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REVIEW

Saikosaponins Targeting Programmed Cell Death as Anticancer Agents: Mechanisms and Future Perspectives

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Abstract: Saikosaponins (SS), which are major bioactive compounds in Radix Bupleuri, have long been used clinically for multicomponent, multitarget, and multipathway therapeutic strategies. Programmed cell death (PCD) induction is among the multiple mechanisms of SS and mediates the anticancer efficacy of this drug family. Although SS show promise for anticancer therapy, the available data to explain how SS mediate their key anticancer effects through PCD (apoptosis, autophagy, ferroptosis, and pyroptosis) remain limited and piecemeal. This review offers an extensive analysis of the key pathways and mechanisms involved in PCD and explores the importance of SS in cancer. We believe that high-quality clinical trials and a deeper understanding of the pharmacological targets involved in the signalling cascades that govern tumour initiation and progression are needed to facilitate the development of innovative SS-based treatments. Elucidating the specific anticancer pathways activated by SS and further clarifying how comprehensive therapies lead to cross-link among the different types of cell death will inspire the clinical translation of SS as cancer treatments.

Keywords: apoptosis, autophagy, ferroptosis, pyroptosis

Introduction

The incidence of cancer is increasing, and cancer poses a growing and critical public health concern because of its elevated mortality rate.[1] Progress in surgery, chemotherapy, radiation, and, more recently, immunotherapy has led to increases in survival rates in recent years. However, five-year survival rates for various cancers still need to improve, especially in less developed countries. The low quality of life of a notable proportion of cancer survivors following cancer treatment places a heavy burden on both patients and their families.[2,3] Therefore, there is an urgent need to clarify the underlying pathogenesis of cancers and determine how best to administer highly cost-effective and comprehensive treatments.

One therapeutic approach to combat cancer involves inducing cancer cell death. Different types of cell death, such as apoptosis, ferroptosis, autophagy, and pyroptosis, can be categorized on the basis of morphological characteristics, the cellular context, and their stimuli. Cell death is classified into the following main types: accidental cell death (ACD) and programmed cell death (PCD).[4] ACD refers to an uncontrolled cell death process that occurs due to severe mechanical, physical, or chemical damage. PCD, known as regulated cell death, can be influenced by genetic modifications or pharmacological agents.[5] Increasing evidence indicates that PCD subroutines are vital elements associated with tumorigenesis.[6] Malignant cells have developed numerous mechanisms to evade PCD pathways, including cancer metastasis and immune evasion.[7] Gaining insight into these mechanisms is essential for addressing many unanswered questions concerning the conditions of various tumour types that underly these mechanisms. Furthermore, the emergence of small-molecule compounds that target these subroutines represents a promising therapeutic approach that has shown potential in treating various human cancers and possibly reducing the incidence of adverse effects due to targeted therapy or chemotherapy.

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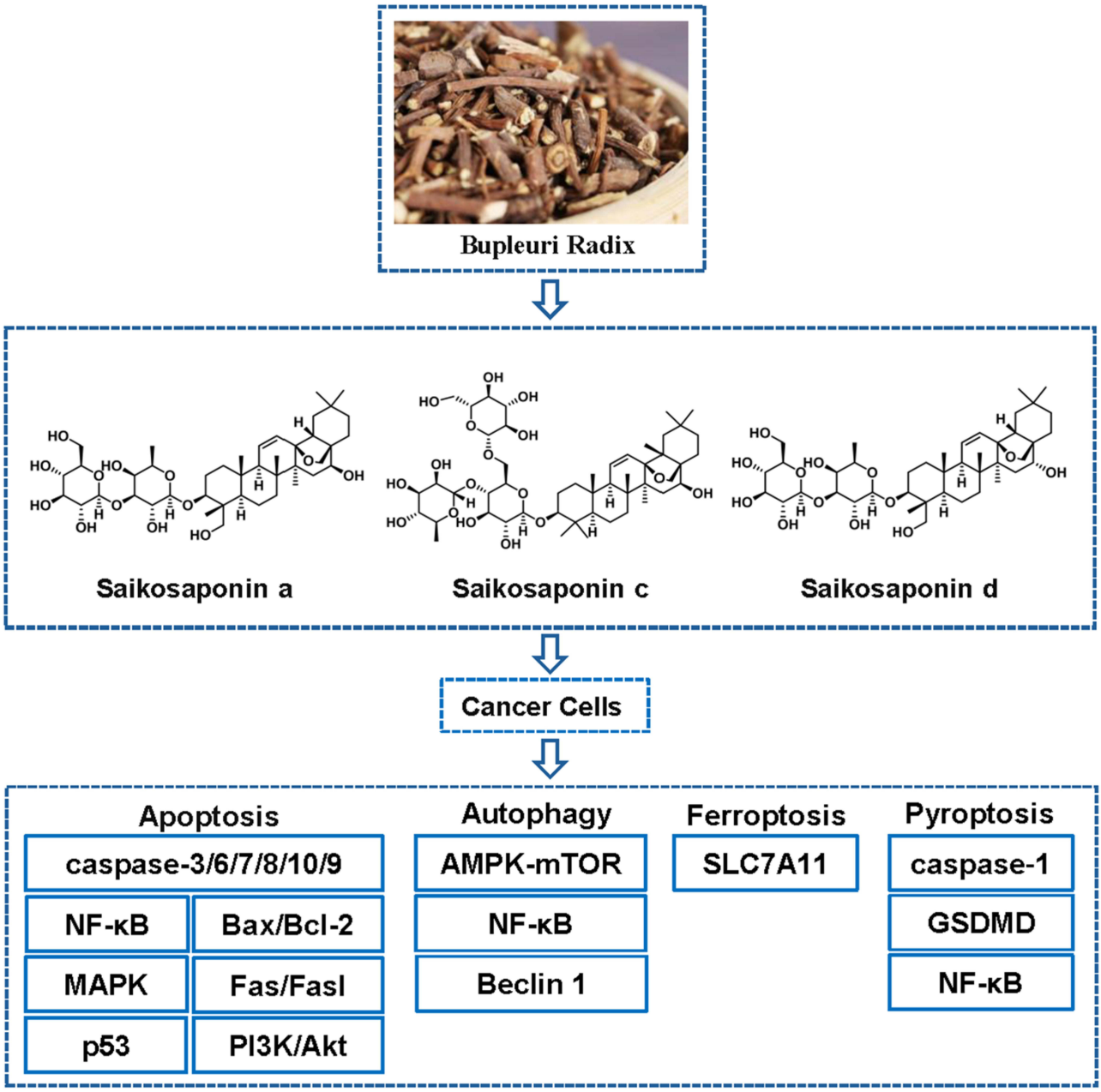
Graphical Abstract



Traditional Chinese Medicine (TCM) is an ancient yet continuously advancing medical practice based on empirical treatments; TCMs include herbal and acupuncture therapies, which are distinguished from other antitumour interventions through their multicomponent, multitarget, and multipathway activity.[8] Radix Bupleuri (RB), which is derived from a species of Bupleurum, is a commonly used herbal treatment with a long history of clinical application for the treatment of fever, chills, inflammation, and tumours.[9] Saikosaponins (SS) are triterpenoid saponins extracted mainly from RB.[10] Each type of SS has unique structural elements, and the main compounds that have been investigated—saikosaponin a (SSa), saikosaponin b (SSb), saikosaponin c (SSc), and saikosaponin d (SSd)—have attracted considerable attention due to their diverse biological activities.[11] The effects of SS against lung carcinoma,[12] prostate cancer,[13] liver cancer,[14]

and breast cancer[15] have been intensively studied, and reports have indicated the promising antitumour potential of SS. Furthermore, PCD is among the versatile mechanisms by which SS induce their anticancer effects.

Although SS show promise for anticancer therapy, the available data on the role of PCD in their key anticancer effects are limited and piecemeal. Therefore, with a focus on four PCD modalities (apoptosis, autophagy, ferroptosis, and pyroptosis), this review comprehensively summarizes the cell death-mediated anticancer effects of SS. A better understanding of cell death mechanisms could lead to the identification of novel targets for RB-derived anticancer compounds with more specific targets and fewer side effects than available treatment.

Apoptosis

Apoptosis has been at the forefront of scientific research for more than five decades since its initial identification as the primary type of PCD in 1972. As a key type of PCD, apoptosis has been thoroughly investigated in various cancers[16] and is acknowledged as the predominant mechanism through which chemotherapeutic agents induce cell death in tumour cells.[17] The apoptosis process is characterized by several features, including shrinkage of the cytoplasm, pyknosis, and blebbing of the plasma membrane, ultimately leading to the creation of apoptotic bodies. These apoptotic bodies are subsequently taken up by neighbouring phagocytic cells and degraded within lysosomes.[18] These hallmark events are crucial indicators used to determine the initiation of the apoptotic pathway in cell death. Overall, apoptosis is an essential intracellular process that plays a pivotal role in maintaining the homeostasis of an organism and regulating cell populations.

Molecular Mechanisms of Apoptosis

Apoptosis occurs predominantly through two well-established pathways: the intrinsic pathway (B-cell lymphoma 2 (BCL-2)-regulated or mitochondrial) and the extrinsic pathway (death receptor).[19] In healthy cells, various antiapoptotic proteins, including BCL-W, BCL-2, BCL-XL, MCL-1, and A1/BFL1, play crucial roles in safeguarding cell survival by inhibiting BAK and BAX, which are crucial mediators of cell death. The apoptosis process begins when BH3-only proteins bind these antiapoptotic proteins, which results in the activation and subsequent release of the effectors BAK and BAX.[20] The intrinsic apoptosis pathway is modulated primarily by BCL-2 family proteins and is engaged when cells suffer irreparable damage to their components. BH3-only molecules display a strong affinity for antiapoptotic BCL-2 molecules, and the binding of BH3-only molecules to BCL-2 frees BAX and BAK.[21] The extrinsic apoptosis pathway is initiated through the binding of death ligands, such as TRAIL, FasL, and TNFα, to their corresponding death receptors (FASR, TNFR1, and TRAIL R1/R2). This binding event leads to the formation of an intracellular complex that promotes cell death, which activates caspase-8, subsequently initiating downstream effectors such as caspases 3 and 7.[6] Furthermore, the intrinsic apoptosis pathway is linked to the death receptor pathway through proteolytic activation mediated by caspase-8.[22] The mitogen-activated protein kinase (MAPK) family consists of three members: c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and p38 MAPK. Increasing evidence has shown that triggering MAPK cascades promotes apoptosis in cells.[23] The intrinsic and extrinsic apoptosis pathways engage in delicate coordination and crosstalk, which leads to a caspase activation cascade. Over the last few decades, new insights into the inner workings of apoptosis pathways have led the way for the creation of specific classes of therapeutics that target the apoptosis pathway.

The Association Between Apoptosis and Cancer

The initial finding that aberrant intrinsic apoptosis can contribute to carcinogenesis came from studies examining BCL2 because this protein was discovered in human follicular lymphomas.[24] In healthy cells harbouring risk-conferring mutations, apoptotic cell death serves as a crucial barrier against carcinogenesis. Evasion from apoptosis not only supports the initiation of tumour formation and maintenance of tumour features but also fosters resistance to therapeutic interventions.[16] Several antiapoptotic proteins, such as BCL-2 and BCL-XL, are frequently overexpressed in cancer cells, which thereby hinders apoptosis and supports the survival of tumour cells. Moreover, the apoptosis process is regulated by a complex array of factors, and the PI3K/Akt/mTOR signalling pathway is crucial for this regulation.[25] Approved drugs such as venetoclax directly target the intrinsic apoptosis pathway, while most therapeutics that induce cell death act

indirectly through inhibitors of growth factor signalling pathways, kinases, mTOR, proteasome components, or histone deacetylases (HDACs).[16] Targeting apoptotic mechanisms in tumour cells is a powerful anticancer approach that leads to improved clinical responses and eliminates the chance of tumour relapse.

SS Induce Apoptosis in Cancer

Apoptosis is the primary mechanism of tumour cell death following treatment with SS. SSd significantly inhibited the growth of HepG2 cells in a dose-dependent manner in vivo, with concentrations of SSd ranging from 5 to 20 μg/mL.[26] A time-dependent increase in expression of the proapoptotic protein Bad and a decrease in expression of the antiapoptotic protein Bcl-2 were noted in vitro.[27] Treatment with SS consistently inhibited the proliferation of lung cancer cells and led to a greater percentage of cells in arrest at G0/G1 phase of the cell cycle, and the observed effects were relative to the administered dose.[28] Furthermore, SS enhanced the interaction between death receptors and their respective ligands, which triggers the caspase 2/8 pathway and activates the death receptor signalling cascade, thereby exerting antitumour effects.[29] SS inhibited cell cycle progression by promoting p53 expression and increasing p21/WAF1 levels. The apoptotic effect induced by SS was attributed to increased levels of APO-1/Fas and its two ligands, membrane-bound Fas ligand (mFasL) and soluble Fas ligand (sFasL), and increased protein levels of Bax.[30]

Currently, SS are being evaluated as ideal sensitizers to chemotherapy and radiotherapy in cancer treatment. In vitro, SSd promoted the accumulation of reactive oxygen species (ROS) in A549 cells, thus increasing sensitivity to cisplatin and triggering apoptosis.[31] In vivo, the administration of SSd (10 mg/kg) via intraperitoneal injection every other day into a HSVtk/Hep3B xenograft tumour mouse model reduced tumour growth and improved sensitivity to HSVtk/GCV.[32] Additionally, treatment with 2–15 μM SSd inhibited Hep3B cell viability, invasion, and migration, enhancing HSVtk/GCV-induced apoptosis by increasing SUMO-specific protease 5 expression and inhibiting Gli1 SUMOylation under hypoxic conditions, in vitro in a concentration- and time-dependent manner.[32] Furthermore, the combination of radiation and SSd (1–3 μg/mL) treatment had a time- and concentration-dependent synergistic effect on apoptosis and inhibited the growth of SMC-7721 cells, potentially via a mechanism involving p53 pathway activation.[33] Treatment with SSd sensitized cisplatin-resistant ovarian cancer cells, resulting in mitochondrial fragmentation and cell cycle arrest, irrespective of p53 status.[34] Overall, these findings indicate that SSd effectively decreases resistance to various chemotherapeutic agents, presenting a substantial opportunity to enhance treatment outcomes for cancer patients.

Autophagy

Autophagy is an essential phagocytic biological mechanism that degrades detrimental proteins or organelles via lysosomal fusion, and this process plays a vital role in sustaining cellular functions and maintaining homeostasis. Excess or damaged organelles and proteins are efficiently engulfed through autophagy, during which double-membraned autophagic vacuoles (autophagosomes) are formed.[35] Autophagy can be divided into three specific subtypes: microautophagy, macroautophagy, and chaperone-mediated autophagy.[36] Although each subtype has unique characteristics, they all converge at a common endpoint, the lysosome, where intracellular cargos are degraded and intracellular contents are recycled. Although autophagy serves a housekeeping function in the absence of stress, it can be activated by a variety of stressors, including protein or organelle damage, nutrient deprivation, oxidative stress, hypoxia, or pathogenic infection.[37] Autophagy is crucial for both cellular physiology and pathology, particularly in the context of malignant tumour development. The excessive promotion of autophagy can result in autophagic cell death,[23,24] also referred to as type II PCD.[38]

Molecular Mechanisms of Autophagy

Autophagy, a highly intricate and tightly regulated cellular process, involves various signalling pathways and autophagy-related genes that guide the initiation, elongation, maturation, and degradation phases. Central to this regulation is the mammalian target of rapamycin (mTOR) protein, which has a substantial influence on the functional dynamics of autophagy. mTOR activity is primarily modulated by two upstream negative regulators: the p53 and AMPK signalling pathways. Under conditions of cellular nutrient deficiency, AMPK and p53 protein activation promotes autophagy by inhibiting mTOR.[39] In contrast, in well-nourished cells, the suppression of AMPK and p53 expression leads to mTOR

activation, thereby inhibiting autophagy.[40] The elongation phase of autophagy is largely facilitated by a complex system of ubiquitination events that involve processes such as ATG12 binding and light chain 3 (LC3) modification. Typically found in the cytoplasm as LC3-I, LC3 undergoes a transformation into its membrane-bound form, LC3-II, during autophagy. This transformation results in the localization of LC3-II to both the membrane of the autophagosome and the outer autophagosome membrane. Consequently, assessing the expression levels of LC3-II through immunoblotting can provide insights into the quantity of autophagosomes present.[41] Moreover, p62/sequestosome-1 (SQSTM1) serves as another crucial factor in the regulation of autophagy and can directly interact with LC3 to facilitate the formation of autophagosomes. Under normal autophagic conditions, p62/SQSTM1 is degraded. However, when autophagy is absent or impaired, p62 accumulates, indicating disruption of the autophagic process.[42,43] Autophagy also plays dual roles in the occurrence and development of tumours.[44]

The Association Between Autophagy and Cancer

The involvement of autophagy in cancer is complex and often debated, as autophagy has dual functions in tumour development and progression, which vary on the basis of the tumour type and stage and genetic alterations present in the tumour.[45] In the precancerous stage, inhibiting autophagy can lead to increased levels of ROS and genomic damage, which subsequently exacerbate endoplasmic reticulum stress (ERS) and DNA damage, ultimately fostering tumour growth. Conversely, under conditions such as oxidative stress or starvation, autophagy can supply nutrients and energy to tumours, supporting cancer cell survival.[45,46] The autophagy process is carefully regulated by a group of autophagy-related genes, including unc51-like autophagy-activating kinase 1 (ULK1), Beclin-1, p62, LC3, and forkhead box O (FoxO), among which ULK1 acts as a promoter and initiator of autophagy.[47] Key signalling pathways, such as the phosphatidylinositol 3-kinase complex 1 (PI3KC1)-protein kinase B (PKB)-mammalian target of rapamycin complex 1 (mTORC1), nuclear factor kappa-B (NF-κB), and Ras-Raf-MAPK pathways, are vital for modulating autophagy and have an impact on tumour progression as well as metastasis. Additionally, the autophagy pathway is linked to pivotal regulatory proteins involved in carcinogenesis, including phosphatase and tensin homologue (PTEN), the tumour suppressor gene p53, the proto-oncogene Bcl-2, and death-associated protein kinase.[48,49] Despite its adaptive and protective roles, autophagy can also induce cancer cell death both in vivo and in vitro.[50] Autophagy promotes autophagic cell death, even eliminating cancer cell resistance to apoptosis during chemotherapy. By removing cytotoxic substances, cells with genomic mutations, and damaged proteins, autophagy serves as a protective mechanism against carcinogenesis.[51]

SS Induce Autophagy in Cancer

Drug resistance remains the principal obstacle to successful cancer therapy, particularly as cancer cells often develop resistance to apoptosis following prolonged chemotherapeutic treatments.[52] In this context, inducing autophagic cell death in tumour cells that are either defective or resistant to apoptosis is a promising alternative strategy for tumour suppression.[53] Consequently, the application of small-molecule autophagic inducers has gained traction in the treatment of various cancers, such as lung, breast, pharyngeal, cervical, and rectal cancers, especially those that are resistant to apoptosis.[54] Caspase-3/-8/-9 activity is essential for drug-induced apoptosis.[55] Intriguingly, even in the absence of Bax/Bak and caspase-3/-7/-8 gene expression, treatment with SSd can initiate caspase-independent cell death through autophagy. These results underscore the potential of SSd as a treatment option for cancers that are resistant to apoptosis.[56] SSd promotes autophagic cell death and autophagy in cells defective in apoptosis by directly inhibiting the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase pump (SERCA) and mTOR, leading to disrupted calcium balance and the activation of ERS.[56] Research on the impact of SSd on cells from autosomal dominant polycystic kidney disease (ADPKD) has indicated that SSd reduces cell growth by promoting autophagy, increasing intracellular Ca^{2+} concentrations, and stimulating the CaMKK-AMPK signalling pathway. These effects also inhibit mTOR signalling and facilitate autophagy.[57] Furthermore, SSd increases the responsiveness of gastric cancer cells to cisplatin, resulting in reduced proliferation and cell migration, the promotion of autophagy, and the induction of apoptosis.[58]

Ferroptosis

Ferroptosis is a newly identified form of oxidative and nonapoptotic PCD. In 2012, the term "ferroptosis" was introduced following a screen for small-molecule compounds that might inhibit the proliferation of RAS-mutant cancer cells.[59] The fundamental concept of ferroptosis likely originated from observations of tumour cell mortality induced by nutrient shortages, especially the depletion of cysteine.[60] The ultimate trigger for ferroptosis is excessive lipid peroxidation, which leads to catastrophic cellular failure. Although ferroptosis and what was once commonly referred to as oxidative stress-induced cell death share several characteristics, ferroptosis possesses distinct attributes that set it apart as a separate cell death pathway. Ferroptosis contrasts with other modes of cell death, such as autophagy and apoptosis, in terms of genetics, morphology, and its molecular mechanisms. Cells undergoing ferroptosis display unique bioenergetic and morphological characteristics. Morphologically, the ferroptosis process involves the absence of cell membrane rupture, plasma membrane blistering, increased mitochondrial membrane density, mitochondrial shrinkage, a reduction in the number of mitochondrial cristae or their disappearance, and a normal nuclear size with uncondensed chromatin. Recent research suggests that factors that initiate lipid peroxidation and trigger ferroptosis include DNA stress, metabolic reprogramming, and ROS production mediated by mitochondria.[61–63]

Molecular Mechanisms of Ferroptosis

Ferroptosis can be triggered via two primary pathways: the extrinsic pathway (transporter-dependent), which involves factors such as decreased glutamine or cysteine and increased iron absorption, and the intrinsic pathway (enzyme-regulated), in which the suppression of glutathione peroxidase 4 (GPX4) plays a central role. The occurrence of ferroptosis is fuelled primarily by an imbalance between antioxidants and oxidants. This imbalance is driven by the abnormal expression and function of diverse redox-active enzymes that are responsible for the generation and detoxification of free radicals, as well as the byproducts of lipid peroxidation. Consequently, the regulation of ferroptosis is complex and involves various levels of control at the posttranscriptional, posttranslational, and epigenetic levels.[64] The molecular elements involved in ferroptosis, such as LPCAT3 and ACSL4, which create membrane lipids that are prone to peroxidation, have been identified.[65] One crucial component that acts as a cystine/glutamate translocator in this process is solute carrier family 7 member 11 (SLC7A11). SLC7A11 is essential for the import of cystine into the cell from the outside environment, which promotes the production of glutathione (GSH) to support GPX4 function.[66,67] Ferroptosis inducers such as erastin contribute to the inhibition of SLC7A11, which leads to the depletion of GSH within the cell, subsequently diminishing GPX4 activity. Any issues that lead to a decrease in GSH may result in the accumulation of lipid ROS, damaging the lipid membrane or proteins and ultimately resulting in ferroptosis.[68,69] Therefore, GSH depletion and decreased GPX4 activity are fundamental to the mechanisms underlying ferroptosis.[70]

The Association Between Ferroptosis and Cancer

Ferroptosis, a concept described less than a decade ago, represents a noteworthy potential strategy for cancer treatment.[59] The link between ferroptosis and cancer was initially established following the groundbreaking identification of chemical compounds that induce ferroptosis, which was driven by the search for innovative cancer therapeutic compounds.[71] Indeed, ferroptosis was first noted in a human foreskin fibroblast line expressing oncogenic Ras during the compound screening of RAS-mutated cancer cells. One study identified agents that trigger ferroptosis, including erastin and Ras-selective lethal small molecule 3 (RSL 3).[72] Recent studies indicate that ferroptosis could play an adaptive role that is essential for the elimination of carcinogenic cells.[73] Because they are generally more metabolically active with higher levels of ROS, cancer cells might exhibit a greater propensity for ferroptosis. However, cancer cells can also acquire additional genetic or epigenetic alterations to counter the induction of ferroptosis by metabolic and oxidative stress. These adaptations might involve increased SLC7A11 expression or upregulation of the antioxidative transcription factor NRF2.[74] Consequently, the sensitivity or resistance of a particular type of cancer to the induction of ferroptosis hinges on its distinct genetic configuration. While the molecular mechanisms involved in ferroptosis in cancer are still the subject of ongoing research, two major tumour suppressor proteins, BAP1 and p53, play crucial roles as ferroptosis effectors.[75]

Furthermore, ferroptosis is closely associated with the resistance of certain cancers to various forms of treatment. Compared with tumour cells with an epithelial phenotype, tumour cells with a mesenchymal phenotype tend to be more resistant to multiple cancer therapies; however, these tumour cells are particularly vulnerable to ferroptosis-inducing compounds. Moreover, pretreatment of cancer cells with agents that trigger ferroptosis can increase the sensitivity of these cells to later immunotherapy.[76] Collectively, these results underscore the potential of targeting ferroptosis as an innovative approach for cancer treatment.[77]

SS Induce/Inhibit Ferroptosis in Disease

An increasing number of studies indicate that ferroptosis and ERS are closely related.[78] Inducers of ferroptosis can activate ERS through the unfolded protein response (UPR) in cellular environments.[79] A previous study demonstrated that SSa affects glutathione metabolism and suppresses the expression of SLC7A11, which induces ferroptosis. SSa activates the PERK/eIF2a/ATF4 signalling pathway, resulting in ERS in hepatoma cells; mechanistically, ferroptosis mediated by ATF3 is essential for the antitumour effects of SSa.[14] Research indicates that the classic oxidative stress pathway serves as a critical element in the initiation of ferroptosis,[80] and it seems logical to propose that cancer cells may have a greater tendency to undergo ferroptosis than noncancerous cells do because of the enhanced metabolic functions and elevated ROS levels of cancer cells.[81] In addition, cancer cells often have increased iron demand, which may further sensitize them to ferroptosis.[82]

Similarly, ferroptosis might also have adapted to support cell survival. Mounting evidence suggests that SS alleviate inflammatory damage to cells through antioxidative stress and ferroptosis mediated through anti-inflammatory mechanisms. One study revealed that SSb2 treatment inhibited ferroptosis via the TLR4/NF-κB signalling pathway in a GPX4-dependent manner. This intervention supported the maintenance of calcium homeostasis, alleviated ERS, and reduced central neuroinflammation.[14,83] Furthermore, SSa was found to lower the concentrations of inflammatory markers, including TNF-α, MPO, and IL-1β, and increase the levels of GPX4 and GSH to alleviate pathological damage to mammary tissue.[84] SSd alleviated acute liver injury resulting from CCl4-induced hepatitis, potentially by curtailing oxidative stress and inhibiting activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, in a cellular model.[85] SSa decreased the levels of iNOS, ROS, and TNF-α and COX-2 and IL-8 expression in human umbilical vein endothelial cells (HUVECs) stimulated with lipopolysaccharide (LPS), consequently inhibiting the inflammatory and oxidative responses triggered by LPS.[86] In vitro observations revealed that SSd increased the inner diameter of C2C12 myotubes, diminished oxidative stress, and elevated the expression of proteins such as p-mTOR, Nrf2, p-AKT, HO-1, and p70S6K.[87] Furthermore, AMPK is recognized as a therapeutic target for various diseases and is involved in processes linked to oxidative stress.[88] One enrichment analysis revealed a notable connection between the targets of RB and the AMPK pathway, implying that RB might exert its effects through the modulation of AMPK pathway function.[89]

Pyroptosis

In 2018, the Nomenclature Committee on Cell Death made an important update to the classification of cellular death mechanisms by reclassifying pyroptosis as a specific type of programmed inflammatory death that is primarily mediated by the gasdermin protein family.[90] Pyroptosis is characterized by a series of cellular changes. Initially, cells undergoing pyroptosis begin to flatten and form protrusions resembling apoptotic bodies that are typically 1–5μm in size; these protrusions are termed focal dead bodies and are observable through time-lapse electron microscopy. Following this initial phase, cell swelling occurs, associated with the creation of pores in the plasma membrane and eventual membrane rupture.[91] These events facilitate the release of intracellular substances, including IL-18 and IL-1β, thereby inducing an inflammatory reaction.[92] Morphologically, pyroptosis is characterized by DNA fragmentation, chromatin condensation, and caspases-3/-7 activation, similar to apoptosis.[90] However, a key distinction between pyroptosis and apoptosis lies in the fact that in pyroptotic cells, the nuclei remain intact and exhibit early-stage positive TUNEL staining.[93]

Molecular Mechanisms of Pyroptosis

Inflammasomes play a vital role in pyroptosis, which can occur through two distinct pathways: the canonical pathway and the noncanonical pathway. The canonical pathway is characterized by its dependence on caspase-1 activation, whereas the noncanonical pathway involves the activity of caspase-4, caspase-5, and caspase-11.[94] The initiation of the canonical pyroptosis pathway starts when cytoplasmic pattern recognition receptors (PRRs) detect damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs). Upon recognizing specific stimuli, nod-like receptors (NLRs), or absent in melanoma 2-like receptors (ALRs), start assembling to form inflammasomes, leading to the activation of caspase-1. Specifically, caspase-1 enzymatically cleaves pro-IL-18 and pro-IL-1β, transforming them into the active inflammatory cytokines IL-18 and IL-1β, respectively.[95] Moreover, the noncanonical pyroptosis pathway relies on the activation of caspase-11, caspase-4, and caspase-5. Upon stimulation by LPS, these caspases can directly interact with the conserved structure of LPS, specifically lipoprotein A. This interaction promotes caspase oligomerization, leading to subsequent activation. This mechanism results in the cleavage of GSDMD and the movement of its N-terminus to the cell membrane, facilitating pore formation.[96] Traditionally, granzymes were understood to primarily induce apoptosis by activating caspase-3 or its substrates.[97] Nevertheless, recent research has indicated that the activation of proapoptotic caspase-3 may also induce pyroptosis through the cleavage of GSDME.[98] Compared to apoptosis, pyroptosis occurs more swiftly and earlier, and unlike apoptosis, pyroptosis is often associated with the release of numerous proinflammatory factors. Along with GSDM family proteins, inflammatory bodies serve as the primary substrates that facilitate cell membrane disruption and trigger the ensuing inflammatory response.[99]

The Association Between Pyroptosis and Cancer

Chronic inflammation has a profound impact on every stage of carcinogenesis, with prolonged exposure to inflammatory conditions increasing the risk of tumour development.[92,100] Pyroptosis, a form of lytic cell death, plays a crucial role in this scenario by promoting the secretion of mature IL-18 and IL-1, which may affect cancer progression.[101] However, pyroptosis is also a dual regulator of tumour development and progression. On the one hand, pyroptosis leads to the release of inflammatory bodies that can hinder tumour cell proliferation and metastasis. This effect is primarily mediated by NLRP3 inflammatory bodies, which secrete inflammatory cytokines that impede tumorigenesis and thereby act as a defence mechanism against cancer. In contrast, the accumulation of inflammatory bodies in the tumour microenvironment can promote tumour growth. Pyroptosis-induced changes in tumour cells also alter the immune landscape within the tumour, resulting in robust antitumour immune responses.[102]

Immune checkpoint regulators such as PD-1 and PD-L1 are essential components of many cancer treatments. The combination of pyroptosis induction with PD-L1 treatment can more effectively suppress tumour growth than can either approach alone.[103] PD-L1 interacts with p-stat3, which subsequently translocates into the nucleus and increases GSDMC expression, leading to hypoxic stress-induced pyroptosis. These cytokines induce not only pyroptosis but also apoptosis and necroptosis, a process that is referred to as PANoptosis.[104–107] In conclusion, innovative pyroptosis-related cancer therapeutic strategies might show promise, as they can overcome chemotherapeutic drug resistance and elicit an anticancer immune response.

SS Induce Pyroptosis in Cancer

Pyroptosis is a distinct form of PCD that is driven primarily by the activation of inflammatory caspases, particularly caspase-1, caspase-4, caspase-5, and caspase-11.[108] Therefore, the importance of promoting pyroptosis lies not only in enhancing the cytotoxic effects of chemical drugs on tumour cells but also in utilizing pyroptosis as a potent mechanism of inflammatory cell death. Pyroptosis disrupts the membrane of tumour cells, leading to the release of cytokines and cellular components into the surrounding environment. This, in turn, promotes the attraction of immune cells and stimulates antitumour immune responses within the tumour microenvironment, which has the potential to significantly enhance cancer treatment.[103] SSd can induce balloon-like swelling accompanied by DNA damage through increased activity of the NF-κB/NLRP3/caspase-1/GSDMD pathway and ROS accumulation, ultimately leading to pyroptosis in lung cancer cells. These findings suggest that targeting pyroptosis could serve as a promising approach in cancer

therapy.[109] In addition to their involvement in inducing pyroptosis via chemotherapeutic agents across various cancer types, Chinese medicinal herbs and their bioactive compounds are regarded as antitumour agents that regulate pyroptosis under different conditions.

Conclusions and Perspectives

The "death signal" is likely not an isolated stimulus; rather, cell death is likely the result of a disruption in the delicate balance between anti-death and pro-death signals. Research into the complex regulatory systems that govern cell death offers a foundational understanding for developing innovative pharmaceuticals and formulating effective approaches for cancer treatment that either encourage or inhibit cell death.[94,110] Cell death pathways, including those involved in apoptosis, pyroptosis, necroptosis, and other types of PCD, are interconnected and cross-linked[111] To this end, we have summarized the mechanisms of SS in these death pathways and constructed a molecular pathway diagram (Figure 1). There are several scenarios in which autophagy indirectly leads to cell death by triggering alternative types of cell death. When stimulated and at elevated levels, autophagy can shift towards apoptosis, beyond which repair mechanisms prove

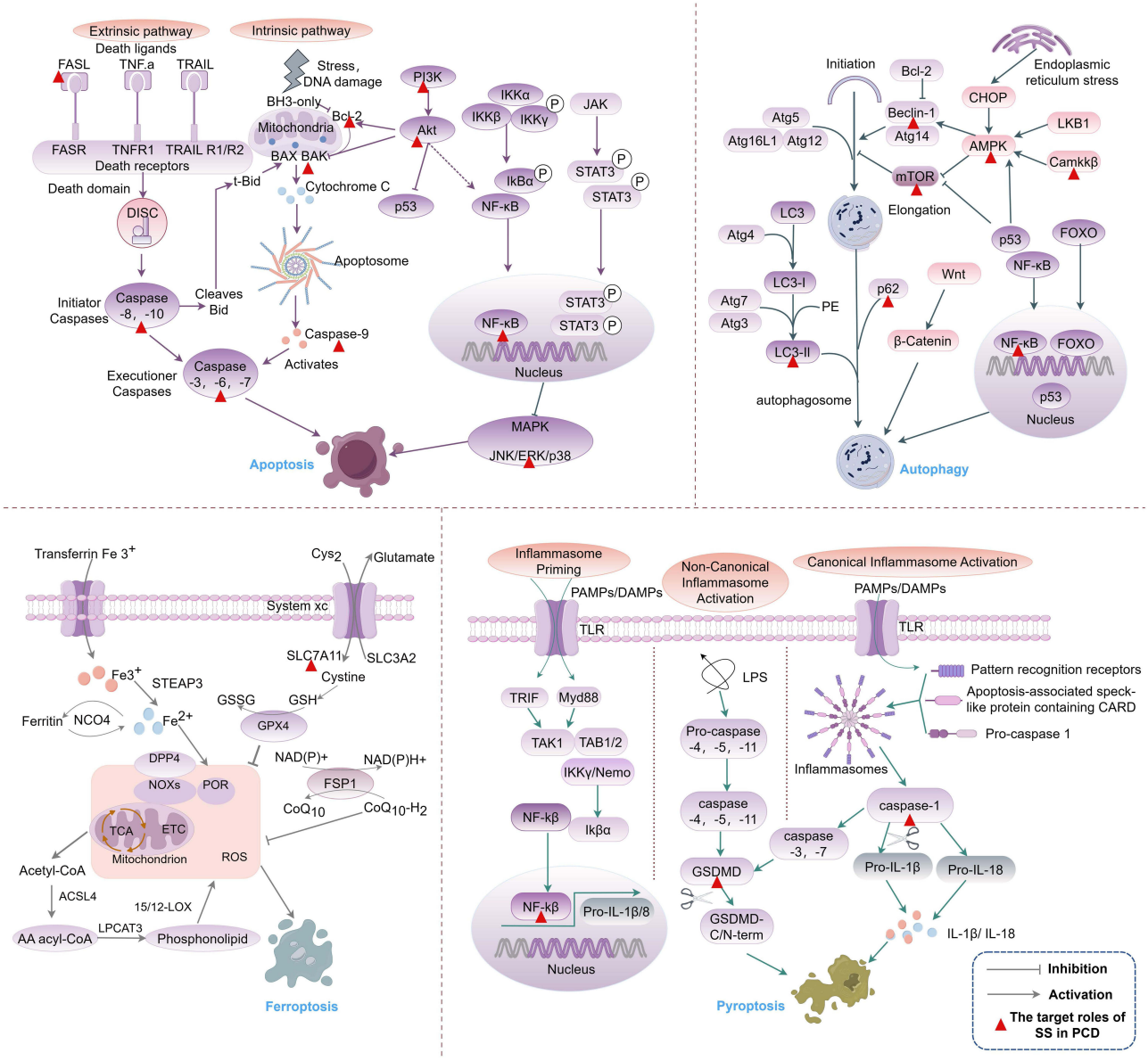


Figure 1 Anticancer mechanism of programmed cell death and the role of saikosaponins (Created by Figdraw).

futile.[112] The interaction among caspase family proteins influences both autophagy and apoptosis. For example, following treatment with radiation and stress signals (SSd), the level of Beclin-1 expression increased in MHCC-97L hepatoma cells, whereas the level of the apoptosis-related protein cleaved caspase-3 was significantly elevated in the group that received the combined treatment compared with the control group.[39] In addition to inducing ferroptosis as the primary cell death process, the absence of GPX4 may also lead to pyroptosis, apoptosis, parthanatos, or necroptosis in certain cells.[113,114] Additionally, some oxysterols, when administered at cytotoxic levels across multiple cell types from diverse species, can initiate oxiapoptophagy–a hybrid process that combines oxidative stress, apoptosis, and autophagy.[115] Oxysterols facilitate caspase-mediated cell death via both the intrinsic and extrinsic apoptosis pathways. The compounds increase the expression of inflammatory factors by generating ROS associated with increased oxidative stress and increase the LC-3II/I ratio during autophagy by modulating the Akt-mTOR signalling pathway.[116,117] Perturbations introduced by different key participants in apoptosis and autophagy can disrupt this delicate balance, shifting the cellular environment from cell generation to death or regulating the transition from a proinflammatory to an anti-inflammatory state. The genes involved play paradoxical roles and undergo crosstalk, so further investigation is needed; the pathways shared by these genes highlight the intricacies of cell death mechanisms. Numerous molecules responsible for mediating transitions between these death processes remain to be discovered and applied to therapeutic development.

In recent years, TCM has garnered increasing acceptance worldwide, and TCM is acknowledged as an abundant source for drug discovery. Additionally, TCM is extensively utilized to alleviate the adverse side effects of chemotherapy and enhance the effectiveness of standard cancer treatments. Despite its general safety, RB has demonstrated safety problems in the clinic, particularly concerning dosage and the duration of administration. Notably, following the oral intake of RB extract, its primary constituents–SSa, SSd, and SSc–are detected in plasma at levels that are considerably lower than those used in current in vitro research, raising concerns about the biological safety of high doses of RB extract.

SS, which are generally safe with low toxicity, have emerged as ideal candidates for enhancing the activity of anticancer drugs. The comprehensive application of SS with other therapies for the sensitization of cancer cells to chemotherapeutics is recognized as a potential strategy against chemoresistance. Owing to their diverse therapeutic potential, SS have gained substantial clinical attention, necessitating further rigorous research. However, the examination of SS in a nontherapeutic context for pharmacological interventions remains notably limited, especially concerning targeted PCD therapies, where clinical studies are nearly nonexistent. This deficiency not only hampers clinical application but also emphasizes the need for prompt action. It is crucial to determine whether a dose that triggers the observed PCD modes in vitro is achievable in vivo. Although numerous laboratory investigations have demonstrated the efficacy of SS in cancer therapy via apoptotic (summarized in Table 1) and nonapoptotic (summarized in Table 2) PCD, in vivo studies are insufficient. Among the various subtypes of SS, each may exhibit distinct variations in biological activity due to differences in molecular structure. To address these issues, future studies should include a wider array of dosage groups and evaluations of hepatorenal function within PCD models to ascertain the maximum safe dose for each SS subtype and to increase the potential of each SS subtype for clinical application. An essential strategy for tailored clinical application and efficient drug development involves the integration of different SS subtypes within a unified study, linking their pharmacological distinctions to their structural differences. Therefore, experiments focused on the interactions between protein targets and SS are necessary. The reasons underlying variations in cell death modalities across different experimental conditions need to be investigated.

In summary, the results of this study indicate that SS function as anticancer compounds and have a range of pharmacological characteristics, including antioxidant and anti-inflammatory effects and their ability to promote apoptosis and autophagy.[150,151] However, the translational value of SS is unclear due to limitations in study design, experimental bias, and insufficient replicability. These concerns stem from the nonphysiological concentrations used in in vitro studies and inconsistent data on the safety and effectiveness of SS as anticancer agents. High-quality clinical trials and a deeper understanding of the pharmacological targets involved in the signalling cascades that govern tumour initiation and progression are needed to facilitate the development of innovative SS-based treatments. For the above purpose, this systematic review has summarized the roles of SS in activating PCD mechanisms and explored potential crosslinking between PCD pathways. Therefore, systemic evaluation of SS metabolites to determine their PCD

Table 1 Apoptotic Effects Induced by SS in Cancers

Reagent	Cancer type	Experimental subjects		Pathway	Reference
		In vivo	In vitro		
Total Bupleurum saponin extract	Colon cancer	/	SW620 and SW480 cells	Bax/Bcl-2/caspase-3/ caspase-9	[118]
Saikosaponin a	Bladder cancer	5637 cell-derived tumour xenografts in athymic nude mice	T24 and 5637 cells	Bax/Bcl-2/caspase-3/ caspase-9	[13]
	Breast cancer		MCF-7 and MDA-MB -231 cells	Caspase-3	[119]
	Cervical cancer	HeLa cell-derived xenograft tumours in nude mice	HeLa cells	PI3K/Akt/Caspase-3	[120]
	Colon carcinoma	BALB/c nude mice bearing LoVo or SW480 colon carcinoma cells	LoVo, HT29, SW480, and SW620 cells	Caspase-4/-3/-8/-2	[121]
		/	HCT116, LoVo, SW48, and SW480 cells	Caspase-2/-8	[29]
	Gastric cancer	/	AGS, HGC-27 and MKN-28 cells	PI3K/Akt	[122]
	Neuroblastoma	/	SK-N-AS cells	Caspase-7/caspase-9/ Bax/Bcl-2/PARP	[123]
	Pancreatic cancer	/	MIA PaCa-2 and BxPC-3 cells	Caspase-3	[124]
Saikosaponin d	Brain cancer	3xTg mice		NF-κB	[125]
		/	PC12 cells	Caspase-9/-3	[126]
		/	PC12 cells	Caspase-3/MAPK.	[127]
		/	U87 cells	PI3K/Akt and ERK	[128]
	Breast cancer	/	HCC1937 cells	Caspase 3/PARP	[15]
		/	MDA-MB-231 cells	p38/MAPK	[129]
	Cervical cancer	/	HeLa cells	NF-κB	[130]
		/	HeLa cells	Caspase-3	[31]
	Colorectal cancer	Model of lung metastasis in BALB/c mice	CT26 and HCT116 cells	Caspase-9/-3/-8/ MAPK.	[131]
	Endometrial cancer	/	Ishikawa cells	MAPK	[132]
	Kidney cancer	/	769-P and 786-O cells	p38/MAPK	[133]
		LPS-treated mice		Caspase-3/Bax/Bcl-2	[134]

(*Continued*)

Table 1 (Continued).

Reagent	Cancer type	Experimental subjects		Pathway	Reference
		In vivo	In vitro		
	Liver Cancer	/	Hep3B cells	Caspase-3	[135]
		/	HepG2 cells	Caspases-3/-7	[26]
		/	HepG2 cells	NF-κB	[130]
		/	HepG2 and Hep3B cells	Bax/Bcl-2/NF-κB	[30]
		/	SMMC-7721 and HepG2 cells	Bcl2	[33]
		/	SMMC-7721 and HepG2 cells	Bcl-2	[27]
		/	SMMC-7721 and MHCC97L	Caspase-3/PARP	[39]
	Lung cancer	/	A549 cells	Fas/Fasl	[28]
		/	A549 cells	JNK	[136]
		/	A549 and H1299 cells	STAT3/caspase-3	[12]
		HCC827/GR cell-derived xenograft tumours in nude mice	HCC827 and HCC827/GR cells	Caspase-3/STAT3/Bcl-2	[137]
	Melanoma	/	A375.S2 cells	JNK/p38/Caspase-9	[138]
	Osteosarcoma	/	143B and MG-63 cells	Bax/caspase-3	[139]
		/	U2 cells	Akt and ERK	[140]
	Pancreatic cancer	/	BxPC3 cells	Caspases-3/-9/JNK	[141]
		/	DU145 cells	Caspase-3/Bax/Bcl-2	[142]
		/	Panc-1 cells	Akt/Bax/Bcl-2/caspase-2	[143]
	Skin cancer	/	HSC-1 cells	Caspase 3/7/MAPK	[144]
	Thyroid carcinoma	Xenograft tumorigenesis model	ARO, 8305C, SW1736 cells	Bax/Bcl-2	[145]
Saikosaponin b4	Colon cancer	/	SW480 and SW620 cells	Caspases-3/-9/Bax	[146]

modalities is necessary and will be vital not only for understanding the therapeutic efficacy of SS but also for discovering SS derivatives as prospective drug candidates.

Future genomic and systems biology approaches, coupled with studies on cell signalling networks, will provide insights into whether specific molecular signatures or pathways facilitate or hinder particular modes of PCD. Elucidating the specific anticancer pathways activated by SS and understanding how comprehensive therapies cause a switch between different cell death processes will inspire clinical translation to improve cancer treatments.

Table 2 Nonapoptotic PCD Induced by SS in Cancers

Cell death pathway	Reagent	Cancer type	Experimental subjects	Target	Reference
Autophagy	Saikosaponin a	Prostate cancer	PC-3, LNCaP, and DU145 cells	Akt-mTOR	[147]
	Saikosaponin d	Breast cancer	MCF-7 cells	CaMKKβ-AMPK-mTOR	[56]
		Cervical cancer	HeLa cells	CaMKKβ-AMPK-mTOR	[56]
		Colorectal cancer	CT26 and HCT116 cells	L3II/p62	[131]
		Gastric cancer	SGC-7901 cells	NF-κB	[58]
		Glioblastoma multiforme	RG-2, U87-MG, and U251 cells	Beclin 1	[148]
		Kidney cancer	UCL93 and OX161 cells	CaMKKβ-AMPK-mTOR	[57]
		Liver cancer	MHCC97L and SMMC-7721 cells	mTOR	[39]
			MHCC97L and SMMC-7721 cells	mTOR	[149]
Ferroptosis	Saikosaponin a	Liver cancer	HepG2 and Huh-7 cells	SLC7A11	[14]
Pyroptosis	Saikosaponin d	Lung cancer	HCC827 and A549 cells	NF-κB/NLRP3/caspase-1/GSDMD	[109]

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Disclosure

All the authors declare that there are no conflicts of interest in this work.

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