

Antineoplastic effects of icaritin: molecular mechanisms and applications

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ABSTRACT

Icaritin (ICA) is a prenylflavonoid natural product extracted from plants of the *Epimedium* genus. The approval of ICA softgel capsules as a class 1.2 traditional Chinese medicine (TCM) innovative drug represents a major advancement offering a novel therapeutic approach for patients with advanced hepatocellular carcinoma (HCC). Class 1.2 TCM innovative drugs generally denote extracts and formulations derived from single plants, animals, minerals, or other substances. ICA exhibits diverse pharmacological effects, encompassing anti-inflammatory, immune-regulatory, anti-oxidation, anti-osteoporosis, anti-depression, and notably anti-cancer properties. This review presents a comprehensive overview of the molecular mechanisms underlying the anti-cancer properties of ICA, and further highlights recent progress in use of ICA in cancer research, discusses present challenges, and examines potential opportunities for ICA application and further development.

Keywords: traditional Chinese medicine, icaritin, hepatocellular carcinoma, anti-tumor, molecular mechanism, application

1. INTRODUCTION

In recent years, the successful marketing of pharmaceuticals targeting novel pathways and the optimization of drug combinations with diverse mechanisms of action have facilitated substantial progress in the field of cancer therapeutics [1]. Nevertheless, despite advancements in immunotherapy and the establishment of treatment standards using immune checkpoint inhibitors, a substantial portion of patients remain unresponsive to this form of treatment, and experience disease progression and eventual mortality. Furthermore, major challenges exist with conventional cancer therapies, including pronounced toxicity and the development of drug

resistance, thereby further complicating cancer treatment [2]. Scholarly interest in the scientific investigation of medicinal plants and herbs is growing, because of their potential multi-targeting properties, cost-effectiveness, minimal toxicity, and widespread availability of bioactive compounds derived from plants [3]. Notably, the search for less toxic and effective treatment options, combined with predictable biomarkers, might lead to personalized and cost-effective treatment [4]. Icaritin (ICA), a medicinal principal bioactive compound, is extracted from the aboveground parts of *Epimedium*. Currently, the limited ICA content in medicinal materials often requires use of multiple chromatographic techniques for separation to ensure its purity [5], and

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necessitates industrial synthesis for pharmaceutical production. ICA exhibits a diverse array of pharmacological effects in conditions amelioration of sexual dysfunction [6], anti-osteoporosis [7], anti-cardiovascular disease [8], and regulation of immune disorders [9].

More importantly, ICA possesses a robust anti-cancer activity profile against multiple cancer types. The main therapeutic application of ICA is advanced cancer treatment. Numerous basic and clinical studies have demonstrated the safety and efficacy of ICA in the treatment of solid tumors as well as hematologic malignancies, including leukemia and lymphoma [10]. Notably, ICA has shown promising results against advanced hepatocellular carcinoma (HCC). ICA capsules have been approved as a class 1.2 traditional Chinese medicine (TCM) innovation drug, thus offering a novel therapeutic avenue for individuals with advanced HCC and heralding a new era in the precise management of advanced HCC with TCM. ICA has been shown to inhibit cancer cell proliferation, induce cancer cell apoptosis and differentiation, modulate cancer cell cycle progression and invasion, suppress angiogenesis and cancer stem/progenitor cell growth, reverse drug resistance, and elicit additional physiological effects [11]. Beyond its well-known anti-cancer properties, ICA plays roles in modulating immune cell responses and the tumor microenvironment, a complex ecosystem that facilitates tumor growth and metastasis while suppressing immune surveillance [12].

This review critically evaluates progress in ICA applications in cancer therapy and examines the mechanistic pathways of ICA that might serve as potential targets for cancer treatment. Moreover, this review summarizes the anti-cancer molecular mechanisms of ICA, such as apoptosis, necrosis, autophagy, senescence, metastasis, angiogenesis, and immunomodulation. The primary aim of this review is to lay a scientific groundwork for predicting the efficacy of combining ICA with antitumor therapy, and improving combined therapy strategies in preparation for potential clinical application.

2. MOLECULAR MECHANISMS OF THE ANTI-CANCER EFFECTS OF ICA

2.1 ICA promotes apoptosis

Apoptosis, a tightly regulated form of programmed cell death, facilitates the systematic removal of damaged cells resulting from DNA damage or developmental processes [13]. In mammalian cells, apoptosis is initiated through caspase-mediated signal transduction pathways, with both endogenous and exogenous triggers [14]. Ultimately, both pathways activate apoptotic caspases, which in turn cause various cellular, morphological, and biochemical changes characteristic of apoptosis [15]. Cancer is well known to avoid apoptosis; therefore, apoptosis-related proteins are attractive cancer therapeutic targets. Research on apoptosis reactivation strategies has focused on understanding how this process is deregulated, to provide novel opportunities for cancer treatment.

2.1.1 ICA promotes apoptosis in HCC. Apoptotic pathway regulators such as B-cell lymphoma-2 (Bcl-2) and Bcl-2 associated X (Bax) (which regulate pro-apoptotic and anti-apoptotic processes, respectively) belong to the Bcl-2 protein family [16]. Variations in the Bcl-2/Bax ratio partly determine cellular sensitivity to death signals, and are crucial in inducing apoptosis [17]. First, ICA induces apoptosis and inhibits proliferation by modulating the mouse double minute 2 (Mdm2)/p53 pathway in HCC cells [18]. Additionally, ICA triggers apoptosis through estrogen receptor (ER)-independent activation and c-Jun N-terminal kinase 1 (JNK1) signaling [19, 20]. Meanwhile, ICA inhibits sphingosine kinase 1 (SphK1) activity, and results in the generation of pro-apoptotic ceramides and activation of JNK1 [21]. Finally, ICA activates the factor-related apoptosis (FAS)-mediated apoptotic pathway, as evidenced by increased caspase-8 activation in HCC cells [22]. In conclusion, ICA mediates apoptosis in liver cancer cells and inhibits tumor growth through multiple mechanisms.

2.1.2 ICA promotes apoptosis in other cancers. ICA also modulates the expression of Bax and Bcl-2, thereby inducing apoptosis in A549 cells [23]. Meanwhile, ICA inhibits the expression of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) through the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK)/mTOR signaling pathway in lung cancer-induced osteoclastogenesis [24]. Additionally, ICA induces apoptosis through p53 activation and the AKT/mTOR pathway in ovarian cancer cells [25, 26]. Reactive oxygen species (ROS) production by ICA in turn leads to a rapid escalation in oxidative DNA damage in cervical cancer cells [27]. ICA and its derivatives decrease the expression of the anti-apoptotic protein Bcl-2 and promote apoptosis in breast cancer [28]. Treatment with ICA activates STAT3 signaling, and elicits a dose-dependent increase in apoptotic proteins (caspase-3 and Bcl-2) [29]. Moreover, ICA modulates the activity of peroxisome proliferator-activated receptor gamma (PPAR γ) through activation of the AMPK signaling pathway [30], and accelerates apoptosis [31]. Recent evidence has suggested that ICA also has extensive apoptotic activity in hematologic malignancies, including chronic myeloid leukemia (CML) [32], acute myeloid leukemia [33], multiple myeloma [34], and lymphoma [35, 36] (Table 1 and Figure 1). Therefore, ICA-induced apoptosis inhibits the proliferation and growth of various cancers.

2.2 ICA induces autophagy

Autophagy is a crucial survival mechanism maintaining organelle functionality, inhibiting the buildup of harmful substances, and supplying essential nutrients for metabolic processes during periods of nutrient deprivation. Although autophagy can suppress tumorigenesis in certain contexts, it predominantly facilitates tumorigenesis [42, 43]. Hence, determining whether the modulation of autophagy should be aimed at augmentation or suppression in the context of cancer therapy across varying conditions is crucial.

Table 1 | Molecular mechanism of ICA-induced apoptosis in various cell lines.

Tumor type	Cell line	Study type	Mechanisms	Reference
HCC	HepG2 cells	<i>In vitro</i>	Inducing apoptosis by increasing the Bax/Bcl-2 ratio and activating caspase-3	[17]
HCC	HepG2 cells	<i>In vitro</i>	Decreasing AFP levels, slowing cell growth, and increasing cell death through Mdm2/p53 pathways	[18]
HCC	HepG2 cells	<i>In vitro</i>	Inducing apoptosis via the JNK1 signaling pathway	[19]
HCC	HepG2 and Huh-7 cells	<i>In vitro</i> and <i>in vivo</i>	Increasing levels of SphK1; sensitive to ICA-induced apoptosis	[21]
HCC	SMMC-7721 HCC cells	<i>In vitro</i>	Triggering cell death through mitochondrial apoptosis via Fas pathways	[22]
Lung cancer	A549 cells	<i>In vitro</i>	ICA-induced cell cycle arrest, increasing ROS-mediated apoptosis	[23]
Lung cancer	A549 cells	<i>In vitro</i>	Blocking osteoclast formation via the AMPK/mTOR signaling pathway	[24]
Lung cancer	A549 cells	<i>In vitro</i>	Inhibiting tumor cell growth and inducing cell death via the PTEN/AKT and ERK pathways	[37]
Ovarian cancer	A2780s and A2780cp cells	<i>In vitro</i>	ICA-induced apoptosis through activating p53 and inhibiting the AKT/mTOR pathway	[25]
Ovarian cancer	Ovarian cancer SKOV3 cells	<i>In vitro</i>	ICA-inhibited cell cycle arrest through blocking the PI3K/AKT signaling pathway	[26]
Oral squamous cell carcinoma	KB and SCC9 cells	<i>In vitro</i>	ICA-induced decrease in tumor cell growth through increasing miR-124 levels	[38]
Oral squamous cell carcinoma	CAL27 and SCC9 cells	<i>In vitro</i> and <i>in vivo</i>	ICA-induced apoptosis by increasing cleaved caspase-3 expression	[29]
Renal cell carcinoma	786-O cells	<i>In vitro</i> and <i>in vivo</i>	Blocking IL-6-induced STAT3 phosphorylation and triggering apoptosis	[39]
Prostate cancer	LNCaP cells	<i>In vitro</i>	Activating PEA3, thereby blocking HER2/AR signaling	[40]
Endometrial cancer	Hec1A cells	<i>In vitro</i>	Increasing Bax, decreasing Bcl-2 expression, and inducing ERK1/2 phosphorylation	[20]
Cervical cancer	HeLa and SiHa cells	<i>In vitro</i>	ICA-induced cell death through increasing oxidative DNA damage and activating apoptotic pathways	[27]
Malignant melanoma	A375, A2058, MEWO, and HS68 cells	<i>In vitro</i>	ICA-induced apoptosis through PARP cleavage	[41]
Gliomas	U87MG and T98G cells	<i>In vitro</i>	ICA-activated AMPK signaling, thereby promoting apoptosis through PPAR γ	[30]
Gliomas	U87MG and U373 cells	<i>In vitro</i>	ICA-induced tumor cell death through NF- κ B suppression	[31]
Chronic myeloid leukemia	K562 cells	<i>In vitro</i> and <i>in vivo</i>	Triggering cell apoptosis and differentiation through MAPK/ERK/JNK and STAT3/AKT signaling	[32]
Acute myeloid leukemia	NB4, HL60, and U937 cells	<i>In vitro</i>	Triggering acute myeloid leukemia cell death through the MAPK/ERK and PI3K/AKT pathways	[33]
Lymphoma	Raji and P3HR-1 cells	<i>In vitro</i>	Triggering cell death and apoptosis	[35]
Lymphoma	SNK-10 and SNT-8 cells	<i>In vitro</i>	Leading to anti-proliferative and pro-apoptotic effects by inhibiting STAT3 and AKT pathways	[36]

In HCC cells, ICA enhances mitophagy and, in synergy with doxorubicin, induces immunogenic cell death and consequently suppresses tumor growth [44].

Meanwhile, ICA, in conjunction with autophagy and mitophagy inhibitors, synergistically enhances anti-cancer efficacy and apoptotic effects via PTEN induced

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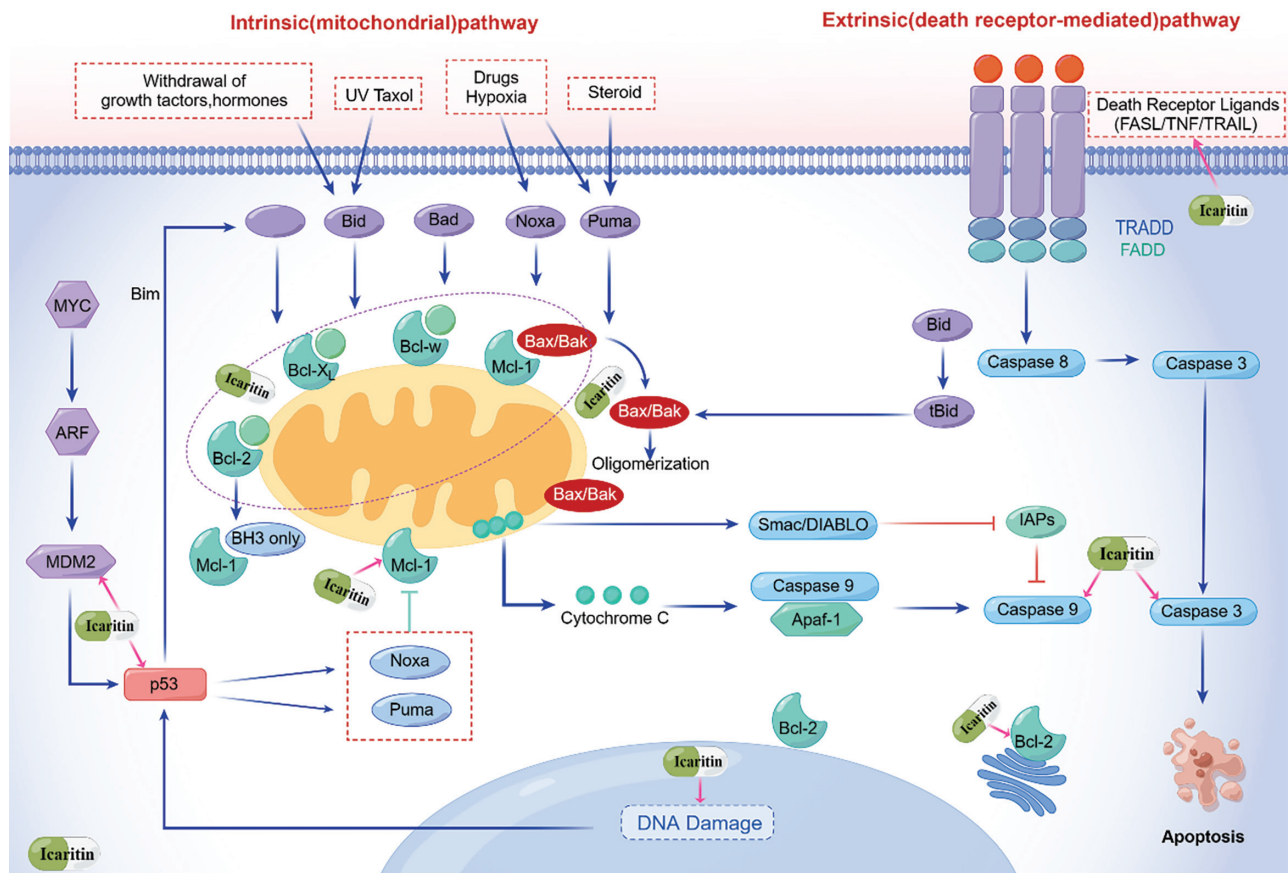


Figure 1 | Molecular mechanism of ICA-induced apoptosis in cancer cells. (1) Regulating Bcl-2 and Bax expression through the mitochondrial apoptosis pathway; (2) inducing the Fas-mediated apoptotic pathway through activating caspase-3 and caspase-8; and (3) inducing DNA damage and ROS generation via p53 signaling pathways.

putative kinase 1 (PINK1)/Parkin-mediated mitophagy in HCC [45]. Additionally, autophagy inhibitor treatment partially alleviates the decrease in cell viability stimulated by ICA, thus suggesting that ICA-induced autophagy contributes to cell death [29, 46]. Previous studies have demonstrated that ICA effectively inhibits BT5637 and T24 bladder cancer cells by suppressing autophagy [47]. ICA has also been reported to activate autophagy and enhance cytotoxicity through the AMPK signaling pathway [48]. These contradictory findings underscore the intricate involvement of ICA in tumor autophagy and the need for further investigation. Furthermore, both augmentation and suppression of autophagy are recognized as potential therapeutic approaches in the treatment of advanced cancers [49].

2.3 ICA induces necrosis

Regulated necrosis, also referred to as programmed necroptosis, can be initiated autonomously without caspase activation [50]. Various stimuli, such as members of the TNF receptor superfamilies, cellular metabolic and genotoxic stress, or the activation of anticancer drugs,

can trigger necrosis. The mitochondrial channel complex plays a critical role in the regulation of necrotic cell death. In response to oxidative stress, opening of mitochondrial permeability transition pores leads to necrosis [51]. ICA induces necrosis primarily by decreasing the mitochondrial membrane potential and facilitating signaling upstream of the mitochondrial permeability transition pore-dependent necrotic pathway by activating JNK in colorectal cancer cells [52]. Research on the inhibition of cancer progression through ICA-induced necrosis is currently limited, and further exploration is required.

2.4 ICA induces senescence

Senescence is a cellular defense mechanism in various physiological and pathological circumstances, such as tissue remodeling, injury, and cancer [53]. ICA has been found to effectively suppress cell proliferation and promote cellular senescence in imatinib-resistant CML cells [54]. In addition, ICA enhances ROS generation and causes DNA damage, thereby leading to cellular senescence and apoptosis [55]. Therefore, the induction of

senescence by ICA holds promise in impeding oncogene activation and curbing the growth of cancer cells.

2.5 ICA induces cell-cycle arrest

The mitotic cell cycle is an essential biological process of tumor cells. The intermitotic phase, the longest phase, consists of four distinct phases: G1 (when the cell grows or enters a quiescent state, G0), S (when DNA synthesis occurs), and G2 (when the cell prepares for mitosis). The shorter M phase is the period in which genetic material is divided, and cytokinesis occurs [56]. The cell cycle is the central mechanism governing cell proliferation, and is regulated and influenced by multiple factors, including key regulators such as cyclins and cyclin-dependent kinases (CDKs), as well as cell growth signaling pathways. The regulation of molecular targets by cell cycle genes is a major topic in the study of cancer drugs [57].

The anti-cancer efficacy of ICA is associated with cell cycle arrest, specifically through G1 and G2-M blocks, as well as its ability to modulate the levels of Bcl-xL, Bcl-2, Mcl-1, and survivin in a dose-dependent manner [30, 39, 40, 58]. Additionally, ICA affects key signaling factors involved in growth and apoptosis regulation, such as cyclins A, B, D, and E; CDK2 and CDK4; and caspase 3 [29, 34, 55, 59-62]. These findings indicate ICA's potential to induce cell cycle arrest in specific phases, such as S phase or G2/M phase. Together, the differential effects of ICA in regulating the cell cycle might be due to differences in cell type (Table 2 and Figure 2). However,

the identification of cell cycle heterogeneity is expected to provide new opportunities for the advancement of precision therapeutic strategies targeting these specific cellular states.

2.6 ICA inhibits metastasis

Tumor metastasis is characterized by intricate processes of cancer cell activation, invasion, and dissemination to distant organs, as well as evasion of immune responses and metastatic growth [65, 66]. Hence, a primary strategy for treating tumors is obstructing this process effectively [67]. ICA has been identified as a promising drug candidate that inhibits tumor cell invasion and epithelial-mesenchymal transformation (EMT) through targeting extracellular matrix metalloproteinases. Moreover, ICA inhibits tumor progression by suppressing signal transducers and activators of transcription 3 (STAT3) activation [68, 69]. ICA inhibits proliferation, migration, and invasion in a dose- and time-dependent manner by targeting various signaling pathways, including the phosphatase and tensin homolog (PTEN)/protein kinase B (Akt)/hypoxia inducible factor-1 α (HIF-1 α) [70], Akt/mTOR [71], nuclear factor kappa-B (NF- κ B) [72], Wnt, and Hedgehog pathways [73]. Furthermore, ICA, by regulating the cGAS-STING signaling pathway, alleviates the inflammatory pre-metastatic niche induced by extracellular microparticles [74]. The promising application of ICA to the treatment of tumor metastasis remains under-explored and necessitates further research.

Table 2 | Molecular mechanisms of ICA-induced cell cycle arrest in various cell lines.

Tumor type	Cell line	Study type	Mechanisms	Reference
Lung cancer	A549 cells	<i>In vitro</i>	Inducing cell cycle arrest at S phase, and decreasing cyclin A and CDK2 expression	[23]
Renal carcinoma	786-O and Renca cells	<i>In vitro</i> and <i>in vivo</i>	Decreasing survivin and cyclin D1 protein expression	[39]
Endometrial cancer	Hec1A cells	<i>In vitro</i>	Increasing p21 and p27 levels, and decreasing cyclin D1 and CDK 4 levels	[20]
Oral squamous cell carcinoma	OSCC cells	<i>In vitro</i>	Decreasing expression of cyclin A2 and cyclin D1	[29]
Glioblastoma	GBM cells	<i>In vitro</i>	Increasing the cell population in the G0/G1 phase of the cell cycle	[30]
Myeloma	Multiple myeloma cells	<i>In vitro</i> and <i>in vivo</i>	Inducing S phase cell cycle arrest by decreasing cyclin A, cyclin B, and CDK2, and increasing cyclin E expression	[34]
Lymphoma	SNK-10 and SNT-8 cells	<i>In vitro</i>	Causing cell cycle arrest at G2/M phase	[36]
Prostate cancer	LNCaP cells	<i>In vitro</i>	Causing substantial G1 phase arrest by affecting cyclin-related proteins	[40]
Prostate cancer	PC-3 cells	<i>In vitro</i>	Inhibiting growth by causing G1 and G2-M arrest	[63]
Breast cancer	4T1 cells	<i>In vitro</i>	ICA-induced G2/M blockage	[58]
Breast cancer	MDA-MB-453 and MCF7 cells	<i>In vitro</i>	ICA-induced cell cycle arrest in G2/M phase, with decreased cyclin B, CDC2, and CDC25C expression	[64]
Colon cancer	COLO-205 cells	<i>In vitro</i>	Suppressing cyclin D1 and cyclin E signaling	[60]
Gastric cancer	GC cells	<i>In vitro</i>	ICA-induced cell cycle arrest at G2	[61]

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Regulation of cell cycle by Icaritin

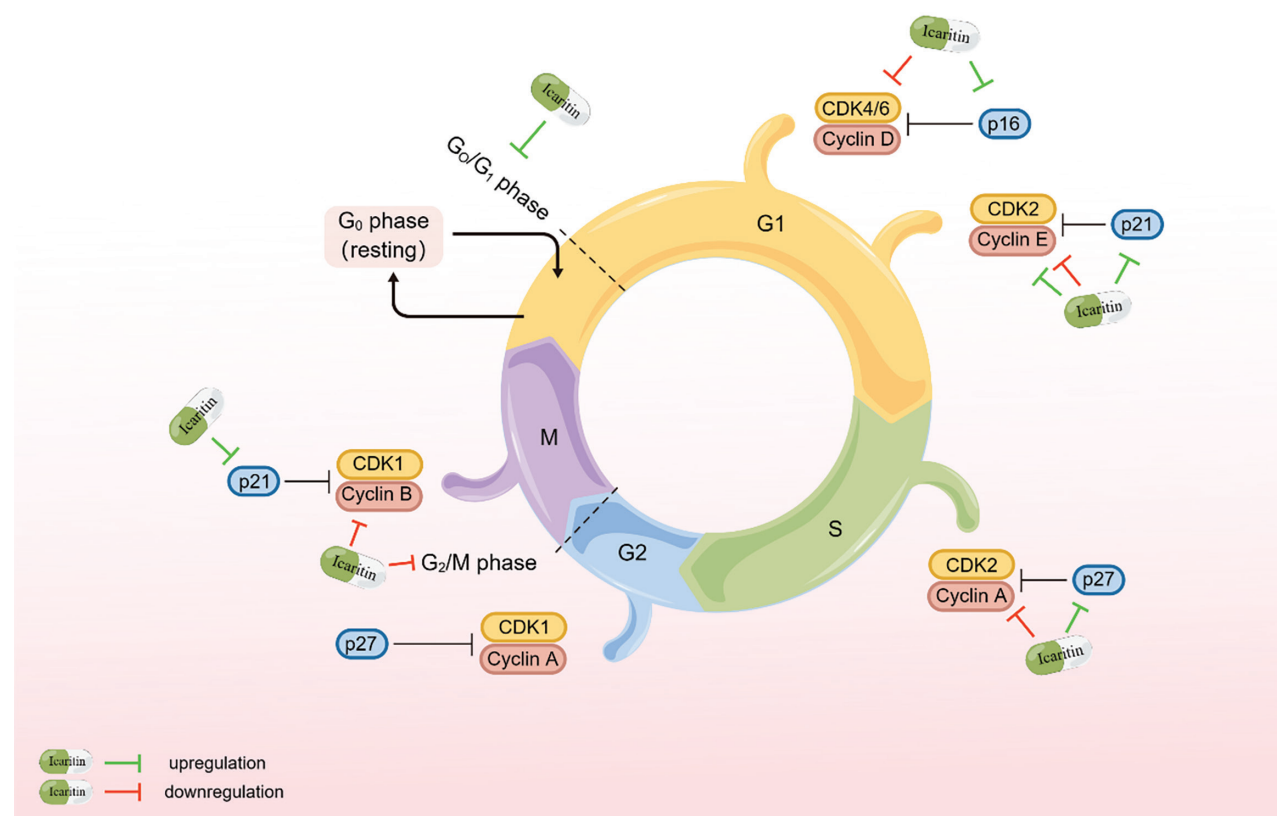


Figure 2 | Molecular mechanism of ICA's regulation of the cell cycle. The anticancer properties of ICA are associated with the induction of cell cycle arrest, specifically through modulation of cell cycle proteins including cyclins A, B, D, and E, as well as cyclin-dependent kinases such as CDK2 and CDK4. This modulation affects cellular proliferation and the regulation of apoptosis.

2.7 ICA inhibits angiogenesis

Tumor cells require substantial quantities of oxygen and nutrients to support their accelerated proliferation and expansion, thus leading to a hypoxic microenvironment unfavorable to aggressive and invasive tumor cell development [75]. However, hypoxia can stimulate tumor cells and stromal cells to exhibit sustained high secretion of vascular endothelial growth factor (VEGF), which in turn initiates multiple angiogenic pathways and ultimately leads to the development of characteristic abnormal vascular networks in tumors [76]. Antiangiogenic therapy is a known effective cancer treatment. ICA has been shown to modulate various targets and extracellular regulated protein kinase (ERK) signaling pathways, thereby inhibiting neovascularization in chick embryo chorioallantois membrane and zebrafish embryos [77, 78]. Additionally, ICA effectively suppresses tumor growth, attenuates STAT3 activation, and diminishes VEGF expression in tumors. Furthermore, the combination of ICA with ionizing radiation further enhances these effects and suppresses angiogenesis [39, 58] and

Multicomponent thermosensitive lipid complexes containing ICA have been found to enhance desmoplastic tumor therapy by stimulating anti-angiogenic effects [79]. Therefore, anti-angiogenic therapy in conjunction with other treatment modalities might potentially act synergistically in achieving therapeutic outcomes.

2.8 ICA inhibits cancer stem cells

Cancer stem cells (CSCs) within distinct tumor microenvironments contribute to intertumoral heterogeneity through diverse mechanisms. These cells have self-renewal capability and can replicate the heterogeneity of the primary tumor [80]. Several studies have shown that metastasis is also initiated by specialized tumor cells with CSC properties [81]. ICA inhibits the proliferation of esophageal CSCs [73] and the malignant progression of liver cancer initiating cells, which are characterized by high malignancy, recurrence rates, and multidrug resistance [82]. In addition, the synergistic effect of ICA combined with anti-angiogenic targeting drugs substantially decreases the burden of CML stem cells,

prevents resistance to anti-angiogenic targeting drugs, and decreases the risk of disease relapse. These findings offer a robust scientific basis for a combination therapy strategy targeting tyrosine kinase inhibitor-resistant stem/progenitor cells [83]. Mounting evidence supports ICA's promise as a therapeutic agent against a range of CSCs and inhibiting tumorigenesis.

2.9 ICA inhibits hormone-related cancer growth

Estrogen, a crucial regulator of the menstrual cycle and human reproduction, plays essential roles in bone mineral density regulation, cholesterol mobilization, and diverse brain functions [84]. However, abnormal estrogen levels have been associated with elevated rates of certain cancers, particularly breast and endometrial cancers [85]. As shown in Table 3, ICA upregulates the mRNA expression of progesterone receptor and presenilin-2 (PS2) in MCF-7 cells, known as ER-responsive genes, thus displaying potential estrogen-independent activity [86]. Additionally, ICA enhances the expression of ERβ and PTEN homologous oncoprotein [68], thus destabilizing ERα protein through activation of aryl hydrocarbon receptor (AhR) signaling, and ultimately inhibiting the proliferation of ERα-positive breast cancer cells [87]. ICA also induces activation of the epidermal growth factor receptor (EGFR)/mitogen-activated protein kinase (MAPK) signaling pathway, and consequently inhibits the proliferation of SKBr3 breast cancer cells [92]. Moreover, combined treatment with ICA, an inhibitor of ER-α36, and cetuximab, an EGFR-targeting agent, effectively suppresses the proliferation of triple-negative breast cancer (TNBC) cells *in vitro* and *in vivo* [89].

Androgen receptor (AR) is ubiquitous in both primary and metastatic prostate cancers, and plays crucial roles in promoting the survival and proliferation of prostate cancer cells [93]. ICA displays slight selective effects on PC-3

cells without influencing ERs, thus indicating a potential structure-activity relationship [63]. Furthermore, ICA disrupts AR expression and consequently inhibits neuroendocrine prostate cancer development. This potent anticancer effect is achieved through the induction of polyomavirus enhancer activator 3 (PEA3), along with human epidermal growth factor receptor 2 (HER2)/AR signaling [40, 90]. The cellular heterogeneity of prostate cancer is a key factor in the failure of current androgen-deprivation therapy. Therefore, ICA is a potential therapeutic agent for prostate cancer.

2.10 ICA inhibits tumorigenesis by modulating ROS signaling

ROS play major roles in various cellular processes, including signaling pathways and cell death. Modulation of ROS levels can lead to divergent outcomes [94]. In advanced stages of tumor progression, metastatic tumors exploit ROS to facilitate the spread of cancer cells [95]. Numerous classical pathways promoting tumor inflammation and cell proliferation have been shown to be activated by ROS [96]. A novel mechanistic explanation for the anticancer properties of ICA involves its ability to stimulate ROS production. ICA has been found to exert anti-colon cancer effects by stimulating ROS production [60]. In imatinib-resistant CML cells, the growth-inhibitory effects of ICA have been observed to coincide with an increase in ROS levels, which might be caused by ROS accumulation inducing DNA damage and triggering of cellular senescence [54]. Moreover, ICA induces DNA damage and consequently promotes senescence in HCC cells [55] and cell death in cervical cancer cells through ROS accumulation [27]. Further studies have shown that ICA attenuates docetaxel-induced skin damage by inhibiting ROS signaling, decreasing microtubule bundles, and blocking autophagy [97]. Indeed,

Table 3 | Regulation of hormone-dependent tumors by ICA.

Tumor type	Cell line	Study type	Mechanism	Reference
Glioblastoma	U87-MG cells	<i>In vitro</i> and <i>in vivo</i>	Upregulating ERβ and PTEN, decreasing downstream targets and p-AKT expression	[68]
Breast cancer	MCF-7 and BUS cells	<i>In vitro</i>	Increasing levels of progesterone receptor and PS2 mRNA	[86]
Breast cancer	MCF-7 cells	<i>In vitro</i> and <i>in vivo</i>	Inhibiting growth of ERα-positive breast cancer cells by activating AhR-signaling	[87]
Breast cancer	SKBr3 cells	<i>In vitro</i>	Regulating SKBr3 cell growth through GPER1-mediated EGFR-MAPK signaling	[88]
Breast cancer	MCF-7 and MDA-MB-231/36 cells	<i>In vitro</i> and <i>in vivo</i>	Decreasing ER-α36 expression, slowing TNBC cell growth, and triggering cell death	[89]
Prostate cancer	LNCaP cells	<i>In vitro</i>	Activating PEA3, thereby blocking HER2/AR signaling	[40]
Prostate cancer	LNCaP cells	<i>In vitro</i> and <i>in vivo</i>	Suppressing neuroendocrine differentiation via the IL-6/STAT3 and aurora kinase A pathways	[90]
Prostate cancer	CRPC cells	<i>In vitro</i>	Promoting degradation of both AR and ARvs by binding AhR	[91]

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numerous chemotherapeutic drugs and natural herbs have shown potential as anti-cancer agents through enhancing ROS levels [98]. Understanding this intricate phenomenon might enable specific targeting of ROS or ROS-regulated pathways with sophisticated drug interventions aimed at cancer cell destruction.

2.11 ICA inhibits tumorigenesis in immunotherapy

In the past decade, major advancements have been achieved in the field of cancer treatment, specifically in the area of immunotherapy [99]. Tumor-associated macrophages in immunotherapy show rapid responsiveness to local stimuli, such as cytokines and therapeutic interventions. These macrophages exhibit a wide range of functions encompassing both pro-inflammatory and anti-inflammatory phenotypes [100, 101]. ICA and its derivatives have shown efficacy in impeding the polarization of macrophages toward the M2/protumor phenotype of tumor-associated macrophages. Combined use of ICA treatment in conjunction with PD-1 inhibitors yields favorable immunotherapy outcomes [69, 102]. Myeloid-derived suppressor cells (MDSCs), a distinct cell population, play crucial roles in immune

evasion and the protection of tumor cells against host immune surveillance. Recent findings have shown that ICA hinders MDSC generation by decreasing extramedullary hematopoiesis and suppressing the production of interleukin-10 (IL-10), IL-6, and TNF- α , thereby hindering tumor immunosuppression and promoting an anti-tumor immune response [103, 104]. In addition, ICA decreases the expression of PD-L1 on MDSCs [105]. The concurrent administration of ICA with CTLA-4 therapy augments the anti-tumor action of ICA [106].

Notably, the influence of ICA on tumor immunity relies on the presence of CD8⁺ T cells, as characterized by elevated infiltration of CD8⁺ T cells and an increase in effector memory T cells [106, 107]. ICA administration through intertumoral CpG injection promotes the infiltration of cytotoxic CD8⁺ T cells with elevated expression of interferon- γ (IFN- γ) and TNF- α [108]. ICA has also been shown to enhance IFN- γ -induced antitumor immune responses through a mechanism dependent on CD8⁺ T cells, thus suggesting the potential for synergistic effects when immune checkpoint therapy is combined with ICA-based antitumor treatments [104]. Moreover, ICA induces mitophagy and apoptosis, thereby triggering immunogenic cell death in both mouse Hepa1-6 and

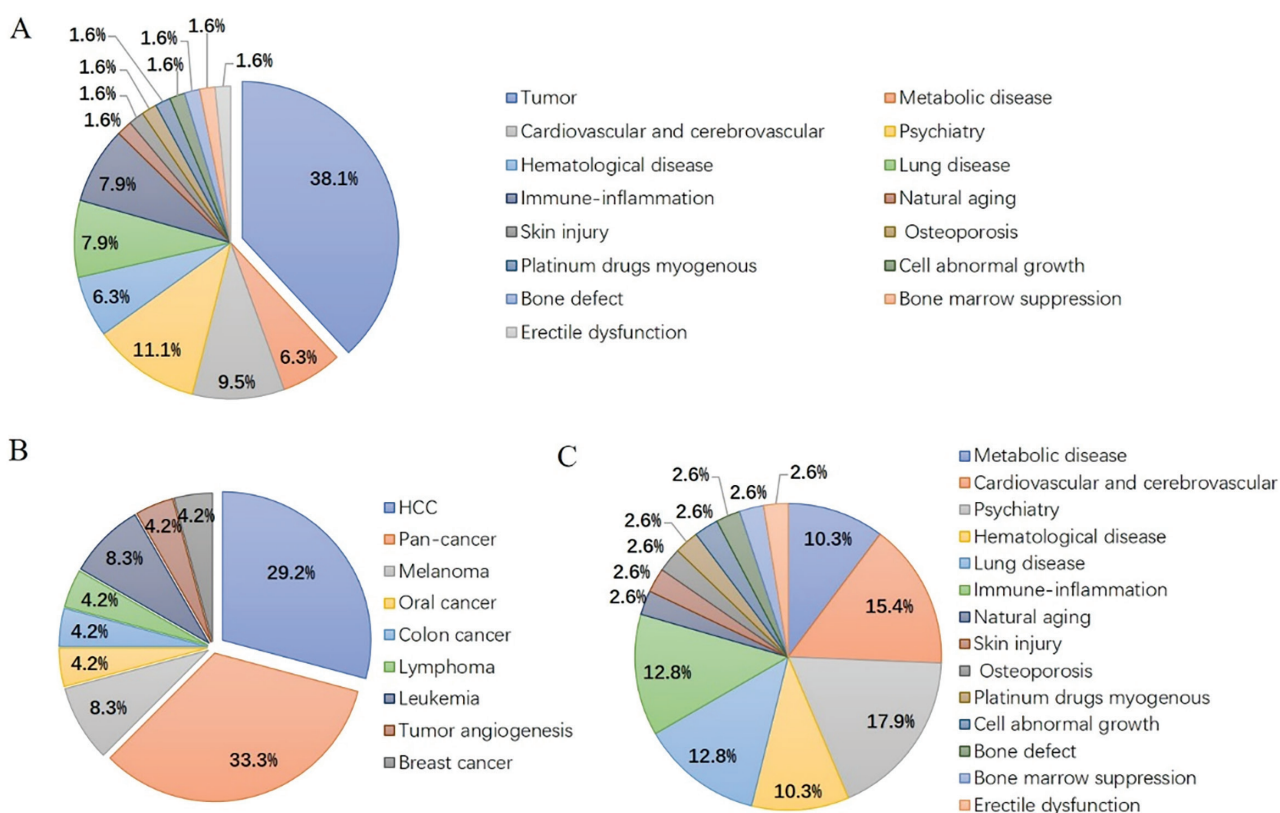


Figure 3 | Analysis of patent data associated with ICA. (A) ICA patent data associated with all diseases. (B) ICA patent data associated with cancers. (C) ICA patent data associated with diseases other than cancer. Patent data are from the innojoy.com website (<https://www.innojoy.com/search/home.html>).

human Huh7 cells [44]. In the context of advanced HCC undergoing treatment with ICA-based immunomodulation therapy, ICA immunotherapy increases the composite biomarker score, which is associated with enhanced survival in HCC [109]. Together, these findings suggest that ICA therapy holds promise as a potent approach to stimulate T cell-mediated antitumor immune responses and serve as an effective immunotherapeutic agent.

3. CLINICAL APPLICATION AND DEVELOPMENT OF ICA

3.1 Clinical applications and trials of ICA

The approval of ICA softgel capsules as a class 1.2 TCM innovative drug marked a major advancement providing a novel treatment option for patients with advanced liver cancer. This landmark achievement was a culmination of extensive clinical studies and foundational research in the field. In single-arm phase I clinical trials, ICA exhibited favorable safety profiles and showed promising long-term survival advantages in patients with advanced HCC. These benefits were associated with its immune-modulatory properties and effects on immune biomarkers, thereby suggesting that ICA

might serve as an innovative oral immunotherapy for advanced HCC [107, 109]. A phase I clinical study demonstrated ICA's good safety and tolerability. Subsequently, a phase II study was aimed at investigating the efficacy of ICA in treating advanced HCC. Preclinical pharmacokinetic/pharmacodynamic and toxicological studies have revealed that ICA effectively suppresses the proliferation of HCC in both laboratory and animal models. Synergistic analysis of the phase II trial data indicated that ICA exhibits high bioavailability and a relatively short half-life, and therefore is a promising candidate for the treatment of HCC. This trial was registered on the ichgcp.net website (<https://ichgcp.net/zh/clinical-trials-registry/NCT01972672>). Additionally, a phase III randomized, double-blind study has compared ICA softgel capsules to Huachashu as a first-line treatment for HBV-associated advanced liver cancer enriched with biomarkers. The interim analysis results were as follows. The administration of ICA softgel capsules significantly increased overall survival and showed a favorable safety profile in a cohort of patients with HBV-related advanced HCC characterized by prospective composite biomarker score enrichment, despite their poor baseline conditions [110]. This trial was registered at clinicaltrials.gov

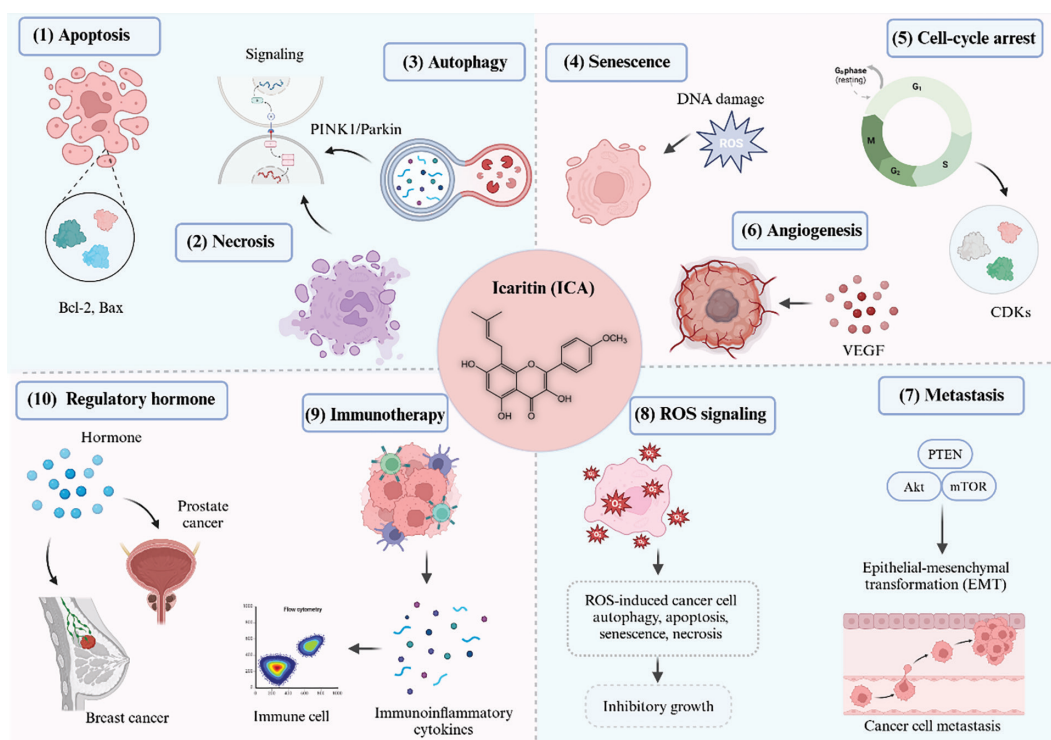


Figure 4 | Diagram of the molecular mechanism of ICA. This review systemically summarized the molecular mechanisms and applications of ICA. (1) ICA regulates the expression of Bcl-2 and Bax, thereby inducing apoptosis; (2) ICA induces necroptosis; (3) ICA regulates autophagy via PINK1/Parkin signaling and other mechanisms; (4) ICA induces DNA damage, thus leading to senescence; (5) ICA induces cell-cycle arrest by regulating CDKs; (6) ICA inhibits tumor angiogenesis; (7) ICA inhibits metastasis via Akt/mTOR signaling; (8) ICA regulates oxidative stress and promotes cell death; (9) ICA is an immune-modulatory agent; (10) ICA regulates hormones, thereby influencing hormone-associated breast or prostate cancer.

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(<https://clinicaltrials.gov/study/NCT03236636>). Moreover, a phase IV study of ICA softgel capsules is underway, and the results are much anticipated.

3.2 Combination of ICA with other drugs

The combination of ICA nanoparticles with ionizing radiation has synergistic killing effects on breast cancer cells [58]. The concurrent administration of ICA and cetuximab has shown synergistic inhibition of cell proliferation and promotion of programmed cell death in TNBC cells [89]. The combination of ICA with ganciclovir, compared with ICA alone, significantly increases induction of apoptosis in extranodal NK/T-cell lymphoma (ENKL) cells [36]. Furthermore, the combination of epirubicin with ICA has effective inhibitory effects on bladder cancer cells [47]. Additionally, the oral tyrosine kinase inhibitor dasatinib combined with SNG1153, a modified form of ICA, has shown efficacy in eradicating infiltrated BCR-ABL⁺ blast cells and increasing overall survival rates [83]. A phase II clinical study of ICA softgel capsules combined with lenvatinib and transarterial chemoembolization has indicated efficacy in the treatment of unresectable, non-metastatic HCC (<https://www.chictr.org.cn/showproj.html?proj=197839>). Hence, comprehensive basic and clinical research on the concurrent use of ICA capsules with other medications is imperative. Such investigations might reveal promising therapeutic synergies achievable through drug combinations.

3.3 Developmental patents for ICA

Preliminary investigations have indicated that, beyond effective inhibition and treatment of cancers, ICA demonstrates notable biological activity against variety of other pathologies, including metabolic, cardiovascular, pulmonary, immune, and neurological disorders. Thus, we examined ICA-associated patents at the [innojoy.com](https://www.innojoy.com) website (detailed findings in [Table S1](#) and [Table S2](#)). Patent classification analysis revealed that patents related to cancers comprised 38.1% of the total, and liver cancer emerged as the predominant type ([Figure 3](#)). Moreover, patents regarding metabolic, cardiovascular, neurological, and immune conditions constituted a substantial proportion among other identified diseases, thus suggesting that ICA research must be extended to advance understanding of its applications and potential effects within the field.

4. CONCLUSIONS AND PERSPECTIVES

In this review, we systemically summarized the molecular mechanisms and applications of ICA discovered over the past 20 years. such as induction of apoptosis, autophagy, necroptosis, and senescence; cell-cycle and hormone regulation; inhibition of metastasis and angiogenesis; oxidative stress regulation; and immune-modulatory properties ([Figure 4](#)). Whereas the inhibitory effects of ICA in various cancer types have been established, and its mechanism of action has been examined, its specific

target and regulatory network remain elusive. For ICA to be effectively used as an anti-cancer medication in future treatments, the molecular targets of ICA must be clearly identified. Hence, an urgent need exists to elucidate the proteins directly targeted by ICA and the associated regulatory networks. By leveraging cutting-edge technologies, researchers can address these challenges to facilitate broader clinical utilization of ICA. In summary, ICA has emerged as a promising anticancer agent that modulates various signaling pathways and tumor microenvironments. ICA also enhances the efficacy of immune checkpoint inhibitors and other targeted therapies, and complements conventional treatments such as radiotherapy and chemotherapy.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Jigang Wang, Qian Zhang and Kun Meng supervised and guided this review. Piao Luo and Hao Zhang was responsible for writing the manuscript and graphic production. Guangqing Cheng, Ping Wang and Yin-Kwan Wong was responsible for the graphic production and revised the manuscript. Junhui Chen, Jingya Fang, Wenhui Li, Bin Liu and Ting Cao revised the manuscript.

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SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from https://amm-journal.org/wp-content/uploads/2025/02/amm20240035_Suppl.zip.

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