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REVIEW ARTICLE

Morphological changes in intracellular small organelles during paraptosis and their relationship with diseases: A review

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Abstract

Paraptosis, a distinct form of programmed cell death, has attracted significant attention in cell biology due to its unique characteristics and potential therapeutic implications. Unlike classical apoptosis or necrosis, paraptosis is induced by specific stimuli and is marked by cell swelling, organelle distension, and the absence of nuclear condensation. This review explores the morphological changes in intracellular small organelles during paraptosis, including mitochondria, endoplasmic reticulum (ER), Golgi apparatus, lysosomes, and autophagosomes. Mitochondria undergo swelling and cristae loss, which impair adenosine triphosphate production and disrupt calcium homeostasis. The ER expands and experiences calcium ion imbalance, triggering ER stress and the unfolded protein response. The Golgi apparatus undergoes vasicularization and structural disassembly, impacting protein glycosylation and secretion. Lysosomal membrane instability leads to the release of acidic hydrolases, exacerbating cellular damage, while autophagosome formation is characterized by the development of double-membrane vesicles and their fusion with lysosomes. These organelle-specific changes are tightly regulated by complex intracellular signaling pathways and provide valuable insights into the mechanisms underlying paraptosis. Understanding these processes offers a theoretical foundation for developing novel therapeutic strategies targeting diseases characterized by dysregulated cell death. In tumor therapy, paraptosis, as a form of immunogenic cell death, can overcome tumor cell resistance to traditional apoptosis-inducing drugs and enhance the efficacy of immunotherapy. In neurodegenerative diseases, mild paraptosis is linked to tumorigenesis, whereas severe paraptosis is associated with neurodegenerative diseases such as Alzheimer's disease. The mechanisms of action and potential therapeutic value of paraptosis in these disease contexts continue to be actively investigated.

Keywords: Paraptosis; Organelle morphological changes; Endoplasmic reticulum stress; Golgi apparatus dysfunction; Intracellular signaling pathways; Tumor therapy; Diseases

1. Introduction

Paraptosis, a distinctive form of programmed cell death, plays a critical role in various physiological and pathological processes, including tumor development.[1] Unlike classical apoptosis or necrosis, paraptosis is triggered by diverse factors, such as metabolic and

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oxidative stress, which activate specific signaling pathways that ultimately disrupt intracellular homeostasis and lead to cell death.[2]

Morphological changes in intracellular small organelles represent not only key observational hallmarks of paraptosis but also critical components for understanding its underlying mechanisms. For instance, mitochondrial swelling and dysfunction are pivotal events that initiate paraptosis,[3] whereas expansion of the endoplasmic reticulum (ER) is closely associated with intracellular calcium ion (Ca^{2+}) dysregulation, further influencing cell survival.[4] Investigating the specific roles of these organelles during paraptosis holds promise for developing novel therapeutic strategies, particularly for treating tumors. This mode of cell death has demonstrated unique advantages in tumor therapy, as it can overcome the resistance of tumor cells to traditional apoptosis-inducing drugs and enhance the efficacy of immunotherapy. In addition, paraptosis may be closely related to neurodegenerative diseases and inflammatory diseases.[5] The mechanisms of action and potential therapeutic value of paraptosis in these diseases continue to be actively investigated.

2. Current research status domestically and internationally

Over recent years, significant progress has been made in understanding the morphological changes of small organelles during paraptosis. Sperandio *et al.*[6] first identified insulin-like growth factor-1 receptor (IGF1R)-induced non-apoptotic cell death, termed paraptosis, and described its hallmark features, including cytoplasmic vacuolation caused by swelling of the ER and mitochondria.

Subsequent studies have revealed that certain compounds, such as δ-Tocotrienol, bortezomib, curcumin, and glabridin, can induce paraptosis in cancer cells. These agents consistently induce cytoplasmic vacuolation, enhance ER stress responses, and inhibit cell proliferation.[7] At the molecular level, the activation of mitogen-activated protein kinases (MAPKs), particularly MEK-2/extracellular signal-regulated kinase (ERK) 2, is essential for triggering the paraptotic process.[8-10] In addition, paraptosis is frequently accompanied by disrupted intracellular ion homeostasis, especially calcium dysregulation, which leads to mitochondrial swelling and dysfunction.[11-14] Emerging evidence also implicates developmental pathways, such as the Wnt signaling pathway, in modulating paraptosis.[15,16]

The modulators of paraptosis include a variety of activators, inhibitors, and regulators that function within the complex signaling network of the cell to collectively govern the initiation and progression of paraptosis. Activators, such as the MAPK/ERK/c-Jun N-terminal kinase signaling pathways and src homology-2 domain-containing protein tyrosine phosphatase-2, promote the process by enhancing signal transduction and cellular stress responses.[17] In contrast, inhibitors such as phosphatidylethanolamine binding protein 1 and ALG-2-interacting protein X exert negative regulation by suppressing relevant signaling pathways or protein functions, thereby preventing paraptosis.[18] Regulators, such as ubiquitin-specific peptidase 10 and caspase-9, act at the intersection of multiple signaling pathways within the cell and can either promote or inhibit paraptosis under certain conditions.[19] The interplay and dynamic balance of these factors determine whether a cell ultimately undergoes paraptosis, providing potential targets for cancer therapy.[20]

At present, mechanistic studies of paraptosis primarily focus on the following aspects: first, ER stress and the excessive generation of reactive oxygen species (ROS) are important factors in inducing paraptosis.[17] For example, curcuminoid B63 induces paraptosis in gastric cancer cells by targeting thioredoxin reductase 1 (TRXR1) to trigger ROS-mediated ER stress and MAPK activation.[21] In contrast, δ-tocotrienol induces paraptosis in melanoma cells by causing mitochondrial calcium overload and ROS production, which activates the MAPK pathway.[22] Second, mitochondrial calcium overload is one of the key features of paraptosis. For instance, morusin induces paraptosis in ovarian cancer cells through mitochondrial calcium overload and ER vacuolization. In addition, the activation of IGF1R and other cell membrane receptors can lead to mitochondrial dysfunction and paraptosis by releasing calcium and copper ions.[23] Proteasome dysfunction is also closely related to paraptosis. Auranofin, for example, induces paraptosis by inhibiting TRXR1 and the proteasome, upregulating the activating transcription factor 4 (ATF4)-CHAC glutathione-specific gamma-glutamylcyclotransferase 1 axis, and depleting glutathione, thereby exacerbating proteotoxic stress. Excessive ROS production can further impair proteasome activity, amplify oxidative stress signals, and ultimately lead to cell death.[24] Finally, the induction of paraptosis also involves the activation of multiple signaling pathways, such as the mammalian target of rapamycin and MAPK pathways. For example, honokiol induces paraptosis in acute promyelocytic leukemia cells by activating these pathways.[25] The activation of Yes-associated protein/transcriptional coactivator with a PDZ-binding domain is also associated with cellular sensitivity to paraptosis, as it regulates the cytoskeleton and the formation of stress granules, thereby affecting the cell's resistance to paraptosis.[26]

In addition, in cancer research, a variety of compounds have been found to kill cancer cells by inducing paraptosis.

For example, elaiophylin induces paraptosis in ovarian cancer cells by activating the MAPK pathway, whereas YRL1091 induces paraptosis in breast cancer cells through the production of ROS and ER stress. These findings provide new insights into overcoming cancer drug resistance. In addition, paraptosis may affect the behavior of immune cells by reshaping the tumor microenvironment, but the specific mechanisms remain unclear. In other disease areas, the role of paraptosis in neurodegenerative and cardiovascular diseases is still in the exploratory stage. However, its association with cellular stress responses suggests that it may play a potential role in these diseases.

Despite these advances, the precise molecular mechanisms underlying paraptosis remain poorly characterized. The interactions between affected organelles and their contribution to this form of cell death require further investigation, highlighting the need for continued exploration of this unique cell death modality. At the same time, translating laboratory research findings into clinical applications presents challenges, such as ensuring that compounds inducing paraptosis selectively kill cancer cells without harming normal cells. Future research should focus on further elucidating the molecular mechanisms of paraptosis, identifying specific biomarkers, developing novel compounds that induce paraptosis, and investigating its role in immunotherapy.

3. Morphological changes of intracellular organelles during paraptosis

Morphological changes in intracellular organelles during paraptosis have long been a focus of cell biology research. From initial exploratory studies to recent in-depth investigations, researchers have uncovered critical insights into the mechanisms of paraptosis and its role in regulating cell death.

3.1. Mitochondrial changes during paraptosis

Mitochondrial morphological changes are a hallmark of paraptosis, including swelling, loss of mitochondrial membrane potential, and fragmentation. Mitochondrial swelling is primarily driven by an influx of Ca^{2+} into the mitochondria. This influx often coincides with ER calcium release, leading to ER expansion and subsequent mitochondrial swelling.[27,28] The decline in mitochondrial membrane potential reflects mitochondrial dysfunction, which compromises adenosine triphosphate production and disrupts Ca^{2+} homeostasis.[29] Mitochondrial fragmentation, regulated by the activation of fission proteins such as dynamin-related protein 1, further disrupts mitochondrial integrity and functionality.[30] Together, these changes contribute to the progression of paraptosis.

The underlying mechanisms of mitochondrial remodeling during paraptosis involve multiple interconnected factors, including Ca^{2+} imbalance, oxidative stress, and altered mitochondrial dynamics.[31] ER stress-induced calcium release activates inositol 1,4,5-triphosphate receptors, promoting calcium flux into the cytoplasm and mitochondria. This calcium overload initiates ER stress and the unfolded protein response (UPR), resulting in protein misfolding, increased osmotic pressure, and eventual cell death. Concurrently, mitochondrial dysfunction leads to excessive production of ROS, exacerbating oxidative stress and amplifying paraptosis.[32] Alterations in mitochondrial dynamics, including disrupted fusion, fission, and motility, further exacerbate mitochondrial damage, destabilize mitochondrial membrane potential, and impair energy production, ultimately activating cell death signaling pathways.[33]

3.2. ER changes during paraptosis

The ER plays a fundamental role in protein synthesis, folding, and post-translational modifications, ensuring proper protein maturation and functionality.[17] It is not only the initiation site of protein synthesis but also a critical hub for maintaining cellular homeostasis.[20] The ER converts linear amino acid sequences into specific three-dimensional conformations, a process known as "correct folding," and participates in post-translational modifications to optimize protein function.[32]

During paraptosis, morphological changes in the ER are hallmark features that characterize this unique form of cell death. These changes primarily include ER swelling and cytoplasmic vacuolization. ER swelling occurs as a result of intracellular Ca^{2+} imbalance, which facilitates calcium efflux from the ER into the mitochondria.[20] The depletion of Ca^{2+} within the ER lumen further exacerbates swelling. As the ER expands, a significant number of vacuoles form in the cytoplasm, primarily originating from the ER, with some contributions from the mitochondria.[33] These vacuoles, which vary in size, are a defining feature of paraptosis and result in significant cytoplasmic reorganization.

The mechanisms underlying ER morphological changes are multifactorial, involving Ca^{2+} dysregulation, oxidative stress, ROS production, and ER stress-induced UPR activation.[34] Ca^{2+} imbalance activates inositol 1,4,5-trisphosphate receptors, promoting calcium efflux from the ER into the cytoplasm and mitochondria.[35] This process not only contributes to ER swelling but also initiates the UPR by accumulating misfolded or unfolded proteins, increasing osmotic pressure, and disrupting proteostasis.[36] ER stress upregulates specific markers, such as binding immunoglobulin protein, ATF4, and CCAAT-enhancer-binding protein homologous protein

(CHOP), further amplifying stress responses and accelerating cellular dysfunction.[37]

In addition, mitochondrial dysfunction exacerbates ROS production, leading to oxidative stress and cytoplasmic damage, which in turn aggravates the morphological changes in the ER.[38]

Proteasome inhibition also plays a pivotal role in paraptosis by impairing the activity of the 20S proteasome, which leads to the accumulation of misfolded proteins in the ER and persistent UPR activation.[39-40] Together, these events highlight the central role of ER dysfunction in the progression of paraptosis.

3.3. Golgi apparatus changes during paraptosis

The Golgi apparatus, a key organelle responsible for protein glycosylation, sorting, and secretion, serves as a vital "logistics center" for intracellular biochemical processes. It ensures the proper modification and delivery of proteins and lipids to their respective cellular destinations.[41] However, during paraptosis, the Golgi apparatus undergoes profound structural alterations, including swelling and disassembly.[41]

Golgi apparatus swelling transforms its characteristic flattened cisternal structure into disordered vesicles and tubular networks.[42] Concurrently, Golgi disassembly occurs, characterized by cleavage of structural proteins such as 130 kDa cis-Golgi matrix protein 1 and giantin. Evidence suggests that caspases, potentially caspase-9, may be involved in this cleavage process, contributing to the breakdown of Golgi architecture.[43]

The underlying mechanisms driving these morphological changes involve Ca^{2+} dysregulation, cytoskeletal disruption, ER stress, and protein misfolding. Ca^{2+} imbalance disrupts Golgi apparatus functionality and structural integrity, leading to its fragmentation.[44] In addition, cytoskeletal destabilization, a hallmark of paraptosis, may cause Golgi apparatus redistribution and fragmentation. This process further activates the UPR, indirectly affecting Golgi morphology and function.[45] ER stress, resulting from protein misfolding, contributes to the destabilization of the Golgi structure by impairing protein trafficking and glycosylation processes.[37]

Recent studies using U251MG cells with fluorescent markers have revealed novel insights into Golgi dynamics during paraptosis. Treatments with glabridin, morusin, and honokiol caused the disintegration of the Golgi apparatus, leading to the formation of larger, spherical structures termed putative paraptosomes. These structures, observed under electron microscopy, consist of a dense network of interwoven membranes and are distinctly separated from the surrounding cytoplasm. This discovery suggests that Golgi-derived materials contribute to paraptosome formation, offering valuable insights into the mechanistic underpinnings of paraptosis and its progression.[45]

In summary, the small organelles interact and are interconnected during paraptosis. In the early stages, mitochondria undergo swelling and loss of cristae structure, leading to the release of Ca^{2+} and a decrease in membrane potential. Mitochondrial dysfunction results in increased ROS production. Ca^{2+} imbalance causes the swelling of the ER, which in turn triggers ER stress and UPR activation, with an increase in ER stress markers. ER dysfunction also leads to the production of ROS. Ca^{2+} dysregulation disrupts the structural integrity of the Golgi apparatus, causing it to fragment. Misfolded proteins contribute to its structural instability, leading to vesicularization and structural disorganization of the Golgi apparatus. In conclusion, these changes are conducive to the progression of paraptosis (Figure 1).

3.4. Lysosomal changes during paraptosis

Lysosomes, often referred to as the digestive factories of the cell, undergo distinctive morphological changes during paraptosis.[5] These changes are intricately linked to the degradation and recycling of intracellular components, including unabsorbed nutrients, damaged organelles, and other cellular debris.[46] In paraptosis, lysosomal alterations play a critical role in cell death and include lysosomal activation, increased membrane permeability, and morphological changes such as swelling and rupture.[47] These events are often accompanied by the release of lysosomal proteases, which further exacerbate cellular damage.[48]

The mechanisms driving these lysosomal changes involve oxidative stress, ROS production, proteasome inhibition, and ion homeostasis disruption. Elevated ROS levels can damage the lysosomal membrane, increasing its permeability and enabling the release of hydrolytic enzymes into the cytoplasm, thereby accelerating cell death.[24,49] Proteasome inhibition leads to the accumulation of misfolded proteins, which activates the UPR and increases osmotic pressure in the ER and lysosomes, resulting in their swelling.[50] Furthermore, imbalances in ion homeostasis, particularly involving Ca^{2+}, destabilize the lysosomal membrane, contributing to its rupture.[51] ER stress and Ca^{2+} overload exacerbate ROS production, destabilizing lysosomal integrity, and function.[52] Together, these interconnected mechanisms emphasize the central role of lysosomes in the progression of paraptosis.

3.5. Autophagosomes changes in paraptosis

Autophagosomes, double-membrane vesicles formed during autophagy, play a crucial role in the degradation

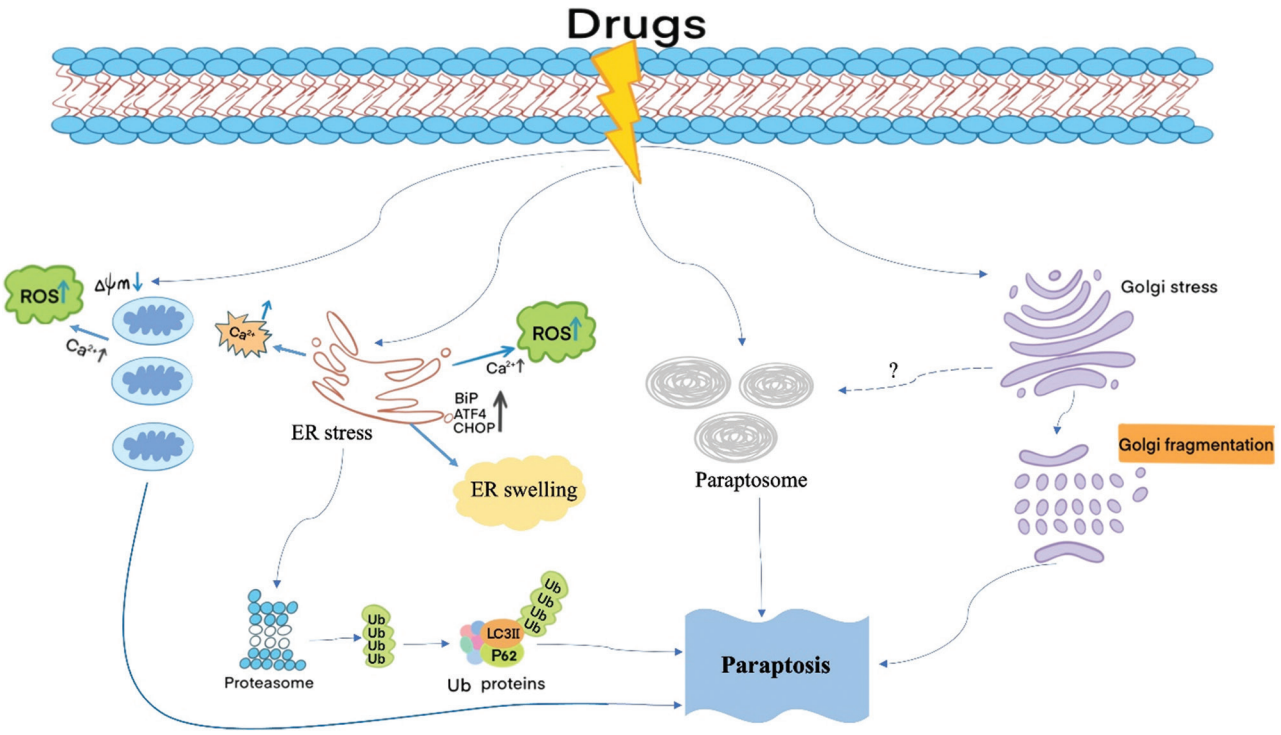

Figure 1. Drugs that induce paraptosis can trigger an ER stress response, leading to ER swelling and Ca^{2+} efflux. This process also activates the unfolded protein response, resulting in increased expression of ER stress markers such as BiP, ATF4, and CHOP. ER stress not only disrupts the normal folding of proteins but also leads to an increase in ROS levels within the ER. The efflux of Ca^{2+} raises the intracellular calcium concentration. In addition, influenced by paraptosis-inducing drugs, Ca^{2+} flow into the mitochondria, causing a decrease in mitochondrial membrane potential, mitochondrial swelling, and the disappearance of cristae. Overloading of Ca^{2+} in the mitochondria can significantly increase ROS levels within the mitochondria. Furthermore, drugs that induce paraptosis can also cause fragmentation of the Golgi apparatus. Under stress, the Golgi apparatus undergoes structural changes, and a high-density morphological structure, possibly originating from the Golgi apparatus, called the paratosome, has been identified. The formation of paratosomes may be associated with Golgi stress, though its specific function requires further investigation. These alterations in organelles collectively lead to an imbalance in cellular protein homeostasis, disruption of energy metabolism, and exacerbation of oxidative stress, ultimately triggering paraptosis. Image created by the authors. Abbreviations: ATF4: Activating transcription factor 4; BiP: Binding immunoglobulin protein; Ca^{2+}: Calcium ion; CHOP: CCAAT-enhancer-binding protein homologous protein; ER: Endoplasmic reticulum; LC3II: Microtubule-associated protein light chain 3; P62: Ubiquitin-binding protein p62; ROS: Reactive oxygen species; Ub: Ubiquitin.

of intracellular material by delivering it to lysosomes for digestion. In the normal process of cell death, the changes in autophagosomes are mainly characterized by an increase in their number and the activation of their function. As double-membrane vesicles, autophagosomes are formed by the extension and closure of a local region of the cytoplasmic membrane (phagophore), which then engulf damaged organelles, protein aggregates, or other substances that need to be degraded within the cell.[52] As autophagosomes mature, they fuse with lysosomes, delivering the enclosed contents for degradation and digestion. This process is an important mechanism for cells to clear damaged components and maintain intracellular homeostasis.[53] During normal cell death, these dynamic changes in autophagosomes help the cell to complete autophagic cell death in an orderly manner while providing energy and nutrients to support cell survival or promote cell renewal and regeneration.[54,55] In paraptosis, autophagosomes undergo morphological changes. For instance, the membrane structure of autophagosomes may become unstable, leading to irregular shapes or even rupture. These morphological alterations are likely associated with changes in the intracellular stress environment, such as oxidative stress and imbalances in Ca^{2+} homeostasis.[47] In paraptosis, the formation of autophagosomes is characterized by the extension and incomplete closure of the cytoplasmic membrane (phagophores).[58] These dilated phagophores then fuse with lysosomes, delivering the enclosed material for digestion (Figure 2).

The morphological changes and mechanisms governing autophagosome formation during paraptosis

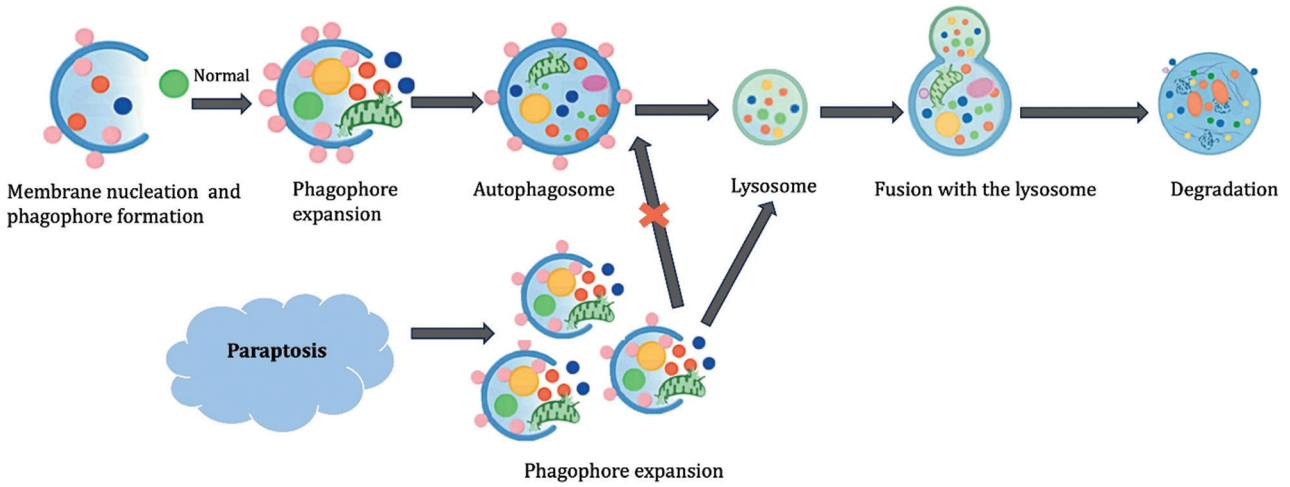

Figure 2. Autophagosome formation and fusion with lysosomes. Under normal conditions, the initiation of autophagy involves the formation of the phagophore and its initial membrane structure. The phagophore gradually expands and eventually forms an autophagosome. The autophagosome then fuses with lysosomes, where hydrolases within the lysosome degrade the contents of the autophagosome, completing the autophagy process. In the case of paraptosis, the formation of autophagosomes is characterized by abnormal expansion and remodeling of the plasma membrane, preventing the formation of normal autophagosomes. These expanded phagophores ultimately fuse with lysosomes, where hydrolases within the lysosome degrade the material inside the phagophore, completing the digestion process. Image created by the authors.

involve several tightly regulated steps. Following an autophagy-inducing signal, phagophores emerge as membrane precursors resembling liposomes. These structures expand to enclose damaged organelles, aged biomolecules, and other cellular components. Once fully formed, the autophagosome fuses with lysosomes, forming autolysosomes, where lysosomal enzymes degrade the inner autophagosomal membrane and its cargo. The degradation products are then recycled back into the cytoplasm for reuse by the cell.[57]

Various factors contribute to autophagosome formation and function, including ion homeostasis disruption, proteasome inhibition, ER stress, and signal transduction pathways, such as IGF1R, MAPK, and c-Jun N-terminal kinase.[58,59] ER stress influences the morphology and function of autophagosomes by activating the UPR and increasing osmotic pressure.[58] Proteasome inhibition leads to the accumulation of misfolded proteins, which further activates the UPR and causes autophagosome swelling.[60] In addition, following the fusion of autophagosomes with lysosomes, the release of lysosomal enzymes exacerbates cellular damage and may, in turn, affect the stability of autophagosomes.[61] Together, these factors interact with each other, collectively driving the morphological changes of autophagosomes and reflecting the complex response mechanisms of cells under stress conditions.[62] The pathways regulate transcription, protein synthesis, and autophagy-related machinery, underscoring the complexity of autophagosomal dynamics in paraptosis.[63]

4. The relationship between paraptosis and diseases

The relationship between paraptosis and diseases, particularly cancer, has garnered significant attention in recent years. Paraptosis is a unique form of programmed cell death characterized by cytoplasmic vacuolization and swelling of the ER and mitochondria, without the activation of caspases. This distinction sets it apart from apoptosis and necrosis, making it a potential alternative pathway for targeting cancer cells that are resistant to traditional apoptosis-inducing therapies.[64]

4.1. Paraptosis as a strategy for cancer treatment

Paraptosis has emerged as a promising strategy for overcoming resistance to cancer therapies. Many tumor cells exhibit resistance to conventional apoptosis-inducing agents, but paraptosis can bypass these resistance mechanisms. For instance, proteasome inhibitors such as bortezomib have been shown to induce paraptosis by causing ER stress and mitochondrial calcium overload.[65] Moreover, combining paraptosis-inducing agents with conventional chemotherapy or radiation can enhance treatment efficacy by increasing sensitivity to these therapies.[65] Several natural compounds and small molecules have been identified as potential paraptosis inducers. For example, honokiol induces paraptosis-like cell death in acute promyelocytic leukemia through the activation of the mammalian target of rapamycin and MAPK signaling pathways.[33] Curcumin and its derivatives

also induce paraptosis by disrupting thiol proteostasis and causing proteasomal dysfunction. Other compounds, such as gambogic acid and certain nanomedicines, have demonstrated similar effects.[26]

4.2. Therapeutic efficacy of paraptosis in cancer treatment when combined with other drugs

In recent years, researchers have actively explored the combination of paraptosis inducers with other drugs to enhance antitumor efficacy and overcome drug resistance. In the case of proteasome inhibitors, bortezomib has achieved success in hematological malignancies, but its monotherapy efficacy in solid tumors is limited, mainly due to the presence of resistance mechanisms. However, when bortezomib is combined with nutlin-3 (an inhibitor of mouse double minute 2 homolog-tumor protein p53 protein interaction), it can synergistically promote cell death through ER stress and proteasome inactivation, thereby overcoming resistance to bortezomib in various solid tumors (such as breast cancer, colorectal cancer, and glioblastoma).[66] In addition, the antihypertensive drug lercanidipine can enhance the efficacy of bortezomib by inducing paraptosis through exacerbated ER stress and mitochondrial calcium overload.[57]

In the combination of nanomedicine and photodynamic therapy (PDT), the carrier-free nanomedicine Pyromor combines the photosensitizer Pyro and the paraptosis inducer morusin through self-assembly.[68] In PDT, Pyromor not only induces apoptosis through photosensitization and ROS generation but also triggers paraptosis by inducing vacuolization of the ER and mitochondria through morusin. This combination strategy significantly enhances the efficacy of PDT and demonstrates good tumor targeting and antitumor effects in *in vivo* experiments.[69]

Natural products and small-molecule drugs have also shown antitumor activity. For example, compounds such as curcumin and gambogic acid can trigger paraptosis by disrupting the thiol redox balance or inducing ER stress. The hypoxia-responsive core-shell nanoparticle (GC@MCS NP), which combines gambogic acid with the photosensitizer chlorin e6, performs well in hypoxia response and ROS generation, further enhancing the induction of paraptosis.[70] Moreover, the combination of immune modulation and paraptosis induction has shown great potential. For example, a complex called CMN containing copper ions, morusin, and the indoleamine 2,3-dioxygenase 1 inhibitor NLG919 can trigger paraptosis through morusin-induced vacuolization of the ER and mitochondria, while simultaneously activating cytotoxic T cells through NLG919 to enhance antitumor immunity.[71]

4.3. Association between tumor cells and paraptosis

Paraptosis has been shown to be associated with various types of tumor cells. The following are some notable research findings: curcumin and its derivatives can trigger paraptosis in malignant breast cancer cells by inducing mitochondrial calcium overload and proteasome dysfunction. Curcuminoid B63 induces paraptosis-like cell death in gastric cancer cells by targeting TRXR1 and activating ROS-mediated pathways.[28] In addition, indirubin-3'-monoxime can induce paraptosis in MDA-MB-231 breast cancer cells by transferring Ca^{2+} from the ER to the mitochondria.[14] In prostate cancer cells, δ-tocotrienol induces paraptosis by triggering ER stress and autophagy.[72] Morusin induces paraptosis-like cell death in epithelial ovarian cancer cells through mitochondrial calcium overload and dysfunction.[31] Hesperidin triggers paraptosis in hepatocellular carcinoma cells by inducing mitochondrial dysfunction and calcium overload.[13] Boletus edulis, an anti-tumor protein isolated from edible matsutake mushrooms, can induce paraptosis in lung cancer A549 cells, involving both apoptosis and autophagy.[73] Ophiobolin A induces paraptosis-like cell death in human glioblastoma cells by reducing the activity of large-conductance calcium-activated potassium channels.[22] Ginsenoside Rh2 induces paraptosis-like cell death in colorectal cancer cells by activating p53.[74] Δ-tocotrienol induces paraptosis in melanoma cells through calcium overload and ROS-associated mitochondrial dysfunction.[22] Jolkinolide B induces ROS-mediated paraptosis and apoptosis by targeting the thioredoxin and glutathione systems.[75] These research findings indicate that paraptosis has the potential to induce cell death in various types of tumor cells.

4.4. Paraptosis inducers currently applied in clinical practice

At present, paraptosis inducers used in clinical settings or with potential for clinical application mainly include the following categories: first, proteasome inhibitors, such as bortezomib, carfilzomib, and ixazomib. These drugs induce paraptosis by inhibiting proteasome activity, thereby triggering ER stress and cytoplasmic vacuolization. They can also be combined with other chemotherapeutic agents to enhance efficacy against solid tumors.[76] Second, natural products and small-molecule compounds, such as morusin, δ-tocotrienol, and gambogic acid, induce paraptosis by causing dilation of the ER and mitochondria or disrupting thiol homeostasis, demonstrating potential anti-tumor activity.[77] Third, the repurposing of clinical drugs, such as loperamide and lercanidipine. The former can overcome bortezomib resistance in colorectal cancer cells through CHOP-mediated paraptosis, while the latter

enhances bortezomib-induced ER stress and mitochondrial calcium overload, thereby inducing paraptosis.[16] Fourth, nanomedicines, such as the Cu^{2+}-coordinated morusin/doxorubicin biological organizer (COMBO) system and GC@MCS NPs, are being explored. The COMBO system combines morusin and doxorubicin to induce paraptosis through ER and mitochondrial dilation, while the GC@MCS NPs deliver gambogic acid and chlorophyll e6 through a hypoxia-responsive hyaluronic acid-nitroimidazole shell, enhancing ROS generation and inducing paraptosis.[78] Finally, other potential inducers, such as integrated stress response inhibitors, can induce paraptosis by enhancing the cytotoxicity of proteasome inhibitors.[64]

4.5. Stress responses involved in paraptosis and their impact on tumors

During the process of paraptosis, various stress responses work in concert to exert profound effects on tumors. First, ER stress is activated due to the accumulation of unfolded proteins, which triggers the UPR. This event, in turn, upregulates transcription factors such as CHOP and leads to the dilation of the ER and mitochondria, ultimately promoting cell death.[79] Second, ROS stress significantly increases, disrupting cellular thiol homeostasis, causing protein misfolding, and exacerbating ER stress. It also activates Ca^{2+} channels in the mitochondria, further damaging mitochondrial function.[80] In addition, Ca^{2+} stress, characterized by the release of Ca^{2+} from the ER into the mitochondria, further impairs the function of organelles. Proteostasis stress manifests as protein misfolding and dysfunction in the degradation system, activating heat shock proteins (HSPs) and the ubiquitin-proteasome system, thereby intensifying intracellular stress responses.[81] Finally, immunogenic stress releases damage-associated molecular patterns (DAMPs), such as HSPs and high mobility group box 1 protein, which activate the immune system and enhance the body's immune response against tumors. Collectively, these stress responses not only promote tumor cell death but also overcome resistance to traditional apoptotic pathways and enhance immune responses, providing new strategies for cancer treatment.[82] However, paraptosis may be accompanied by potential side effects in clinical applications, necessitating further research to optimize its therapeutic efficacy.

4.6. The relationship between paraptosis and other diseases

Beyond cancer, paraptosis has been implicated in the pathogenesis of other diseases. For instance, in neurodegenerative diseases, the disruption of intracellular protein homeostasis may lead to a cell death process similar to paraptosis.[83] This suggests that paraptosis could be a potential therapeutic target in these conditions as well. In addition, cell death induced by paraptosis can activate the immune system by releasing DAMPs, which stimulate an immune response. This immune activation can transform non-immunogenic tumors into immunogenic ones, thereby enhancing anti-tumor immunity.[84]

Despite its potential, the specific mechanisms of paraptosis and its role in various diseases are still under investigation. Future research should focus on elucidating the signaling pathways involved in paraptosis and developing targeted therapies that can selectively induce this form of cell death in cancer cells. Furthermore, exploring the combination of paraptosis with immunotherapy may further enhance its therapeutic potential.

In summary, the research and application of paraptosis inducers are continuously progressing, showing unique advantages in cancer therapy, especially in overcoming resistance to traditional chemotherapy, which holds significant importance.

5. Challenges in paraptosis research

At present, we have gained some understanding of the morphological characteristics, signaling pathways, and key molecular markers of paraptosis. However, the biological connection between mitochondrial and ER changes is not yet clarified. Moreover, due to the overlapping morphological features with other cell death pathways (such as apoptosis, necroptosis, autophagic cell death, and ferroptosis), the identification and study of paraptosis face numerous challenges. In the following sections, we will discuss these challenges, including the biological connection between mitochondrial and ER changes, the perspectives on overlapping morphological features, limitations of technical methods, cellular heterogeneity, crossovers with novel cell death pathways, and the lack of unified diagnostic criteria.

5.1. Biological connection between mitochondrial and ER changes

Mitochondrial swelling and loss of membrane potential, as well as ER expansion, are key morphological changes during paraptosis. These changes are interconnected and regulate downstream signaling through mechanisms such as Ca^{2+} transfer, oxidative stress, and the UPR. ER expansion may be triggered by the accumulation of unfolded proteins and the release of Ca^{2+}, which subsequently leads to mitochondrial calcium overload and dysfunction. These changes may act as both triggers and consequences of paraptosis, forming a complex cause-and-effect relationship. Moreover, pathways such as protein kinase R-like ER kinase and inositol-requiring-1–X-box

binding protein 1 play important roles in regulating ER expansion.[2] However, the specific molecular mechanisms need further investigation to clarify their functions in paraptosis.

5.2. Overlapping morphological features

The typical morphological features of paraptosis include cytoplasmic vacuolization, cell swelling, mitochondrial swelling, and nuclear integrity.[5] However, these features significantly overlap with those of other cell death pathways, making identification challenging. For example, the formation of autophagosomes in autophagic cell death may be confused with the vacuolization in paraptosis; early necroptosis may also exhibit similar vacuolization; and apoptosis, under certain stress conditions, may also be accompanied by partial vacuolization. Moreover, with the discovery of novel programmed cell death pathways (such as ferroptosis and pyroptosis), the complexity of morphological identification has further increased.

5.3. Limitations of technical methods

The detection and study of paraptosis are constrained by current technical methods. First, its key dynamic characteristics (such as vacuolization of the ER and Golgi apparatus) require observation through transmission electron microscopy, as conventional optical microscopes cannot clearly resolve the details of these subcellular structures. Second, cell death is a dynamic process, yet current research primarily relies on static observations at fixed time points, making it difficult to capture the entire process of characteristic changes. In addition, paraptosis currently lacks specific biochemical markers, and relying solely on morphological features can easily lead to misjudgments.

5.4. Heterogeneity of cell types and experimental conditions

Different cell lines may exhibit varying death responses to the same stimulus. For example, certain cancer cell lines may be more prone to paraptosis, while epithelial cells may tend to undergo apoptosis.[71] Moreover, changes in drug concentration, duration of action, or microenvironment factors (such as nutritional status) may induce mixed-type cell death, complicating the morphological features.[85] This heterogeneity of cell types and experimental conditions further increases the complexity of paraptosis research.

5.5. Crossover with novel cell death pathways

In recent years, the discovery of novel programmed cell death pathways, such as ferroptosis and pyroptosis, has made the morphological identification of paraptosis more complex. For example, early ferroptosis may be accompanied by cytoplasmic vacuolization,[86] and the characteristic plasma membrane rupture in pyroptosis[87] may be confused with paraptosis. The crossover between these novel cell death pathways and paraptosis increases the difficulty of morphological classification.

5.6. Lack of unified diagnostic criteria

The research history of paraptosis is relatively short (first named in 2000), and its morphological criteria have not yet been fully standardized. Differences in the description of vacuole size and distribution across various studies lead to a high degree of subjectivity in diagnosis. Moreover, the lack of specific molecular markers presents an additional challenge in paraptosis research.

6. Strategies and future directions

In response to the challenges outlined above, the following strategies and future research directions are proposed:

6.1. Multimodal technology integration

Combining electron microscopy (to clarify subcellular structures), live-cell imaging (to dynamically track vacuole formation), and molecular marker detection can improve the accuracy of paraptosis identification.

6.2. Advances in the research of specific molecular markers for paraptosis

At present, there are no clear and widely accepted specific molecular markers for paraptosis. Its identification primarily relies on morphological features and indirect evidence from the exclusion of other cell death pathways. Several potential candidate markers and signaling pathways have been proposed, but further validation is still required. For example, abnormal activation of the IGF1R/protein kinase B pathway, sustained activation of the MAPK/ERK pathway, and downregulation of ALG-2-interacting protein X protein expression may all be associated with paraptosis. The P35 protein, derived from baculovirus and acting as a caspase inhibitor, does not directly regulate paraptosis but may indirectly promote the shift of cells toward paraptosis by inhibiting the apoptotic pathway. However, the lack of specificity of these molecules limits their application as independent markers.[17]

These specific molecular markers and pathways, once validated, can improve the accuracy of paraptosis identification.

6.3. Functional validation experiments

Genetic knockouts or inhibitors targeting specific death pathways, followed by observation of the morphological features of the remaining cell death phenomena, can help distinguish paraptosis from other types of cell death.

6.4. Standardized morphological assessment system

Establishing a quantitative scoring system based on vacuole origin (ER/Golgi apparatus), mitochondrial morphology, and nuclear changes can reduce subjective judgment errors.

In summary, distinguishing paraptosis from other types of cell death in experiments requires a comprehensive approach that integrates morphological observation, molecular marker detection, functional validation, and exclusion strategies. Researchers can use transmission electron microscopy to confirm vacuolization originating from the ER and Golgi apparatus and exclude autophagosomes or lysosomal vacuoles. In addition, detecting specific molecular markers of other cell death pathways (such as caspase activation in apoptosis, mixed lineage kinase domain-like phosphorylation in necroptosis, or lipid peroxidation markers in ferroptosis) can help rule out possibilities other than paraptosis. Moreover, using signaling pathway inhibitors or gene-editing tools to validate the specific phenotype of paraptosis is an essential experimental approach.

The following Table 1 summarizes the differences between paraptosis and other modes of cell death, including apoptosis, necroptosis, and ferroptosis.

7. Current status and challenges of paraptosis in clinical research

7.1. Clinical trials of paraptosis-inducing compounds

At present, no drugs with a clear mechanism for inducing paraptosis have entered clinical trials. However, some compounds (such as the copper ion carrier elesclomol,

Table 1. Summary of the differences between paraptosis and other modes of cell death, including apoptosis, necroptosis, and ferroptosis

Feature	Paraptosis	Apoptosis	Necroptosis	Ferroptosis
Morphological features	Cytoplasmic vacuolization, increased cell volume, mitochondrial swelling, and intact nucleus	Cell shrinkage, nuclear condensation, nuclear fragmentation, formation of apoptotic bodies, and intact cell membrane	Cell swelling, cell membrane rupture, organelle swelling, and nuclear dissolution (in the late stage)	Decreased mitochondrial cristae, increased membrane density, intact cell membrane (early stage) → rupture of the cell membrane (late stage), absence of nuclear condensation
Signal transduction pathway	IGF1R/Akt pathway, caspase-independent activation	Caspase-dependent (activation of caspase-3/7), mitochondrial pathway (Bcl-2/Bax), death receptor pathway	Phosphorylation of RIPK1/RIPK3/MLKL, caspase-independent, dependent on TNF/IFN signaling	Iron-dependent lipid peroxidation, GPX4 inactivation, regulation by ACSL4 and System Xc
Detection methods	Transmission electron microscopy (to observe vacuoles in the endoplasmic reticulum and Golgi apparatus), lack of specific markers (other cell death pathways need to be excluded)	TUNEL staining (for DNA fragmentation), Annexin V/PI double staining, caspase-3/7 activity detection	p-MLKL immunofluorescence/Western blot, necrostatin-1 (inhibitor) blockade validation, PI staining (membrane integrity)	Lipid peroxidation markers (MDA, 4-HNE), iron chelator (DFO) inhibition validation, GPX4 activity detection
Key molecular markers	Vacuolization (endoplasmic reticulum/Golgi apparatus), absence of caspase activation	Activation of caspase-3/7, cytochrome c release, PARP cleavage	Phosphorylation of RIPK1/RIPK3/MLKL, release of HMGB1	Accumulation of lipid ROS, upregulation of ACSL4 expression, depletion of glutathione
Inflammatory response	Typically, no significant inflammation	No inflammation	Strong inflammatory response (release of DAMPs)	Moderate inflammation (induced by lipid peroxidation products)
Physiological and pathological significance	Involved in the development, tumor resistance	Programmed cell death (physiological clearance), tumor suppression	Inflammatory-related diseases (infections, ischemia-reperfusion injury)	Moderate inflammation (induced by lipid peroxidation products)

Abbreviations: ACSL4: Acyl-CoA synthetase long-chain family member 4; Akt: Protein kinase B; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; DAMPs: Damage-associated molecular patterns; DFO: Deferoxamine mesylate; GPX4: Glutathione peroxidase 4; HMGB1: High mobility group box 1 protein; IFN: Interferon; IGF1R: Insulin-like growth factor-1 receptor; MDA: Malondialdehyde; MLKL: Mixed lineage kinase domain-like; PARP: Poly (ADP-ribose) polymerase; PI: Propidium iodide; RIPK: Receptor-interacting protein kinase; ROS: Reactive oxygen species; TFN: Transferrin; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling; 4-HNE: 4-Hydroxynonenal.

disulfiram, and IGF1R inhibitors) have shown the potential to induce cell death through the paraptosis pathway in preclinical studies. Clinical trials of these compounds are primarily based on other known mechanisms (such as apoptosis or ferroptosis) and have not been specifically designed to verify the contribution of paraptosis.[17]

7.2. Clinical data on paraptosis in human diseases

Paraptosis may be implicated in various human diseases, including cancer, neurodegenerative diseases, and cardiovascular diseases. For example, paraptosis-like features have been observed in chemotherapy-resistant tumor samples, suggesting that it may serve as an alternative cell death pathway to escape apoptosis.[17] In addition, ER vacuolization and mitochondrial swelling found in the brain tissue of Alzheimer's disease patients may be related to paraptosis.[88] However, these studies are preliminary and lack specific biomarkers and validation with clinical samples.

7.3. Limitations of translating *in vitro* studies to clinical therapies

The clinical translation of paraptosis faces numerous challenges, including the simplification of model systems, technical bottlenecks in detection and identification, off-target effects, toxicity concerns, as well as issues with drug delivery and pharmacokinetics. For example, *in vitro* studies cannot simulate the complex tumor microenvironment *in vivo*, which may lead to an overestimation of the paraptosis-inducing effects.[17] Moreover, the lack of specific biomarkers limits the ability to diagnose and monitor paraptosis in clinical settings.[2]

8. Conclusion

This review has comprehensively examined the morphological changes in intracellular small organelles during paraptosis, highlighting the specific alterations and molecular mechanisms underlying the involvement of mitochondria, the ER, Golgi apparatus, lysosomes, and autophagosomes. As a non-classical form of programmed cell death, paraptosis is distinct from apoptosis and necrosis, with hallmark features such as cell swelling and organelle distension.[66] These morphological changes are pivotal for understanding how cells respond to external and internal stimuli.

Among the organelles, mitochondrial changes are particularly prominent. During the early stages of paraptosis, mitochondria exhibit swelling and loss of cristae structure, likely driven by elevated Ca^{2+} concentrations.[27] As paraptosis progresses, increased mitochondrial outer membrane permeability facilitates the release of pro-apoptotic factors such as cytochrome c, exacerbating cellular damage. Mitochondrial dysfunction also impairs adenosine triphosphate production, further compromising cell viability.[66]

The ER, a central hub for protein synthesis, folding, and calcium storage, undergoes significant expansion during paraptosis. This expansion disrupts Ca^{2+} homeostasis, triggering ER stress and the UPR, which can lead to autophagy or paraptosis.[18] The interplay between ER stress and paraptosis provides critical insights into how cells adapt to various stressors. However, the causal relationship between these morphological changes and downstream signaling pathways is not yet fully understood. Loss of mitochondrial membrane potential may affect downstream signaling through ROS-mediated oxidative stress or imbalance in Ca^{2+} homeostasis. In addition, vacuolization of the ER may be related to the UPR, involving pathways such as inositol-requiring-1–X-box binding protein 1 and protein kinase R-like ER kinase–CHOP. Moreover, interactions between mitochondria and the ER (such as Ca^{2+} transfer and lipid exchange) may exacerbate cellular stress responses. Future research needs to integrate multi-dimensional techniques to reveal the precise regulatory mechanisms of these changes.

The Golgi apparatus, often referred to as the "logistics center" of the cell, undergoes vesicularization and structural irregularities during paraptosis. These alterations hinder normal protein and lipid trafficking, contributing to cellular environment imbalances and accelerating cell death.[42,44] Lysosomes, in turn, exhibit decreased membrane stability, leading to the leakage of acidic hydrolases into the cytoplasm. This leakage acidifies the intracellular environment, further damaging organelles and amplifying cell death.[49]

Autophagosome formation during paraptosis involves the development of double-membrane vesicles, which enclose damaged organelles and biomolecules for degradation.

These morphological changes are not merely passive responses but represent tightly regulated events mediated by complex intracellular signaling networks. They unveil novel mechanisms of cell death and provide a theoretical foundation for designing targeted cell protection strategies under specific pathological conditions.

Finally, the challenges in the morphological identification of paraptosis mainly stem from overlapping features with other cell death pathways, technical limitations, and unclear classification criteria. Future research should focus on integrating technologies, developing specific markers, and conducting multi-dimensional analyses to

establish a more precise identification system. Clarifying the role of paraptosis in diseases and developing targeted intervention strategies will be crucial for future research. Through multidisciplinary collaboration, it is hoped that current technical bottlenecks can be overcome, further advancing the study of paraptosis. Future studies will likely investigate the interplay between paraptosis and other cell death pathways, as well as elucidate the biological connection between mitochondrial and ER changes and explore approaches to modulate organelle-specific changes for disease treatment. At present, no drugs with a clear mechanism for inducing paraptosis have entered clinical trials. In cancer therapy, paraptosis, as an immunogenic form of cell death, can overcome the resistance of tumor cells to traditional apoptosis-inducing drugs and enhance the efficacy of immunotherapy. Moreover, paraptosis is closely related to cardiovascular diseases, neurological disorders, and inflammatory diseases. Its mechanisms of action and potential therapeutic value in these conditions are continuously being investigated.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

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Ethics approval and consent to participate

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Availability of data

The data that support the findings of this study are available from the corresponding author through email upon reasonable request.

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