Breast Cancer: Targets and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

The Role of The Xihuang Pill in Inhibiting Triple-Negative Breast Cancer Through Immunogenic Cell Death

Xingchao Xu[1,2], XiangQi Li[2]

[1]The First Clinical Medical School, Shandong University of Traditional Chinese Medicine, Jinan, Shandong, People's Republic of China; [2]The Second Affiliated Hospital of Shandong First Medical University, Tai'an, Shandong, People's Republic of China

Correspondence: XiangQi Li, The Second Affiliated Hospital of Shandong First Medical University, Tai'an, Shandong, 271000, People's Republic of China, Tel +86 18505386835, Email drlixqi@hotmail.com

Abstract: Modern Western medicine uses surgeries, chemotherapy, targeted therapy, and radiotherapy to inhibit breast cancer cells through pathways such as apoptosis, necrosis, and autophagy. However, for triple-negative breast cancer (TNBC), a highly aggressive subtype lacking targeted therapies, conventional chemotherapy often leads to severe side effects and drug resistance, thereby limiting its clinical efficacy. Immunogenic cell death (ICD) can prompt dying tumor cells to release antigens and activate anti-tumor immune responses, converting "cold tumors" into "hot tumors". This opens up a new direction for overcoming tumor drug resistance and enhancing the efficacy of traditional therapies. Additionally, Xihuang Pill (XHP) has the potential for immunomodulation and chemotherapy sensitization. Studies have found that as an ICD inducer, XHP can trigger the production of three key damage-associated molecular patterns (DAMPs): HSP membrane translocation, massive ATP release, and HMGB1 secretion. The main active components of XHP include bilirubin, volatile oil, pentacyclic triterpenoids (such as boswellic acid), and steroids (such as hyodeoxycholic acid). These components possess anti-tumor effects, as well as functions in immunomodulation, efficacy enhancement, and toxicity reduction. This article elaborates on the role and mechanism of XHP in treating TNBC from aspects such as formula analysis, the mechanism of immunogenic cell death, the anti-tumor immune effects of XHP, and its relationship with immunogenic cell death. The aim is to provide a theoretical basis for the clinical application of XHP in treating TNBC through the immunogenic cell death pathway and to promote the secondary development of this medicine.

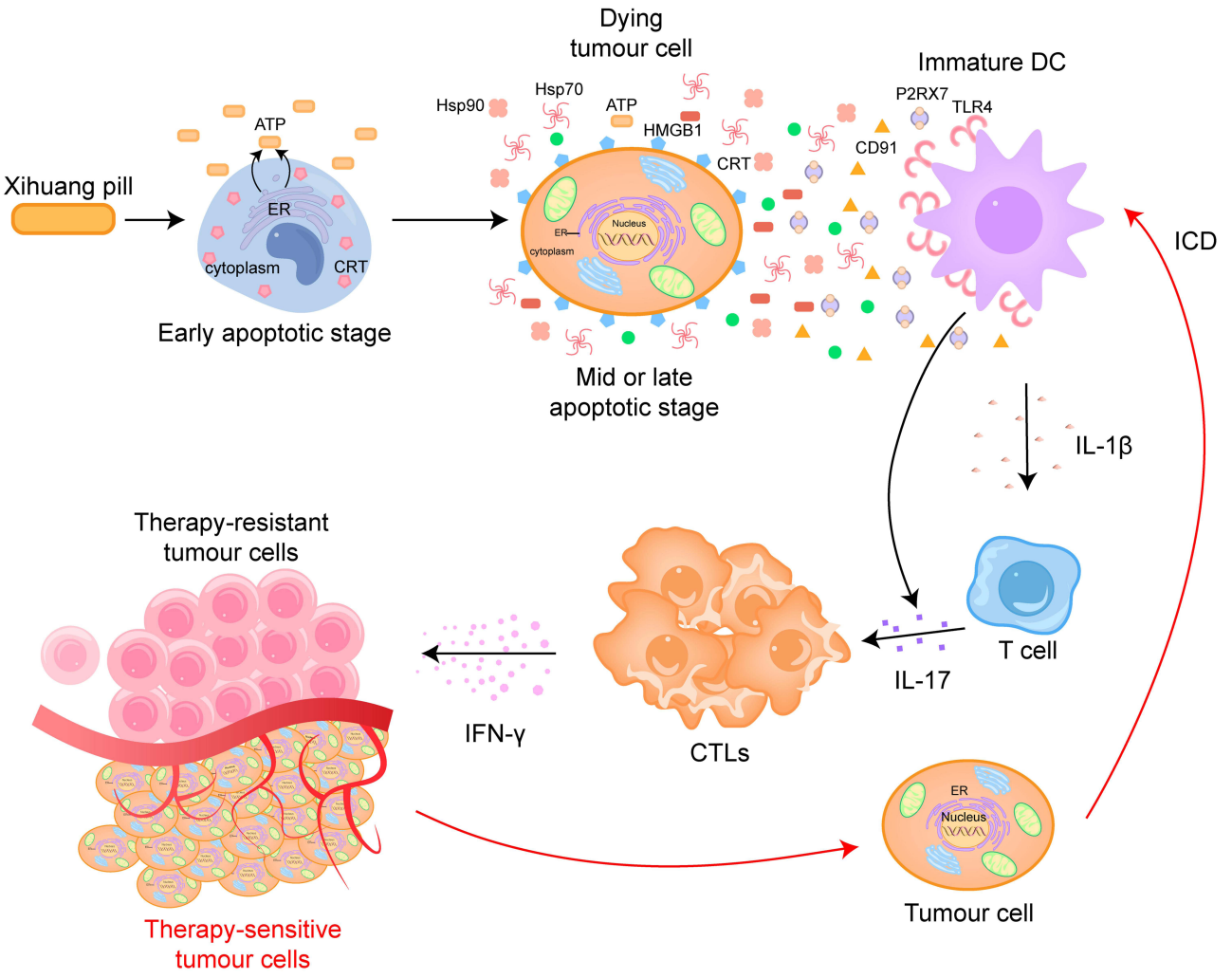
Keywords: Xihuang pill, triple-negative breast cancer, mechanism, immunogenic cell death

Background

In breast cancer patients, triple-negative breast cancer (TNBC) accounts for 15–20% of all breast cancer cases.[1] Due to the lack of effective targeted therapy targets, chemotherapy remains the primary treatment modality for TNBC in current clinical practice. However, there is significant heterogeneity in TNBC patients' response to chemotherapy: approximately 30% of patients are forced to discontinue treatment in the early stage of chemotherapy due to severe adverse reactions (eg, grade III-IV myelosuppression, gastrointestinal reactions).[2] These patients are more prone to distant metastasis and have lower overall survival rates compared with other breast cancer subtypes.[3] Chemotherapy not only causes significant physical suffering to patients but also induces drug resistance, which further limits clinical efficacy, making it a critical challenge that urgently needs to be addressed in the diagnosis and treatment of TNBC.

Immunogenic cell death (ICD), as a distinctive form of cell death, it often accompanied with the stimulation of autophagic mechanisms in tumor cells and endoplasmic reticulum (ER)-stress pathways.[4] During cell death, cancer cells release ATP and expose heat-shock protein (HSP) and calreticulin (CRT) to the outside of the cell membrane. In addition, secondary necrosis causes the nucleus's high-mobility group box 1 (HMGB1) to be released into the extracellular area due to enhanced nuclear membrane permeability.[5] Damage-associated molecular patterns (DAMPs) may attach to

Received: 6 June 2025
Accepted: 30 August 2025
Published: 5 September 2025



Scheme 1 The ICD mechanism diagram demonstrates that in the preapoptotic stage, cancer cells exposed to the Xihuang pill display calreticulin (CRT) on the outer leaflet of their plasma membrane, and in the apoptotic stage, they produce adenosine triphosphate (ATP). Furthermore, when their membranes permeabilize during secondary necrosis, ICD-undergoing cells release the nuclear protein high-mobility group box 1 (HMGB1). To CD91, P2RX7, and TLR4, respectively, CRT, ATP, and HMGB1 bind. This encourages the recruitment of dendritic cells (DCs), which are encouraged by ATP, the uptake of tumor antigens by DCs, which is prompted by CRT, and the best possible presentation of antigens to T cells, which is prompted by HMGB1.

Abbreviations: HMGB1, high-mobility group box 1; DC, dendritic cell; CTL, cytotoxic T lymphocyte; IFN, interferon; IL, interleukin; TLR, toll-like receptor.

antigen-presenting cells' (APCs) pattern recognition receptor (PRR), altering the tumor immunological milieu in the process[6] (Scheme 1).

The Xihuang pill (XHP), a traditional Chinese anti-tumor medicine is hailed as "the number one anti-cancer medicine". Its main components include frankincense, myrrh, bezoar, and musk. Previous studies have revealed that the main chemical constituents of Xihuang Pill include triterpenoids (β-boswellic acid, 3-acetyl-β-boswellic acid, 11-carbonyl-β-boswellic acid, 11-carbonyl-β-acetyl-boswellic acid, α-boswellic acid, and 3-acetyl-α-boswellic acid), hyodeoxycholic acid, bilirubin, and volatile oil components such as (1S-endo)-2-methyl-3-methylene-2-(4-methyl-3-pentenyl) bicyclo, heptane, 10α-hydroxyoplopan, muscone.[7] Studies have shown that Ma[8] used W256 breast cancer cells to establish a tumor-bearing rat model, and after intervention with the use of the volatile oil and ethanol extract of the Xihuang pill, they discovered that it might strengthen the tumor-bearing mice's immune system, regulate the expression of tumor-related inflammatory factors, and exert an anti-tumor effect. Dai[9] determined that its mechanism of action against prostate cancer is associated with inhibiting the AR/mTOR-signaling pathway, restraining the proliferation of LNCaP cells, and inducing apoptosis in cancer cells.

The main active compound in frankincense, 11-keto-β-boswellic acid (KBA), exists in the β-configuration. Schmiech[10] verified that it also has α-isoforms in nature, which showed cytotoxicity against three triple-negative breast cancer (TNBC) cell lines in vitro; that it was capable of apoptosis of MD-AMB-231 xenografts in vivo; and that its β-isoform and acetylated forms exhibited greater inhibitory effect on the proliferation of TNBC cells. Besides boswellic acid, hyodeoxycholic acid and bilirubin, the main active components of bezoar have also been found to possess anti-tumor effects. Zhang[11] using the transporter-recruitment, prodrug technique we devised and produced four kinds of conjugate cytarabine and bile acids, among which they uncovered porcine deoxycholic acid; this showed its effective anti-proliferative activity in HepG-2 hepatoma cells, proving that porcine deoxycholic acid possesses anti-cancer activity and that it also constitutes the anti-tumor activity site of the Xihuang pill. Yang[12] uncovered the potential antitumor activity of bilirubin against HepG-2 cells and developed bifunctional albumin-based nanoparticles of glutathione (GSH)-responsive bilirubin loading in the treatment of liver cancer. Z-guggulsterone (Z-GS) is an active compound extracted from the gum resin of myrrh. Studies by Tian H[13] have shown that in addition to inhibiting the viability of NSCLC cells in vitro and suppressing the growth of Lewis lung carcinoma (LLC) tumors in mice in vivo, Z-GS also upregulates the expression of PD-L1 in NSCLC both in vivo and in vitro. This indicates that Z-GS, as a candidate anti-tumor drug, can be used in combination with PD-1/PD-L1 antibodies for the treatment of NSCLC.

Meanwhile, studies have shown that muscone, an active component in Xihuang Pill, can regulate key molecules such as heat shock protein (HSP), adenosine triphosphate (ATP), and high mobility group box 1 (HMGB1).[14,15] These key markers are exactly the core damage-associated molecular patterns (DAMPs) in the process of ICD, which can reshape the tumor immune microenvironment by activating antigen-presenting cells and promoting tumor antigen presentation.[16] Based on this, this review will systematically elaborate on the drug composition, efficacy of Xihuang Pill and its molecular mechanism in tumor immunogenic cells, and further explore its application potential in the clinical treatment of triple-negative breast cancer, so as to provide new ideas and methods for the treatment of TNBC.

Analysis of Xihuang Pill Prescription

Prescription Composition and Active Components of Xihuang Pill

Xihuang Pill is composed of 4 Chinese medicinal ingredients: Frankincense, Myrrh, Bezoar, and Musk (Figure 1). Its anti-tumor activity relies on the synergistic effects of various specific components, as detailed below:

Frankincense

Frankincense is extracted from the resins of Boswellia carteri Birdw. and its congener Boswellia abhaurdajiana Birdw. (family Burseraceae). It is used as a spice in religious rituals and in traditional medicine for treating bacterial infections, inflammation, and cancer.[17] Its main active components are triterpenoids, including 11-keto-β-boswellic acid (KBA), 11-keto-β-acetylboswellic acid (AKBA), α-boswellic acid (αBA), β-boswellic acid (βBA), 3-acetyl-α-boswellic acid (AαBA), and 3-acetyl-β-boswellic acid (AβBA). Among these, only KBA and AKBA have been clearly confirmed to possess anti-tumor activity.[7]

Studies have shown that the Hostanska team et al[18] discovered that ethanol extracts of frankincense gum resin can induce apoptosis in 5 leukemia cell lines (HL-60, k562, U937, MOLT-4, THP-1) and 2 brain tumor cell lines (LN-18, LN-229), demonstrating broad-spectrum anti-tumor effects. Agrawal[19] confirmed that boswellic acids exert anti-angiogenic and tumor cell apoptosis-inducing effects by reducing peritoneal angiogenesis and microvascular density and upregulating the expression of Bax and caspase3. Schmiech et al[10] further confirmed that the α-isomer of KBA exhibits strong cytotoxicity against 3 drug-resistant TNBC cell lines (MDA-MB-231, BT-549, HCC1937), and its β-isomer and acetylated forms show stronger inhibitory effects on TNBC cell proliferation. In gastric cancer, AKBA can dose-dependently upregulate PTEN and downregulate p-AKT and COX-2, blocking the PTEN/Akt/COX-2 pathway. When combined with cisplatin, it enhances the sensitivity of cancer cells to cisplatin by downregulating p53, reversing drug resistance.[20,21]

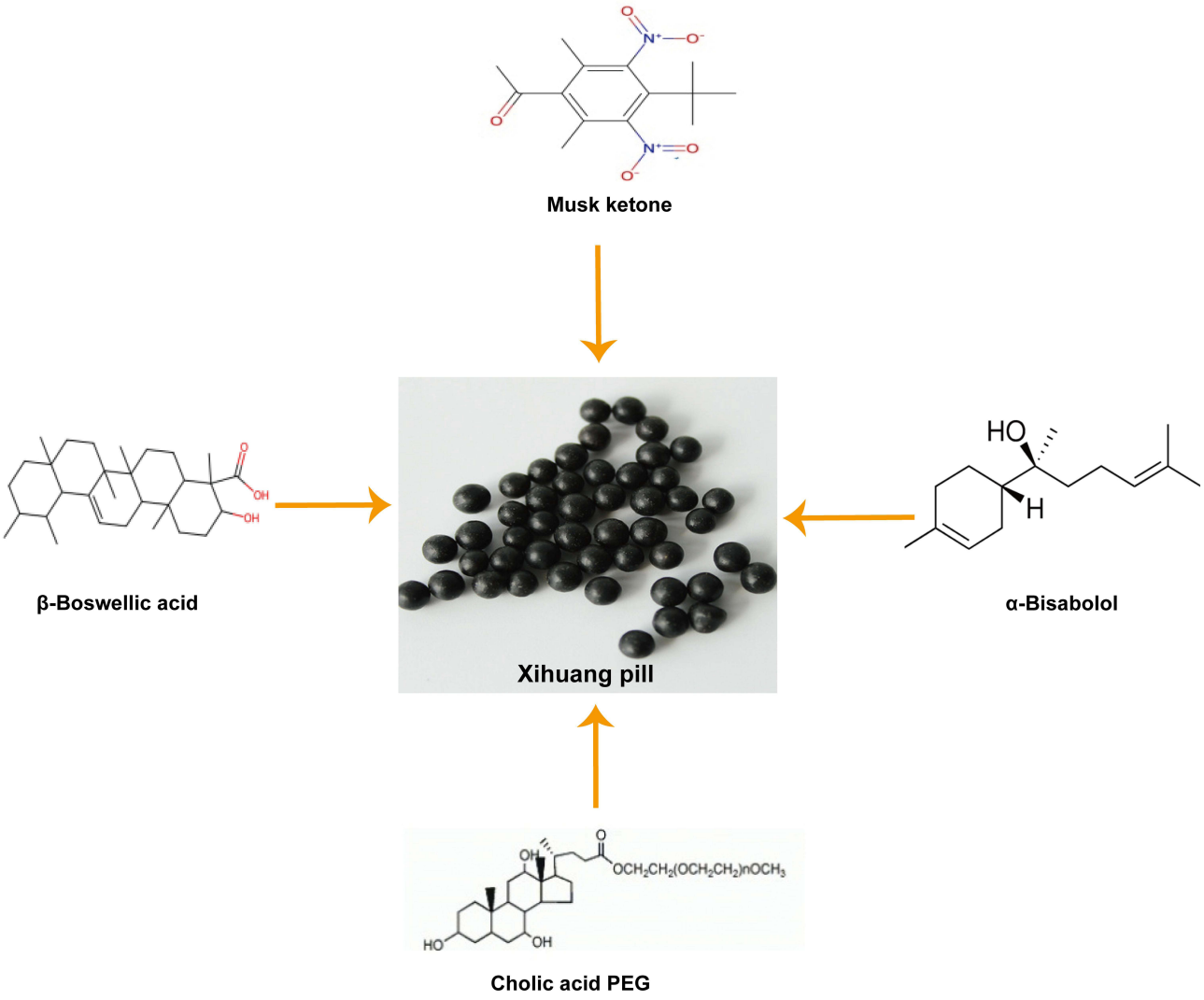


Figure 1 The main components of Xihuang Pill.

Myrrh

Myrrh is derived from the dried gum resin of shrubs or small trees of the Burseraceae family, traditionally used for treating urinary system diseases, liver diseases, and visceral tumors.[22] Its active substances include volatile oil components (eg, β-caryophyllene), curcumin, and its isomer Z-demethoxycurcumin. Studies have shown that β-caryophyllene can inhibit lysosomal leakage and oxidative stress, protect the heart and its lysosomal structure, and block the intrinsic apoptotic pathway.[23] Curcumin targets the farnesoid X receptor, exerting strong inhibitory and anti-inflammatory effects on tumor cells. In SENCAR mice, it inhibits skin tumorigenesis by regulating the MAPK and NF-κB pathways.[24,25] Z-demethoxycurcumin can inhibit the AKT signaling axis, reducing the secretion of vascular endothelial growth factor (VEGF), thereby limiting tumor angiogenesis.[26] Curcumin significantly enhances the chemosensitivity of multidrug-resistant breast cancer cells (MCF-7/DOX) to doxorubicin in vitro and in vivo by inhibiting the expression of Bcl-2 and P-glycoprotein, reversing the drug-resistant phenotype.[27]

Musk

Musk is derived from the dried secretion of the scent glands of mature male musk deer such as Moschus berezovskii, Moschus sifanicus, or Moschus moschiferus. Traditionally used for inducing resuscitation and promoting blood

circulation, its anti-cancer effect is mainly associated with immune regulation.[28] Muscone is the main active component, accounting for over 50% of musk volatile oil.

Qi[29] confirmed through in vitro experiments on hepatoma cells and nude mouse models that muscone can increase the apoptosis and autophagy rates of hepatoma cells by activating endoplasmic reticulum stress responses (eg, the PERK/eIF2α pathway), and autophagy regulation is closely related to the AMP kinase/mTOR complex 1 signaling pathway. Wang[28] systematically reviewed that muscone has multi-target effects in cancer, neurological diseases (eg, stroke), and cardiovascular diseases, with mechanisms involving the regulation of cell apoptosis, inflammatory responses, and oxidative stress.

Bezoar

Bezoar is a calculus in the gallbladder of Bovidae animals. Due to ethical and resource constraints, artificial bezoar is now mostly used, with components similar to natural bezoar, including bilirubin, bile acids (hyodeoxycholic acid, chenodeoxycholic acid, etc.), and amino acids.[30] Its active substances are mainly hyodeoxycholic acid, bilirubin, and total extracts of artificial bezoar.

Studies have shown that chenodeoxycholic acid and ursodeoxycholic acid exert significant cytotoxicity on ovarian cancer cells by inducing apoptosis and reducing PKC activity.[31] Yang[32] found that bilirubin has potential anti-tumor activity against HepG-2 cells, and glutathione (GSH)-responsive bilirubin-loaded nanoparticles based on bifunctional albumin can improve its targeting and efficacy, providing a new strategy for liver cancer treatment. Khan[33] found that bezoar can inhibit the viability of melanoma A375 cells in vitro (IC50=45.2 μM), promote apoptosis (flow cytometry showed a 2.3-fold increase in apoptosis rate), arrest the G2/M phase cell cycle, and reduce migration and invasion abilities. Artificial bezoar can regulate the MEOX2/PI3K/AKT/mTOR pathway, increase p-AKT/AKT and p-mTOR/mTOR levels, inhibit the proliferation, invasion, and migration of nephroblastoma cells, and simultaneously induce lymphocyte transformation, enhance macrophage phagocytosis, and improve the body's non-specific immunity.[34]

Volatile Oil

Volatile oil is a mixture of volatile components from frankincense, myrrh, and musk in Xihuang Pill, mainly including muscone, (1S-endo)-2-methyl-3-methylene-2-(4-methyl-3-pentenyl) bicycloheptane, 10α-hydroxypinene, etc.[7]

Ma [8] confirmed through a W256 breast cancer-bearing rat model that the volatile oil and ethanol extract of Xihuang Pill can upregulate the levels of IL-2 and IFN-γ in peripheral blood, increase the proportions of CD3+ T cells, CD8+ T cells, and B7-1 cells in the T lymphocyte population, enhance immune clearance function, and the tumor inhibition rate in the high-dose group reaches 42.6%.

Common and Specific Characteristics of Active Components in Xihuang Pill

Common Mechanisms

Immune regulation: Boswellic acids (eg, β-boswellic acid) inhibit the NF-κB pathway, reducing the release of pro-inflammatory factors such as TNF-α and IL-1β.[35] Bezoar improves immune activity by inducing lymphocyte transformation and enhancing macrophage phagocytosis.[34] Volatile oils upregulate Th1-type cytokines (IL-2, IFN-γ) to enhance cellular immunity. Regulation of cell death: Bezoar can promote caspase3 activation in A375 cells, hyodeoxycholic acid can induce S-phase arrest.[36] Cross-talk of signaling pathways: All regulate tumor-related pathways such as PI3K/AKT and MAPK, eg, AKBA inhibits Akt phosphorylation,[21] artificial bezoar regulates PI3K/AKT/mTOR,[37] and curcumin inhibits the MAPK pathway.

Specific Differences

Frankincense (KBA/AKBA): Focuses on direct cytotoxicity, with additional effects on reversing chemotherapy resistance (eg, cisplatin, doxorubicin), especially effective against TNBC, lung cancer, and gastric cancer. Myrrh (curcumin): Emphasizes reversing multidrug resistance and anti-angiogenesis, with prominent effects on breast cancer and skin cancer.

Musk (muscone): Induces tumor cell death through endoplasmic reticulum stress and autophagy, while enhancing non-specific immunity, showing broad-spectrum anti-tumor effects. Bezoar and volatile oils: Centered on immune regulation; bezoar focuses on anti-oxidation and targeting the PI3K/AKT pathway, while volatile oils directly enhance

T cell activity, synergistically improving anti-tumor immunity. Mechanism of Immunogenic Cell Death and Tumor Immune Regulation.

Definition and Signature Events of ICD

ICD is a unique form of cell death distinct from traditional apoptosis or necrosis, characterized by dying cells actively releasing damage-associated molecular patterns (DAMPs) to activate the body's adaptive immune response and form long-term anti-tumor immune memory.[38] Unlike homeostasis apoptosis with low immunogenicity, ICD relies on the activation of pathways such as endoplasmic reticulum stress and autophagy, ultimately triggering immune responses through the following signature events:

Calreticulin (CRT) translocation: Moves from the endoplasmic reticulum to the cell membrane surface, serving as an "eat me" signal recognized by dendritic cells (DCs) to promote tumor antigen uptake.[39] Adenosine triphosphate (ATP) release: Secreted extracellularly through an autophagy-dependent pathway, acting as a "find me" signal to recruit DCs and T cells to the tumor microenvironment.[40] High-mobility group box 1 (HMGB1) release: Secreted by secondary necrotic cells, functioning as an "activation signal" to promote DC maturation and antigen presentation via the TLR4/MyD88 pathway.[41] All three are indispensable: absence of CRT exposure prevents DC recognition of tumor cells; insufficient ATP release hinders immune cell recruitment; and defective HMGB1 secretion leads to failure of T cell activation, none of which can induce complete ICD.

Functional and Regulatory Networks of Key DAMPs

Calreticulin (CRT)

CRT is a 46kDa Ca^{2+}-binding protein containing an N-terminal lectin domain, a P domain, and a C-terminal Ca^{2+}-binding domain, with an ER retention sequence (KDEL) at the carboxyl terminus. It participates in protein folding and Ca^{2+} homeostasis regulation in the ER; in ICD, CRT binds to CD91 (LRP1) on the DC surface via its N-terminal lectin domain, promoting cross-presentation of tumor antigens and activating B cells and macrophages.

Chemotherapeutic drugs (eg, anthracyclines) trigger endoplasmic reticulum stress by activating the PERK-eIF2α-ATF4 pathway, prompting CRT to dissociate from KDEL receptors and translocate to the cell membrane via the PI3k-mediated distal secretory pathway and PERK-regulated proximal secretory pathway; this process depends on caspase8-mediated BCAP31 cleavage and BAX/BAK protein activation. Studies have shown that in UV-induced apoptosis, CRT can synergize with phosphatidylserine to enhance DC phagocytosis and significantly promote B cell maturation and immunoglobulin class switching.[42]

Adenosine Triphosphate (ATP)

Dual-signaling roles: Low-concentration ATP (<1 μM) recruits immune cells to the injury site by activating the P2Y2 receptor on monocytes.[43] High-concentration ATP (>100μM) activates the P2X7 receptor on DCs, promoting NALP3-ASC inflammasome assembly and driving IL-1β secretion, which provides necessary signals for $CD8^{+}$T cell polarization.[44]

Metabolic balance: Extracellular ATP can be hydrolyzed to ADP by CD39 and further converted to adenosine by CD73. Adenosine inhibits T cell activity via the A2A receptor, forming an "ATP-adenosine" immune regulatory axis. High expression of CD39/CD73 in the tumor microenvironment leads to adenosine accumulation, weakening the immune activation effect of ICD. Research evidence: Clearing extracellular ATP can completely eliminate chemotherapy-induced anti-tumor immunity, while inhibiting CD73 can enhance ATP-mediated DC recruitment.[45]

High-Mobility Group Box 1 (HMGB1)

In normal cells, HMGB1 acts as a non-histone chromatin-binding protein involved in transcriptional regulation. In ICD, secondary necrosis causes nuclear membrane rupture, releasing HMGB1 extracellularly to function via binding to TLR2, TLR4, and RAGE. HMGB1 binding to TLR4 activates the MyD88/NF-κB pathway, promoting DC maturation and IL-12 secretion; binding to TLR2 upregulates MHC-I molecule expression, enhancing antigen presentation. Apetoh et al 17704786 found that HMGB1 released by tumor cells after systemic or local chemo-radiotherapy can activate the TLR4-MyD88 pathway. Clinical data show that the TLR4 Asp299Gly mutation affects HMGB1 binding to its receptor, thereby adversely affecting the prognosis of breast cancer patients.

Heat Shock Proteins (HSPs)

Dual roles of HSP70: As a DAMP, HSP70 binds to DCs via the CD91 receptor, promoting cross-presentation of tumor antigen-MHC-I complexes and activating $CD8^{+}$T cells. It also acts as an endogenous ligand of TLR4, stimulating innate immunity via the TLR4/MyD88 pathway. However, its high expression may induce Treg cell infiltration, inhibiting immune responses.

HSP90 and ICD regulation: HSP90 participates in endoplasmic reticulum stress and NF-κB pathway regulation by stabilizing proteins such as PERK and IKK. Its inhibitors (eg, L80) can enhance the sensitivity of TNBC cells to ICD inducers. After doxorubicin chemotherapy for breast cancer, the transcription factor brn-3b is upregulated, promoting the secretion of target protein HSP. Phosphorylated HSP-27 inhibits apoptosis via death domain-associated protein (DAXX), and HSP-27 inhibits apoptosis by binding to cytochrome C, inducing tumor cell drug resistance and promoting actin polymerization, enabling breast cancer cell metastasis.[46] These mechanisms provide a theoretical basis for the regulation of DAMPs by components of Xihuang Pill.

Regulation of Tumor Immunity by Xihuang Pill Through ICD

Regulation of Key ICD Molecules by Components of Xihuang Pill

Immunogenic cell death (ICD) inducers are generally classified into type I and type II (Figure 2). Type I ICD inducers have distinct spatial targeting, mainly acting on three key regions: the plasma membrane, cytosol, and nucleus. At the plasma membrane level, they activate signal transduction by acting on transmembrane proteins or ion channels, promoting the translocation of DAMPs such as CRT to the membrane surface to provide an "eat me" signal. In the cytosol, they regulate proteins such as Bcl-2 family and Bax/Bak to activate the intrinsic apoptotic pathway and activate autophagy-related proteins to promote ATP release. In the nucleus, they target DNA replication and repair-related

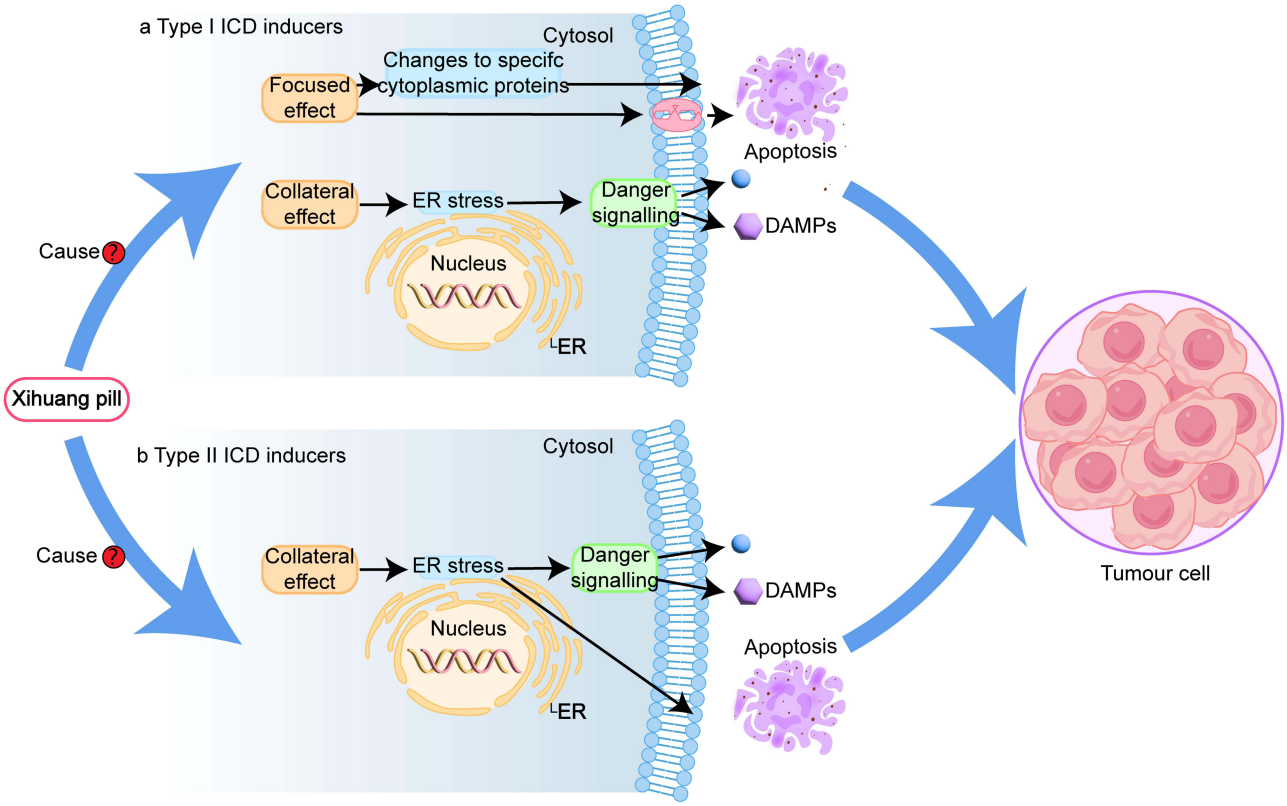


Figure 2 The Xihuang pill belongs to the category of immunogenic cell death (ICD) inducers, either type I or type II. a | The main or focused areas of impact of the majority of type I ICD inducers are the plasma membrane (certain transmembrane proteins or channels), the cytosol (some cytosolic proteins), and the nucleus (proteins involved in DNA replication and repair). b | The endoplasmic reticulum (ER) is the location of targeted effects in the form of primary ER stress in the case of type II ICD inducers. Their major ability to generate ER stress is what allows them to trigger apoptosis, danger signaling, and the production of damage-associated molecular patterns (DAMPs).

https://doi.org/10.2147/BCTT.S545150

proteins, induce DNA damage, activate tumor suppressor genes such as p53, and promote HMGB1 release. This multi-site synergistic effect achieves a "killing-immune activation" dual effect.

Type II ICD inducers are characterized by primary endoplasmic reticulum stress, with targeted effects focused on the endoplasmic reticulum. They induce the accumulation of unfolded proteins by interfering with endoplasmic reticulum function, activating unfolded protein response pathways such as PERK-eIF2α-ATF4-CHOP. As stress intensifies, molecules such as CHOP are upregulated, triggering apoptosis via the mitochondrial pathway, accompanied by DAMP signals such as CRT exposure, ATP secretion, and HMGB1 release. Additionally, calcium ions and ROS released by the endoplasmic reticulum further amplify immunogenicity and enhance the antigen-presenting function of dendritic cells. This "endoplasmic reticulum stress→apoptosis→DAMPs release" cascade reaction gives them unique potential in tumors resistant to type I inducers.

Although no studies have yet confirmed which type Xihuang Pill belongs to, our preliminary studies have shown that muscone can increase cell viability after LPS/ATP stimulation, reduce the production of interleukin (IL)-1β/18, and inhibit the activation of caspase-1, NOD-like receptor pyrin domain protein 3 (NLRP3) inflammasome, and Gasdermin D.[15] High-mobility group box 1 is associated with bilirubin.[47] Limonene and β-pinene regulate heat shock protein 70.[48] Therefore, in the future, we need to verify whether Xihuang Pill and its main active components specifically belong to which type of immunogenic inducer through in vivo and in vitro experiments.

Overall Remodeling of the Tumor Immune Microenvironment by Xihuang Pill

The high-dose Xihuang Pill group can increase serum IL-2 and IFN-γ levels in TNBC-bearing mice by 2.3-fold and 1.9-fold, respectively, while the low-dose group reduces IL-6 and IL-10 levels by 40% and 35%, reversing the drift of Th1 to Th2.[49]

The mechanism is related to inhibiting Treg cell infiltration (reducing the proportion of $Foxp3^+$ cells) and promoting M1 macrophage polarization (increasing iNOS expression). After Xihuang Pill treatment, the infiltration density of $CD3^+$T cells and $CD8^+$CTLs in tumor tissues increases by 1.7-fold and 2.0-fold, respectively, and the $CD4^+/CD8^+$ ratio decreases from 1.8 to 1.2, approaching the normal level. The DC maturation rate ($CD11c^+CD86^+$) increases to 65% (28% in the model group), enhancing antigen presentation ability. β-boswellic acid from frankincense inhibits the NF-κB pathway, reducing TNF-α and IL-1β levels in tumor tissues by more than 50%. Bezoar reduces the risk of DNA damage and mutation by scavenging reactive oxygen species (ROS levels reduced by 38%), inhibiting tumor progression.

Security and Limitations

Based on the pharmacokinetic study of Xihuang Pill,[50] its safety in the treatment of breast cancer is reliably guaranteed. In breast cancer models, the pharmacokinetic parameters (AUC, Cmax) of key components (β-BA, AKBA) showed no statistical difference from those in the normal group, and the clearance rate was stable, indicating that the disease state did not lead to accumulation risk. Targeted distribution reduces non-target toxicity: active components are specifically enriched in immune organs such as the liver and spleen (eg, the concentration of β-BA in the spleen is doubled), which promotes immune activation (related to ICD). At the same time, their distribution in the heart and brain is reduced, optimizing the therapeutic window. The metabolic and excretory pathways are safe: the urinary excretion rate of the prototype drug is low (0.5–2%), with liver metabolism as the dominant pathway. No evidence of excretion disorders or renal injury was found in pathological states, which supports the safety basis for long-term ICD treatment.

Although the safety of Xihuang Pill has provided a basic guarantee for clinical application, the complexity of its multi-component synergistic effects still brings two key problems: first, the ambiguity of action targets. It is necessary to further separate and verify the components to clarify the independent functions and interactive mechanisms of each active component in the regulation of immunogenic cell death (ICD); second, existing studies are mostly limited to in vitro cell experiments and animal models, lacking large-scale and high-quality clinical data support, especially insufficient elaboration on specific upstream and downstream targets in ICD regulation. Therefore, future research needs to systematically combine in vivo and in vitro experiments with clinical trials to comprehensively verify its mechanism of action and clinical efficacy, so as to promote its precise application.

Summary and Outlook

Triple-negative breast cancer (TNBC) lacks effective targeted therapy targets, so chemotherapy remains the main current treatment modality. However, in clinical practice, there are prominent issues such as severe side effects, frequent drug resistance, and poor patient prognosis. As a traditional anti-cancer Chinese medicine, Xihuang Pill has shown significant therapeutic potential in immune regulation and the regulation of immunogenic cell death (ICD). Its pharmacokinetic studies have confirmed that key active components have no accumulation risk in breast cancer models, can be targetedly enriched in immune organs such as the liver and spleen, and have safe metabolic and excretory pathways, providing a reliable guarantee for exerting therapeutic effects through the ICD pathway in the long term.

In the future, it is necessary to focus on conducting large-scale, high-quality clinical trials to systematically verify the specific ICD-related targets and upstream and downstream signaling associations of oral Xihuang Pill in TNBC patients. This research direction is expected to lay a more solid theoretical and clinical foundation for the treatment of TNBC with Xihuang Pill through the ICD pathway, thereby promoting its precise clinical application and secondary development.

Abbreviations

TNBC, Triple-negative breast cancer; ICD, Immunogenic cell death; DAMPs, Damage-associated molecular patterns; HSP, Heat-shock protein; CRT, Calreticulin; HMGB1, High-mobility group box 1; APCs, Antigen-presenting cells; PRR, Pattern recognition receptor; XHP, Xihuang pill; KBA, 11-keto-β-boswellic acid; AKBA, 11-keto-β-acetylboswellic acid; αBA, α-boswellic acid; βBA, β-boswellic acid; AαBA, 3-acetyl-α-boswellic acid; AβBA, 3-acetyl-β-boswellic acid; VEGF, Vascular endothelial growth factor; DCs, Dendritic cells; TLR, Toll-like receptor; COX-2, Cyclooxygenase-2; PTEN, Phosphatase and tensin homolog; AKT, Protein kinase B; NF-κB, Nuclear factor-κB; MAPK, Mitogen-activated protein kinase; mTOR, Mechanistic target of rapamycin; PERK, Protein kinase R-like endoplasmic reticulum kinase; eIF2α, Eukaryotic translation initiation factor 2α; ATF4, Activating transcription factor 4; CHOP, C/EBP homologous protein; IL, Interleukin; IFN-γ, Interferon-γ; Treg, Regulatory T cell; CTL, Cytotoxic T lymphocyte; LPS, Lipopolysaccharide; NLRP3, NOD-like receptor pyrin domain protein 3; GSDMD, Gasdermin D; ROS, Reactive oxygen species; MHC, Major histocompatibility complex; PI3K, Phosphatidylinositol 3-kinase; BAX/BAK, Bcl-2-associated X protein/Bcl-2 homologous antagonist/killer; BCAP31, B cell receptor-associated protein 31; CD, Cluster of differentiation; AR, Androgen receptor; SENCAR, Sensitivity to carcinogenesis; Z-GS, Z-guggulsterone; NSCLC, Non-small cell lung cancer; PD-L1, Programmed death-ligand 1; CYP3A4, Cytochrome P450 3A4; MEOX2, Mesenchyme homeobox 2; Foxp3, Forkhead box P3; iNOS, Inducible nitric oxide synthase; PARP, Poly-ADP ribose polymerase; JNk, c-Jun N-terminal kinase; PCNA, Proliferating cell nuclear antigen; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; caspase, Cysteine aspartate-specific protease; GSH, Glutathione; AUC, Area under the curve; Cmax, Maximum concentration; LD50, Median lethal dose.

Acknowledgments

We thank LetPub (www.letpub.com.cn) for its linguistic assistance during the preparation of this paper.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The author(s) of this paper state that they have received financial assistance for the purpose of research, authorship, and/or publishing. The current research was funded in part by the Tai'an Science and Technology Innovation Development Project (grant no. 2021NS382), the Shandong Province Medical and Health Technology Development Project (grant no. 2019WS406), the National Natural Science Foundation of China (grant no. 82274538), and the Shandong Province Traditional Chinese Medicine Science and Technology Project (grant no. Q-2022108).

Disclosure

There is no conflict of interest.

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