receptor-γ; ERK: extracellular regulated protein kinase; JNK: c-Jun N-terminal kinase; AP-1: activator protein 1; pMHC: peptide- major histocompatibility complex; TCR: T cell receptor; IL-6: interleukin-6; gp130: glycoprotein 130; TYK2: protein tyrosine kinase 2; JAK: Janus kinase; STAT: signal transducer and activator of transcription; TGF-β: transforming growth factor-β; TGF-βR: transforming growth factor-β receptor; RORγt: retinoic acid-related orphan receptor γt.

claimed to have anti-fibrotic abilities and reduce intestinal permeability in colitis models, but the specific mechanisms are unclear[100,116].

Altogether, andrographolide or its derivatives can effectively improve the inflammatory phenotype of UC models *in vivo* and *in vitro*; however, in view of the complexity of the immune system and the multiple targets of these drugs, additional studies are required to clarify the effects of these compounds on the regulation of the patient's immune function. In addition, specific anti-inflammatory targets and how andrographolide and its derivatives act on these targets also need to be clarified.

Conclusions

This review summarizes the pharmacological effects of andrographolide and its derivatives in gastrointestinal protection. Numerous studies have provided evidence on the potential use of andrographolide in gastrointestinal disorders. Andrographolide has prophylactic and therapeutic effects in gastrointestinal disorders such as GU, gastric cancer, colorectal cancer, and inflammatory bowel disease via its ability to regulate apoptosis and inflammation. The beneficial results of andrographolide provide solid evidence that novel discoveries of protective natural compounds are a promising direction for the treatment of gastrointestinal tract disorders. Unfortunately, one of the factors limiting andrographolide effectiveness is its poor solubility. Therefore, its chemical structure needs to be modified to improve its water solubility and optimize delivery systems. Indeed, in animals with gastrointestinal tract disorders, studies determining the treatment efficacies of andrographolide derivatives have been published. It is hoped that in the near future, more studies regarding the clinical efficacy of andrographolide derivatives in gastrointestinal tract disorder treat-

ment will be available, which may push the development of andrographolide derivatives as novel medication for gastrointestinal tract disorders.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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