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Review Article

Pyroptosis: A promising target for lung cancer therapy

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ABSTRACT

Pyroptosis is a type of programed cell death that differs from apoptosis, ferroptosis, or necrosis. Numerous studies have reported that it plays a critical role in tumorigenesis and modification of the tumor microenvironment in multiple tumors. In this review, we briefly describe the canonical, non-canonical, and alternative mechanisms of pyroptotic cell death. We also summarize the potential roles of pyroptosis in oncogenesis, tumor development, and lung cancer treatment, including chemotherapy, radiotherapy, targeted therapy, and immunotherapy. Pyroptosis has double-edged effects on the modulation of the tumor environment and lung cancer treatment. Further exploration of pyroptosis-based drugs could provide novel therapeutic strategies for lung cancer.

Introduction

Lung cancer is associated with the highest global mortality rate.[1] Anti-tumor therapies, such as chemotherapy, targeted therapy, and immunotherapy, have been developed over the past decade. However, even though anti-tumor treatment can be successful during the initial period, therapy resistance eventually occurs, resulting in disease recurrence or progression.[2] Tumors exist within a tumor microenvironment (TME) that has been adapted and substantially modified owing to interactions between tumor cells and non-cancerous tissues. Identifying how cells in the TME react is of great importance.

In recent years, programmed cell death (PCD), which plays a vital role in cancer therapy, has been gradually understood. There are several forms of PCD including apoptosis, necroptosis, and ferroptosis. Among these, pyroptosis is the most common pro-inflammatory cell death pathway, can elicit the most robust immune response, and is associated with distinct morphological features, such as cell swelling, chromatin condensation, and the loss of cell membrane barrier functions.[3] As shown in Fig. 1, the word pyroptosis was proposed by Cookson et al.[4] in 2001 to describe a form of caspase-1-dependent pro-inflammatory PCD based on the Greek roots *pyro* (fire/fever) and *ptosis* (falling). It was clearly understood as a defined concept in 2015[5–7] and consentaneously redefined in 2018.[8] Finally, scientists have defined pyroptosis as a type of PCD in which plasma membrane pores are formed, mediated by gasdermin (GSDM) protein family members, frequently in response to inflammatory caspase activation.[8] An understanding of the mechanisms underlying cell death is indispensable for investigations of the TME, as this process is tightly associated with anti-cancer therapy.

This review focuses on the molecular mechanisms underlying pyroptotic cell death, as well as the association between pyroptosis and oncogenesis, tumor development, and the clinical outcomes of lung cancer treatment. We also discuss recent research achievements in the immunological effects of pyroptosis on lung cancer. This review will help researchers and physicians understand the roles of pyroptosis in lung cancer tumorigenesis and develop and identify novel therapeutic strategies for this disease.

Mechanisms of pyroptosis

There are two crucial pathways that lead to the activation of pyroptosis with several alternatives (Table 1). In these two pathways, pyroptosis is initiated in cells depending on gasdermin D (GSDMD), which involves caspase-1 (canonical) or caspase-4/5/11 (non-canonical) path-

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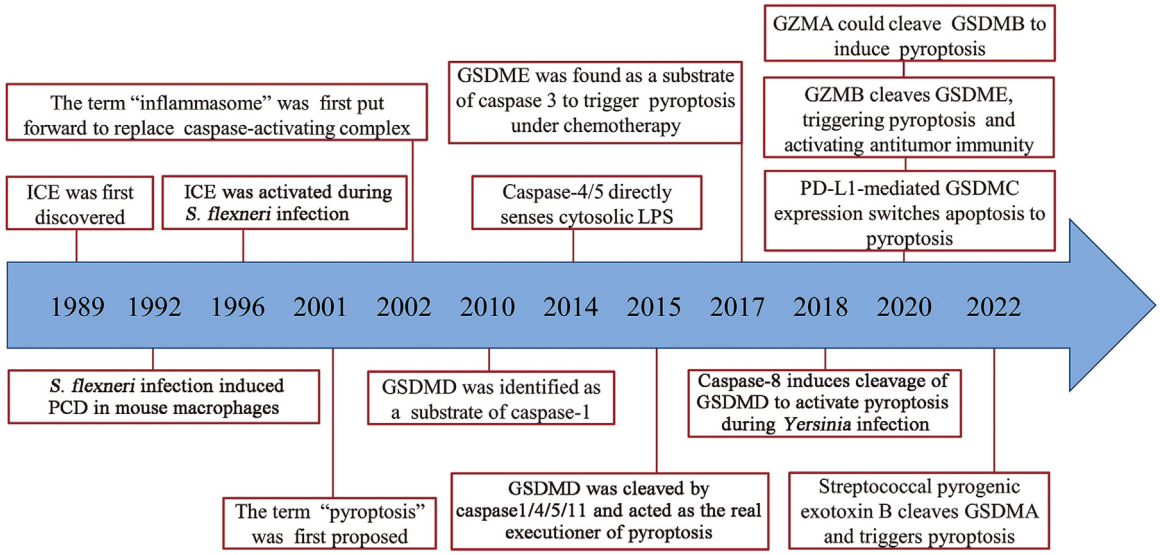

Fig. 1. Timeline of pyroptosis research. GSDMA: gasdermin A; GSDMB: gasdermin B; GSDMB: gasdermin B; GSDMC: gasdermin C; GSDMD: gasdermin D; GSDME: gasdermin E; gasdermin E; GZMA: granzyme A; GZMB: granzyme B; ICE: interleukin-1 beta converting enzyme; LPS: lipopolysaccharide; PCD: programed cell death; PD-L1: programed cell death protein ligand 1; *S. flexneri*: *Shigella flexneri*.

Table 1
Comparison of different pyroptotic pathways.

Pathways	Trigger	Sensor	Adaptor	Effector	GSDM	IL-1β and IL-18	References
Canonical	*Bacillus anthracis*	NLRP1	ASC	Caspase-1	GSDMD	+	17
	DAMPs and PAMPs	NLRP3					16
	PAMPs	NLRC4 (NAIP)					18,19
	dsDNA	AIM2					20
	Toxin/Rho GTPase	Pyrin					21
Non-canonical	LPS	–	–	Caspase-4/5/11	GSDMD	–	13
Alternative	*Yersinia*	–	–	Caspase-8	GSDMD	–	27
	Activated NK cells or cytotoxic T cells	–	–	Caspase-3, GZMB	GSDME	–	25,29
	Hypoxia and PD-L1	–	–	Caspase-8	GSDMC	–	28
	–	–	–	Caspase-1, GZMA	GSDMB	–	30,31

AIM2: absent in melanoma 2; ASC: apoptosis-associated speck-like protein containing CARD; CARD: caspase activation and recruitment domain; DAMPs: danger-associated molecular patterns; dsDNA: double-stranded DNA; GSDM: gasdermin; GSDMB: gasdermin B; GSDMC: gasdermin C; GSDMD: gasdermin D; GSDME: gasdermin E; GZMA: granzyme A; GZMB: granzyme B; IL: interleukin; LPS: lipopolysaccharide; NAIP: nucleotide oligomerization domain (NOD)-like receptor (NLR) family apoptosis inhibitory protein; NK: natural killer; NLRC: nucleotide oligomerization domain (NOD)-like receptor (NLR) family CARD containing; NLRP: nucleotide oligomerization domain (NOD)-like receptor (NLR) family pyrin domain-containing protein; PAMPs: pathogen-associated molecular patterns; PD-L1: programed cell death protein ligand 1; –: not available.

ways, whereas the other members of the GSDM family (A/B/C and E) participate in alternative pathways.[9,10] GSDM is composed of an active pore-forming region of the N-terminus, a flexible linker region, and a region of the C-terminus that binds to a domain of the N-terminus to suppress its activation. The cleavage of the linker, induced by caspases or granzymes, results in the release of a region of the GSDM N-terminus, leading to the generation of a large pore with a diameter of 10–20 nm.[11,12]

Canonical pathway

Canonical pyroptotic death is activated via inflammasome assembly, with subsequent GSDMD cleavage and interleukin (IL) secretion (IL-1β/18), as shown in Fig. 2. Inflammasomes comprise assembled signaling complexes composed of sensors, adaptors, and effectors.[13] Pattern recognition receptors (PRRs), known as inflammasome sensors, recognize pathogen-associated molecular patterns and danger-associated molecular patterns. A subset of nucleotide oligomerization domain (NOD)-like receptors (NLRs) and Toll-like receptors (TLRs) participates in pyroptosis as sensors,[14] and NLR family pyrin domain-containing protein (NLRP) 1, NLRP3, NLR family caspase recruitment domain (CARD)-containing protein 4 (NLRC4), absent in melanoma 2 (AIM2), and pyrin are relatively well-studied inflammasome sensors at present,[15] as we summarzed in Table 1. NLRP3 senses various stimuli, such as electrolyte imbalances, toxins, pathogens, mitochondrial dysfunction, and metabolic changes.[16] Meanwhile, NLRP1 is activated after N-terminus cleavage by the anthrax lethal factor protease.[17] Moreover, NLRC4 inflammasome assembly is stimulated by cytosolic flagellin or components of the type 3 secretion system (T3SS) and the recruitment of NLR family apoptosis inhibitory protein (NAIP).[18,19] AIM2 can recognize impaired double-stranded DNA,[20] whereas pyrin is activated when small GTPases of the host Ras homologue (RHO) family are inactivated.[21] Subsequently, activated PRRs assemble with apoptosis-associated speck-like protein containing CARD (ASC, adaptor) and pro-caspase-1 (effector) to form an inflammasome assembly. After cleavage of the inflammasome assembly and pro-caspase-1, caspase-1 is activated and cleaves GSDMD, pro-IL-1β, and pro-IL-18, causing membrane pores to form, mediated by N-GSDMD, and the release of pro-inflammatory cytokines (IL-1β or IL-18).

Non-canonical pathway

Non-canonical pyroptosis pathways rely on caspase-4/5 (humans) and caspase-11 (mice), rather than caspase-1 (Fig. 2).[22] Intracellu-

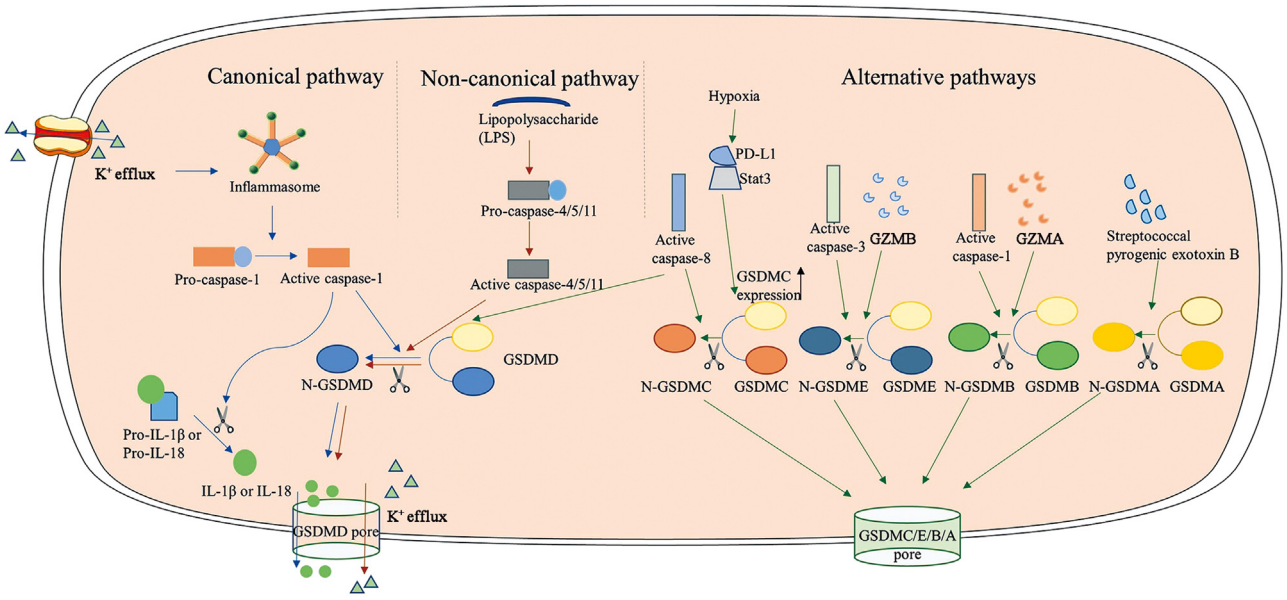

Fig. 2. Mechanisms of pyroptosis. Pyroptosis mechanisms can be divided into three types, canonical, non-canonical, and alternative. In the canonical pathway, inflammasome assembly is triggered by intracellular signals, which cleave GSDMD and pro-IL-1β/18 into N-GSDMD and IL-1β/18, respectively. IL-1β/18 is released through pores formed by N-GSDMD. In the non-canonical pathway, caspase-4/5/11 cleaves GSDMD into N-GSDMD after LPS stimulation. The latter forms membrane pores, leading K^+ efflux, which mediates inflammasome formation and induces pyroptosis. In the alternative pathway, caspase-8 cleaves GSDMD or GSDMC to induce pyroptosis. Under hypoxic conditions, PD-L1 facilitates self nuclear translocation through interactions with p-Stat3, which cleaves GSMDC and switches apoptosis to pyroptosis. Moreover, caspase-3/GZMB cleaves GSDME, caspase-1/GZMA cleaves GSDMB, and streptococcal pyrogenic exotoxin B cleaves GSDMA to activate pyroptosis. GSDM: gasdermin; GSDMA: gasdermin A; GSDMB: gasdermin B; GSDMC: gasdermin C; GSDMD: gasdermin D; GSDME: gasdermin E; GZMA: granzyme A; GZMB: granzyme B; IL: interleukin; LPS: lipopolysaccharide; PD-L1: programed cell death protein ligand 1; p-Stat3: phosphorylated-signal transducer and activator of transcription 3.

lar lipopolysaccharide (LPS) triggers direct caspase-4/5/11 activation to cleave GSDMD to form N-GSDMD.[5,11,22,23] Notably, in contrast to caspase-1, caspase-4/5/11 cannot cleave pro-IL-1β or pro-IL-18, but membrane pores shaped by N-GSDMD lead to K^+ efflux, which results in formation of the NLRP3 inflammasome and further activates the NLRP3/caspase-1 pathway to induce pyroptosis.[6]

Alternative pathways

Apoptosis is tightly correlated with pyroptosis as the two processes share caspases. Accordingly, crosstalk between the two is frequently observed. Pyroptosis-related caspase-1 mediates apoptosis in the absence of GSDMD.[24] Similarly, apoptosis-related caspase proteins can induce pyroptosis under certain conditions, such as those associated with targeted therapy or chemotherapy.[25,26] Multiple studies have elaborated on the role of other GSDM proteins in alternative pyroptosis pathways (Fig. 2). For example, pyroptosis is activated through the cleavage of GSDMD[27] or GSDMC,[28] mediated by caspase-8. Under hypoxic conditions, PD-L1 facilitates self nuclear translocation by interacting with phosphorylated-signal transducer and activator of transcription 3 (p-Stat3), which further promotes the cleavage of GSMDC and switches apoptosis to pyroptosis.[28] Caspase-3 and granzyme B (GZMB) cleave gasdermin E (GSDME),[25,29] and caspase-1 and granzyme B (GZMA) are involved in the cleavage of GSDMB.[30,31] A recent study first reported that streptococcal pyrogenic exotoxin B cleaves GSDMA and triggers pyroptosis.[32]

Role of pyroptosis in lung cancer

As a mode of PCD, pyroptosis is involved in tumorigenesis and antitumor defense in lung cancer. Further research has shown that the roles of pyroptosis in cancers are variable and contingent on cellular types, genetic characteristics, and the duration of pyroptosis activation. Clarifying the role of pyroptosis in lung cancer might help to understand its biology and further improve treatment strategies for lung cancer.

Involvement of pyroptosis in lung cancer development and progression

Recent studies have revealed that several pyroptosis components are involved in tumor oncogenesis, including NLRP3 inflammasome, AIM2, and IL-1β; involved in tumor progression including NLRP3 inflammasome, GSDMD, and GSDMC.

The NLRP3 inflammasome, a key molecule in the canonical pathway, plays an important role in lung cancer oncogenesis.[33,34] Duan et al.[34] demonstrated that human bronchial epithelial cells (BEAS-2B) were changed significantly in terms of morphology after stimulation with LPS and coal tar pitch extract, and the NLRP3 inflammasome was found to be involved in the malignant transformation of BEAS-2B cells. Benzopyrene or LPS induces lung tumorigenesis in mice, which can be inhibited by the loss of NLRP3.[33] Human AIM2 protein, which recognizes impaired double-stranded DNA, is upregulated in non-small cell lung cancer (NSCLC) and promotes oncogenesis in an inflammasome-dependent manner.[35] Further, the knockdown of *AIM2* hampers NSCLC development by promoting mitochondrial fusion and reducing reactive oxygen species (ROS).[36] IL-1β, a product of pyroptosis, mediates local inflammation and promotes tumorigenesis; as such, its therapeutic blockade with canakinumab was found to reduce the incidence of lung cancer in patients with atherosclerosis.[37] Overall, these findings indicate that pyroptosis plays a vital role in inflammation-associated lung oncogenesis.

In addition to oncogenesis, the NLRP3 inflammasome also enhances the proliferation and metastasis of A549 cells by activating serine/threonine kinase (Akt) and extracellular-regulated protein kinase (ERK) 1/2 signaling.[38] Moreover, GSDMD expression was found to be upregulated in NSCLC and correlated with larger tumors and a higher tumor stage.[39] Additionally, high GSDMD expression is associated with inferior survival in stage II lung adenocarcinoma (LUAD).[39] The knockdown of *GSDMD* attenuates cell proliferation, promotes cancer cell apoptosis through mitochondrial pathways, and inhibits epidermal growth factor receptor/Akt signaling.[39] Similarly, GSDMC expression was found

to be upregulated in LUAD and correlated with poor survival.[40] Under hypoxic conditions, based on bioinformatic tools, PD-L1 was determined to interact with p-Stat3 and facilitate self nuclear translocation, which was found to further promote the expression of GSMDC and the switching from apoptosis induced by tumor necrosis factor α (TNF-α) to pyroptosis, mediated by nuclear PD-L1.[28] These results support the hypothesis that pyroptotic molecules are involved in lung cancer development and progression.

Effects of pyroptosis on inhibiting lung cancer growth

Pyroptosis components, including GSDMD and GSDME, also participated in inhibiting growth of lung cancer cells. There is a positive correlation between the expression of $CD8^+$ T cell marker genes and GSDMD, and deficiency of GSDMD can reduce the cytolytic capacity of $CD8^+$ T cells.[41] It is contradictory with high GSDMD expression in NSCLC patients with inferior survival.[39] This may be attributed to that GSDMD has different biological functions in different cancer cell types, which requires further studies. In terms of GSDME, its expression level in most lung cancer specimens is lower than that in paired normal tissues.[42] However, some studies have reported inconsistent results.[43,44] Further research with a larger sample size is needed to conclude on the roles of GSDME expression in lung cancer. Notably, all of these studies reported that higher GSDME expression levels correlate with superior survival in patients with NSCLC.[42–44] Regarding GSDMA/B, there are limited data on its role in lung cancer, which is worthy of further exploration.

Novel treatment strategies to induce pyroptosis in lung cancer

Activation of pyroptosis in cancer cells as a new treatment strategy

Given that inflammasomes, caspases, and GSDM proteins play key roles in mediating pyroptotic cell death, drugs that can activate these components have the potential to be used as new cancer treatments (Fig. 3). The NLRP3 inflammasome triggers the conversion of dormant procaspase to active caspase via proteolytic cleavage, which converts GSDM into the active GSDM N-terminal domain. Cucurbitacin B, a natural triterpenoid derived from a *Cucurbitaceae* plant, upregulates the levels of ROS/Ca^{2+} and triggers GSDMD-dependent pyroptosis via NLRP3, suppressing NSCLC growth *in vitro* and *in vivo*.[45] In addition, polyphyllin VI induces pyroptosis by activating the ROS/NLRP3/GSDMD pathway in NSCLC.[46] Compound 8, a novel chalcone analog, triggers caspase-3-mediated pyroptosis via ROS modulation and further inhibits the proliferation of NSCLC cell lines.[47] Moreover, a novel piperlongumine analog effectively induces pyroptosis through ROS-mediated nuclear factor-κB (NF-κB) inhibition.[48] In addition, simvastatin treatment suppresses the proliferative and migratory capabilities of NSCLC cells via NLRP3/caspase-1-mediated pyroptosis.[49] In 2020, Tang et al.[50] found that the inhibition of maternal embryonic leucine zipper kinase (MELK) upregulates caspase-3 expression and induces GSDME-dependent pyroptosis. Dasatinib was also found to increase the expression of GSDMD and GSDME and induce pyroptotic cell death in lung cancer cells.[51] Furthermore, the secretoglobin (SCGB) 3A2 chaperones LPS into cells, and induces pyroptosis via the non-canonical inflammasome pathway in NSCLC cells, which might serve as a novel anti-cancer therapeutic.[52,53]

Although the mechanism is yet to be fully elucidated, switching from apoptosis to pyroptosis under certain conditions might contribute to anti-tumor effects. L61H10, a new thiopyran derivative, induces pyroptosis, shifting from apoptosis via inhibiting the NF-κB signaling pathway.[54] Similarly, 13d, emerging as a novel NF-κB inhibitor, was shown to lead to an apoptosis-to-pyroptosis switch that correlates with the inhibition of NF-κB.[55] Surprisingly, multiple lung cancer cells display the co-occurrence of and interplay between apoptosis and pyroptosis during tyrosine kinase inhibitor exposure, which indicates that pyroptosis partially contributes to the response to molecular-targeted drugs.[26] One recent study showed that apurinic/apyrimidinic endonuclease 1 (APE1) inhibition induces apoptosis, pyroptosis, and necroptosis in A549 and NCI-H460 lung cancer cells, which increases their sensitivity to both cisplatin and erlotinib.[56] Additionally, sea hare hydrolyzates, extracted from sea hares, stimulate M1 macrophages, activate caspase-1 and IL-1β, and induce pyroptotic/necroptotic NSCLC cell death.[57]

Pyroptosis in cytotoxic chemotherapies

Pyroptosis is a double-edged sword for cancer chemotherapy. On one hand, pyroptosis in normal tissue mediates the toxicity of chemotherapeutic drugs. On the other hand, pyroptosis in cancer cells produces anti-tumor effects and is a potential mechanism underlying the effects of chemotherapeutic agents. As such, balancing the contradictory effects of pyroptosis might help to optimize the clinical application of chemotherapeutic drugs.

Previous studies have demonstrated that chemotherapy induces apoptosis by activating caspase-3.[58,59] In 2017, Wang et al.[25] reported that chemotherapy also induces pyroptosis by activating caspase-3 and cleaving GSDME, which is silenced by promoter methylation in most cancers. Furthermore, pyroptosis activation was observed in lung cancer cells with high GSDME expression upon exposure to chemotherapeutic agents.[25] The switch from apoptosis to pyroptosis in cancer chemotherapy thus depends on the expression of GSDME.

The activation of GSDME-dependent pyroptosis in tumors induced by chemotherapeutic drugs generates anti-tumor effects, and the degree of activation differs between drugs. *In vitro* experiments have shown that paclitaxel and cisplatin trigger pyroptosis in LUAD through caspase-3/GSDME activation in a slightly different manner.[60] Compared to that of paclitaxel, cisplatin has a stronger ability to activate caspase-3 and generate N-GSDME. Caspase-3 inhibitors or GSDME knockdown suppress cisplatin-induced, but not paclitaxel-induced, pyroptosis. These findings suggest that cisplatin induces caspase-3/-7 activation and GSDME cleavage more efficiently than paclitaxel, resulting in a stronger pyroptosis-promoting effect.

GSDME-dependent pyroptosis is involved in overcoming chemoresistance. One study revealed that the ablation of miR-556-5p induces pyroptotic cell death in cisplatin-resistant NSCLC cells by promoting NLRP3 expression and caspase-1 cleavage, which suggests a novel strategy to sensitize NSCLC cells to cisplatin.[61] Another study reported that the knockdown of long non-coding RNA-X inactive specific transcript (lncRNA-XIST) hampers cancer cell growth and exerts cisplatin sensitivity-enhancing effects by facilitating both apoptosis and pyroptosis in NSCLC cells.[62] It was further illustrated that the downregulation of lncRNA-XIST reduces NSCLC cell proliferation by activating miR-335/superoxide dismutase 2 (SOD2)/ROS pathway-mediated pyroptotic cell death.[63]

To some extent, pyroptosis also contributes to the adverse effects of cytotoxic chemotherapies. For example, $Gsdme^{-/-}$ mice do not exhibit chemotherapy-associated tissue damage and show better chemotherapy tolerance.[25] Furthermore, pyroptosis via caspase-3 and GSDME is involved in doxorubicin (DOX)-induced cardiotoxicity.[64,65] A subsequent study also demonstrated that chemotherapy-induced nephrotoxicity is induced by caspase-3/GSDME signaling.[66] These results suggest that targeting GSDME in specific organs might reduce the toxicity of cytotoxic chemotherapies. In summary, cytotoxic chemotherapies can induce pyroptotic cell death in both normal tissues and tumor cells, which is associated with adverse effects, anti-tumor effects, and sensitization to chemotherapeutic drugs.

Pyroptosis in radiotherapy

Pyroptosis might play a role in the adverse effects during radiotherapy. The AIM2 inflammasome, which recognizes impaired double-stranded DNA, contributes to radiation-associated DNA damage. The knockout of AIM2 was found to protect mice from radiation-induced gastrointestinal and hematological toxicity caused by caspase-1-mediated

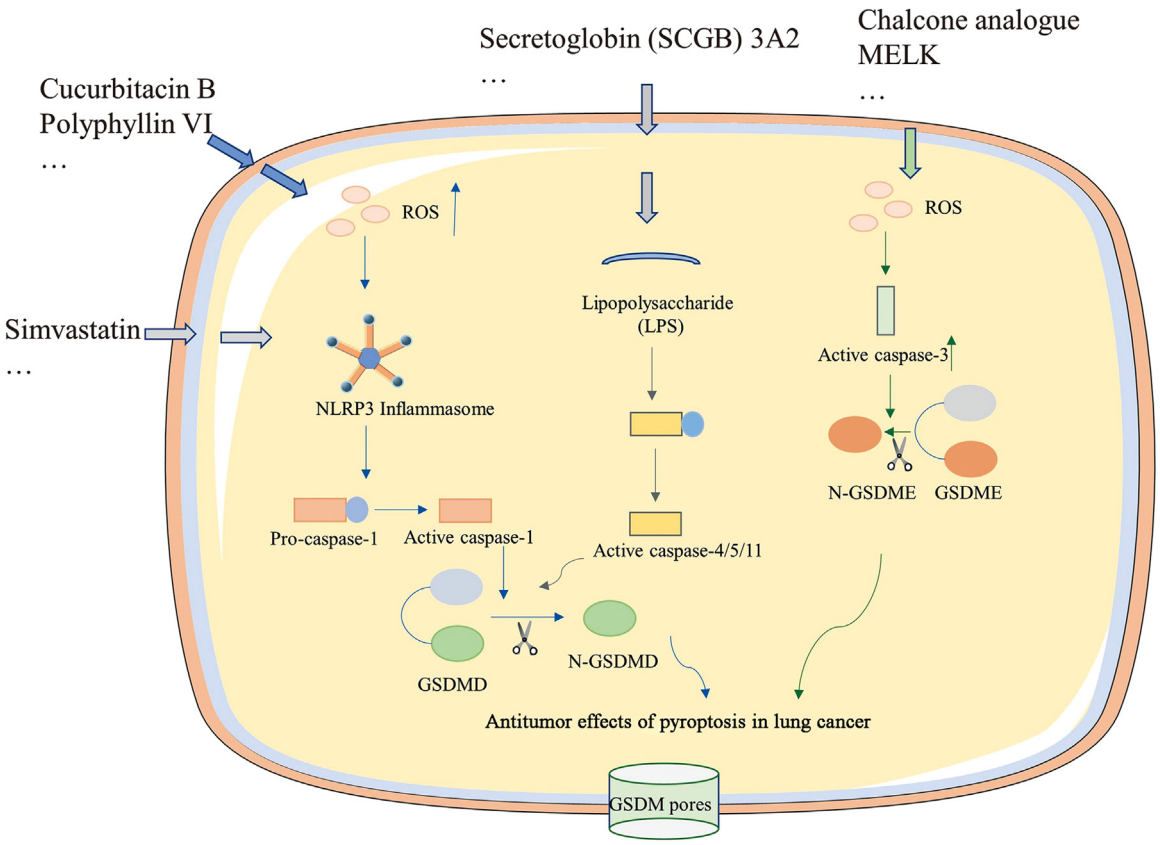

Fig. 3. Pyroptosis-mediated therapy in lung cancers. Drugs that can activate the NLRP3 inflammasome, caspases (such as caspase 1/4/5/11/3), and GSDM proteins (such as GSDMD and GSDME) in the pyroptotic pathway exert pyroptosis-mediated antitumor effects in lung cancer. GSDM: gasdermin; GSDMD: gasdermin D; GSDME: gasdermin E; LPS: lipopolysaccharide; MELK: maternal embryonic leucine zipper kinase; NLRP3: nucleotide oligomerization domain (NOD)-like receptor family pyrin domain-containing protein 3; ROS: reactive oxygen species.

pyroptotic cell death.[67] Moreover, NLRP3 inflammasome-mediated pyroptosis is triggered in macrophages derived from the bone marrow during radiotherapy and is suppressed after NLRP3 deletion.[68] Overall, pyroptosis in normal tissues constitutes a side effect of radiotherapy, and NLRP3 and AIM2 inflammasomes could be potential therapeutic targets to diminish irradiation-induced injuries.

Pyroptosis in targeted therapy

Pyroptosis has been proven to be a potential mechanism underlying the response to targeted therapy in patients with lung cancer associated with druggable molecular targets. Lu et al.[26] observed typical pyroptotic cell morphological changes (balloon-like bubbles) in multiple lung cancer cell lines during targeted therapy, including A549 cells with *KARS*^{G12S} positivity, PC9 cells positive for an *EGFR* 19del alteration, and NCI-H3122 cells with an *EML4–ALK* fusion. Moreover, GSDME overexpression was found to sensitize cells to targeted therapy *in vitro*, whereas GSDME knockout impaired this efficacy, which could be reused after exogenous GSDME expression. Similarly, *BRAF* and *MEK* inhibitors promote the cleavage of GSDME and activate pyroptosis, and GSDME-deficient tumors are associated with impaired anti-tumor effects.[69] These findings indicate that therapies that activate pyroptosis might be a potential strategy to enhance efficacy and overcome resistance during targeted therapy. For example, circ7312 mediates osimertinib resistance, and its inhibition was found to enhance drug efficacy by activating pyroptosis.[70] Further, inhibiting apurinic/apyrimidinic endonuclease 1 induces apoptosis, pyroptosis, and necroptosis in lung cancer cells, which increases drug sensitivity to cisplatin and erlotinib.[56] In conclusion, the activation of pyroptosis might potentiate the efficacy of targeted therapy and exert a synergistic effect.

Pyroptosis-related immunological effects in lung cancer

Chronic inflammation promotes tumorigenesis, whereas acute inflammation facilitates the recruitment of immune cells and modifies the TME.[71] The infiltration of immune cells into tumor tissues is a prerequisite for anti-tumor immunity.[72] Although the mechanism underlying the activation of anti-tumor immunity mediated by pyroptotic tumor cells remains unclear, pyroptosis in the TME activates acute inflammation with some anti-tumor immunity, which provides a strategy to “warm up the cold tumor”, as shown in Fig. 4. Moreover, the secretion of multiple inflammatory chemokines to recruit T cells into cancer tissues might play a crucial role in promoting anti-tumor immunity induced by pyroptosis.

Pyroptosis-related components are closely associated with the infiltration and function of multiple immune cells in the TME. Peng et al.[42] observed that with cisplatin treatment, the overexpression of GSDME increased the number of tumor-infiltrating CD3$^+$ T cells in the TME and that the levels of TNF-α and interferon γ (IFN-γ) were increased in both NSCLC tissues and the blood. Similarly, in tumors with low GSDME expression (such as breast and colorectal cancer), tumor-infiltrating lymphocytes (TILs) are more likely to be recruited when GSDME expression is upregulated. In addition, GSDME expression promotes the functions of tumor-infiltrating CD8$^+$ T lymphocytes and natural killer (NK) cells, which produce IFN-γ and TNF.[29] Moreover, GSDME is positively correlated with macrophages and CD4$^+$ and CD8$^+$ T lymphocytes in LUAD specimens.[73] Treatment with decitabine (DAC), one of the most commonly used DNA methyltransferase inhibitors, results in the dysregulation of the methylated modifications of DNA and restores the functions of silenced genes.[74] GSDME expression in macrophages and tumor cells was found to be upregulated following DAC treatment. The combination

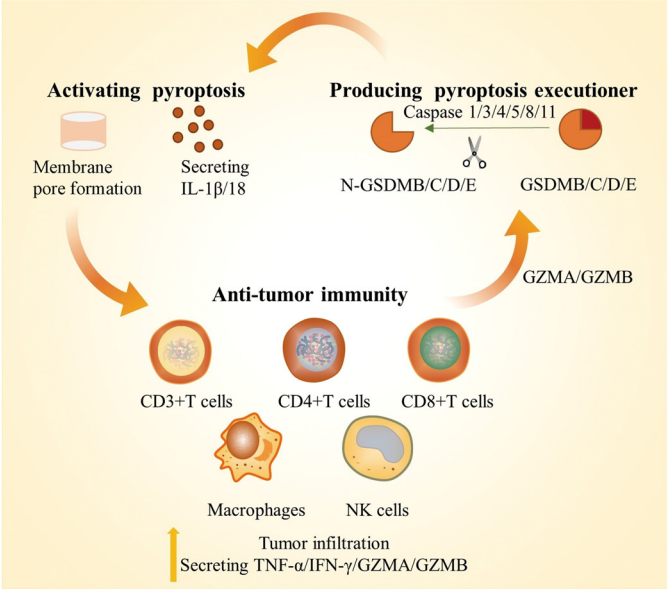

Fig. 4. Association between pyroptosis and immunological effects. GSDM: gasdermin; GSDMB: gasdermin B; GSDMC: gasdermin C; GSDMD: gasdermin D; GSDME: gasdermin E; GZMA: granzyme A; GZMB: granzyme B; IFN-γ: interferon γ; IL: interleukin; NK: natural killer; TNF-α: tumor necrosis factor α.

of DAC with chemotherapeutic drugs induces severe pyroptosis in cancer cells with low GSDME expression, augmenting the anti-tumor immunological effects of chemotherapy.[75] Based on these findings, respirable inhalable microspheres loaded with DAC and DOX, with the capacity to induce cell pyroptosis, were examined for orthotopic lung cancer treatment with fewer systemic adverse reactions.[76] Moreover, an increase in GSDMD cleavage was observed in human active $CD8^+$ T cells, and GSDMD expression was determined to be associated with $CD8^+$ T cell markers in a public database.[41] The phenomenon that GSDMD deficits weaken the cytotoxicity of $CD8^+$ T cells demonstrates that GSDMD is indispensable for $CD8^+$ T cell anti-tumor responses in lung cancer.[41]

Multiple pyroptosis-based models or signatures have been constructed, and these were demonstrated to be related to immune infiltration in lung cancer. For example, a pyroptosis-related five-lncRNA signature (colorectal neoplasia differentially expressed [CRNDE], HERV-H LTR-associating 3 [HHLA3], MIR193b-365a host gene [MIR193BHG], long intergenic non-protein coding RNA 941 [LINC00941], and long intergenic non-protein coding RNA 1843 [LINC01843]) was found to correlate with the infiltration of $CD4^+$ memory T cells, $CD8^+$ T cells, and macrophages in LUAD.[77] Moreover, other signatures based on pyroptosis-related lncRNAs are also associated with prognosis and the TME in lung cancer.[78] In addition to lncRNAs, multiple models based on pyroptosis-related genes are related to survival and inflamed immunotypes in lung cancer.[79–85] These findings provide a novel perspective for predicting immune responses and developing targeted drugs.

The development of immune checkpoint blockade therapies has dramatically changed the treatment strategies for lung cancer; however, the response rates are still not satisfactory.[86,87] Generally, a high population of $CD8^+$ T lymphocytes in tumors is associated with superior clinical outcomes with PD-1 blockade therapy.[88,89] P2RX7 (P2X7 receptor), known as an adenosine triphosphate (ATP)-gated ion channel, is involved in the activation of NLPR3 and caspase-1.[90] HEI3090, a positive modulator of P2X7R (P2X7 receptor), regulates dendritic cells expressing P2X7R to produce IL-18, which facilitates the recruitment and activation of NK cells and $CD4^+$ T cells and the production of IFN-γ within tumors.[91] A combination of HEI3090 and a PD-(L)1 inhibitor showed remarkable antitumor efficacy both *in vitro* and *in vivo*, which can be partly attributed to increased expression of major histocompatibility complex I (MHC-I) and PD-L1 on tumor cells stimulated by IFN-γ. Given the effect of pyroptosis on the TME, studies on the pyroptosis system can provide valuable information to enhance the efficacy of immunotherapy. Introducing an N-terminal form of GSDM with an activator selectively into tumor cells to augment the infiltration of T cells might thus be an alternative therapeutic strategy.

At an ineffective dose, nanoparticle-conjugated gasdermin plus Phe-BF3 was found to increase sensitivity to PD-1 inhibitors in 4T1 cancers.[92] Three NSCLC patients experienced a survival benefit from a novel therapeutic regimen comprising low-dose DAC plus camrelizumab, suggesting the potential efficacy of combining DAC with PD-(L)1 inhibitors for NSCLC.[93] Combined chemotherapy with immune checkpoint inhibitors displays remarkable clinical efficacy, with a higher response rate and longer progression-free survival and overall survival.[94] Pyroptosis induced by chemotherapeutic drugs might stimulate T cell recruitment in tumors, enhancing the response to immune checkpoint inhibitors. However, each coin has two sides. Immune checkpoint inhibitors reactivate immune cells, which can secrete GZMA and GZMB, cleave GSDM to induce pyroptosis in normal cells, and recognize normal cells sharing antigens expressed in tumors and cause cell lysis.[95] Both can account for immune-related adverse events to some extent.

Conclusion

In this review, we summarize the roles of pyroptosis in lung cancer and propose future directions. Many prognostic biomarkers based on pyroptosis-related genes have been identified, but the effects of pyroptosis on tumorigenesis, progression, and prognosis are not consistent in lung cancer. Pyroptosis components increase the proliferative and migratory abilities of lung cancer cells, but also promote an antitumor immune microenvironment. The dual mechanism of promoting and inhibiting tumor development remains unclear. Moreover, the toxic and antitumor effects of pyroptosis during therapy often occur simultaneously. Optimizing treatment effectiveness and minimizing side effects is thus worth exploring. Considering the immunological effects of pyroptosis in lung cancer, the approach of enhancing the efficacy of immunotherapy by activating pyroptosis should be explored further in lung cancer.

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Conflicts of interest

None.

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