Pharmaceutical Sciences, 2023, 29(1), 20-36
doi:10.34172/PS.2022.25
https://ps.tbzmed.ac.ir/

Review Article

The Role of *Andrographis paniculata* in Modulating the Immune Response in Cancer-Associated Chronic Inflammation, Angiogenesis, and Metastasis

Rengganis Ulvia[1], Andayana Puspitasari Gani[2,3], Retno Murwanti[3,4*]

[1]*Faculty of Pharmacy, Universitas Gadjah Mada, Jl. Sekip Utara, Sleman, Yogyakarta 55281, Indonesia.*
[2]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Jl. Sekip Utara, Sleman, Yogyakarta 55281, Indonesia.*
[3]*Medical Plants and Natural Products Research Center, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Sleman, Yogyakarta 55281, Indonesia.*
[4]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Jl. Sekip Utara, Sleman, Yogyakarta 55281, Indonesia.*

Article Info

Article History:
Received: 25 Feb 2022
Accepted: 03 Jun 2022
ePublished: 09 Jun 2022

Keywords:
-*Andrographis paniculata*
-Angiogenesis
-Anticancer
-Immunomodulatory
-Metastasis
-Tumor microenvironment

Abstract

The immune system plays an essential role in cancer pathogenesis through providing an inflammatory immune response. Chronic inflammation causes tumor growth, angiogenesis, and metastasis facilitated by interactions between tumor, immune, and stromal cells in the tumor microenvironment (TME). Various inflammatory mediators and growth factors secreted by cells in the TME exert a synergistic effect on cancer promotion. Thus, the development of cancer therapies that lead to inhibition of the activity of immune cells, cytokines, chemokines, and cancer-inducing growth factors is a promising therapeutic strategy. *Andrographis paniculata* (*A. paniculata*) is an ethnomedicinal plant with immunomodulatory and anticancer activity. *A. paniculata* can also inhibit the resistance of chemotherapy agents associated with TME as adjuvant chemotherapy. This review focuses on the mechanism of *A. paniculata* in suppressing cancer-associated chronic inflammation, angiogenesis, and metastasis through modulation of the immune response. The results show that *A. paniculata* exerts anticancer effects directly targeting cancer cells, inhibiting cancer growth by modulating immune responses. *A. paniculata* exerts anticancer effects by inhibiting the production of cytokines, growth factors, and chemokines via the nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways. In addition, this review provides a new hypothesis regarding the potential of *A. paniculata* to serve as an anticancer agent that can inhibit cancer cell proliferation at the angiogenesis and metastatic stages through regulating inflammation due to interactions between cancer cells, immune cells, and stromal cells in the TME.

Introduction

The immune response in inflammation can either suppress or trigger cancer growth. Acute inflammation has an anticancer effect derived from the activity of immune cells, including natural killer (NK) cells, dendritic cells (DC), M1 polarized macrophages, N1 polarized neutrophils, and effector T cells (CD8+ cytotoxic T cells and effector CD4+ T cells).[1,2] Immune cell activity in the acute inflammatory response to cancer growth includes direct killing, phagocytosis, and secretion of proinflammatory cytokines. Chronic inflammation affects the TME, which exerts an immunosuppressive response that causes cancer cells to grow and promotes metastasis and angiogenesis. The TME comprises the surrounding immune cells, fibroblasts, lymphocytes, blood vessels, extracellular matrix, inflammatory cells of the bone marrow, and various signaling molecules. The interaction between cancer cells and cells in the TME influences cancer development.

Immune cells that play a role in chronic inflammation are tumor-associated macrophage (TAM)-associated M2 polarized macrophages, tumor-associated neutrophil-associated N2 polarized neutrophils (TAN), Treg regulatory T cells, and myeloid-derived suppressor cells (MDSC).[1,2] The activity of immune cells in chronic inflammation in the TME includes the secretion of proinflammatory cytokines, chemokines, and growth factors that promote cancer cell growth. The proinflammatory cytokines are produced mainly by activated macrophages and are involved in the upregulation of inflammatory cytokines, including interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α). Chemokines are a large family of cytokines with chemotactic activity. Chemokines and their

*Corresponding Author: Retno Murwanti, E-mail: retno_murwanti@ugm.ac.id

receptors are expressed by cancer cells and cells around the TME that play a role in leukocyte activation, angiogenesis, metastasis, and cell proliferation.[3] Chemokines and their receptors have become targets for cancer immunotherapy. Some examples of chemokines and their receptors that play a role in cancer development are (1) CXC-chemokine ligand 16 (CXCL16) and its receptor CXC-chemokine receptor 6 (CXCR6) serve as a potent angiogenic mediators, and (2) CC-chemokine receptor 7 (CCR7) mediates tumor cell migration to lymph nodes through binding to CC-chemokine ligands 19 and 21 (CCL19 and CCL21).[4,5] In some tumors, the expression of CXCR4 gives cancer cells the ability to migrate and metastasize to CXCL12-secreting organs.[6,7] Growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor-beta (TGF-β) stimulate the proliferation, invasion, and angiogenesis of cancer cells.[8]

The production and expression of inflammatory mediators such as cytokines, chemokines, and growth factors are regulated by the transcription factors nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), and Janus kinase (JAK)/signal transducer and activator of transcription (STAT). The NF-κB signaling pathway controls the expression of genes such as TNF-α, IL-6, VEGF, B-cell lymphoma pro-apoptotic protein (Bcl-2), Bcl-XL, and Bcl-Xs. JAK/STAT signaling pathways,[9] especially those including transcription factor STAT3, facilitate cell cycle progression by activating cyclin-dependent kinases (CDKs) and inducing VEGF expression, and the transcription factor STAT5 inhibits apoptosis by increasing the production of the anti-apoptotic protein Bcl-xL.[10] The MAPK signaling pathway p38-MAPK also plays a central role in producing the inflammatory cytokines IL-1β, TNF-α, and IL-6.[11] These signaling pathways promote cellular proliferation through apoptosis resistance, angiogenesis, invasion, and metastasis. Chronic inflammation associated with cancer can also be caused by chemotherapy treatment. Several chemotherapeutic agents induce inflammation, leading to TME-associated resistance and metastasis.[12] These insights encourage therapeutic approaches that modulate the immune response to cancer-associated chronic inflammation in the development of chemotherapeutic agents or adjuvant chemotherapy.

A. paniculata, known as the "king of bitters," is an ethnomedicinal plant widely used traditionally in Indonesia, India, Thailand, China, Pakistan, Bangladesh, Philippines, Hong Kong, and Malaysia. *A. paniculata* contains various phytochemical compounds, including diterpenoids, flavonoids, and polyphenols.[13] The main phytochemicals of *A. paniculata* are andrographolide, 14-deoxy-11,12-didehydroandrographolide, and neoandrographolide (Figure 1). Several flavonoid compounds from *A. paniculata* have been isolated, and their activities are known to be those of polymethoxy flavones and flavanones.[14-16] *A. paniculata* has been used for generations as a traditional medicine to treat various diseases, including cancer, and its use has been supported by scientific evidence. Currently, synthetic anticancer drugs have side effects such as immunosuppression; toxicity to the heart, kidneys, and liver; alopecia; nausea; and vomiting.[17] Therefore, herbal plants without such side effects, such as *A. paniculata*, have become an alternative cancer treatment or cancer adjuvant therapy. The advantage of *A. paniculata* compared with other herbal plants is that *A. paniculata* is not only proven to have anticancer activity on various cancer cells, but its bioactive metabolite andrographolide when combined with chemotherapeutic agents (doxorubicin, gemcitabine, cisplatin, and topotecan) can increase their effectiveness. Moreover, andrographolide could reduce the side effects of the chemotherapeutic agent bleomycin.[18-22] Another advantage is that both *A. paniculata* extract and its bioactive compound andrographolide can inhibit various inflammatory mediators and signaling pathways that play a role in cancer angiogenesis and metastasis. In addition, these activities can be an alternative in suppressing the potential for chemotherapy resistance, which will be discussed in this review.

Natural products are currently widely used for

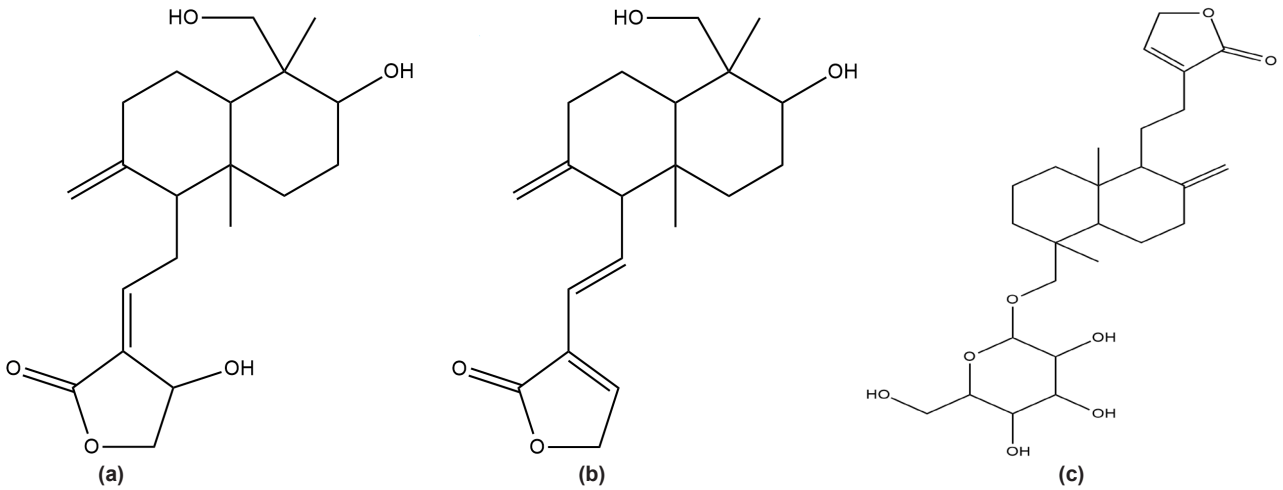

Figure 1. Chemical structures of **(a)** Andrographolide; **(b)** 14-deoxy-11,12-didehydroandrographolide; **(c)** Neoandrographolide.

cancer therapy, namely for the induction or inhibition of inflammatory activity through specific signaling pathways.[23] Most of the immunomodulatory agents with anticancer activity are divided according to two mechanisms of action: the first through blocking the initiation of cancer cells and the second through inhibiting the proliferation of cancer cells in the early stages of cancer angiogenesis and metastasis.[24,25] This review describes the immunomodulatory and anticancer mechanisms of *A. paniculate* and focuses on discussing the mechanism of *A. paniculata* in modulating the immune response to suppress cancer growth. In addition, it provides a new hypothesis regarding the activity of *A. paniculata* as a suppressor of cancer cell proliferation at the angiogenesis and metastatic stages and its potential to inhibit chemotherapeutic agent resistance through the regulation of inflammation in the TME.

Pharmacological Activities

The pharmacological activities of *A. paniculata* extract and its bioactive metabolites as immunomodulatory, anticancer, anti-hyperglycemic, antipyretic, antibacterial, anti-malarial, anti-diarrheal, filaricidal, hepatoprotective, anti-inflammatory, anti-allergic, anti-parasitic, anti-ulcerogenic, antiviral, anti-hyperlipidemic, antioxidant, analgesic, renoprotective, anti-fertility, and anti-platelet agents have been demonstrated *in vitro* and *in vivo*.[15,26,27]

The role of the immune system in cancer

The immune system is divided into innate and adaptive immune systems, which function to maintain tissue homeostasis from exposure to harmful agents such as infectious organisms (viruses, bacteria, etc.), or toxic compounds.[28] The form of defense of the innate and adaptive immune system is an inflammatory response produced by the activity of immune cells. The innate immune system functions as a short-term first defense include phagocytic cells such as macrophages, neutrophils, natural killer cells, and various receptors such as the Tall Like Receptor (TLR).[29] The adaptive immune system is an antigen-specific system mediated by T cells and B cells with highly specialized receptors.[30,31] This immune system explicitly targets the types of pathogens that cause an infection and has been previously identified. In conclusion, the adaptive immune system works more slowly than the innate immune system. The advantage of adaptive immune system is being able to "remember" the type of pathogen. When the same pathogen has exposed the body, the adaptive immune system can respond quickly.[32]

The immune response to cancer has the dual potential of suppressing or promoting cancer development. This activity depends on the inflammatory response produced by immune cells. The mediators of the inflammatory response in the initiation of cancer is divided into extrinsic and intrinsic factors.[2] Extrinsic factors are pathogenic infections, autoimmune attacks, UV exposure, excessive alcohol consumption, smoking, and obesity. Conversely, cancer-inducing mutations cause intrinsic factors to activate various inflammatory cells associated with cancer development. These extrinsic and intrinsic factors have been reported to form a immunosuppressive TME that is favorable for cancer development. Several immune system inflammatory mediators and growth factors play a role in the promotion and suppression of cancer. Their functions and roles are described below.

IL-6

IL-6 is a proinflammatory cytokine secreted by monocytes in response to infection and tissue injury. IL-6 increases proliferation and inhibits apoptosis through binding its receptor (IL-6Rα) and coreceptor glycoprotein 130 (gp130), thereby activating the transcription factors STAT1 and STAT3 in the JAK/STAT signaling pathway.[33] STAT3 is involved in the proliferation and suppression of apoptosis in breast tumor cells through upregulating the target genes cyclin D1, c-Myc, Mcl-1, Bcl-2, and Bcl-xL. The protumorigenic effect of IL-6 has been shown in various types of cancer, including gastric cancer, lung cancer, and colorectal cancer.[34-36] When NOD scid gamma female mice received intraductal mammary gland injections of IL-6-overexpressing T47D (ER+) cells (xenograft model), there was a significant increase in p-STAT33 in primary tumors accompanied by increased tumor growth. Activation of the IL-6/STAT3 pathway significantly increased lung metastases by approximately 5-fold. These results indicate that activating the IL-6/STAT3 pathway in ER+ tumors promotes the spread of metastases.[37]

TNF-α

TNF-α is an inflammatory cytokine with two types of functions in tumor development: antitumor and protumor. This cytokine has two receptors: TNF-α receptor-1 (TNF-αR1) and TNF-αR2. TNF-α, an antitumor cytokine that triggers apoptosis through TNF-αR1, forms a signaling complex I with TNF receptor-associated domain protein (TRADD), receptor interactions protein kinase (RIPK)-1, and TNF receptor-associated factor (TRAF) 2 (sometimes TRAF2 can be activated by TRAF 5). Signaling complex I recruits the adapter protein Fas-associated death domain protein (FADD), forming a death-inducing signaling complex (DISC) (also known as complex 2). DISCs can recruit and promote automatic catalytic activation of pro-caspases 8 and 10 (also known as FADD [Fas-associated death domain]-like IL-1β–converting enzyme [FLICE] and FLICE 2); caspases 8 and 10 are activated and then activate effector caspases, such as caspases 3 and 6, until apoptosis occurs. TNF-α acting as a protumor cytokine plays a role in cell proliferation, invasion, and angiogenesis of cancer cells. The binding between TNF-α and TNF-αR1 induces cell proliferation, cell activation, and differentiation by activating the transcription factors NF-κB, AP-1, and Elk-1. TNF-αR2 can regulate cell proliferation, cell-to-cell interactions, and migration through Etk phosphorylation, activation of PI3K and Akt pathways, and activation of

VEGFR2 receptors, thereby increasing angiogenesis.[38]

IL-1β

IL-1β is an IL-1 family cytokine produced and secreted by immune cells, fibroblasts, and cancer cells. To produce a response, IL-1β binds to its receptor IL-1R1. IL-1β mediates the process of angiogenesis and metastasis of cancer cells through interactions with VEGF. Several studies have shown that angiogenesis and VEGF depend on IL-1.[39] IL-1β interacts with IL-1R1 on endothelial cells and activates p38 MAPK and MAPK activated protein kinase 2, inducing cell migration.[40] IL-1β also induces the production of IL-6, TGF-β, TNF-α, and epidermal growth factor (EGF) in breast cancer cells.[41] IL-1β activates extracellular signal-regulated protein kinase (ERK) 1/2, activator protein (AP)-1, and matrix metalloproteinases (e.g., MMP-9) in invasive breast ductal carcinoma.[42] IL-1β can act as an anticancer agent through induction of Th1 and Th9 responses to produce IL-9, IL-2, and IFN-γ, which play a role in inhibiting cancer growth. IL-1β also activates CD4 and CD8 to exert anticancer effects.[43]

VEGF

VEGF is a growth factor in cancer cell angiogenesis in a family consisting of placental growth factor (PGF), VEGFA/VEGF, VEGFB, VEGFC, and VEGFD. VEGFs binds to three endothelial receptor tyrosine kinases with different affinities, such as VEGFR1, VEGFR2, VEGFR3, as well as co-neuropilin receptors and heparan sulfate proteoglycans.[44] VEGF is a crucial mediator of angiogenesis in cancer, and is regulated by oncogenes; various growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF), and TNF; IL-1 cytokines; and hypoxia. In addition to acting as a mitogenic signal for vascular endothelial cells, VEGF also induces anti-apoptotic factors that protect against vascular apoptosis and mediate the secretion and activation of enzymes that degrade the extracellular matrix. The blood vessels that form around the tumor are irregular, have blind ends, are tortuous and disorganized, and have high interstitial pressure, which results in hypoxia and further VEGF production.[45] VEGF is also immunosuppressive through inhibiting T cell function, increasing recruitment of Treg cells and MDSCs, and inhibiting DC cell differentiation and activation. The binding of the VEGF ligand to the VEGFR2 receptor inhibits T cell activity and increases the activity of immunosuppressive Treg cells. VEGF and VEGFR2 activate MDSCs via the JAK2/STAT3 signaling pathway, whereas the VEGF ligand and VEGFR1 receptor interaction inhibit DC cell maturation.[44] Various studies have shown that the binding of VEGF to VEGFR1 blocks the activation of the transcription factor NF-κB and results in inhibition of DC maturation in murine models.[46-48] VEGF promotes the development of TAMs, which can inhibit T cell activation and proliferation by releasing IL-10, TGF-β, and prostaglandins.[49,50]

TGF-β

TGF-β exerts a tumor suppressor effect in the early stages of cancer through inhibiting cell cycle progression and inducing apoptosis. In the late stages of cancer, TGF-β exerts a promotion, invasion, and metastasis effect. Receptors play an essential role in apoptosis by signaling via the suppressor of mothers against decapentaplegic (SMAD) pathway. The TGF-β ligand binds to the TGF-β RII receptor and activates TGF-β RI to form a hetero-tetrameric receptor complex. Ligand binding and subsequent oligomerization trigger TGF-β RII to phosphorylate TGF-β RI on serine or threonine residues. In the canonical pathway, activated receptor complexes phosphorylate R-SMAD (SMAD2 or SMAD3), which forms a heteromeric complex with SMAD4, which then translocates to the nucleus to regulate the expression of target genes.[51] Non-canonical pathways that play a critical role in cancer cell invasion and metastasis occur through activation of the RAS/MAPK, PI3K/Akt, RHO/Rho-associated protein kinase (ROCK), Jagged/Notch, WNT/β-catenin, and mammalian target of rapamycin (mTOR) signaling pathways.[8,52]

Chemokines

Chemokines are the largest cytokine subfamily and can be further subdivided into four main classes, depending on the location of two cysteine residues (C). They are, namely, CC chemokines, CXC chemokines, chemokines, and CX3C chemokines. In cancer development, chemokines have dual protumor and antitumor roles.[53] Several protumor chemokines have been identified, including CCL2, CCL3, and CCL5, which can increase tumor invasion and metastasis. CCL2 activates the JAK/STAT and p38 MAPK signaling pathways and influences vascularization and metastasis by inducing MMP-9. CCL2, and CCL5 and recruiting MDSCs and macrophages into the TME. CCL18 also plays a role in invasion and metastasis, but this depends on the type of cancer. CXCL12 and VEGF promote angiogenesis in vascular endothelial cells.[54] CXCL12 and its receptor CXCR4 induce cancer cell invasion and metastasis. Gefitinib-resistant A549 cells expressed high levels of CXCR4+ and showed increased phosphorylation of Akt, mTOR, and STAT3 (Y705). The antitumor effect of chemokines is through the activity of several chemokines such as CXCL8, CXCL9, and CXCL10. CXCL8 enhances the immunogenicity of cancer cells by translocating calreticulin to the cell surface. CXCL9 and CXCL10 inhibit angiogenesis in endogenous tumors.[54,55] CXCL10 inhibits FGF-induced angiogenesis formation by CXCL8 *in vivo* and *in vitro*.[56,57]

The activity of the immune system on the growth of cancer cells in the form of an inflammatory response is divided into acute inflammation and chronic inflammation. Acute inflammation can prevent cancer facilitated by NK cells, DC, M1 polarized macrophages, N1 polarized neutrophils, CD8+ cytotoxic T cells, and CD4+ T cells.[1,2] These cells trigger the secretion of cytokines (IL-12, IL-15, IFN-γ, and IL-1) that induce an inflammatory response to kill cancer

cells. A schematic illustration of the role of the immune system in the development of cancer is shown in Figure 2. The mechanism of each immune cell in acute inflammation in inhibiting cancer growth is as follows: NK cells produce granzymes and perforins, DC activated by tumor antigens induce the production of cytokines (IL-12, IL-15, and IFN-γ), mature DCs activate B cells, CD8+ T cells, and CD4+ T cells, and activate M1 macrophages and N1 neutrophils to phagocytose tumor cells.[2] During infection M1-TAM and N1-TAN produce the cytokines TNF-α, IL-6, IL-1β. Continuous production of these proinflammatory cytokines will cause chronic inflammation and induce DNA damage through the formation of reactive oxygen and nitrogen species (RONS). This condition will recruit tumor-inducing immune cells consisting of TAM, TAN, Treg regulatory T cells, and MDSC. These cells stimulate the secretion of cytokines (IL-10, IL-6, and PGE-2), CXC motif chemokine ligand (CXCL1, CXCL6, and CXCL8) and CC motif chemokine ligand (CCL5 and CCL22), and growth factors (TGF-β, VEGF, EGF, and platelet-derived growth factor [PDGF]) that contribute to the formation of a proangiogenic and protumor microenvironment.[2] In this TME, cancer cells also prevent tumor antigen presentation on DC. Continuous chronic inflammation will cause angiogenesis and metastasis in cancer cells associated with the TME.

Many studies have reported that inflammation is related to angiogenesis or neovascularization. This activity is mediated by tumor-inducing immune cells in the TME. Tumor-inducing immune cells, especially TAM and MDSC, secrete proangiogenic factors such as VEGF, EGF, PDGF, TGF-B, and cytokines (IL-8 and IL-10) that trigger angiogenesis.[2,58] This new blood vessel formation recruits inflammatory cells to the site of inflammation, allowing it to continue. Cancer cells can produce proangiogenic factors and inflammatory mediators through themselves or from the extracellular matrix, so cancer cells are constantly surrounded by inflammatory cells.[59] In the TME, cancer cells, immune cells, and other stromal cells work together to form blood vessels and promote endothelial cell proliferation. This condition is the initiation of metastases.

The main requirement for cancer cells to metastasize is that they migrate to other sites in the body. Cancer cells must leave their primary site, invade surrounding tissues, and intravasate into blood and lymph vessels. Immune cells that play a role at this stage are TAM and TAN, which affect the organization of the extracellular matrix (ECM), cancer cell motility, and the formation of blood and lymph vessels.[60] Tumor cells, immune cells, and stromal cells in the TME can affect the ECM by secreting ECM-remodeling enzymes (MMPs, cathepsins, and other proteases). ECM remodeling can support the invasion of tumor cells that

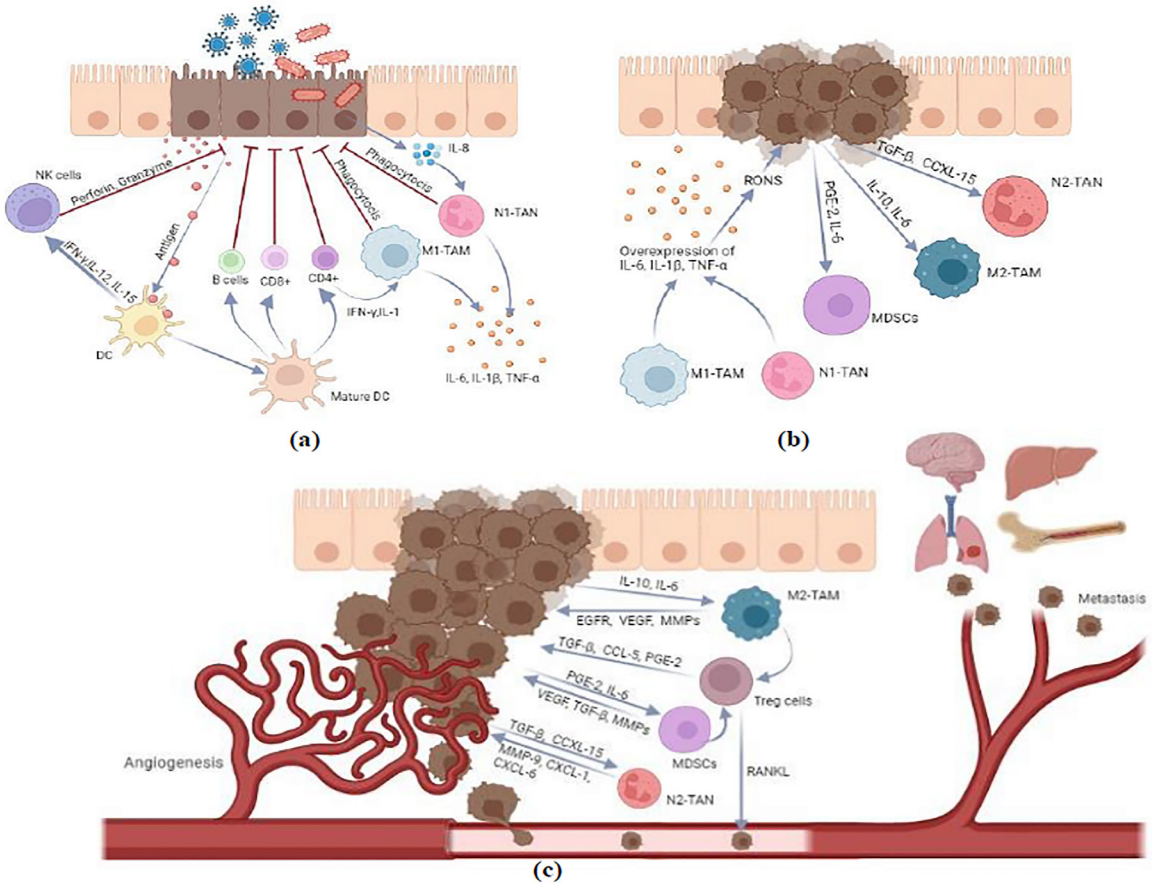

Figure 2. Schematic illustration of the role of the immune system in cancer development. **(a)** acute inflammation; **(b)** Chronic inflammation; **(c)** Angiogenesis and metastasis.

change the shape of the surrounding tissue and increase the release of growth factors.[61,62] The main route of migration of cancer cells to other places is through the blood and lymph vessels, called intravasation. After intravasation, circulating cancer cells must defend against immune cells such as NK cells to colonize distant sites. Treg cells can increase the survival of cancer cells in circulating blood vessels and lymph through the production of receptor activator of NF-κB ligand (RANKL).[63] The next stage is establishing a pre-metastatic "niche," which is facilitated by bone marrow-derived cells (BMDCs) recruited to vital organs such as lung, liver, brain, and bone to provide a response to VEGFA and cancer cell-derived growth factors. Before the arrival of tumor cells in specific organs and after the establishment of a pre-metastatic "niche," cancer cells must extravasate, that is, migrate through modulation and attachment to the endothelium. Immune cells that play a role in the extravasation stage are neutrophils that release extracellular neutrophil traps (NETs). The final step is colonization and persistent growth of new cancer cells.[60]

Immunomodulatory effects of A. paniculata and its bioactive metabolites

Several *in vitro* and *in vivo* studies reported the immunomodulatory activity of *A. paniculata* extract and its bioactive metabolites to modulate the immune system, as summarized in Table 1. Regarding the immunomodulatory effects of *A. paniculata*, one study indicated that *A. paniculata* ethanolic extract (1–16 µg/ml) and andrographolide (0.5–8 µg/ml) inhibited in a dose-dependent manner the nitric oxide (NO) and PGE2 production resulting from NF-κB and MAPK signaling pathways induced by lipopolysaccharide (LPS) in RAW 264.7 cells.[64] *A. paniculata* also exerts an effect on oxidative stress and inflammation in dermal fibroblasts (human dermal fibroblasts, adult [HDFa]). The production of reactive oxygen species (ROS) that play a role in oxidative stress was reduced in hydrogen peroxide-treated HDFa after incubation with methanolic extract of *A. paniculata* (5 µg/mL) and 14-deoxyandrographolide (1 µg/mL). The methanolic extract of *A. paniculata* (5 µg/mL) and andrographolide (5 µg/mL) exerted an anti-inflammatory effect by decreasing the expression and production of the inflammatory mediators TNF-α and IL-6 in HDFa cells stimulated by LPS and TNF-α, respectively.[65] The production of TNF-α decreased after administration of *A. paniculata* extracts (16.4 µM and 18.7 µM) in a human monocytic cell line (THP-1) stimulated with LPS.[66]

The results of previous *in vitro* studies reported that 19 diterpenoid lactone compounds isolated from *A. paniculata* decreased the production of TNF-α and IL-6 in LPS-treated RAW 264.7 cells.[67] Andrographolide significantly inhibited NO production in LPS-treated RAW 264.7 cells, with an IC_{50} value of 13.4 µM.[68] Andrographolide concentrations of 6.25, 12.5, and 25 µg/ml significantly reduced IL-1β, IL-6, and TNF-α production and expression via NF-κB/MAPK signaling pathway in LPS-induced RAW 264.7 cells.[69] Andrographolide activates the AMP protein kinase (AMPK) pathway.[70] Andrographolide activates the AMPK signaling pathway that can inhibit the NF-κB/MAPK signaling pathway and the production of IL-1β, IL-6, inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), and cyclooxygenase (COX)-2 in LPS-treated macrophages.[71,72] AMPK activation also provides anti-inflammatory effects on numerous immune cells, including mast cells, T cells, neutrophils, and macrophages.[73,74]

Andrographolide suppressed expression of COX-2 and inhibited NF-κB in RAW 264.7 cells stimulated by Toll-like receptor 3 (TLR3) agonists (e.g., Poly [I: C]) and TLR4 agonists (e.g., LPS). Andrographolide also suppresses the toll-interleukin-1 receptor domain-containing adapter inducing interferon-β (TRIF) signaling pathway by targeting TANK-binding kinase 1 (TBK1). The TLR adapter molecules TRIF and TBK1 are kinases involved in the TRIF-dependent TLR signaling pathway. TRIF will facilitate TLR4 and TLR3 to activate transcription factors interferon regulatory factor (IRF3) and NF-κB. Phosphorylated IRF3 will be translocated into the nucleus to bind to an IFN-stimulated response element in the promoter regions of the genes interferon-γ-inducible protein 10 (IP-10) and IFN-β. This IRF3-regulated gene plays a crucial role in antibacterial and antiviral immune responses.[75]

In the atherosclerosis rat model, *A. paniculata* extract doses of 0.6 and 2.4 mg/kg body weight (BW) significantly reduced the number of Th17 cells. Moreover, *A. paniculata* extract at 1.2 mg/kg BW significantly increased the number of Treg cells. The ratio of Treg cells to Th17 cells significantly increased after administration of *A. paniculata* extract at 0.6 and 2.4 mg/kg BW.[76] Ethyl acetate extract of *A. paniculata* (0.78, 1.56, 3.12, and 6.25 mg/kg BW) reduced TNF-α, IL-12, macrophage inflammatory protein (MIP)-2, and NO in serum and peritoneal macrophages isolated from BALB/c mice treated with LPS.[77] Andrographolide at 200 mg/kg BW and *A. paniculata* extract at 100, 200, and 400 mg/kg BW given orally once a day for 28 days significantly inhibited the NF-κB signaling pathway through the upregulation of Iκβα phosphorylation, p65 protein expression, and nuclear translocation in male Wistar rat treated with fine particulate matter (PM2.5) at 8 mg/kg BW.[78]

As shown in previous studies, *A. paniculata* and its bioactive metabolites also enhance the immune response. Water extract of *A. paniculata* (200 and 400 µg/ml) can stimulate the proliferation of human peripheral blood mononuclear cells (PBMCs) and the production of IFN-γ and TNF-α.[79] *A. paniculata* ethanol extract (1 µg/mL) can stimulate the proliferation of lymphocyte cells by approximately 38% compared with control lymphocyte cells without treatment.[80] Extract of *A. paniculata* (100 mg/kg BW) increased the number of lymphocytes and macrophages significantly.[81]

Table 1. The mechanisms of immunomodulatory effects of *A. paniculata* and its bioactive metabolites.

Sample	Study design	Subject	Modulation	Mechanism	Ref.
Ethanol extract of *A. paniculata*	*In vitro*	LPS-stimulated RAW 264.7 cells	↓	↓NO, PGE-2 Inhibits phosphorylation of p38, JNK, ERK (MAPK pathway), and IκBα.	64
Andrographolide	*In vitro*	LPS-stimulated RAW 264.7 cells	↓	↓NO	87
Diterpenoid Lactone	*In vitro*	LPS-stimulated RAW 264.7 cells	↓	↓ TNF-α and IL-6	88
Andrographolide	*In vitro*	LPS-stimulated RAW 264.7 cells	↓	↓TNF-α, IL-6, and IL-1β ↓TNF-α, IL-6, and IL-1β mRNA expression Inhibits phosphorylation of IκBα, p38, JNK, ERK 1/2	89
Neoandrographolide, Dehydroandrographolide, 5, 7, 2′, 3′-sitosterol	*In vitro*	RAW 264.7 cells	↓	↓NO	90
Andrographolide	*In vitro*	Cigarette smoke extract-stimulated RAW 264.7 cells	↓	↓TNF-α dan IL-1β	91
Andrographolide	*In vitro*	LPS-stimulated RAW 264.7 cells	↓	Inhibits of NF-κB/MAPK pathway, activation of AMPK pathway	92
Andrographolide	*In vitro*	Poly [I: C] and LPS-stimulated RAW 264.7 cells	↓	Inhibits TLR from TRIF and TBK1 pathway, ↓IRF3 and ↓ IFN-β, IP-10 mRNA expression	93
Methanol extract of *A. paniculata* and 14-deoxyandrographolide	*In vitro*	LPS-stimulated HDFa	↓	↓IL-6, TNF-α	94
A. paniculata extract and andrographolide	*In vitro*	LPS-stimulated human monocytic cell line THP-1	↓	↓TNF-α	66
Ethyl acetate extract of *A. paniculata*	*In vivo*	LPS-stimulated BALB/c mice	↓	↓TNF-α, ↓IL-12, ↓MIP-2, ↓NO	77
A.paniculata extract	*In vivo*	Atherosclerosis rat model	↓	↓Th17 cell ↑Treg cell	95
A.paniculata extract and andrographolide	*In vivo*	PM2.5-stimulated rat	↓	↓ NF-κB p65 and IκBα phosphorylation	78
Water extract of *A. paniculata*	*In vitro*	Peripheral blood mononuclear cells (PBMCs)	↑	↑PBMC proliferation, ↑ TNF-α, IFN-γ	79
Ethanol Extract of *A. paniculata*	*In vitro*	Lymphocyte cells	↑	↑Lymphocyte cell proliferation	80
Extract of *A. paniculata*	*In vivo*	Type 2 diabetic rats	↑	↑Macrophage, lymphocytes	81

Anticancer effects of A. paniculata and its bioactive metabolites

A. paniculata ethanolic extract has anticancer activity in neuroblastoma cancer cells (IMR-32) and colon cancer cells (HT-29) with an IC_{50} value of 200 µg/mL. An aqueous ethanol and acetone extract of *A. paniculata* 200 µg/mL also exerted anticancer activity on HT-29 cancer cells.[82] A methanolic extract of *A. paniculate* (1.0 µg/mL) inhibited the growth of leukemia cancer cells (P388).[83] MCF-7 breast cancer cells treated with *A. paniculata* extract (111 and 222 ppm) significantly inhibited cell proliferation by 47.98% and 30.50%, respectively, compared with the control group.[84] *A. paniculata* hydroalcoholic extract at a dose of 100 µg/mL can inhibit the growth of an ovarian cancer cell line (ovcar-5) by as much as 51.12%.[85] A water extract of *A. paniculate* (850.3 g/mL) significantly reduced EGF receptor (EGFR) and Akt mRNA expression in human EC-109 cells. The suspected compounds involved were panicolin and moslosooflavone, which were confirmed with liquid chromatography–mass spectrometry (LC-MS).[86]

The major phytochemicals of *A. paniculata* are andrographolide, 14-deoxy-11,12-didehydroandrographolide, and neoandrographolide, which each have anticancer activity on various cancer cells.

It was shown that the 10 µM concentration of andrographolide could significantly induce apoptosis. Moreover, it can significantly decrease IL-6 protein and reduce phosphorylation of STAT3 and ERK in PC-3

and DU145 cells.[96] Andrographolide (5, 10, and 25 μM) significantly inhibited proliferation and invasion of nasopharyngeal carcinoma (NPC) cells in a dose- and time-dependent manner. Andrographolide inhibited the transcriptional activity of NF-κB in TNF-α induced NPCs. Inhibition of NF-κB by andrographolide decreased the expression of surviving cyclin-D1 and EGFR, which play a role in cell proliferation and survival. Furthermore, andrographolide also decreased MMP-9, intercellular adhesion molecule 1 (ICAM-1), and VEGF expression, leading to metastasis and invasion of cancer. Andrographolide also induced the G2/M cell cycle arrest.[97]

Andrographolide (10 μM) suppressed clonogenicity, induced apoptosis, and promoted G1/S cell cycle arrest. Andrographolide inhibits the TLR4/NF-κB signaling pathway through decreased expression of the proteins TLR4, myeloid differentiation primary response 88 (MyD88), p-Iκβα, and p-p65 in B16 cells. This study also demonstrated the anti-melanoma effect of andrographolide with siRNA for TLR4 knockdown in B16 cells. The results showed no significant difference between TLR4 knockdown B16 cells administered 10 M andrographolide. In the NF-κB pathway, andrographolide inhibits the expression of Bcl-6, an antitumor gene, and CXCR4 as a promoter of the NF-κB target gene that activates transcription factors.[98] Andrographolide at 100 μM induces DNA damage through increasing the expression of phosphor-H2AX in HepG2 and HeLa cells. Andrographolide at 100 μM induced the G2/M phase in HepG2 cells.[99] Andrographolide at 10 μg/mL significantly induced apoptosis in Jurkat cells through decreasing the activation of the P13K/Akt signaling pathway.[100]

14-deoxy-11.12-didehydroandrographolide had antiproliferative activity on THP-1 and Jurkat leukemia cells with IC_{50} values of 35,209 ± 2.209 μg/ml and 30,062 ± 1,241 μg/ml, respectively. 14-deoxy-11,12-didehydroandrographolide also decreased glutathione (GSH) levels by 19.76% and increased procaspase-3 expression in THP-1 cells, indicating redox-mediated cell death.[101] 14-deoxy-11,12-didehydroandrographolide showed cytotoxic activity on PANC-1 and PSN-1 pancreatic cancer cells with IC_{50} values of 10.0 and 9.27 μM, respectively.[102] 14-deoxy-11,12-didehydroandrographolide has cytotoxic activity on leukemia cancer cells (U937) with an IC_{50} value of 13 μM. These compounds can also induce apoptosis in a concentration-dependent manner and increase the activation of caspase-3 and caspase-9. Cell cycle analysis revealed that 14-deoxy-11.12-didehydroandrographolide induced G_0/G_1 cell cycle arrest.[103] 14-Deoxy-11,12-didehydroandrographolide is cytotoxic to T47D breast cancer cells. induces stress on the endoplasmic reticulum (ER), and causes the autophagosomes of the T47D. 14-Deoxy-11,12-didehydroandrographolide also upregulates LC3-II. The signaling pathway involved is GADD45A/p38 MAPK/DDIT3.[104]

Neoandrographolide is one of the major compounds from *A. paniculate*. It also has a cytotoxic effect on several human cancer cells, including PC-3 prostate cancer cells (IC_{50} 12.7 ± 0.34 μM), lung cancer cells A549 (IC_{50} 5.53 ± 0.34 μM), and colon cancer cells SW-620 (IC_{50} 9.5 ± 0.89 μM). In this study, the same cancer cell was also treated with a neoandrographolide derivative compound with a modified carbohydrate structure, namely 4′,6′-benzylidene neoandrographolide. This compound had more potent cytotoxic activity than neoandrographolide on SW-620, PC-3, and A549 cancer cells, with IC_{50} values of 6.2 ± 0.21, 2.65 ± 0.78, and 1.75 ± 0.33 μM, respectively. Administration of 4′,6′-benzylidene neoandrographolide also increased cellular ROS production and induced apoptosis through increased expression of caspase-3 Bax and decreased expression of Bcl-2.[105]

A. paniculata hot water extract (1600 mg/kg) decreased the number of regulatory T cells (CD4+, CD25+ and Foxp3+) in lymph nodes and spleen. IL-10 and IL-12 cytokines decreased significantly in leukocytes isolated from the spleen of C57BL/6 mice induced by 4-nitroquinoline 1-oxide (NQO) at 60 μg/mL. This extract also worked synergistically with cisplatin + 5-fluorouracil in inhibiting tumor growth. The side effects of chemotherapy agents can also be reduced by administering an aqueous extract of *A. paniculata*.[79] Andrographolide at 25 and 100 mg/kg BW inhibited the expression of p-p65, c-Jun, p-Akt, osteopontin, and downregulated cyclin D1 and VEGF in the orthotopic NOD/SCID mouse model induced with MDA-MB-231 cells. This study showed that andrographolide downregulates the expression of p-p65, c-Jun, p-Akt, osteopontin, VEGF, and Flk 1 in anticancer and antiangiogenic activity.[106]

The mechanisms of anticancer *A. paniculata* and its bioactive metabolites were listed in Table 2.

Table 2. The mechanisms of anticancer *A. paniculata* and its bioactive metabolites.

Sample	Study design	Subject	Effect	Ref.
A. paniculata ethanolic extract	*In vitro*	IMR-32 and HT-29 cells	IC_{50} value: 200 μg/mL	82
A. paniculata aqueous, ethanol and acetone extract	*In vitro*	HT-29 cells	IC_{50} value: 200 μg/mL	82
A. paniculata methanolic extract	*In vitro*	P388 cells	1.0 μg/mL mL inhibit growth of P388 cells	83
A. paniculata hydroalcoholic extract	*In vitro*	ovcar-5 cells	100 μg/ml can inhibit 51.12% ovcar-5 cells	85

Table 2. Continued.

Water extract of A. paniculata	*In vitro*	EC-109 cells	↓EGFR and Akt mRNA expression	86
Andrographolide	*In vitro*	DU145 and PC-3 cells	↑apoptosis and ↓ IL-6 ↓phosphorylation of STAT3 and ERK	96
Andrographolide	*In vitro*	IMDA-MB-231 cells	20 μM ↓ viability, proliferation, migration, ↑ apoptosis, cell cycle arrest G2/M phase	106
Andrographolide	*In vitro*	NP cells	5, 10, 25 μM ↓proliferation an invasion in time and dose-dependent, ↓ NF-κB, survivin, cyclin D1, EGFR, MMP-9, VEGF, and ICAM-1, ↑cell cycle arrest G2/M phase	97
Andrographolide	*In vitro*	A375 and C8161 cell	↓ proliferation ↑cell cycle arrest G2/M phase, apoptosis, ↑ JNK and p-p38	107
Andrographolide	*In vitro*	B16 cells	IC_{50} : 10 μM ↑cell cycle arrest G1/S phase, apoptosis, ↓TLR4, MyD88, pIκBα, pp65, Bcl-6 and CXCR4	98
Andrographolide	*In vitro*	HepG2 cells	↑cell cycle arrest G2/M phase, ↑phospho-H2AX	99
Andrographolide	*In vitro*	Jurkat cells	10 μg/mL ↑apoptosis, ↓ PI3K/Akt ↑p38 MAPK	100
Andrographolide	*In vitro*	MDA-MB-231 cells	↑GRP-78 and IRE-1 ↑ BAX, XBP-1, and CHOP	108
14-deoxy-11 12-didehydroandrographolide	*In vitro*	THP-1 and Jurkat cells	IC_{50} 35,209 ± 2.209 g/ml and 30,062 ± 1,241 μg/ml	101
14-deoxy-11 12-didehydroandrographolide	*In vitro*	PANC-1 and PSN-1 cancer cells	IC_{50} 10.0 μM and 9.27 μM	102
14-deoxy-11 12-didehydroandrographolide	*In vitro*	U937 cells	IC_{50} 13 μM ↑apoptosis, caspase-3, and caspase-9 ↑G0/G1 cell cyle arrest	103
14-deoxy-11 12-didehydroandrographolide	*In vitro*	T47D cells	↑stress on the endoplasmic reticulum (ER) and autophagosomes, ↑LC3-II. ↑GADD45A/p38 MAPK/DDIT3	104
Neoandrographolide	*In vitro*	PC-3 cells A5-49 cells SW-620 cells	IC_{50} 12.7 ± 0.34 μM IC_{50} 5.53 ± 0.34 μM IC_{50} 9.5 ± 0.89 μM	105
4′,6′-benzylidene neoandrographolide	*In vitro*	PC-3 cells A5-49 cells SW-620 cells	IC_{50} 6.2 ± 0.21 μM IC_{50} 2.65 ± 0.78 μM IC_{50} 1.75 ± 0.33 μM ↑cellular ROS production ↑apoptosis, caspase 3 Bax ↓Bcl-2	105
Hot water extract of *A.paniculata*	In vivo	C57BL/6 mice carcinogen-induced esophageal tumorigenesis	1600 mg/kg ↓regulatory T cells (CD4+, CD25+ and Foxp3+) ↓ IL-10, IL-12 ↓side effect induced by chemotherapy agent	79
Andrographolide	In vivo	C57BL/6J mice induced B16 cells	5 μg/g reduced tumor volume, ↓cell proliferation and angiogenesis ↑apoptosis	98
Andrographolide	In vivo	NOD/SCID mouse model induced MDA-MB-231 cells	25 mg/KgBW and 100 mg/KgBW andrographolide ↓OPN, p-Akt, c-Jun, and p-p65, cyclinD1 and VEGF	106

The potential of A. paniculata in inhibition of metastasis and angiogenesis

Andrographolide inhibits A549 non-small human lung cancer invasion and migration by decreasing MMP-7 expression via the PI3K/Akt/AP-1 signaling pathway.[109] Andrographolide downregulates MMP-9 expression by inhibiting NF-κB in H3255 lung cancer cells. These effects will inhibit the migration and invasion of cancer cells.[110] Andrographolide inhibits 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced migration and invasion of MCF-7 breast cancer cells by reducing MMP-9 activation through inhibiting the ERK1/2, P3K/Akt, AP-1, and NF-κB signaling pathways.[111] Andrographolide inhibits the expression of VEGF and hypoxia-inducible factor-1 (HIF-1α), which play an essential role in angiogenesis and metastasis in non-small cell lung cancer cells.[112] An aqueous extract of *A. paniculata* inhibited the migration of esophageal cancer cells and endothelial cells by suppressing MMP-2 activity.[113] Andrographolide inhibits angiogenesis by blocking the VEGFA-induced activation of VEGFR2 and the MAPK signaling pathway in Hep3B cells.[114] Andrographolide significantly decreased the expression of hVEGF-A165 and VEGF, EGFR, cyclin A, and cyclin B, which exerted an antiangiogenic effect.[115]

The potential of A. paniculata in inhibition of chemotherapy resistance

Chemotherapy induces signaling pathways that inhibit and kill cancer cells and stimulate signals that lead to metastasis. One mechanism for cancer resistance to chemotherapy is mediated by the interaction of tumor cells with the TME, which induces stromal cells to produce cytokines, chemokines, and growth factors. Inflammation will form the TME, which plays a vital role in chemotherapy resistance to metastasize cancer cells. Several chemotherapy drugs, cisplatin, paclitaxel, 5-fluorouracil, and doxorubicin, are known to induce inflammation leading to metastasis of cancer cells.[12]

Cisplatin can induce MAPK, ERK, and epithelial-mesenchymal transition (EMT) signaling pathways in cancer that play a role in cancer cell invasion and metastasis. (12) Paclitaxel regulates the inflammatory mediators IL-1β, IL-8, IL-6, and VEGFA via the AP-1 and NF-kB signaling pathways.[11,116,117] Previous studies have shown that paclitaxel increases the production of cytokines via TLR4 in breast cancer cells. Paclitaxel induces the Ras/Raf/mitogen-activated protein kinase/ERK kinase (MEK)/ERK pathway activation in human breast cancer and lymphoma. Paclitaxel activates the TLR4-MyD88-ERK signaling pathway closely associated with tumor growth, development, invasion, and chemoresistance.[118] 5-fluorouracil activated the p38 MAPK signaling pathway in murine macrophage cells by increasing the production of IL-1β, IL-6, and TNF-α.[119] Other studies have shown that 5-fluorouracil upregulates NF-κB, MAPK, and thymidylate synthase in breast cancer cells. Administration of doxorubicin to various cancer cells increased the production of prostaglandin E2 and IL-1β. Doxorubicin also activates NF-κB through p53 induction in neuroblastoma cells. Doxorubicin increased the expression of IL-1β and IL-6 mRNA in murine macrophages, which was mediated by p38 MAPK.[120]

All inflammatory mediators, growth factors, and signaling pathways involved in chemotherapy-induced inflammation play an essential role in regulating the TME leading to resistance, invasion, metastasis, and angiogenesis of cancer cells. As indicated in previous reports, *A. paniculata* suppresses inflammation through inhibiting the production of inflammatory mediators and related signaling pathways. Thus, *A. paniculata* can be used as an adjunct to chemotherapy to inhibit the development of resistance.

Tables 1 and 2 show that *A. paniculata* has immunomodulatory and anticancer activity. The activity of *A. paniculata* in inhibiting inflammatory cytokines from various signaling pathways in immune cells can be developed as an anticancer agent. As in previous reports, immunomodulatory-anticancer agents can be obtained through two mechanisms; the first is blocking the initiation of cancer cells and the second is inhibiting the proliferation of cancer cells at the stage of angiogenesis and cancer metastasis. The first mechanism of inhibiting the initiation of cancer is a form of acute inflammation, whereas the second mechanism of inhibiting angiogenesis and metastasis can be in the form of suppressing the activity of pro-tumor immune cells, cytokines, chemokines, and growth factors. The molecular mechanism of *A. paniculata* in modulating the immune response in cancer-associated chronic inflammation, angiogenesis, and metastasis is shown in Figure 3.

The mechanism of *A. paniculata* in inhibiting inflammation can be the basis for developing immunomodulatory-anticancer agents through the second mechanism. It is also supported by previous studies showing that *A. paniculata* inhibits angiogenesis and metastasis through suppressing the production of MMP-7, MMP-9, MMP-2, VEGF, EGFR, cyclin A, cyclin B, and HIF-1α through the PI3K/Akt/AP-1, NF-kB, ERK1/2, and MAPK signaling pathways. Andrographolide is the primary bioactive metabolite that plays a role in inhibiting the inflammatory response. Interestingly, Table 1 provides information that A. paniculata can also enhance the immune response. These activities can be used as the basis for developing immunomodulatory-anticancer agent therapy. The first mechanism is to block the initiation of cancer cells in the form of an acute inflammatory response. The previous report also stated that the activity of the immune response in the form of acute inflammation could inhibit the development of cancer cells.

Several chemotherapy drugs such as cisplatin, paclitaxel, 5-fluorouracil, and doxorubicin can increase inflammation through various pathways such as NF-kB, MAPK, ERK, and TLR that play a role in the resistance, metastasis, invasion, and angiogenesis of cancer cells. *A. paniculata* is known to have a mechanism that inhibits these signaling pathways,

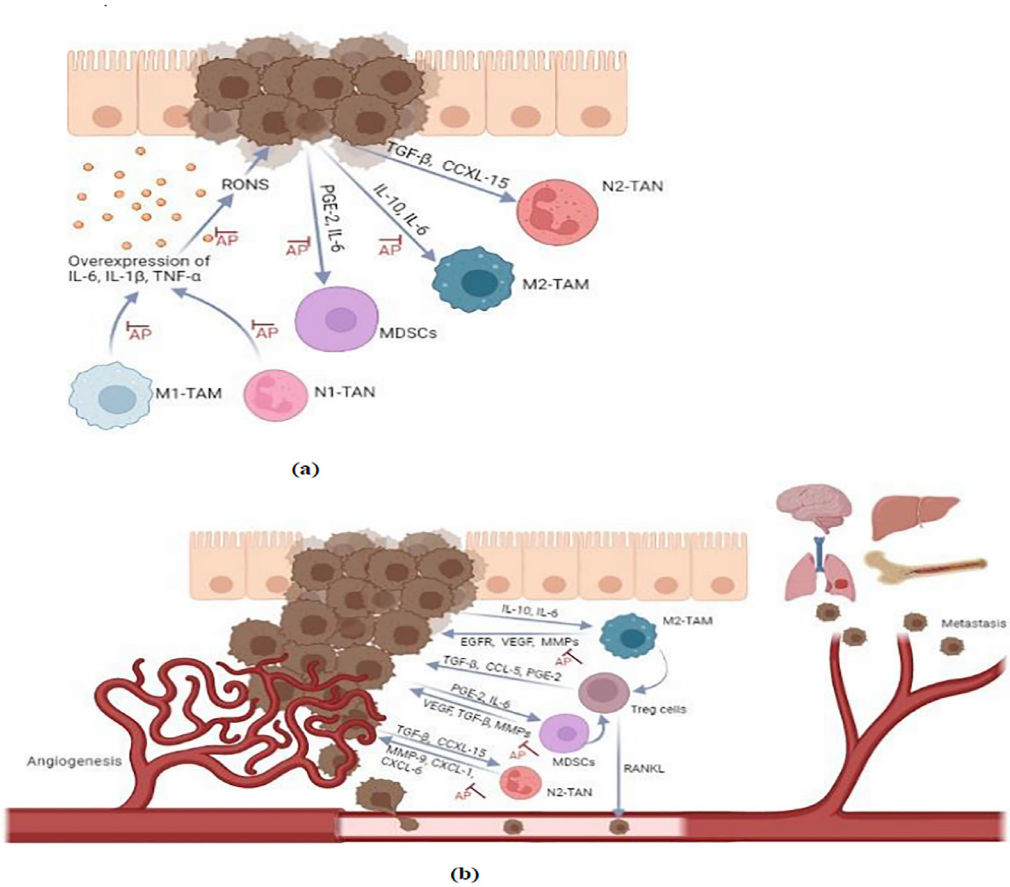

Figure 3. The molecular mechanism of *A. paniculata* in modulating the immune response in **(a)** chronic inflammation; **(b)** angiogenesis and metastasis; *A. paniculata* (AP).

so combination chemotherapy with *A. paniculata* can be an alternative in suppressing potential chemotherapy resistance. *A. paniculata* also can be a chemotherapy adjuvant through overcoming chemotherapy resistance associated with TME regulation.

Much research on *A. paniculata* and its bioactive metabolites as immunomodulatory and anticancer agents has been performed in the last ten years. However, there are still many problems to be solved. First, this study shows that *A. paniculata* extract suppresses the immune response and stimulates an immune response in the form of inflammation. The activity of *A. paniculata* in promoting the inflammatory response can be developed as an anticancer agent at the stage of cancer initiation. Meanwhile, the effect of *A. paniculata* in suppressing the inflammatory response can use for cancer therapy in cancer-related chronic inflammation, angiogenesis, and metastasis. Its action in stimulating and suppressing the immune response in inflammation may be dose-dependent, so to be developed as an anticancer agent, further research is needed to determine the dose of *A. paniculata*. Second, the bioactive metabolite that plays a role in inhibiting inflammation is andrographolide, so further research is needed on compounds that play a role in immunostimulant activity using a bioassay-guided isolation method. If the active compounds are known, it will make it easier to develop products by maximizing the extraction method to obtain these compounds. Third, the use of *A. paniculata* as an anticancer agent and inhibition of chemotherapy resistance associated with the TME needs to be explored. Research on the effect of *A. paniculata* on other components of the TME, such as TAM, MDSCs, and TAN, is necessary.

Conclusion

A. paniculata and its bioactive metabolites have immunomodulatory and anticancer activities. *A. paniculata* suppresses cancer-associated chronic inflammation, angiogenesis, and metastasis through the modulation of immune responses. *A. paniculata* and its bioactive metabolites can be developed as anticancer agents that inhibiting the proliferation of cancer cells at the angiogenesis and metastatic stages. *A. paniculata* can also be developed as an adjuvant chemotherapy that inhibits chemotherapy-induced TME-associated inflammation.

Acknowledgements

This work was supported by Rekognisi Tugas Akhir (RTA), Directorat of Research, Universitas Gadjah Mada, Indonesia with contract number 3143/UN1.P.III/DIT-LIT/PT/2021.

Author Contributions

RU participated in the study concept and design, data collection, analysis, and interpretation of results, wrote the paper, and drafted the manuscript. RM and APG participated in the development of study concept and design, editing the manuscript, critical interpretation, and scientific guidance throughout the development of the paper. All authors approved the final version of the manuscript.

Conflict of Interest

The authors have no conflict of interests to declare.

References

1. Lei X, Lei Y, Li JK, Du WX, Li RG, Yang J, et al. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. Cancer Lett. 2020;470:126-33. doi:10.1016/j.canlet.2019.11.009
2. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, et al. Inflammation and tumor progression: signaling pathways and targeted intervention. Signal Transduct Target Ther. 2021;6(1):263. doi:10.1038/s41392-021-00658-5
3. Mollica Poeta V, Massara M, Capucetti A, Bonecchi R. Chemokines and chemokine receptors: new targets for cancer immunotherapy. Front Immunol. 2019;10:379. doi:10.3389/fimmu.2019.00379
4. Zlotnik A, Burkhardt A, Homey B. Homeostatic chemokine receptors and organ-specific metastasis. Nat Rev Immunol. 2011;11:597-606. doi:10.1038/nri3049
5. Yu X, Zhao R, Lin S, Bai X, Zhang L, Sun L, et al. CXCL16 induces angiogenesis in autocrine signaling pathway involving hypoxia-inducible factor 1α in human umbilical vein endothelial cells. Oncol Rep. 2015;35(3):1557-65. doi: 10.3892/or.2015.4520
6. Chow MT, Luster AD. Chemokines in cancer. Cancer Immunol Res. 2014;2(12):1125-31. doi: 10.1158/2326-6066
7. Darash-Yahana M, Pikarsky E, Abramovitch R, Zeira E, Pal B, Karplus R, et al. Role of high expression levels of CXCR4 in tumor growth, vascularization, and metastasis. FASEB J. 2004;18(11):1240-2. doi:10.1096/fj.03-0935fje
8. Witsch E, Sela M, Yarden Y. Roles for growth factors in cancer progression. Physiol Bethesda Md. 2010;25(2):85-101. doi:10.1152/physiol.00045.2009
9. Xia L, Tan S, Zhou Y, Lin J, Wang H, Oyang L, et al. Role of the NFκB-signaling pathway in cancer. OncoTargets Ther. 2018;11:2063-73. doi:10.2147/OTT.S161109
10. Brooks AJ, Putoczki T. JAK-STAT Signalling pathway in cancer. Cancers. 2020;12(7):1971. doi:10.3390/cancers12071971
11. Collins TS, Lee LF, Ting JP. Paclitaxel up-regulates interleukin-8 synthesis in human lung carcinoma through an NF-kappaB- and AP-1-dependent mechanism. Cancer Immunol Immunother CII. 2000;49(2):78-84. doi:10.1007/s002620050605
12. Vyas D, Laput G, Vyas AK. Chemotherapy-enhanced inflammation may lead to the failure of therapy and metastasis. OncoTargets Ther. 2014;7:1015-23. doi:10.2147/OTT.S60114
13. Chao HP, Kuo CD, Chiu JH, Fu SL. Andrographolide exhibits anti-invasive activity against colon cancer cells via inhibition of MMP2 activity. Planta Med. 2010;76(16):1827-33. doi:10.1055/s-0030-1250039
14. Chao WW, Kuo YH, Lin BF. Anti-inflammatory activity of new compounds from *Andrographis paniculata* by NF-kappaB transactivation inhibition. J Agric Food Chem. 2010;58(4):2505-12. doi:10.1021/jf903629j
15. Hossain MdS, Urbi Z, Sule A, Rahman KMH. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. Sci World J. 2014;2014:274905. doi:10.1155/2014/274905
16. Koteswara Rao Y, Vimalamma G, Venkata Rao C, Tzeng YM. Flavonoids and andrographolides from *Andrographis paniculata*. Phytochemistry. 2004;65(16):2317-21. doi:10.1016/j.phytochem.2004.05.008
17. Aslam MS, Naveed S, Ahmed A, Abbas Z, Gull I, Athar MA. Side effects of chemotherapy in cancer patients and evaluation of patients opinion about starvation based differential chemotherapy. J Cancer Ther. 2014;5(8):817-22. doi:10.4236/jct.2014.58089
18. Guo H, Zhang Z, Su Z, Sun C, Zhang X, Zhao X, et al. Enhanced anti-tumor activity and reduced toxicity by combination andrographolide and bleomycin in ascitic tumor-bearing mice. Eur J Pharmacol. 2016;776:52-63. doi:10.1016/j.ejphar.2016.02.032
19. Lin HH, Shi MD, Tseng HC, Chen JH. Andrographolide sensitizes the cytotoxicity of human colorectal carcinoma cells toward cisplatin via enhancing apoptosis pathways in vitro and in vivo. Toxicol Sci Off J Soc Toxicol. 2014;139(1):108-20. doi:10.1093/toxsci/kfu032
20. Zhou J, Ong CN, Hur GM, Shen HM. Inhibition of the JAK-STAT3 pathway by andrographolide enhances chemosensitivity of cancer cells to doxorubicin. Biochem Pharmacol. 2010;79(9):1242050. doi:10.1016/j.bcp.2009.12.014.
21. Bao GQ, Shen BY, Pan CP, Zhang YJ, Shi MM, Peng CH. Andrographolide causes apoptosis via inactivation of STAT3 and Akt and potentiates antitumor activity of gemcitabine in pancreatic cancer. Toxicol Lett. 2013;222(1):23-35. doi:10.1016/j.toxlet.2013.06.241
22. Hodroj MH, Jardaly A, Abi Raad S, Zouein A, Rizk S. Andrographolide potentiates the antitumor effect of topotecan in acute myeloid leukemia cells through an intrinsic apoptotic pathway. Cancer Manag Res. 2018;10:1079-88. doi:10.2147/CMAR.S160924

23. Aravindaram K, Yang NS. Anti-inflammatory plant natural products for cancer therapy. Planta Med. 2010;76(11):1103-17. doi:10.1055/s-0030-1249859
24. Nandakumar V, Singh T, Katiyar SK. Multi-targeted prevention and therapy of cancer by proanthocyanidins. Cancer Lett. 2008;269(2):378-87. doi:10.1016/j.canlet.2008.03.049
25. Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, et al. Chemopreventive and therapeutic effects of curcumin. Cancer Lett. 2005;223(2):181-90. doi:10.1016/j.canlet.2004.09.041
26. Rajanna M, Bharathi B, Shivakumar BR, Deepak M, Prashanth D, Prabakaran D, et al. Immunomodulatory effects of *Andrographis paniculata* extract in healthy adults – An open-label study. J Ayurveda Integr Med. 2021;12(3):529-34. doi:10.1016/j.jaim.2021.06.004
27. Mehta S, Sharma AK, Singh RK. Pharmacological activities and molecular mechanisms of pure and crude extract of *Andrographis paniculata*: An update. Phytomedicine Plus. 2021;1(4):100085. doi:10.1016/j.phyplu.2021.100085
28. Medzhitov R. Inflammation 2010: new adventures of an old flame. Cell. 2010;140(6):771-6. doi: 10.1016/j.cell.2010.03.006.
29. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat Immunol. 2010;11(5):373-84. doi:10.1038/ni.1863
30. Pancer Z, Cooper MD. The evolution of adaptive immunity. Annu Rev Immunol. 2006;24:497-518. doi:10.1146/annurev.immunol.24.021605.090542.
31. Yuandani, Jantan I, Rohani AS, Sumantri IB. Immunomodulatory effects and mechanisms of curcuma species and their bioactive compounds: a review. Front Pharmacol. 2021;12:643119. doi:10.3389/fphar.2021.643119
32. Abbas AK, Licthtman AH, Pillai S. Basic Immunology: Functions and disorders of the immune system. Amsterdam: Elsevier; 2019.
33. Hodge DR, Hurt EM, Farrar WL. The role of IL-6 and STAT3 in inflammation and cancer. Eur J Cancer Oxf Engl. 1990. 2005;41(16):2502-12. doi:10.1016/j.ejca.2005.08.016.
34. Heichler C, Scheibe K, Schmied A, Geppert CI, Schmid B, Wirtz S, et al. STAT3 activation through IL-6/IL-11 in cancer-associated fibroblasts promotes colorectal tumour development and correlates with poor prognosis. Gut. 2020;69(7):1269-82. doi:10.1136/gutjnl-2019-319200.
35. Ke W, Zhang L, Dai Y. The role of IL-6 in immunotherapy of non-small cell lung cancer (NSCLC) with immune-related adverse events (irAEs). Thorac Cancer. 2020;11(4):835-9. doi:10.1111/1759-7714.13341
36. Sun X, Qu Q, Lao Y, Zhang M, Yin X, Zhu H, et al. Tumor suppressor HIC1 is synergistically compromised by cancer-associated fibroblasts and tumor cells through the IL-6/pSTAT3 axis in breast cancer. BMC Cancer. 2019;19(1):1180. doi:10.1186/s12885-019-6333-6
37. Siersbæk R, Scabia V, Nagarajan S, Chernukhin I, Papachristou EK, Broome R, et al. IL6/STAT3 signaling hijacks estrogen receptor α enhancers to drive breast cancer metastasis. Cancer Cell. 2020;38(3):412-23.e9. doi:10.1016/j.ccell.2020.06.007
38. Waters JP, Pober JS, Bradley JR. Tumour necrosis factor and cancer. J Pathol. 2013;230(3):241-8. doi:10.1002/path.4188.
39. Dinarello CA. Why not treat human cancer with interleukin-1 blockade? Cancer Metastasis Rev. 2010;29(2):317-29. doi:10.1007/s10555-010-9229-0
40. Jagielska J, Kapopara PR, Salguero G, Scherr M, Schütt H, Grote K, et al. Interleukin-1 assembles a proangiogenic signaling module consisting of caveolin-1, tumor necrosis factor receptor-associated factor 6, p38-mitogen-activated protein kinase (MAPK), and MAPK-activated protein kinase 2 in endothelial cells. Arterioscler Thromb Vasc Biol. 2012;32(5):1280-8. doi:10.1161/ATVBAHA.111.243477
41. Baker KJ, Houston A, Brint E. IL-1 Family members in cancer; two sides to every story. Front Immunol. 2019;10:1197. doi:10.3389/fimmu.2019.01197
42. Ma L, Lan F, Zheng Z, Xie F, Wang L, Liu W, et al. Epidermal growth factor (EGF) and interleukin (IL)-1β synergistically promote ERK1/2-mediated invasive breast ductal cancer cell migration and invasion. Mol Cancer. 2012;11(1):79. doi:10.1186/1476-4598-11-79
43. Rébé C, Ghiringhelli F. Interleukin-1β and Cancer. Cancers. 2020;12(7):1791. doi:10.3390/cancers12071791
44. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to modulate antitumor immunity. Front Immunol. 2018;9:978. doi:10.3389/fimmu.2018.00978
45. Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. Oncology. 2005;69(Suppl 3):4-10. doi:10.1159/000088478
46. Voron T, Marcheteau E, Pernot S, Colussi O, Tartour E, Taieb J, et al. Control of the immune response by pro-angiogenic factors. Front Oncol. 2014;4:70. doi:10.3389/fonc.2014.00070
47. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med. 1996;2(10):1096-103. doi: 10.1038/nm1096-1096
48. Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, et al. Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. J Immunol. 1950. 1998;160(3):1224-32.
49. Lapeyre-Prost A, Terme M, Pernot S, Pointet AL, Voron T, Tartour E, et al. Immunomodulatory activity of VEGF in cancer. Int Rev Cell Mol Biol. 2017;330:295-342. doi: 10.1016/bs.ircmb.2016.09.007

50. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet Lond Engl. 2001;357(9255):539-45. doi:10.1016/S0140-6736(00)04046-0
51. Syed V. TGF-β Signaling in Cancer. J Cell Biochem. 2016;117(6):1279-87.
52. Lamouille S, Derynck R. Cell size and invasion in TGF-beta-induced epithelial to mesenchymal transition is regulated by activation of the mTOR pathway. J Cell Biol. 2007;178(3):437-51. doi:10.1083/jcb.200611146
53. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu Rev Immunol. 2014;32:659-702. doi:10.1146/annurev-immunol-032713-120145
54. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. Nat Rev Immunol. 2017;17(9):559-72. doi:10.1038/nri.2017.49
55. Romagnani P, Annunziato F, Lasagni L, Lazzeri E, Beltrame C, Francalanci M, et al. Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. J Clin Invest. 2001;107(1):53-63. doi:10.1172/JCI9775
56. Angiolillo AL, Sgadari C, Taub DD, Liao F, Farber JM, Maheshwari S, et al. Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. J Exp Med. 1995;182(1):155-62. doi:10.1084/jem.182.1.155
57. Strieter RM, Kunkel SL, Arenberg DA, Burdick MD, Polverini PJ. Interferon γ-Inducible Protein-10 (IP-10), a member of the C-X-C chemokine family, is an inhibitor of angiogenesis. Biochem Biophys Res Commun. 1995;210(1):51-7. doi:10.1006/bbrc.1995.1626
58. Albini A, Bruno A, Noonan DM, Mortara L. Contribution to tumor angiogenesis from innate immune cells within the tumor microenvironment: implications for immunotherapy. Front Immunol. 2018;9:527. doi:10.3389/fimmu.2018.00527
59. Stockmann C, Schadendorf D, Klose R, Helfrich I. The impact of the immune system on tumor: angiogenesis and vascular remodeling. Front Oncol. 2014;4:69. doi:10.3389/fonc.2014.00069
60. Blomberg OS, Spagnuolo L, de Visser KE. Immune regulation of metastasis: mechanistic insights and therapeutic opportunities. Dis Model Mech. 2018;11(10):dmm036236. doi:10.1242/dmm.036236
61. Ghajar CM, Bissell MJ. Extracellular matrix control of mammary gland morphogenesis and tumorigenesis: insights from imaging. Histochem Cell Biol. 2008;130(6):1105-18. doi:10.1007/s00418-008-0537-1
62. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Model Mech. 2011;4(2):165-78. doi:10.1242/dmm.004077
63. Tan W, Zhang W, Strasner A, Grivennikov S, Cheng JQ, Hoffman RM, et al. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL–RANK signalling. Nature. 2011;470(7335):548-53. doi:10.1038/nature09707
64. Baek K-S, Cho JY, Hwang JH, Kim Y, Choe H, Kim M-Y, et al. NF-κB and MAPK-targeted anti-inflammatory activity of andrographis paniculata extract. Am J Biomed Sci Res. 2021;11(5):452. doi:10.34297/AJBSR.2021.11.001677
65. Mussard E, Jousselin S, Cesaro A, Legrain B, Lespessailles E, Esteve E, et al. *Andrographis paniculata* and its bioactive diterpenoids protect dermal fibroblasts against inflammation and oxidative stress. Antioxidants. 2020;9(5):432. doi:10.3390/antiox9050432
66. Low M, Khoo CS, Münch G, Govindaraghavan S, Sucher NJ. An in vitro study of anti-inflammatory activity of standardised *Andrographis paniculata* extracts and pure andrographolide. BMC Complement Altern Med. 2015;15(1):18. doi:10.1186/s12906-015-0525-7
67. Gan L, Zheng Y, Deng L, Sun P, Ye J, Wei X, et al. Diterpenoid lactones with anti-inflammatory effects from the aerial parts of *Andrographis paniculata*. Molecules. 2019;24(15):2726. doi:10.3390/molecules24152726
68. Hanh T, My N, Cham P, Quang T, Cuong N, Huong T, et al. Diterpenoids and flavonoids from *Andrographis paniculata*. Chem Pharm Bull (Tokyo). 2020;68:96-9. doi:10.1248/cpb.c19-00662
69. Li Y, He S, Tang J, Ding N, Chu X, Cheng L, et al. Andrographolide inhibits inflammatory cytokines secretion in LPS-Stimulated RAW264.7 cells through suppression of NF-κB/MAPK signaling pathway. Evid-Based Complement Altern Med. 2017;2017:8248142. doi:10.1155/2017/8248142
70. Kim N, Lertnimitphun P, Jiang Y, Tan H, Zhou H, Lu Y, et al. Andrographolide inhibits inflammatory responses in LPS-stimulated macrophages and murine acute colitis through activating AMPK. Biochem Pharmacol. 2019;170:113646. doi:10.1016/j.bcp.2019.113646
71. Chang M-Y, Ho F-M, Wang J-S, Kang H-C, Chang Y, Ye Z-X, et al. AICAR induces cyclooxygenase-2 expression through AMP-activated protein kinase-transforming growth factor-beta-activated kinase 1-p38 mitogen-activated protein kinase signaling pathway. Biochem Pharmacol. 2010;80(8):1210-20. doi:10.1016/j.bcp.2010.06.049
72. Jeong HW, Hsu KC, Lee J-W, Ham M, Huh JY, Shin HJ, et al. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. Am J Physiol Endocrinol Metab. 2009;296(4):E955-964. doi:10.1152/ajpendo.90599.2008
73. Day EA, Ford RJ, Steinberg GR. AMPK as a therapeutic target for treating metabolic diseases. Trends Endocrinol Metab TEM. 2017;28(8):545-60. doi:10.1016/j.tem.2017.05.004
74. Wang J, Li Z, Gao L, Qi Y, Zhu H, Qin X. The regulation

effect of AMPK in immune related diseases. Sci China Life Sci. 2018;61(5):523-33. doi:10.1007/s11427-017-9169-6

75. Kim A-Y, Shim H-J, Shin H-M, Lee Y, Nam H, Kim S, et al. Andrographolide suppresses TRIF-dependent signaling of toll-like receptors by targeting TBK1. Int Immunopharmacol. 2018;57:172-80. doi:10.1016/j.intimp.2018.02.019
76. Hendrata AP, Handono K, Kalim H, Fitri L. *Andrographis paniculata* can modulate the ratio of Treg to Th17 cells in atherosclerotic rats. Clin Nutr Exp. 2018;20:20-9. doi:10.1016/J.YCLNEX.2018.05.002
77. Chao W-W, Kuo Y-H, Hsieh S-L, Lin B-F. Inhibitory effects of ethyl acetate extract of *Andrographis paniculata* on NF-κB trans-activation activity and LPS-induced acute inflammation in mice. Evid Based Complement Alternat Med. 2011;2011:enep120. doi:10.1093/ecam/nep120
78. Yao H, Zhao J, Zhu L, Xie Y, Zhao N, Yao R, et al. Protective effect of the effective part of *Andrographis paniculata* (Burm.f.) Nees on PM2.5-induced lung injury in rats by modulating the NF-κB pathway. J Ethnopharmacol. 2021;280:114420. doi:10.1016/j.jep.2021.114420
79. Yue GG-L, Li L, Lee JK-M, Kwok H-F, Wong EC-W, Li M, et al. Multiple modulatory activities of *Andrographis paniculata* on immune responses and xenograft growth in esophageal cancer preclinical models. Phytomedicine Int J Phytother Phytopharm. 2019;60:152886. doi:10.1016/j.phymed.2019.152886
80. Churiyah, Pongtuluran OB, Rofaani E, Tarwadi. Antiviral and immunostimulant activities of *Andrographis paniculata*. HAYATI J Biosci. 2015;22(2):67-72. doi:10.4308/HJB.22.2.67
81. Radhika P, Annapurna A, Nageswara Rao S. Immunostimulant, cerebroprotective & nootropic activities of *Andrographis paniculata* leaves extract in normal & type 2 diabetic rats. Indian J Med Res. 2012;135(5):636-41.
82. Rajeshkumar S, Nagalingam M, Ponnanikajamideen M, Vanaja M, Malarkodi C. Anticancer activity of *Andrographis paniculata* leaves extract against neuroblastoma (Imr-32) and human colon (Ht-29) cancer cell line. World Journal of Pharmaceutical Sciences. 2015;4(6):1667-5
83. Churiyah, Pongtuluran OB, Rofaani E, Tarwadi. Antiviral and immunostimulant activities of *Andrographis paniculata*. HAYATI J Biosci. 2015;22(2):67. doi:10.4308/hjb.22.2.67
84. Sholihah MM, Indarto D, Pramana TY. The inhibitory effect of *Andrographis paniculata* extract on proliferation of breast cancer cell line. IOP Conf Ser Mater Sci Eng. 2019;546(6):062029. doi:10.1088/1757-899X/546/6/062029
85. Singh S, Mehta A, Baweja S, Ahirwal L, Mehta P. Anticancer Activity of *Andrographis paniculata* and *Silybum marianum* on five human cancer cell lines. J Pharmacol Toxicol. 2013;8:42-8. doi:10.3923/jpt.2013.42.48
86. Cheung MK, Yue GGL, Gomes AJ, Wong ECW, Lee JKM, Kwok FHF, et al. Network pharmacology reveals potential functional components and underlying molecular mechanisms of *Andrographis paniculata* in esophageal cancer treatment. Phytother Res. 2022;36(4):1748-60. doi:10.1002/ptr.7411
87. Hanh TTH, My NTT, Cham PT, Quang TH, Cuong NX, Huong TT, et al. Diterpenoids and Flavonoids from *Andrographis paniculat*a. Chem Pharm Bull (Tokyo). 2020;68(1):96-9. doi:10.1248/CPB.c19-00662
88. Gan L, Zheng Y, Deng L, Sun P, Ye J, Wei X, et al. Diterpenoid lactones with anti-inflammatory effects from the aerial parts of *Andrographis paniculata*. Mol Basel Switz. 2019;24(15):E2726. doi:10.3390/molecules24152726
89. Li Y, He S, Tang J, Ding N, Chu X, Cheng L, et al. Andrographolide inhibits inflammatory cytokines secretion in LPS-stimulated RAW264.7 cells through suppression of NF-κB/MAPK signaling pathway. Evid Based Complement Alternat Med. 2017;2017:8248142. doi:10.1155/2017/8248142
90. Wang Y, Jiao J, Yang Y, Yang M, Zheng Q. Screening and identification for immunological active components from andrographis herba using macrophage biospecific extraction coupled with UPLC/Q-TOF-MS. Mol Basel Switz. 2018;23(5):E1047. doi:10.3390/molecules23051047
91. Zhang X-F, Ding M-J, Cheng C, Zhang Y, Xiang S-Y, Lu J, et al. Andrographolide attenuates oxidative stress injury in cigarette smoke extract exposed macrophages through inhibiting SIRT1/ERK signaling. Int Immunopharmacol. 2020;81:106230. doi:10.1016/j.intimp.2020.106230
92. Kim N, Lertnimitphun P, Jiang Y, Tan H, Zhou H, Lu Y, et al. Andrographolide inhibits inflammatory responses in LPS-stimulated macrophages and murine acute colitis through activating AMPK. Biochem Pharmacol. 2019;170:113646. doi:10.1016/j.bcp.2019.113646.
93. Kim A-Y, Shim H-J, Shin H-M, Lee YJ, Nam H, Kim SY, et al. Andrographolide suppresses TRIF-dependent signaling of toll-like receptors by targeting TBK1. Int Immunopharmacol. 2018;57:172-80. doi:10.1016/j.intimp.2018.02.019
94. Mussard E, Jousselin S, Cesaro A, Legrain B, Lespessailles E, Esteve E, et al. *Andrographis paniculata* and its bioactive diterpenoids protect dermal fibroblasts against inflammation and oxidative stress. Antioxid Basel Switz. 2020;9(5):E432. doi:10.3390/antiox9050432
95. Hendrata AP, Handono K, Kalim H, Fitri LE. *Andrographis paniculata* can modulate the ratio of Treg to Th17 cells in atherosclerotic rats. Clin Nutr Exp. 2018;20:20-9. doi: 10.1016/J.YCLNEX.2018.05.002
96. Chun JY, Tummala R, Nadiminty N, Lou W, Liu C, Yang

J, et al. Andrographolide, an herbal medicine, inhibits interleukin-6 expression and suppresses prostate cancer cell growth. genes cancer. 2010;1(8):868-76. doi:10.1177/1947601910383416

97. Peng T, Hu M, Wu TT, Zhang C, Chen Z, Huang S, et al. Andrographolide suppresses proliferation of nasopharyngeal carcinoma cells via attenuating NF-κB pathway. BioMed Res Int. 2015;2015:735056. doi:10.1155/2015/735056
98. Zhang Q-Q, Zhou D-L, Ding Y, Liu H-Y, Lei Y, Fang H-Y, et al. Andrographolide inhibits melanoma tumor growth by inactivating the TLR4/NF-κB signaling pathway. Melanoma Res. 2014;24(6):545-55. doi:10.1097/CMR.0000000000000117
99. Cheung MTW, Ramalingam R, Lau KKK, Chiang MWL, Chiu SK, Cheung HY, et al. Cell type-dependent effects of andrographolide on human cancer cell lines. Life Sci. 2012;91(15-16):751–60. doi:10.1016/j.lfs.2012.04.009
100. Yang T, Yao S, Zhang X, Guo Y. Andrographolide inhibits growth of human T-cell acute lymphoblastic leukemia Jurkat cells by downregulation of PI3K/AKT and upregulation of p38 MAPK pathways. Drug Des Devel Ther. 2016;10:1389-97. doi:10.2147/DDDT.S94983
101. Raghavan R, Cheriyamundath S, Madassery J. 14-Deoxy-11,12-didehydroandrographolide inhibits proliferation and induces GSH-dependent cell death of human promonocytic leukemic cells. J Nat Med. 2014;68(2):387-94. doi:10.1007/s11418-014-0815-2
102. Lee S, Morita H, Tezuka Y. Preferentially cytotoxic constituents of *Andrographis paniculata* and their preferential cytotoxicity against human pancreatic cancer cell lines. Nat Prod Commun. 2015;10(7):1153-8.
103. Sarkar S, Gopal PK, Chakraborty B, Paul M, Chowdhury C, Paul S. 14-Deoxy-11,12-Didehydroandrographolide: A novel compound isolated from *Andrographis paniculata* Nees. induces robust apoptosis in leukemic cells. Pharmacogn Mag. 2019;15(62):135. doi:10.4103/pm.pm_466_18
104. Tan HK, Muhammad TST, Tan ML. 14-Deoxy-11,12-didehydroandrographolide induces DDIT3-dependent endoplasmic reticulum stress-mediated autophagy in T-47D breast carcinoma cells. Toxicol Appl Pharmacol. 2016;300:55-69. doi:10.1016/j.taap.2016.03.017
105. Sharma V, Qayum A, Kaul S, Singh A, Kapoor KK, Mukherjee D, et al. Carbohydrate Modifications of neoandrographolide for improved reactive oxygen species-mediated apoptosis through mitochondrial pathway in colon cancer. ACS Omega. 2019;4(24):20435-42. doi:10.1021/acsomega.9b01249
106. Kumar S, Patil HS, Sharma P, Kumar D, Dasari S, Puranik VG, et al. Andrographolide inhibits osteopontin expression and breast tumor growth through downregulation of PI3 kinase/Akt signaling pathway. Curr Mol Med. 2012;12(8):952-66. doi:10.2174/156652412802480826
107. Liu G, Chu H. Andrographolide inhibits proliferation and induces cell cycle arrest and apoptosis in human melanoma cells. Oncol Lett. 2018;15(4):5301-5. doi:10.3892/ol.2018.7941
108. Banerjee M, Chattopadhyay S, Choudhuri T, Bera R, Kumar S, Chakraborty B, et al. Cytotoxicity and cell cycle arrest induced by andrographolide lead to programmed cell death of MDA-MB-231 breast cancer cell line. J Biomed Sci. 2016;23:40. doi:10.1186/s12929-016-0257-0
109. Lee Y-C, Lin H-H, Hsu C, Wang C-J, Chiang T, Chen J-H. Inhibitory effects of andrographolide on migration and invasion in human non-small cell lung cancer A549 cells via down-regulation of PI3K/Akt signaling pathway. Eur J Pharmacol. 2010;632:23-32. doi:10.1016/j.ejphar.2010.01.009
110. Luo W, Liu Y, Zhang J, Luo X, Lin C, Guo J. Andrographolide inhibits the activation of NF-κB and MMP-9 activity in H3255 lung cancer cells. Exp Ther Med. 2013;6(3):743-6. doi:10.3892/etm.2013.1196
111. Chao C-Y, Lii C-K, Hsu Y-T, Lu C-Y, Liu K-L, Li C-C, et al. Induction of heme oxygenase-1 and inhibition of TPA-induced matrix metalloproteinase-9 expression by andrographolide in MCF-7 human breast cancer cells. Carcinogenesis. 2013;34(8):1843-51. doi:10.1093/carcin/bgt131
112. Lin H-H, Tsai C-W, Chou F-P, Wang C-J, Hsuan S-W, Wang C-K, et al. Andrographolide down-regulates hypoxia-inducible factor-1α in human non-small cell lung cancer A549 cells. Toxicol Appl Pharmacol. 2011;250(3):336-45. doi:10.1016/j.taap.2010.11.014
113. Yue GG-L, Lee JK-M, Li L, Chan K-M, Wong EC-W, Chan JY-W, et al. Andrographis paniculata elicits anti-invasion activities by suppressing TM4SF3 gene expression and by anoikis-sensitization in esophageal cancer cells. Am J Cancer Res. 2015;5(12):3570-87.
114. Shen K, Ji L, Lu B, Xu C, Gong C, Morahan G, et al. Andrographolide inhibits tumor angiogenesis via blocking VEGFA/VEGFR2-MAPKs signaling cascade. Chem Biol Interact. 2014;218:99-106. doi:10.1016/j.cbi.2014.04.020115.
115. Tung Y-T, Chen H-L, Tsai H-C, Yang S-H, Chang Y-C, Chen C-M. Therapeutic Potential of Andrographolide Isolated from the Leaves of *Andrographis paniculata* Nees for Treating Lung Adenocarcinomas. Evid-Based Complement Altern Med ECAM. 2013;2013:305898. doi:10.1155/2013/305898
116. Volk LD, Flister MJ, Bivens CM, Stutzman A, Desai N, Trieu V, et al. Nab-paclitaxel efficacy in the orthotopic model of human breast cancer is significantly enhanced by concurrent anti-vascular endothelial growth factor a therapy. Neoplasia. 2008;10(6):613–23. doi:10.1593/neo.08302
117. Volk LD, Flister MJ, Chihade D, Desai N, Trieu V, Ran S. Synergy of nab-paclitaxel and bevacizumab

in eradicating large orthotopic breast tumors and preexisting metastases. Neoplasia. 2011;13(4):327-38. doi:10.1593/neo.101490

118. Wu J, Guan M, Wong PF, Yu H, Dong J, Xu J. Icariside II potentiates paclitaxel-induced apoptosis in human melanoma A375 cells by inhibiting TLR4 signaling pathway. Food Chem Toxicol. 2012;50(9):3019-24. doi:10.1016/j.fct.2012.06.027
119. Logan RM, Stringer AM, Bowen JM, Gibson RJ, Sonis ST, Keefe DMK. Serum levels of nfkappab and proinflammatory cytokines following administration of mucotoxic drugs. Cancer Biol Ther. 2008;7(7):1139-45. doi:10.4161/cbt.7.7.6207
120. Elsea CR, Roberts DA, Druker BJ, Wood LJ. Inhibition of p38 MAPK suppresses inflammatory cytokine induction by etoposide, 5-fluorouracil, and doxorubicin without affecting tumoricidal activity. PLoS One. 2008;3(6):e2355. doi:10.1371/journal.pone.0002355