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Review

Role of tumor cell pyroptosis in anti-tumor immunotherapy

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ABSTRACT

Peripheral tumor-specific $CD8^+$ T cells often fail to infiltrate into tumor parenchyma due to the immunosuppression of tumor microenvironment (TME). Meanwhile, a significant portion of tumor-specific $CD8^+$ T cells infiltrated into TME are functionally exhausted. Despite the enormous success of anti-PD-1/PD-L1 immune-checkpoint blockade (ICB) treatment in a wide variety of cancer types, the majority of patients do not respond to this treatment largely due to the failure to efficiently drive tumor-specific $CD8^+$ T cell infiltration and reverse their exhaustion states. Nowadays, tumor cell pyroptosis, a unique cell death executed by pore-forming gasdermin (GSDM) family proteins dependent or independent on inflammatory caspase activation, has been shown to robustly promote immune-killing of tumor cells by enhancing tumor immunogenicity and altering the inflammatory state in the TME, which would be beneficial in overcoming the shortages of anti-PD-1/PD-L1 ICB therapy. Therefore, in this review we summarize the current progresses of tumor cell pyroptosis in enhancing immune function and modulating TME, which synergizes anti-PD-1/PD-L1 ICB treatment to achieve better anti-tumor effect. We also enumerate several strategies to better amply the efficiency of anti-PD-1/PD-L1 ICB therapy by inducing tumor cell pyroptosis.

1. Introduction

Cancer is one of the leading causes of adult death worldwide, with an estimated 10 million deaths in 2020 (Siegel et al., 2020, p. 202; Sung et al., 2021). Although several attempts have been made to improve the prognosis of oncology patients lately, fewer of these strategies have ultimately resulted in significant benefits on oncology patients. Among these, surprisingly, anti-PD-1/PD-L1 ICB treatment (such as Atezolizumab, Avelumab, and Durvalumab) have been discovered to gain great success in certain tumor types (Hodi et al., 2010; O'Day et al., 2010), including non-small cell lung carcinoma (NSCLC) (Rittmeyer et al., 2017), urothelial carcinoma (Powles et al., 2018), triple negative breast cancer (TNBC) (Iwata et al., 2019), and Merkel cell carcinoma (Kaufman et al., 2018). Since then, immunotherapy has gained widespread attention and quickly become the first-line therapy for certain cancer. However, as the understanding of anti-PD-1/PD-L1 ICB treatment has grown, we found that the efficiency of this treatment only reached about 20% due to the immunosuppressive mechanisms of TME, which led to rare $CD8^+$ T cell infiltration and unmodifiable progression of tumor-specific $CD8^+$ T cell exhaustion (Chen & Mellman, 2017; Philip & Schietinger, 2021). Therefore, attempts have been made to enhance the anti-tumor effect of ICB treatment through combining it with TME modulators (Kirchhammer et al., 2022). Recent studies found that tumor cell pyroptosis synergized with ICB treatment to enhance anti-tumor immunotherapy, which may be partly explained by the increase of $CD45^+$ immune cell infiltrated in TME (Zhou et al., 2020). In this review, we summarize the current progresses of tumor cell pyroptosis in modulating TME. Besides, the potential role of tumor cell pyroptosis in enhancing anti-PD-1/PD-L1 ICB treatment efficiency and our perspective for application have also been discussed.

2. Tumor immunotherapy and its current challenge

The "cancer-immunity cycle" is the main way in which our immune system surveils tumor cells. At the very beginning of this cycle, tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs), generated from ongoing tumor cell death, are captured by immature migratory dendritic cells (DCs) in situ (Chen & Mellman, 2013). These DCs undergo maturation after stimulated by pathogen-associated molecular patterns (PAMPs)/damage-associated molecular patterns (DAMPs) as well as

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inflammatory cytokines, and then migrate into tumor draining lymph nodes (TdLNs), where they prime naïve tumor-specific $CD8^+$ T cells by presenting peptide-MHC-I (pMHC-I) (Galluzzi et al., 2017). These naïve $CD8^+$ T cells undergo clonal expansion in TdLNs and differentiate into tumor-specific memory-like T cells (T_{TSM}), which further differentiate into exhausted precursor T cells (Tpex) expressing high level of migratory markers (such as S1PR1, S1PR5) for their recruitment into blood upon persistent antigen stimulation (Huang et al., 2022). After recruited into TME, circulating Tpex will transform into exhausted T cells (Tex) with low anti-tumor effect and proliferative capacity (Huang et al., 2022). Although the cytotoxicity of Tex is significantly reduced, these cells can still induce target tumor cell death via the specific recognition of TCR, while dead tumor cells will release additional tumor antigens as well as PAMPs/DAMPs, further motivating anti-tumor immune response (Chen & Mellman, 2013) (Fig. 1). In order to amplify the massive expansion and effector function of tumor-specific cytotoxic T cells (CTLs), anti-PD-1/PD-L1 antibodies, the mainstay of ICB treatment, emerged as the first line of anti-tumor immunotherapy. Based on the researches of chronic viral infection, anti-PD-1/PD-L1 antibodies primarily promote the proliferation of stem-like cells, referred to as Tpex, and drive their differentiation into terminal Tex from transitory state (He et al., 2016; Hudson et al., 2019; Im et al., 2016). Recently though, our group defined T_{TSM} in tumor draining lymph nodes (TdLNs) as the major responder to anti-PD-1/PD-L1 ICB treatment in tumorigenesis (Huang et al., 2022). It is worthwhile to note that ICB monotherapy treatment usually fails to achieve ideal tumor control due to the incapability to bypass or alter the progression of T cell exhaustion in tumorigenesis (Huang et al., 2022; Liu, Xia, et al., 2021; Siddiqui et al., 2019) as well as chronic viral infection (He et al., 2016; Hudson et al., 2019; Im et al., 2016). Moreover, tumor cells escape host immune surveillance by creating an immunosuppressive environment involving hypoxia, high level of potassium, nutrient competition, regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs), immune checkpoint ligands like PD-L1/2, and inhibitory cytokines like TGF-β/IL-10, which further reduces CTL infiltration into the tumor parenchyma and exacerbates T cell functional exhaustion. Unfortunately, none of these can be reversed by anti-PD-1/PD-L1 treatment alone (Motz & Coukos, 2013). Therefore, finding TME modulators that can bypass the exhaustion progress or promote the tumor infiltration of tumor-specific $CD8^+$ T cells will greatly enhance anti-PD-1/PD-L1 ICB treatment and is expected to be a novel era of tumor immunotherapy.

3. The role of pyroptosis in tumor immunotherapy

The antitumor efficacy of inducing tumor cell immunogenicity death has been affirmed by a large amount of previous studies (Galluzzi et al., 2017). As a mega hit type of cell death, pyroptosis has been demonstrated to be closely related to various current mainstream tumor immunotherapy. Therefore, tumor cell pyroptosis is expected to be a potential candidate to enhance immunotherapy. Pyroptosis, a unique cell death pattern executed by pore-forming GSDM family proteins, is often, but not always completed by the activation of inflammatory caspases (Galluzzi et al., 2018). GSDMs contain six members, including GSDMA, GSDMB (expressed in human rather than mice), GSDMC, GSDMD, GSDME and PJVK (Liu, Xia, et al., 2021). Until now, GSDMB-, GSDMC- and GSDME-induced tumor cell pyroptosis have been reported to exert anti-tumor effects (Hou et al., 2020; Liu et al., 2020; Zhang et al., 2021, pp. 8, 2020; Zhou et al., 2020). GSDMB was found to be directly cleaved by granzyme A (GzmA) secreted by CTLs or natural killing cells (NKs) (Zhou et al., 2020). As for GSDMC, it was shown that TNFRSF/TNF signaling activated caspase-8 to cleave GSDMC and induced tumor cell pyroptosis (Hou et al., 2020; Zhang et al., 2021, p. 8). GSDME has also been found to be cleaved by caspase-3 or granzyme B (GzmB) in vitro (Liu et al., 2020; Zhang et al., 2020). It is noteworthy that GSDMB and GSDME can be cleaved by GzmA or GzmB (produced by CTL and NKs) respectively, thus establishing a link between anti-tumor immunity and tumor cell pyroptosis. However, GzmB was very inefficient at cutting GSDME *in vivo*, thus only indirectly cleaved GSDME by activating pro-caspase-3 in target cells (Zhang et al., 2020). Meanwhile, the majority of tumor cells silence caspase-3 and GSDME expression to evade immune surveillance. Therefore, GzmA-expressing CTLs or NKs might be better candidates to execute tumor cells pyroptosis in TME. However, previous studies ignored the anti-tumor effect of GzmA-producing CTLs using GzmA knockout mice (Froelich et al., 2009; Voskoboinik et al., 2015), likely due to the lack of GSDMB or its homolog expression in mice. Intriguingly, Huang et al. also reported that chimeric antigen receptor (CAR)-T cells induced GSDME-dependent tumor cell pyroptosis via the activation of pro-caspase-3 (Liu et al., 2020). They also found out that conventional tumor-specific $CD8^+$ T cells only eliminated tumor cell by apoptosis (which contrasts with the results obtained by Lieberman et al.), despite the fact that CAR-T cells could induce target cell pyroptosis (Liu et al., 2020). This may be due to the higher affinity of CAR with antigens enforced by presence of multiple co-stimulatory signals, so that binding of CAR-T cells to the tumor cells produces more GzmB and perforin, enough to counteract the membrane repair mechanisms of target cells. Additionally, the reason why tumor-specific $CD8^+$ T cells could induce GSDME-associated pyroptosis in the results of Lieberman et al. was that the tumor cell linages they used highly expressed GSDME, allowing little amount of GzmB to exert strong pro-pyroptotic effect. All these results strongly confirmed the importance of GzmA instead of GzmB in inducing tumor cell pyroptosis. Moreover, interferon-γ (IFN-γ) in TME was found to induce GSDMB expression, suggesting that tumor-specific CTLs could induce tumor cell pyroptosis not only by secreting GzmA to specifically cleave GSDMB, but also via releasing IFN-γ to upregulate GSDMB expression (Zhou et al., 2020). On the other hand, pyroptosis of tumor cell also affects the distribution of $CD45^+$ cells in the TME. According to the results from Shao et al., pyroptotic microenvironment showed more $CD8^+$ T cells, $CD4^+$ T cells, NK and macrophages, while neutrophil, myeloid-derived suppressor cells (MDSCs) and monocytes were greatly decreased (Wang et al., 2020). Consequently, the immunosuppressive microenvironment is changed into immunogenic environment in pyroptotic tumor, which makes it easier for tumor-specific $CD8^+$ T cell and pro-inflammatory cell infiltration.

The key to the further expansion of "cancer-immunity cycle", as described in the previous section, is the massive release of tumor cell components into TME after immunogenic cell death (ICD). This was corroborated by the results of Huang et al., who found that tumor cells released cellular components (such as ATP, HMGB1, etc.) under pyroptotic state, and that these components could act as DAMPs to trigger a macrophage-mediated inflammatory storm (Galluzzi et al., 2017; Liu et al., 2020). ATP released after tumor cell pyroptosis can bind to the purinergic receptor P2Y2 (P2RY2) and purinergic receptor P2X7 (P2RX7) on DCs, mediating the chemotaxis of immature DCs to TME and DC activation, respectively (Elliott et al., 2009; Ghiringhelli et al., 2009). Meanwhile, pyroptotic tumor cell-derived high-mobility group box 1 (HMGB1) mediated the maturation of immature DCs by binding with various pattern recognition receptors (PRRs) including toll-like receptor (TLR)-2, TLR-4 and advanced glycosylation end product-specific receptor (AGER; also known as RAGE) (Sims et al., 2010). Other pyroptotic products of tumor cells, such as annexin A1 (ANXA1), calreticulin (CALR), etc. had the potential to exert adjuvant-like effects on tumor immunity (Galluzzi et al., 2017). It has also been shown that chemotherapy-induced ICD of mouse tumor cells resulted in the release of type I interferon from surrounding tumor cells, which might be stimulated by the binding of dead cell-derived RNA (acts as DAMP) to TLR-3 receptor (Sistigu et al., 2014). At the same time, type I interferon promotes the secretion of CXCL10 in large quantities by tumor cells in an autocrine or paracrine manner, thereby recruiting tumor-specific CTLs from the blood to perform anti-tumor effect (Sistigu et al., 2014). By analogy, pyroptotic tumor cell-derived RNA may also potentially mediated the production of CXCL10 by surrounding tumor cells using similar mechanisms shown above, supporting the massive recruitment of

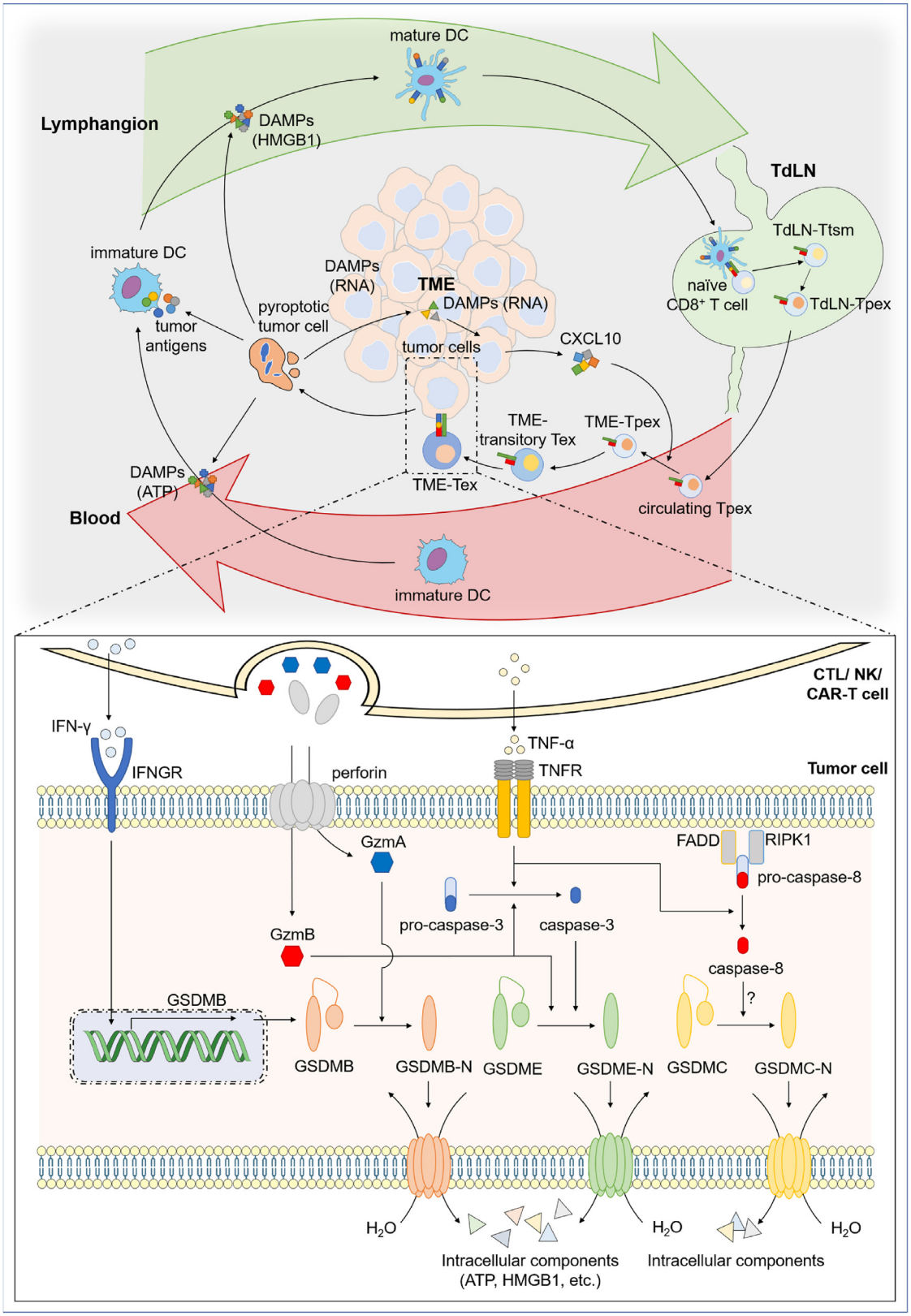

Fig. 1. Immature DCs enter the tumor tissue via the circulation, followed by the maturation of these cells after DAMP stimulation, together with the uptake and presentation of tumor antigens in the TME. These mature DCs will then home to TdLN via the draining lymphangion and prime tumor-specific $CD8^+$ T cells, which are continuously stimulated by the tumor antigens to differentiate into Ttsm and reside in situ. Once Ttsm are formed, these cells are capable of rapidly responding to the tumor antigens entering the TdLN and differentiate into TdLN-Tpex that high express circulating-associated markers (CXCR3, S1PR1, S1PR5). When tumor cells express high levels of GSDMB, GSDMC, or GSDME, tumor-specific $CD8^+$ T cells can induce tumor cell pyroptosis by releasing GZMA, GZMB or TNF-α/IFN-γ. The pyroptotic tumor cells can release intracellular components such as ATP, HMGB1 and nucleic acids into TME to promote TME localization of immature DCs, DC maturation, as well as T cell recruitment, respectively, and ultimately act as a TME modulator to enhance the effect of anti-PD-1/PD-L1 ICB treatment.

tumor-specific $CD8^+$ T cells. In addition, cellular contents released from pyroptotic tumor cells could be internalized by phagocytes, thus transforming TME from initially immunosuppressive into an inflammatory microenvironment via the secretion of inflammatory cytokines and chemokines, finally allowing more tumor-specific CTLs to infiltrate into tumor tissue and exert stronger anti-tumor effect (Denk & Greten, 2022; Morris et al., 2022). Consequently, all of these results indicated that tumor cell pyroptosis might act as an ideal TME modulator to reprogram the immunosuppressive environment (Fig. 1). Similar to pyroptosis, tumor cell necrosis (an inflammatory cell death mediated by RIPK3 and MLKL) also releases tumor antigens, DAMPs and inflammatory cytokines, etc. thereby modulating the TME (Frank & Vince, 2019). Necrosis is thought to be independent of the activity of caspases and is characterized by cellular swelling, organelle dysfunction, extensive mitochondrial damage, and plasma membrane rupture (Galluzzi et al., 2018). However, pyroptosis is thought to be always dependent on but not only dependent on the activity of caspases and is characterized by cellular swelling, organelle dysfunction, extensive mitochondrial damage, and cell membrane rupture (Galluzzi et al., 2018). Tumor necrosis is often associated with aggressive tumor development and metastasis and is thought to be an indication of poor prognosis in patients with breast, lung, and kidney cancer (Caruso et al., 2012; Richards et al., 2011). However, since tumor-specific CD8 T cell-derived GzmA and GzmB can cleave GSDMB and GSDME in target cells, respectively, tumor cell pyroptosis can be expanded by anti-tumor immunity, which continuously activates DCs and promotes the progression of pyroptosis, thus tumor cell pyroptosis is more likely to act as a TME modulator than necrosis.

It is worth mentioning that the prognosis of cancer patients can be improved via tumor cell pyroptosis far beyond simply reducing tumor cell load, as a recent study figured out that complete clearance of tumor mass could be achieved by inducing pyroptosis in only ~15% of 4T1 cells (Wang et al., 2020). Pyroptotic tumor cells act more like a 'pool' for tumor antigens or cellular components to turn immunosuppressive TME into immunogenic microenvironment. Thus, tumor immunotherapy can be greatly boosted in pyroptotic status. Surprisingly, GSDMB-induced tumor cell pyroptosis has already been proved to re-sensitize the so called 'cold' tumors that would otherwise not respond to anti-PD-1/PD-L1 ICB treatment (Zhou et al., 2020). As mentioned in the previous section, the current predicament of anti-PD-1/PD-L1 ICB treatment is the insufficient response to poorly immunogenic tumors and the fact that it can only quantitatively expand tumor-specific T cells without altering their eventual fate towards exhaustion. While tumor cell pyroptosis, as a TME modulator, turns 'cold' tumor into 'hot' tumor that can recruit anti-PD-1/PD-L1 expanded tumor-specific $CD8^+$ T cells, so that ICB treatment can function properly in non-responsive tumors. Moreover, tumor-specific $CD8^+$ T cells are more likely to be fully activated by pyroptotic DCs due to their better antigen encounter and maturation. Whether this could alter the trajectory of tumor-specific $CD8^+$ T cells before the formation of epigenic scar and completely become a subpopulation of cells with stronger effector function and homeostatic proliferation capacity (similar to that of in acute infection) deserves further exploration (Abdel-Hakeem et al., 2021; Rudloff et al., 2023; Tonnerre et al., 2021; Yates et al., 2021; Yousif & Ghoneim, 2021). Anyway, tumor cell pyroptosis will eventually shine as a TME modulator together with anti-PD-1/PD-L1 ICB treatment to change the future of tumor immunotherapy.

4. The application of pyroptosis in immunotherapy

Since tumor cell pyroptosis has been regarded as a potent adjuvant for anti-PD-1/PD-L1 ICB treatment, there has been increasing attempts to improve the prognosis of tumor patients through tumor cell pyroptosis. According to a series of tumor cell pyroptosis from Dr. Feng Shao (Wang et al., 2017; Zhou et al., 2020), tumor cells usually silenced GSDME and GSDMB proteins. Therefore, we believed that the strategies of actively delivering intact GSDM family proteins or their N-terminal to induce tumor cell pyroptosis were more feasible. One way to achieve this is through the delivery of GSDM-N to tumor cells using extracellular vesicles-based GSDMD-N mRNA delivery system (EVTx) (Xing et al., 2023) or recombinant adeno-associated virus (rAAV) packaging (Lu et al., 2021). Additionally, the oncolytic parapoxvirus ovis (ORFV) has been shown to reduce the ubiquitination of GSDME in target cells and subsequently trigger pyroptosis (Lin et al., 2023). This delivery system has the advantage of preferentially accumulating in tumor tissues and upregulating GSDME in low GSDME-expression tumor cells (Lin et al., 2023). Moreover, deliver GSDM family proteins into tumor cells by biological or nanomaterials has also been extensively studied and will been put into use in the near future (Chen et al., 2022; Wang et al., 2020). The second strategy is to induce tumor cell pyroptosis via epigenetic modulation, such as the demethylation of genes expressing GSDM family proteins in tumor cells (as GSDM family proteins are normally epigenetically silenced in these cells) (Akino et al., 2007; Kim et al., 2008). However, tumor-specific delivery of these drugs and specific demethylation of GSDM family protein-related genes are issues that must be addressed before this method can truly be put into the clinic. The third strategy involves isolating DCs from cancer patients and co-cultured with cell lysis of pyroptotic tumor in vitro, since DCs play a momentous role in inducing pyroptosis-associated anti-tumor immune response. Meanwhile, further identifying the features and mechanisms upon DC maturation and activation during pyroptotic states will promote the development of DC vaccines for tumor. This strategy achieves pyroptosis-like effect in tumor cells that silencing GSDM family protein expression and exerts stronger as well as more direct anti-tumor immunity in contrast to convention tumor vaccines. However, it is still in the theoretical stage and requires further investigation due to technical and other constraints. Overall, tumor cell pyroptosis can be a powerful TME modulator in enhancing anti-PD-1/PD-L1 ICB treatment and has significant implications for the development of novel anti-tumor treatment.

5. Conclusion and future perspective

The immunosuppressive microenvironment of tumor can hinder the effective infiltration of tumor-specific $CD8^+$ T cells as well as lead to their terminal exhaustion. Although anti-PD-1/PD-L1 ICB treatment has shown remarkable efficacy in certain tumor types, its responsive rate remains low (around 20%) (Philip & Schietinger, 2021). Furthermore, anti-PD-1/PD-L1 monotherapy cannot reverse or bypass the exhaustion process of tumor-specific $CD8^+$ T cells, underscoring the importance of identifying TME modulators as adjunct to anti-PD-1/PD-L1 ICB therapy (Philip & Schietinger, 2021). Tumor cell pyroptosis, a unique cell death executed by GSDM family proteins, can modulate TME by redistributing tumor-killing cells, thereby enhancing the infiltration of $CD45^+$ cells to synergize with anti-PD-1/PD-L1 ICB treatment. Given the low cutting efficiency of GzmB to GSDME and silenced GSDME expression in most tumor types, targeting on the GzmA-GSDMB axis may be the best approach to induce tumor cell pyroptosis and modulate the TME. Notably, there are still several remaining obstacles that are needed to be overcome or elucidated in order to regulate the GzmA-GSDMB axis. Firstly, it is necessary to fully understand the mechanism behind how tumor cell pyroptosis synergizes with anti-PD-1/PD-L1 ICB treatment, including the cell subsets to exert anti-tumor effect under pyroptotic situation and the role of anti-PD-1/PD-L1 ICB treatment in this process. Second, as GzmA has not been consider the primary cytotoxic molecular for tumor-specific $CD8^+$ T cells, it would be of great theoretical and clinical value to identify the characteristics of $GzmA^+$ tumor-specific CTLs, which could be used to expand this cell population in vitro and apply it to T cell therapy. Finally, it is intriguing to detect whether all different isoforms of GSDMB can induce pyroptosis, as this would enable us to choose the optimal GSDMB isoform overexpression strategy via oncolytic virus expression system, mRNA vaccine, nanoparticles delivery system etc. Based on evidence from both the tumor-bearing mice models and human TCGA data, we are optimistic that inducing tumor cell

pyroptosis can be a promising strategy to synergize with anti-PD-1/PD-L1 ICB therapy and enhance anti-tumor effect.

Conflict of interest

All authors declare no conflicts of interests with this manuscript.

Declaration of competing interest

All authors disclosed no relevant relationships.

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