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Mini-Review

Leveraging immunogenic cell death to potentiate immune checkpoint therapy in cancer

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ABSTRACT

Immune checkpoint therapy (ICT) is exceptionally clinically attractive because it offers not only durable responses, but also better quality of life than many other treatments. In addition to neoantigens that are required for tumors to be recognized and targeted by cytotoxic T cells after ICT, the tumor microenvironment has been considered as a major determinant for the tumor responsiveness to ICT. In general, tumors that are not inflamed and do not elicit a response from the immune system do not respond to ICT. Therefore, creating an immunogenic tumor microenvironment is critical for achieving optimal ICT response. Immunogenic cell death (ICD) is a unique form of stress-induced cell death that drives inflammatory response in tumors and culminates with adaptive immunity. In this mini-review, we will first briefly introduce the mechanistic and functional features of ICD. We will then summarize the published studies in regard to how we can apply ICD-inducing strategies to enhance the effectiveness of ICT for cancer treatment.

KEYWORDS: immune checkpoint therapy (ICT), immunogenic cell death (ICD), combination therapy, T cell activation.

INTRODUCTION

Immune checkpoint therapy (ICT) is a rapidly growing field of treatment where drugs are used to block immune checkpoint pathways. Currently, the main focus of research has been on the PD-1 and CTLA-4 checkpoint pathways, which are both inhibitory receptors commonly found on activated T cells [1]. These pathways, when activated by cancer cells or tumor-microenvironmental ligands, inhibit the antitumor immune response of T cells. While the field of immunotherapy has been around since the mid-20th century, one of the first insights into the world of ICT occurred in 1996 when antibodies that blocked CTLA-4 were tested on mice and found to increase antitumor response [2].

Benefits of ICT

Since 2011, the FDA has approved the use of seven different inhibitors of the two aforementioned pathways and PD-L1, the ligand of PD-1 [3] to treat a plethora of advanced solid tumors, such as metastatic melanoma, lung, and renal carcinoma [4]. The rapid approval of these inhibitors is a testament to their effectiveness in treating cancer patients by instigating the adaptive immune response. For example, two of these FDA-approved inhibitors, ipilimumab (the first and only CTLA-4 inhibitor) and nivolumab (a PD-1 inhibitor), have increased the median survival rate of patients with metastatic melanoma. Before, only 25% of patients survived more than a year, but now with these treatments, over 60% of patients survive past two years [5]. ICT has also proven to be effective in certain cancers that are not as responsive to chemotherapy, such as mismatch repair defect (MMRD)/microsatellite instability (MSI) [6]. Additionally, according to the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire, these treatments

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have been shown to improve or maintain quality of life (QOL) in melanoma patients [7]. Using measurements of five functional and nine symptom subscales, patients experienced no clinically meaningful changes between scores before and after treatment.

Drawbacks of ICT

Although ICT is having increasingly positive clinical results in patients, the therapy only benefits some individuals. For example, it is not as effective in patients with non MSI-H/dMMR colorectal cancer [8]. Even in cancers that are generally treated with ICT, 40-50% of patients may show resistance to the therapy [9]. And despite the improved QOL, there are still toxicities known as immune-related adverse events [10], with around 10-18% of reported patients experiencing grade 3-4 adverse events during anti-CTLA 4 therapies and 7-12% in anti-PD1 therapies [11]. Because of this, reliable biomarkers within the tumor microenvironment (TME) are key in determining whether to go forth with ICT for individual patients so as to not cause unnecessary harm. Commonly used predictive biomarkers are PD-L1 and tumor mutation burden (TMB), with the latter measuring the total number of somatic mutations within a tumor [12]. A small portion of the nonsynonymous mutations, such as single nucleotide variants or frameshift mutations that cause insertions and deletions [13], produce neoantigens, which are foreign peptides expressed on the surface of tumor cells. Neoantigens are what hosts use to signal T cell receptors (TCR) to initiate the adaptive immune response, allowing the immune system to differentiate between cancer cells and self. While only a small portion of the mutations counted in the TMB actually create neoantigens, more mutations usually equate to an increased neoantigen load, which in turn increases the likelihood of an immune response during ICT. This can be seen in tumors with MMRD, which have high TMBs due to increased frameshift mutations. They have been shown to be effectively treated using PD-1 checkpoint blockers [14].

ICT is also most effective in hot tumors, which are inflamed tumors full of cytokines and T cells, allowing for the antitumor response. Generally, melanoma and non-small cell lung cancer [15] fall under this category. In contrast, cold tumors are the opposite, and they lack the proper immune response that is conducive toward ICT. This can arise from different factors within the TME, such as a deficiencies in the amount of antigens or signaling that leads to activation of T cells. Because cold tumors do not respond well to ICT, there has been significant research emphasis on how to turn cold tumors hot. One way is through immunogenic cell death (ICD), which is the adaptive immune response elicited by certain dying cells. When successfully triggered in dying cancer cells, it can help tumors with T cell activation defects produce an antitumor response.

How ICD works

When normal body cells undergo apoptosis, they are ignored by the immune system, which makes ICD unique in that it triggers the immune response. ICD inducers for cancer generally involve triggering cells to undergo apoptosis or necroptosis (programmed necrosis), although other types of ICD, such as pyroptosis (programmed cell death against pathogens) exist as well. For a long time, it was believed that apoptosis was strictly nonimmunogenic, while only necroptosis elicited an immune response. However, we now know that apoptosis can be immunogenic, depending on the molecules secreted by the dying cells. This comes from the release of danger-associated molecular patterns (DAMPs) (Fig. 1), which are adjuvants that serve as danger signals that bind to pattern recognition receptors (PRR) and stimulate antigen presenting cells (APC) [16], ultimately eliciting both the innate and adaptive immune response. Cells undergoing necroptosis, whether induced or innate, release these DAMPs as well. However, because of the inflammatory nature of necroptosis, this form of cell death has been known to promote tumor growth and metastasis as well [17]. Besides this adjuvancy, there must also be antigency for ICD to occur. This comes from the presence of neoepitopes, the antigenic determinants of neoantigens related to the TMB in tumor cells.

In order for dying cells to express the required DAMPs, current ICD inducers trigger reactive oxygen species production (ROS) and endoplasmic reticulum (ER) stress beyond what tumor cells normally experience inside the harsh TME, which triggers the unfolded protein response (UPR) [18]. The UPR

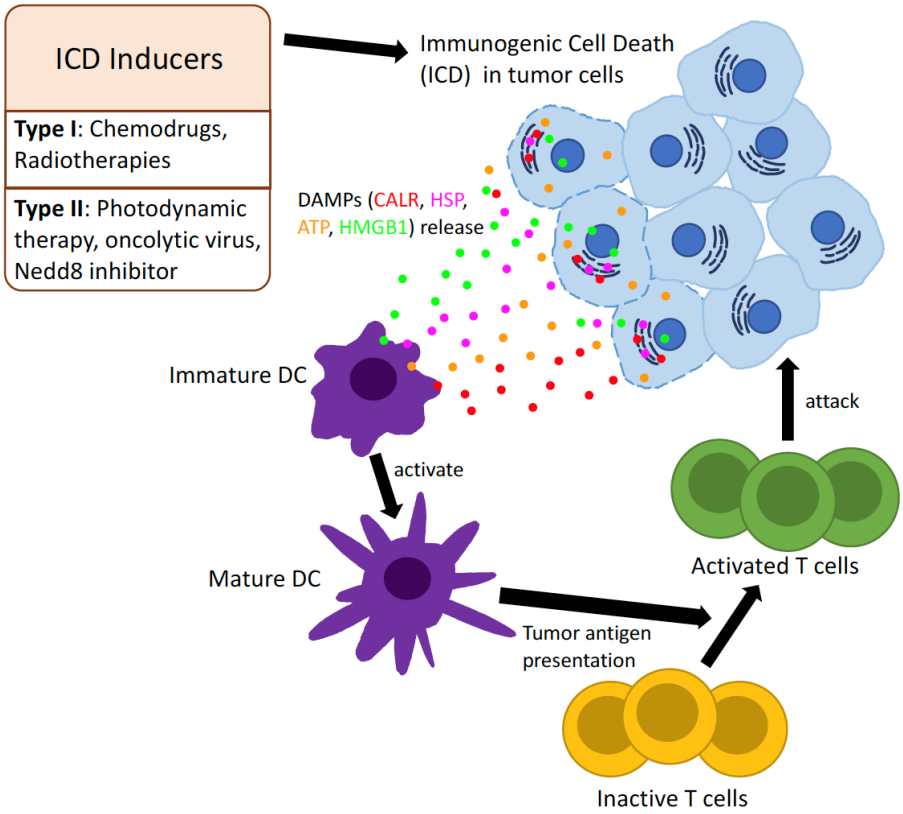

Fig. 1. Schematic summarization of immunogenic cell death (ICD) inducers and the mechanism of ICD-induced immuno-attacking of tumor cells. DAMPS: danger-associated molecular patterns. CALR: calreticulin. DC: dendritic cells. HSP: heat shock proteins.

causes several ER chaperones to become expressed on the cell's surface, such as calreticulin (CALR) and heat shock proteins, which act as DAMPs. CALR is one of the most important hallmarks of ICD. When translocated from the ER to the cell membrane during the early stages of ICD, it becomes an "eat me" signal by binding to receptors on dendritic cells (DC), telling the phagocytes to engulf the dead cell. Additionally, the expression of CALR on the surface (ecto-CALR) increases proportionally throughout apoptosis [19]. In a similar manner, heat shock proteins, namely HSP70 and HSP90, have the goal of being detected by APC once expressed on the cell's surface. However, while they are often expressed during ICD, it is not completely clear whether these proteins are required.

Other DAMPs that are markers for ICD but not ER chaperones include ATP and high mobility group box 1 (HMGB1). When ATP is secreted by the dying cell during the blebbing stage of apoptosis, whether actively or passively, it serves as a "find me" signal. For this to happen, autophagy must occur, which is the pre-mortem lysosomal breakdown of cytoplasmic proteins and organelles. Because autophagy is what allows the ATP to be released, cells treated with autophagy inhibitors have been shown to be unable to elicit the proper immunological responses [20], and a combination of autophagy and cell death releases the most ATP [21]. Ultimately, ATP signals cytokines and inflammasomes (the release of which is triggered from receptors on DCs) that lead to the adaptive immune response [19]. HMGB1 is another DAMP, ordinarily bound to DNA within the nucleus. During late-stage apoptosis, when the nuclear and cellular membranes are permeable post-mortem, the protein is passively released and binds to receptors on DCs. This releases inflammatory cytokines and promotes the cross-presentation of antigens from DCs to T-cells. It is important to note that, while DAMPs play a crucial role in ICD, they have the potential to

promote tumor growth as well. Certain DAMPs, such as the HMGB1 and S100 proteins, can trigger the release of other molecules that lead to inflammation and carcinogenesis [22].

ICD induction in cancer

The expression of ecto-CALR, ATP, and HMGB1 is generally used to determine whether a drug can be used as an ICD inducer. Furthermore, there are two types of ICD inducers, type 1 and type 2 (Fig. 1), with the former most commonly used. The main difference between the types of inducers boils down to how they go about triggering apoptosis. For type 1 inducers, the primary target is not the ER, so ER stress and the subsequent release of the necessary DAMPs is caused indirectly. This collateral results from a barrage on other, non-ER parts of the cell in an effort to cause apoptosis, such as cytosolic proteins, the plasma membrane, and mitochondria [19]. Commonly used type 1 inducers include anthracyclines, bortezomib, cyclophosphamide, oxaliplatin, and radiotherapy [18]. On the other hand, type 2 inducers, such as oncolytic viruses (OV) and photodynamic therapy, specifically target the ER through ROS-mediated ER stress. In recent years, OVs have been a promising new ICD inducer for not only having the ability to directly attack tumor cells, but also being able to trigger antitumor and antiviral responses [23]. They take advantage of the decreased antiviral nature of tumors due to their interferon-signaling defects that normally promote tumorigenesis [24]. One major benefit of ICD in cancer treatment is its ability to produce and remember a tumor-specific immune response, creating a cancer vaccine that can identify any tumor cell within the body [25]. This immunological memory drives the research in finding novel ICD inducers and ways to increase their efficiency. For example, nanoparticles can be used to improve the effects of different ICD inducers, assisting in chemotherapy, radiotherapy and phototherapy. For the former, by delivering cytotoxic drugs to the tumor, liposomes, and other types of nanoparticles can reduce the toxicity that arises from targeting healthy cells [26]. It has also been shown that combining two ICD inducers [27], or ICT with ICD [28], leads to improved outcomes in patients. For example, treatments of oxaliplatin and cyclophosphamide, both type 1 ICD drugs, assist in effectiveness of subsequent CTLA-4 and PD-1 blockades [28]. Antibody-drug conjugates (ADC), a fast-growing class of biological drugs involving a monoclonal antibody attached to an antitumor therapeutic agent *via* a chemical linker, can also help with this combination. This is the case for an anti-HER2 anthracycline-based ADC that induces ICD and contributes to PD-1 blockade [29].

Of note, our group recently identified MLN4924 (pevonedistat, a Nedd8 activating enzyme E1 [NAE] inhibitor) as a potent therapy to target (MMRD)/(MSI) cancers [30]. We found that MMRD induces protein-destabilizing mutations that consequently lead to proteome instability in MSI tumors, resulting in an abundance of misfolded protein aggregates. To compensate, MSI cancer cells use a Nedd8-mediated degradation pathway to facilitate clearance of misfolded proteins and avoid intolerable ER stress and cell death. Blockade of this Nedd8 clearance pathway with MLN4924 leads to substantial accumulation of misfolded protein aggregates and subsequently increase of ER stress, ultimately inducing ICD in MMRD cancer cells. To leverage this ICD, we combined MLN4924 treatment with inhibition of PD-1 and found the combination was synergistic, with significantly improved efficacy over PD-1 inhibition alone.

CONCLUSION

While interest within these fields of immunotherapy has increased greatly over the past decade, there is no doubt that it will continue to grow. Regarding ICT, there are already seven FDA-approved anti-PD-1 and anti-CTLA-4 drugs, although toxicity is still an issue. Additionally, because ICT is most effective in inflamed, hot tumors, knowing how to promote an immune response in cold tumors is important. This is where ICD inducers come into play; an effective treatment leads to the release of DAMPs which activate the immune system to target tumor cells, which are originally resistant to therapies. ICD can be induced in different ways, from chemotherapy, to radiotherapy, and even the use of OVs or the Nedd8 inhibitor as demonstrated in our study. As future research progresses, we will be able to find more ways to use ICT and ICD in highly effective, low toxicity manners, especially using combination therapies.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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