Application of pyroptosis in tumor research (Review)

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Abstract. As a potent clinical strategy, cancer therapy has sparked an academic boom over the past few years. Immune checkpoint inhibitors (ICIs) have been demonstrated to be highly successful. These achievements have progressed cancer treatment and have made an indelible mark on cancer. However, the inherent complexity of cancer means that only part of the population can benefit from this treatment. Pyroptosis is a new suicidal cellular mechanism that induces inflammation by releasing immunogenic cellular components. Inflammatory signaling cascades mediated by pyroptosis commonly inspire numerous cell lysis in immune diseases. Contrariwise, this consequence may be a promising target in cancer research. Therefore, the present study briefly described programmed cell death processes and their potential roles in cancer. Because of the rapid development of bioengineering in cancer, the present study also examined the associated scaffolding available for cancer, highlighting advances in tumor engineering approaches. Ultimately, an improved understanding of pyroptosis and tumor scaffolding might shed light on a combination that can be manipulated for therapeutic purposes.

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1. Introduction

The cells in the body keep updating themselves throughout life. Alterations in cell niches caused by physical wounds, chemical stimulation and genetic hallmarks cause mutations, and these mutations lead to cancer initiation (1). Cancer has a high mortality rate worldwide (2), resulting in the loss of 8.97 million lives, even with advances in medical technology (3). Cancer treatments, including surgery, chemotherapy and radiotherapy, have brought positive results. However, the generalized application of these measures is often restricted due to severe adverse side effects and insufficient therapeutic effects. Immunotherapy for cancer has become an area of great interest to researchers sue to recent clinical success (4). Representative antibodies against cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death 1 in the removal of multiple malignant cancer types, including melanoma, small cell lung cancer, and head and neck squamous cell carcinoma, are making milestone advances in cancer treatment (5,6). Chimeric antigen receptor T cells also provide beneficial antitumor impacts (7). However, such means still fail to implement the fact that it is possible to treat all individuals. To date, scientists are still struggling to come up with new treatment strategies.

Pyroptosis, a type of inflammatory programmed cell death, induces systematic inflammation by releasing pro-inflammatory intracellular contents (8). With the advancement of research, the recognition of pyroptosis in cancer is becoming more apparent. Once inflammasomes activate pyroptosis, it typically provokes a cascade of reactions, including swelling of tumor cells, plasma membrane lysis, chromatin fragmentation and exposure of proinflammatory contents under the influence of caspase-1/4/5/11 (9). This pyroptosis mediated by gasdermin facilitates immune cell activation and infiltration and results in a robust inflammatory response and marked tumor regression (10). Tumors are often called cold tumors due to resistance to immune checkpoint inhibitors (ICIs) and small amounts of T-cell infiltration (11). From the perspective of tumor treatment, induction of pyroptosis may be a potential choice for the recruitment actions of immune cells. These immune effects directly cause 'cold' tumors that become 'hot' tumors with high levels of T cell infiltration, with the aim to regulate the tumor microenvironment (12).

However, methods to regulate pyroptosis still need to be investigated by scientists. Researchers focus on various multifunctional controlled drug distribution systems comprising of

different polymers to trigger pyroptosis (13,14). Also, these efforts drive the rapid development of bioengineering in cancer treatment (15). The present review discusses the features and mechanisms of pyroptosis, and evaluates the clinical value in the oncology of pyroptosis. Furthermore, the present review shares the recent accomplishments in biological engineering associated with pyroptosis in cancer research and provides a future outlook.

2. Characteristics of pyroptosis

In the 1990s, researchers observed cell suicide releases a burst of inflammatory cytokines in macrophages exposed to salmonella (16,17). However, this subversive form of programmed cell death (PCD) was termed pyroptosis until 2002 (18). As another conserved type of PCD, apoptosis, which is mainly initiated by caspase-3 and the microenvironment, has been the subject of intensive research for the past 3 decades (19). These efforts enhanced the comprehensive understanding of features in apoptosis, including nuclear condensation, membrane blebbing, caspase-dependent and DNA fragmentation. It is interesting to note that pyroptosis also contains these characteristics and was initially regarded as apoptosis for this reason. To some extent, this overlap delayed the research process. Moreover, the overlap was not resolved until a clear definition of pyroptosis was given in 2007 (20). With subsequent research, the definite difference in morphological and biochemical characteristics between the apoptosis and pyroptosis has been demonstrated (Table I) (21).

Regarding morphology, pyroptosis presents discriminative alternations, such as pore formation, cell swelling and osmotic lysis with cytosolic contents leakages (22). External challenges promote the activation of caspases and the release of granzymes in cells. Finally, gasdermin D (GSDMD) was cleaved to form a transmembrane pore (23), which results in an unbalance of transmembrane ion fluxes (24). Following that, incoming water molecules trigger the plasma membrane break event. Cytoplasmic swelling induces osmotic cell lysis by releasing pro-inflammatory cytokines (IL-1β and IL-18), leading to the recruitment of immune cells (25). From a biochemical point of view, caspase-1, 4, 5 and 11 act as initiators and effectors in pyroptosis, which differs from apoptotic caspases (26,27). Collectively, these characteristics distinguish pyroptosis and apoptosis, and accelerate our understanding of them.

3. Molecular mechanisms of pyroptosis

Canonical inflammasome pathway. The canonical inflammasome complex is usually composed of a cytosolic sensor called pattern recognition receptors (PRRs), an adapter protein named apoptosis-associated speck-like protein (ASC) containing a C-terminal caspase activation and recruitment domain (CARD), an N-terminal pyrin domain and inflammatory caspases (Fig. 1A) (28,29). PRRs [a nucleotide-binding domain or leucine-rich repeat receptors (NLRs) or absent in melanoma 2 (AIM2)-like receptors] are capable of recognizing pathogen-associated molecular patterns and danger-associated molecular patterns (DAMPs) (30,31). Then, ASC acts as a connector between sensors and the effector protein-caspase-1 (32). Finally, inflammasome

assembly activates caspase-1-dependent canonical pyroptosis by GSDMD-mediated pore formation (33). Meanwhile, the cleavage of caspase-1 results in the maturation of IL-1β and IL-18 (34). Notably, caspase-1 plays an essential role in the canonical pyroptosis pathway. Among these inflammasome subtypes, NLRP3 inflammasome containing NLR-protein-3 is considered to be associated with pyroptosis to sense a wide range of stimuli (35,36). Some evidence hints that therapies targeting the NLRP-3 inflammasome might hold potential for treatment of various types of cancer, including non-small cell lung cancer, breast cancer and colorectal cancer (37-39).

Non-canonical inflammasome pathway. The non-canonical pyroptosis pathway differs from the canonical route because of an activation without the requirement for inflammasomes (40). Lipopolysaccharides (LPS) secreted by the majority of gram-negative bacteria are directly bound to the N-terminal CARD of caspase, which activates caspase-4/5 in humans or caspase-11 in mice (41,42). The activated caspases cleave GSDMD into N-GSDMD to perforate the cell membrane and drive pyroptosis (43). Additionally, N-GSDMD transfers positive feedback to NLRP3 or AIM2 inflammasomes via the efflux of K^+ (44). This signal induces NLRP3/caspase-1 activation, also leading to the maturation of IL-1β and IL-18 (45).

Gasdermin D as the key effector of pyroptosis. The pore-forming family is made up of five members, including GSDMA, GSDMB, GSDMC, GSDME and GSDMF (46), that can exert their function by the release of the N-terminus, such as in group A *Streptococcus*-driven GSDMA cleavage, lymphocyte-derived granzyme A-mediated GSDMB cleavage, caspase-8-mediated GSDMC cleavage, caspase-1/4/5/11-mediated GSDMD cleavage and caspase-3-mediated GSDME cleavage (47-52). Among them, GSDMD is considered to have a classic role because it is a generic substrate for inflammatory cases and a downstream effector of multiple inflammasomes (53-55). The cleaved GSDMD-N assembles into ring-shaped oligomers, permeabilizing the membrane (56). Both canonical and non-canonical pathways are finally subject to GSDMD, triggering pyroptosis (57).

4. Antitumor potential of pyroptosis

Cell death is mediated by accidental cell death (ACD) or regulated cell death (RCD) (58). RCD is mainly focused on by researchers due to uncontrollable ACD. An increasing number of complex molecular controls, including ferroptosis, necroptosis and pyroptosis, have been identified (59,60). Intrinsic signal-mediated death processes are involved in the transformation, growth, invasion and metastases of malignant cells, which play a guiding role in the treatment of human cancers (61). As a unique inflammatory death, pyroptosis is renowned for its particular mechanism and destructive lethality (57). Advances involving targets and products of pyroptotic pathways including the GSDM family over the past few years have revolutionized the status of pyroptosis in treating different types of cancer (62). A number of cancer research attempts by scientists focusing on GSDMD have also yielded positive results. Yan *et al* (63) enhanced the comprehensive understanding of cisplatin in patients with

Table I. Distinction between pyroptosis and apoptosis.

A, Morphology

Characteristics	Apoptosis	Pyroptosis
Programmed cell death	Yes	Yes
Apoptotic bodies	Yes	No
Pyroptotic bodies	No	Yes
Nuclear condensation	Yes	Yes
Chromatin condensation	Yes	Yes
Membrane blebbing	Yes	Yes
DNA fragmentation	Yes	Yes
Pore formation	No	Yes
Cell swelling	No	Yes
Osmotic lysis	No	Yes
Membrane integrity	Yes	No
Mitochondrial integrity	No	Yes

B, Biochemistry

Characteristics	Apoptosis	Pyroptosis
Caspase-1 activation	No	Yes
Caspase-4 activation	No	Yes
Caspase-5 activation	No	Yes
Caspase-11 activation	No	Yes
Caspase-3 activation	Yes	Yes
Caspase-6 activation	Yes	Yes
Caspase-7 activation	Yes	No
Caspase-8 activation	Yes	Yes
Caspase-9 activation	Yes	Yes
Caspase-10 activation	Yes	No

C, Influence

Characteristics	Apoptosis	Pyroptosis
Inflammation	No	Yes

triple-negative breast cancer, demonstrating that treatments are mediated by the GSDMD. Yuan et al (64) demonstrated that cucurbitacin B can inhibit non-small cell lung cancer with GSDMD-independent pyroptosis. These findings highlight that pore formation induced by GSDMD has a destructive effect on tumor cells. Furthermore, one study revealed that immune therapy targeting GSDME in colon cancer has a good effect (65). It has also been indicated that the presence of GSDME in tumors increases the recruitment of immune cells (66). Thus, inducting and activating GSDME may potentially be of clinical value.

Researchers have demonstrated that GSDME-based measures can convert the microenvironment of tumors with elevated levels of infiltrating immune cells, assisting in improving the response to immunotherapy (67-69). From a therapeutic perspective, pyroptosis is a notable choice for cancer treatment. However, pyroptosis is restricted in biomedical applications for the severe side effects caused by the chemotherapeutic drugs. Finding a safe and effective method in combination with immunotherapy is essential.

Immune checkpoints (IC) are cell-surface proteins controlling the initiation, duration and magnitude of immune responses (70). Tumor development is generally caused by IC-related immune evasion. Thus, patients with cancer benefit to a large extent from the application of ICI. Pyroptosis is positively associated with immune infiltration and immune characteristics in 30 types of cancer and directly modulates the expression of immune checkpoint molecules (71). This is consistent with current cancer treatment strategies to convert cold tumors into a hot ones. Clinical evidence has also revealed the potential value of pyroptosis in predicting immunotherapy responses and a theoretical rationale for combining pyroptosis inducers and immunotherapy in cancer treatment (72). Pyroptosis works in the same way as ICIs to strengthen tumor immunity, exerting a powerful potential in the treatment of cancer (73).

5. Role of pyroptosis in inducing immunogenic cell death

Immunogenic cell death (ICD) is a unique form of stress-driven cell death that is typically mediated by DAMPs (74). Applying stressors, including pathogens, viruses and chemotherapeutic drugs, may be a novel treatment method to initiate adaptive immunity (75). Notably, pyroptosis seem to act as a potent stressor to trigger ICD due to exposure to a large amount of cell debris. From the perspective of immune regulation, cellular debris provides dendritic cells with antigens and inflammatory stimuli, and then activates CD8+ T cells to trigger an immune response called antigen cross-priming (76). Meanwhile, the process by which the contents of tumor cells are released with pro-inflammatory signals leads to the efficient immune destruction of cancer cells (77). This type of cascade could reprogram the tumor immune microenvironment into an immune stimulation state through the activation of DAMPs following osmotic lysis, ultimately inhibiting the spread and expansion of the tumor cells (78).

6. Strategies of pyroptosis in cancer therapy

Chemical drugs. Application of chemical drugs is a good choice for patients with cancer. Although a number of clinical drugs, including cisplatin, doxorubicin and dihydroartemisinin, have been approved by the Food and Drug Administration, the pace of research has not slowed down (79-81). More efforts are focused on determining the mechanism of cancer treatment and solving the problem of drug-resistant tumors. Therapies inducing pyroptosis in tumor cells are a current solution. For example, doxorubicin, a common chemotherapy drug, is strongly associated with caspase-3-mediated GSDME activation and JNK phosphorylation-based activation (82). Zhang et al (82) revealed that the GSDME was cleaved under doxorubicin treatment, resulting in the death of breast cancer cells. Abnormal activation of the pyroptosis-related protein caspase3 increases our understanding of doxorubicin. Cisplatin is another potential drug widely used for the treatment of various solid cancers, such as testicular, ovarian, head and
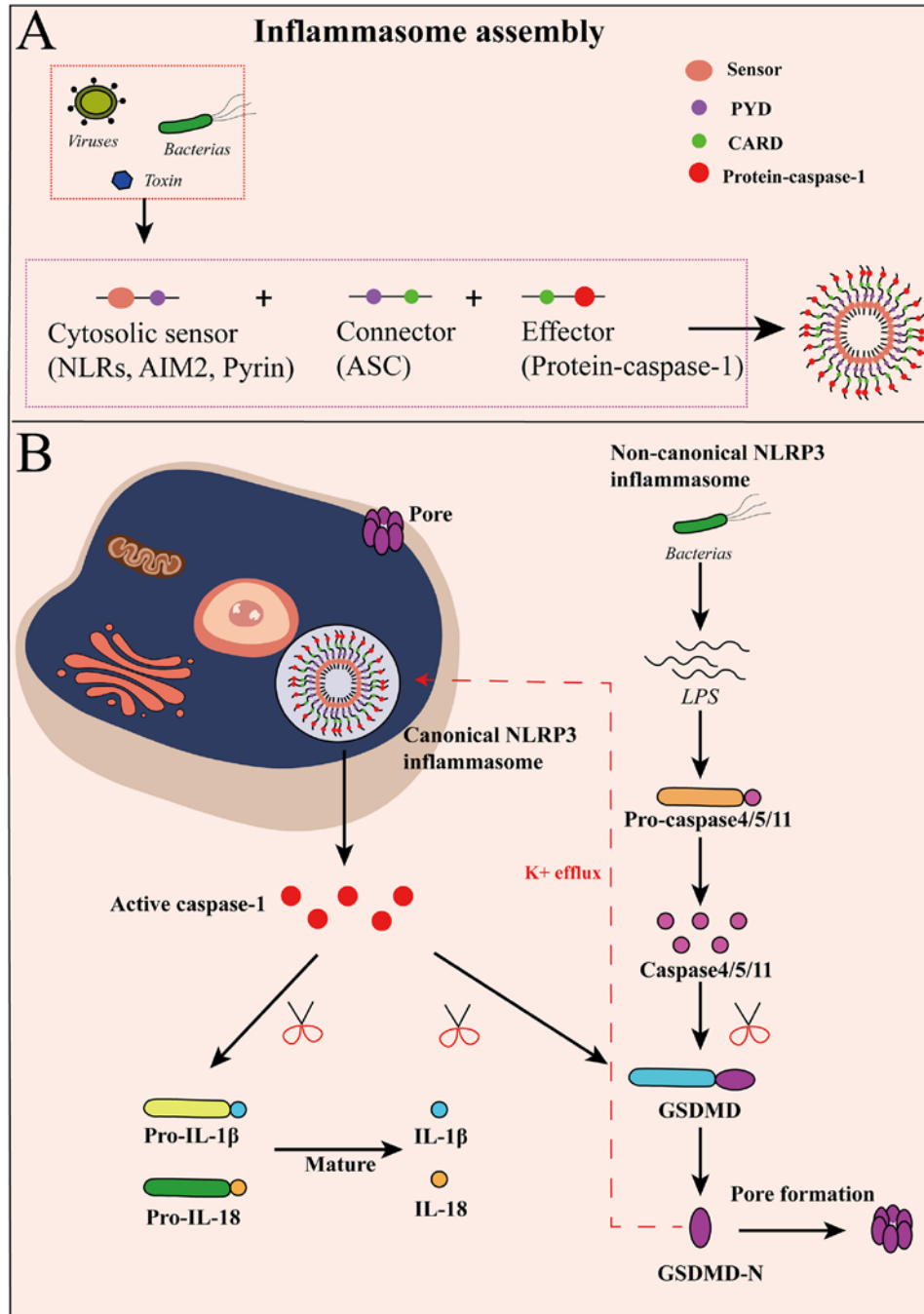
Figure 1. Canonical inflammasome pathway of proptosis. (A) Assembly of the inflammasomes. (B) Role of inflammasomes in pyroptosis. PYD, pyrin domain; CARD, caspase activation and recruitment domain; NLR, leucine-rich repeat receptors; AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein; GSDMD, gasdermin D.

neck, bladder, lung, cervical cancer, melanoma and lymphoma, among others (83). However, the discovery of treatment routes such as apoptosis and ferroptosis does not completely explain the excellent effects of cisplatin in cancer treatment. Notably, pyroptosis sheds new light on the therapeutic effects of cispl-atin. With the development of research, Yan *et al* (63) revealed that cisplatin can activate the NLRP3/caspase-1/GSDMD pyroptosis pathway by upregulating maternally expressed gene 3 (MEG3). Overall, these clues point to novel targets for treat-ment of tumors in the future. However, pyroptosis is restricted in biomedical applications due to the severe side effects caused by the chemotherapeutic drugs used. Therefore, finding a safe

and effective method in combination with immunotherapy is essential.

Nanoparticles. Nanoparticles (NPs) are generally defined as a particulate matter of ≤100 nm (84). Due to the diversity of size, composition, physical properties and chemical properties in NPs, NPs could be conceived as a functional scaffolding system combining targeting, biological responding and drug-releasing purposes. The pyroptosis strategies based on NPs promises to overcome several challenges related to low on-target bioavailability, poor therapeutic drug accumulation and adverse reactions (85).

Recently, the potential of NPs to modulate biological pyroptosis has been recognized gradually by researchers (15). Ploetz *et al* reported that hybrid metal-organic framework NPs consisting of iron (Fe^{3+}) and trimesic acids provide an external trigger for the induction of pyroptosis, according to the extracellular pH (86). Such particular NPs creating a controllable platform for iron delivery turned out to be a success. This success of the tumor treatment model is attributed to the physical induction of pyroptosis, which activates the tumor destruction mechanism. Particle effects triggered by physical stimuli such as sound, light and electricity combined with pyroptosis will help to promote the progress of tumor treatment (87). Except for the physical excitation mode in cancer, the creation of chemically induced cell pores is also relatively common (88). For instance, some ultrasmall NPs with diameters <10 nm act as Trojan horses and are successfully introduced into the pyroptotic cells, releasing extracellular LPS into cells through endocytosis and, in turn, inducing GSDMD-N-terminal membrane pores (89). Such chemically-caused cell inflammatory death is also a success. Notably, NPs-induced pyroptosis with this chemical form can maintain the continuous pyroptosis process and control the severity of pyroptosis (15). Consequently, the distribution system of NPs combined with drugs may be particularly important in the induction of pyroptosis. A previous study attempted to construct a nanoparticle carrier loading with indocyanine green and decitabine to induce activation of pyroptosis for photo-activated cancer cell pyroptosis and solid tumor immunotherapy (90). This induction method is more comprehensive and more effective in combining physical and chemical control. This study detected that selective accumulation of NPs in tumor activate a sharp increase of cytoplasm Ca^{2+} concentration after low-dose NIR photo-activation. Subsequently, the activation of caspase-3 reinforces cleavage with GSDME, triggering a systemic antitumor immunity by pyroptosis. This type of comprehensive understanding of NPs could be used as an effective tool to cause pyroptosis. From the standpoint of a trigger, nanoparticles play a function in the cell by endocytosis, then mediate the pyroptosis mode by physical and chemical methods (15). This method of mediating the formation of pores inside the cell seems to be more direct, faster and more efficient compared with chemical drugs.

Hydrogel delivery system. Although NPs have the benefits of targeted administration, there are still some limitations that make them unsuitable for long-term administration, such as bursting release, poor biological adhesion and irreversible deformation (91). Recently, hydrogels formed by cross-linked polymer networks have been widely utilized for cancer treatment. As a type of degradable multifunctional scaffold, hydrogels have been instrumental in providing intelligent drug delivery systems for cancer immunotherapy (92). The polymer, composed mainly of water, imitates highly hydrophilic biological tissues, a free-standing viscoelastic mesh, and has mechanical properties in ranges suitable for living tissues (93). These unique features give hydrogel the perfect drug delivery capabilities. Some hydrogel-based investigations have also shown applying hydrogel-mediated pyroptosis is a good choice in cancer treatment (94-96). The research using hydrogel as a scaffold for tumor cell pyroptosis opens up a new perspective.

Scientists reported the superiority of cellulose nanofiber-based hydrogels as a release drug stent (97,98). Another paper also pointed out the feasibility of embedding 5-FU on pyroptosis induction in cellulose nanofiber-based hydrogels (99). It was discovered that an important pyroptotic phenomenon was present in breast cancer cells via the caspase 1 cleavage, indicating pyroptosis-based immunotherapy with a hydrogel carrier is potential. However, to date, there has not been much research on hydrogel in the area of pyroptosis-based treatment of cancer.

Since more attention has been focused on treating diseases with traditional hydrogel in the past, hydrogel-based therapies in cancer are a compelling trend. Intelligent hydrogels that react to external stimuli, such as pH, light and temperature, show a solution-gel transition (100-102). This controlled shapeshift favors the development of hydrogel in cancer for controlled drug release, local treatment, fewer side-effects and easy administration. In recent years, the thermosensitive hydrogel has been applied in local cancer therapy. Temperature-controlled gels made of natural polymers such as chitosan, cellulose and hyaluronic acid also performed well, but these results seem to only meet the ideal expectations with immune intervention (103,104). Researchers are dedicated to finding a novel therapy combining the application of the immune checkpoint and thermosensitive hydrogel. For instance, the strategy based on thermosensitive hydrogel by releasing the nitric oxide donor and anti-CTLA-4 micelles achieved good feedback in tumor immunotherapy. The results demonstrated that hydrogel enhanced drug retention, and ultimately activated immune modulation within the tumor injection site (105). This discovery has expanded our understanding of their influence, particularly in relation to their role in drug liberation. Drugs that provoke pyroptosis closely associated with hydrogel will provide a new perspective.

7. Conclusions

Pyroptosis is an inflammatory form of cell death that relies on the formation of pores in the plasma membrane by proteins of the GSDM family (106). Plasma membranes play an essential role in the maintenance of homeostasis in mammalian cells. Thus, disrupting the integrity of the cell membrane will certainly end the life of the cell (107). A previous study has revealed that ~20 units of GSDMD-N construct a large oligomeric ring-shaped pore that directly leads to an elevation of the intracellular osmotic pressure, cell swelling and, eventually, bursting of the cell (108,109). These intracellular liberated substances soon activate a robust immune response, recruit immune effector cells, and are involved in numerous physiological pathological processes (110). As an important strategy for tumor survival and development, immune evasion of the tumour has emerged as a hot topic in antitumor research (111,112). Notably, pyroptosis is more effective at activating immune responses and pushing cancerous cells to the verge of death (113). The continuous dying of cancerous cells contributes to an even more violent cascade of inflammation through exposure to much of the contents of the cell (114). This will inevitably cause the body to become excessively inflamed. Consequently, how to manage and use this double-edged sword is especially important. Further exploration of the control

mechanism of pyroptosis and the search for effective ways to regulate pyroptosis may provide new ideas for treating related tumors (27).

It must be recognized that numerous medications that induce pyroptosis do suppress the tumor, but also bring side effects. There is increasing interest among researchers in drug delivery systems because of their controlled-release properties. This opens up new possibilities for the clinical application of stents based on pyroptosis drugs in the future. Although there are few studies on the stents combined with pyroptosis, it must be a new research trend in the future. For instance, injectable hydrogels are a suitable choice because they are a controlled release system to control the degree of pyroptosis, and may confine diseased tissue to a restricted area. With the slow degradation of the gels, the drugs in the gels are released slowly, which favors the formation of the GSDMD-mediated pore and the rupture of the tumor cells. The present study hypothesizes that the initial exposure of tumor cell contents at the distal end of the gel accelerates the immune clearance of tumor cells. Meanwhile, the side effects caused by excessive inflammation are avoided to a certain extent. It can be categorized as pyroptosis activated by external transmission signals with a reaction zone. Another strategy for inducing pyroptosis is inherent in nanomolecular technology. NPs charging various medicines cause a process of destruction from within. When the nanoparticles enter the cancer site, they are ingested by cancer cells, releasing the drug internally and reacting directly to the activation of the pyroptotic pathway. This strategy of internally induced pyroptosis also leads to the destruction of cancer cells and the clearance of immune responses triggered by the contents of damaged cells. Collectively, stent-based efforts to induce pyroptosis may provide new perspective in future research.

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Competing interests

The authors declare that they have no competing interests.

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