LETTER TO EDITOR / CARTA AL EDITOR

Immunogenic Cell Death: An opportunity for Clinical Oncology?

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Apoptosis was initially seen as a kind of silent cell death with non-induction of the immune response[1]. However, in the recent past, it has been seen that death induced by either infections or action of certain agents can elicit a specific immune response, namely immunogenic cell death (ICD)[2]. This ICD activates the immune system against antigens associated with deceased cells with the concomitant exposure and releasing of the so-called damage-associated molecular patterns (DAMPs) by dying cells[3]. Four principal DAMPs related to ICD have been identified (but not limited to): the endoplasmic reticulum (ER) chaperone calreticulin (CRT), heat shock proteins (HSPs), adenosine triphosphate (ATP) and high mobility group box-1 (HMGB-1)[4].

Today, DAMPS has been shown to play a spatiotemporal role among the ICD process. For instance, CRT is a 46 kDa Ca^{2+}-binding chaperone that is normally found in the lumen of ER, where it acts in Ca^{2+} homeostasis/signaling regulation and proper folding of proteins[5]. During induction of ICD, CRT is exposed on the outer surface of the plasmatic membrane early, in a pre-apoptotic stage, and serves as an "eat me" signal, stimulating phagocytes to engulf portions of dead cells. CRT translocation occurs together with ERp57 but not of other ER proteins as a result of ER stress, which modulates the phosphorylation of eukaryotic translation initiation factor 2α (eiF2α). Different reports have indicated that knockdown of CRT or ERp57 suppress the phagocytosis and the immunogenicity of cell death, indicating the critical role that these proteins play in ICD. The main receptor for CRT on myeloid cells is LDL receptor-related protein 1 (LRP1; also known as CD91).

Heat shock proteins are also a class of chaperone proteins involved in the synthesis, correct folding, subcellular compartment transport, and degradation of intracellular proteins[6]. These proteins are over-expressed under stress conditions, and they can be translocated to the plasma membrane surface, and they can also be released into the extracellular environment. The mainly HSPs involved in ICD is HSP70 and HSP90.

On the other hand, ATP releasing constitutes a "find me" signal for chemotaxis in ICD. ATP can attract macrophages and activates the NLRP3 inflammasome, stimulating the final production of interleukin 1β (IL-1β), due to its action on cell surface purinergic receptors[7]. ATP release could occur for different mechanisms, but premortem autophagy is the primary mechanism that sustains high ATP levels in cells undergoing ICD. In fact, autophagy-deficient cancer cells fail to elicit therapy-relevant immune responses *in vivo*.

Finally, High Mobility Group Box-1 (HMGB-1), is a non-histone chromatin-binding protein considered as alarmin or molecular damage signal[8]. Extracellular HMGB-1 mediates numerous functions, including the activation of the endothelium and recruitment and maturation of cells of the immune system among which are the dendritic cells. ICD inductors elicit the passive release of HMGB-1 in a late-stage, which enhance antigen presentation. Secreted HMGB-1 can bind to advanced glycation end products (RAGE), TLR2, and TLR4. However, TLR4 has been identified as the principal receptor for HMGB-1 that mediates anti-tumoral immune responses via chemotherapy-induced ICD. The binding of HMGB-1 to TLR4 on DC was shown to enhance the processing of phagocytic cargo, facilitate antigen presentation, and increase intracellular levels of pro-IL-1β. Importantly, knockdown of HMGB-1 or tumor cells deficient of HMGB-1 exhibit diminished capacity to induce ICD and anti-tumor immune responses.

In addition to hallmarks above mentioned, secreted type I interferon, extracellular annexin A1 (ANXA1), and nucleic acids have also been described. During ICD process cytokines have been detected. The majority of those can be pro-inflammatory, which are involved in the increase of MHC class I on antigen-presenting cells expression, T cells differentiation, and NK cells activation. However, its putative role in mediating the ICD merits further investigation. (Figure 1)

Beside great advances in understanding the molecular and cellular events involved in ICD, new clinical perspectives toward a more rational combination to treat cancer arise from this phenomenon. For instance, some standard anticancer drugs induce tumoral cell death with concomitant immunogenic signals. Most of these include chemotherapeutic agents within them: Anthracyclines (doxorubicin, epirubicin, idarubicin), Oxaliplatin (a platinum derivate), Cyclophosphamide (an DNA-alkylating agent), bortezomib (a proteasomal inhibitor)[9], mitoxantrone (an anthracenedione), bleomycin (a glycopeptide antibiotic) and bortezomib (a proteasomal inhibitor) that have been employed in the clinic for several years. In addition to the chemotherapeutic agents, specific forms of irradiation[10], high hydrostatic pressures[11], some oncolytic viruses[12], and microtubular inhibitor patupilone[13,14] have also been shown to trigger ICD. However, predicting the ability of a compound to induce or enhance ICD taking into account its structure, chemical properties or functional similarities is not yet possible.

Accordingly, among the rational strategies currently testing in clinical oncology to obtain better overall response against cancer are combinations of ICD inducers with immunomodulatory agents. Mainly, combinations with immunostimulatory cytokines, immune checkpoint inhibitors, adoptive immunotherapy, oncolytic viruses or anticancer vaccines would be a promising choice.

Definitively, the use of ICD bone fide inducing drugs find a great opportunity in cancer treatment with putative great clinical benefit to patients with this disease. However, *a priori* identifying of novel anticancer ICD inducers is today a serious challenge to accomplish on the bench.

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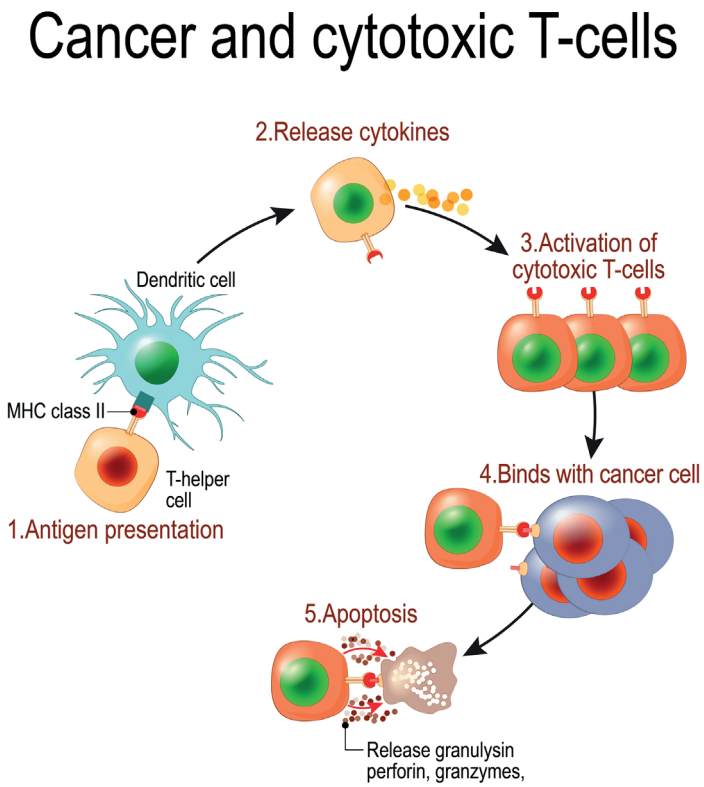

Figure 1. Cancer and cytotoxic T-cells. T lymphocyte kills cancer cells. T-cell immune responses, release the perforin and granzymes, and attack cancerous cells. Through the action of perforin, granzymes enter the cytoplasm of the target cell, and lead to apoptosis cell death.

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