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REVIEW

Molecular mechanisms underlying Immunogenic Cell Death: Overview on damage-associated molecular patterns and the stress of the endoplasmic reticulum

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ABSTRACT

Therapeutic regimes aimed to increase the immunogenic potential of cancer cells making them less immunoevasive have received great attention recently. In this context, the induction of immunogenic cell death has emerged as a novel promising strategy for effective cancer therapy. ICD is hallmarked by the emission of damage-associated molecular patterns (DAMPs) acting as danger signals in a precise spatiotemporal configuration. The DAMPs most prominently involved in the perception of cell death as immunogenic include: surface-exposed calreticulin, extracellular ATP, extracellular high mobility group box 1 (HMGB1) protein, type I IFN, extracellular dying cell-derived nucleic acids, and extracellular Annexin A1 (ANX A1). These ICD-associated danger signals operate on a series of receptors expressed by the innate immune cells to stimulate the presentation of tumor antigens to T cells. This results in the elicitation of tumor-specific adaptive immune responses that can control tumor growth and even eradicate residual cancer cells. ICD has been found to depend on the concomitant induction of reactive oxygen species (ROS) and activation of endoplasmic reticulum (ER) stress. Recent evidence places the activation of the unfolded protein response (UPR), and especially, the protein kinase R-like endoplasmic reticulum kinase (PERK)-mediated arm of the UPR at the core of many of the scenarios where ICD occurs. Here we provide an overview of the current understanding of the basic molecular mechanisms that underlie ICD. In this review, we focus on the crucial role of DAMPs, and the importance of ER stress and ROS in regulating the immunogenicity of dying cancer cells.

Keywords: immunogenic cell death, damage-associated molecular patterns, endoplasmic reticulum stress, cancer.

RESUMEN

Mecanismos moleculares de la Muerte Celular Inmunogénica: Una perspectiva desde los patrones moleculares asociados a daño y el estrés del retículo endoplasmático. Los regímenes terapéuticos que incrementan el potencial inmunogénico de las células tumorales para superar su fenotipo inmunoevasivo han alcanzado una gran relevancia en los últimos años. En este contexto, la inducción de la Muerte Celular Inmunogénica (ICD) emerge como una novedosa y promisoria estrategia para la terapia efectiva contra el cáncer. La ICD se caracteriza por la emisión de Patrones Moleculares Asociados al Daño (DAMPs) que actúan como señales de peligro en una configuración espaciotemporal precisa. Los DAMPs más significativamente involucrados en la percepción de la muerte celular como inmunogénica incluyen: la calreticulina expuesta en la superficie celular, el ATP extracelular, la proteína extracelular de alta movilidad del grupo caja 1 (HMGB1), los IFN de tipo I, los ácidos nucleicos extracelulares derivados de células moribundas y la anexina A1 extracelular (ANX A1). Estas señales de peligro asociadas a la ICD actúan sobre receptores expresados por las células del sistema inmune innato y como resultado ocurre la estimulación de la presentación de los antígenos tumorales a las células T. Esto genera una respuesta inmune adaptativa específica contra el tumor que puede controlar el crecimiento tumoral, e incluso, erradicar las células tumorales residuales. La ICD depende de la inducción concomitante de especies reactivas de oxígeno (ROS) y del estrés del retículo endoplasmático (ER). Las evidencias más recientes sitúan a la activación de la Respuesta a Proteínas No plegadas (UPR) y particularmente a la rama mediada por la proteína quinasa R similar a la quinasa del retículo endoplásmico (PERK) en el centro de muchos de los escenarios en los que se produce la ICD. Aquí ofrecemos una visión general de la comprensión actual de los mecanismos moleculares básicos que subyacen en la ICD. En esta revisión, nos centramos en la función crucial de los DAMPs y en la importancia del estrés del RE y de las ROS en la regulación de la inmunogenicidad de las células tumorales moribundas.

Palabras clave: muerte celular inmunogénica, patrones moleculares asociados al daño, estrés del retículo endoplasmático, cáncer.

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Introduction

In 1994, Polly Matzinger introduced a major change to the paradigm of Self/Non-Self antigens distinction made by the immune system. She proposed the so called Danger Model, which stated that Antigen Presenting Cells (APCs) are activated by endogenous cellular alarm signals exposed from distressed or injured cells, rather than by the recognition of non-self-molecules [1]. This model implied that a large spectrum of molecules related to dangerous events, including cells undergoing non-physiological

1. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol. 1994;12:991-1045.

2. Matzinger P. The danger model: a renewed sense of self. Science. 2002; 296:301-5.

forms of cell death, can stimulate an immune response [2].

Subsequent studies during the upcoming years reassured the basis of this model, as they described a form of cell death induced by antineoplastic agents that was indeed immunogenic [3-5]. Thus, the term Immunogenic Cell Death (ICD) emerged during the last decade, and in 2018, the Nomenclature Committee on Cell Death (NCCD) provided an updated classification of cell death subroutines, where ICD was included [6]. Currently, the term is used to describe a variant of regulated cell death that is sufficient to activate a potent adaptive immune response against altered self-antigens/cancer-derived neo-epitopes, in the case of tumor cells, or against pathogen-derived antigens during an infection, expressed by dying cells [7, 8].

There is an increasing range of stimuli that can drive ICD, but as a matter of fact, the potential of a certain agent to elicit this form of cell death cannot be predicted based on their structural or functional characteristics. This becomes evident with the case of oxaliplatin and cisplatin, two platinum-based compounds that are similar to each other but differ in their ICD-inducing capacity [9, 10]. Thus, ICD can be elicited by a diverse set of stimuli that include some chemotherapeutics (e.g., anthracyclines [3, 5], bortezomib [4], mitoxantrone [5], among others), pathogens (such as viral and bacterial infections) [8], physical cues (including irradiation [5], high hydrostatic pressures [11], hypericin-based photodynamic therapy (PDT) [12], nanopulse stimulation [13]), as well as necroptosis-inducing agents [14, 15].

The key and most crucial event during ICD is the spatiotemporally coordinated release of molecules that are typically retained within healthy cells. The endoplasmic reticulum (ER) stress has been considered as a vital prerequisite for danger signaling and subsequently ICD induction. Stressed or dying cells expose on their surface or release these 'danger' signals known as Damage Associated Molecular Patterns (DAMPs), which are recognized and interpreted by the innate immune cells as warnings of the danger faced by the organism [7, 16, 17]. Recognition occurs by dendritic cells (DCs), macrophages and monocytes thanks to their phagocytic, purinergic, and pattern recognition receptors (PRRs). So far, the main DAMPs mechanistically linked to the perception of cell death as immunogenic include, but are not limited to: surface-exposed calreticulin [5, 18], extracellular ATP [19, 20], extracellular high mobility group box 1 (HMGB1) protein [21, 22], type I IFN [23, 24], extracellular dying cell-derived nucleic acids [25, 26], and extracellular Annexin A1 (ANX A1) [27]. Such DAMPs facilitate the recruitment of APCs such as DCs to the tumor bed, where recognition by their cognate receptors favors the uptake of cell corpses and leads to DCs activation and maturation, and a more efficient dying cell-derived antigen uptake and processing [28, 29]. These loaded DCs migrate to draining lymph nodes, and present those antigens to T cells, which in turn results in a potent adaptive immune response, associated with the establishment of immunological memory (Figure) [7, 30].

ICD has been studied the most in the context of chemotherapeutics and their immunomodulatory effects elicited during cancer cell death. The cell stress/death caused by these ICD-inducing agents prompts the emission of DAMPs in a regulated manner, which is in turn immunogenic. This implies that if those dying cancer cells were to be used as a vaccine, in the absence of any adjuvant they would have the ability to stimulate an immune response that can control tumor growth and even eradicate residual cancer cells [7]. Even though the emission of several of the abovementioned DAMPs constitutes a parameter somewhat sufficient to make accurate predictions on the ICD-inducing abilities of a certain stimulus, the gold standard approach to detect *bona fide* ICD relies on vaccination experiments. Thus, dying tumor cells act in a prophylactic scenario that prevents tumor growth when mice are challenged with syngeneic live cancer cells from the same cell line [7, 31, 32].

Only the highly immunoevasive and mutagenic neoplastic cells are the ones capable of evading immunesurveillance and so generate clinically relevant tumors [33, 34]. Tumor cells not only shift the tumor microenvironment to their benefit [35, 36], but also use other mechanisms to become immunoevasive, which include downregulation of tumor-associated antigens and major histocompatibility complex (MHC) class I expression [37, 38]. It is now clear that the immune system plays a critical role not only during tumorigenesis but also in the tumor's response to therapy. Therefore, there is an increasing need for therapies that not only directly tackle the innate and adaptive arms of the immune system, but also subvert the otherwise immunoevasive phenotype characteristics of cancer cells *per se*.

The aim of this review is to gain a deeper perspective of the basic molecular mechanisms involved in ICD, the crucial role of DAMPs in this process, as well as important aspects involved in the relationship ER stress-ICD. We considered the most relevant papers published up to date in the field of ICD and analyzed the converging points demonstrated by the main research groups on this topic. Noteworthy, ICD possesses great potential as part of novel anticancer therapies, and plays a central role in many of the current efforts to achieve efficient and durable treatments targeting cancer.

DAMPS and their role in ICD

For its perception as immunogenic, ICD as a regulated cell death depends on two factors: its antigenicity and adjuvanticity [8]. On one hand, dying cells must display antigens that have not previously elicited a central or peripheral tolerance. Such neoepitopes can emerge either during cell infection through pathogen-encoded genes, or during oncogenesis, given the mutational load that cancer cells are subject to [39]. When cancer cells undergo ICD, tumor antigens are recognized by T cells and, hence, a specific antitumor immunity response is orchestrated [7, 30]. On the other hand, adjuvanticity relies on DAMPs emission, as it communicates a state of danger in the organism [8]. DAMPs are responsible for recruitment and activation of the essential cellular components for initiation of adaptive immune responses. This is so essential that defective DAMPs emission pathways abrogate ICD induction by agents that would otherwise efficiently trigger such type of

3. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, *et al*. Caspase-dependent immunogenicity of doxorubicin induced tumor cell death. J Exp Med. 2005;202:1691-701.

4. Demaria S, Santori F, Ng B, Liebes L, Formenti SC, Vukmanovic S. Select forms of tumor cell apoptosis induce dendritic cell maturation. J Leukoc Biol. 2005;77:361-8

5. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, *et al*. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med. 2007;13:54-61.

6. Galluzzi L, Vitale I, Aaronson S, Abrams J, Adam D, Agostinis P, *et al*. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 2018;25:486-541.

7. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013;31: 51-72.

8. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. Nat Rev Immunol. 2017;17:97-111.

9. Tesniere A, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, *et al*. Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene. 2009;29: 482-91.

10. Martins I, Kepp O, Schlemmer F, Adjemian S, Tailler M, Shen S, *et al*. Restoration of the immunogenicity of cisplatin-induced cancer cell death by endoplasmic reticulum stress. Oncogene. 2011;30:1147-58.

11. Fucikova J, Moserova I, Truxova I, Hermanova I, Vancurova I, Partlova S, *et al*. High hydrostatic pressure induces immunogenic cell death in human tumor cells. Int J Cancer. 2014;135:1165-77.

12. Garg AD, Krysko DV, Vandenabeele P, Agostinis P. Hypericin-based photodynamic therapy induces surface exposure of damage-associated molecular patterns like HSP70 and calreticulin. Cancer Immunol Immunother. 2012;61:215-21.

13. Nuccitelli R, McDaniel A, Anand S, Cha J, Mallon Z, Berridge JC, *et al*. Nano-Pulse Stimulation is a physical modality that can trigger immunogenic tumor cell death. J Immunother Cancer. 2017;5:32.

14. Aaes TL, Kaczmarek A, Delvaeye T, De Craene B, De Koker S, Heyndrickx L, *et al*. Vaccination with Necroptotic cancer cells induces efficient anti- tumor immunity. Cell Rep. 2016;15:274-87.

15. Yang H, Ma Y, Chen G, Zhou H, Yamazaki T, Klein C, *et al*. Contribution of RIP3 and MLKL to immunogenic cell death signaling in cancer chemotherapy. Oncoimmunology. 2016;5:e1149673.

16. Garg AD, Nowis D, Golab J, Vandenabeele P, Krysko DV. Immunogenic cell death, DAMPs and anticancer therapeutics: an emerging amalgamation. Biochim Biophys Acta. 2010;1805:53-71.

17. Krysko DV, Garget AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P, *et al*. Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer. 2012; 12: 860–875.

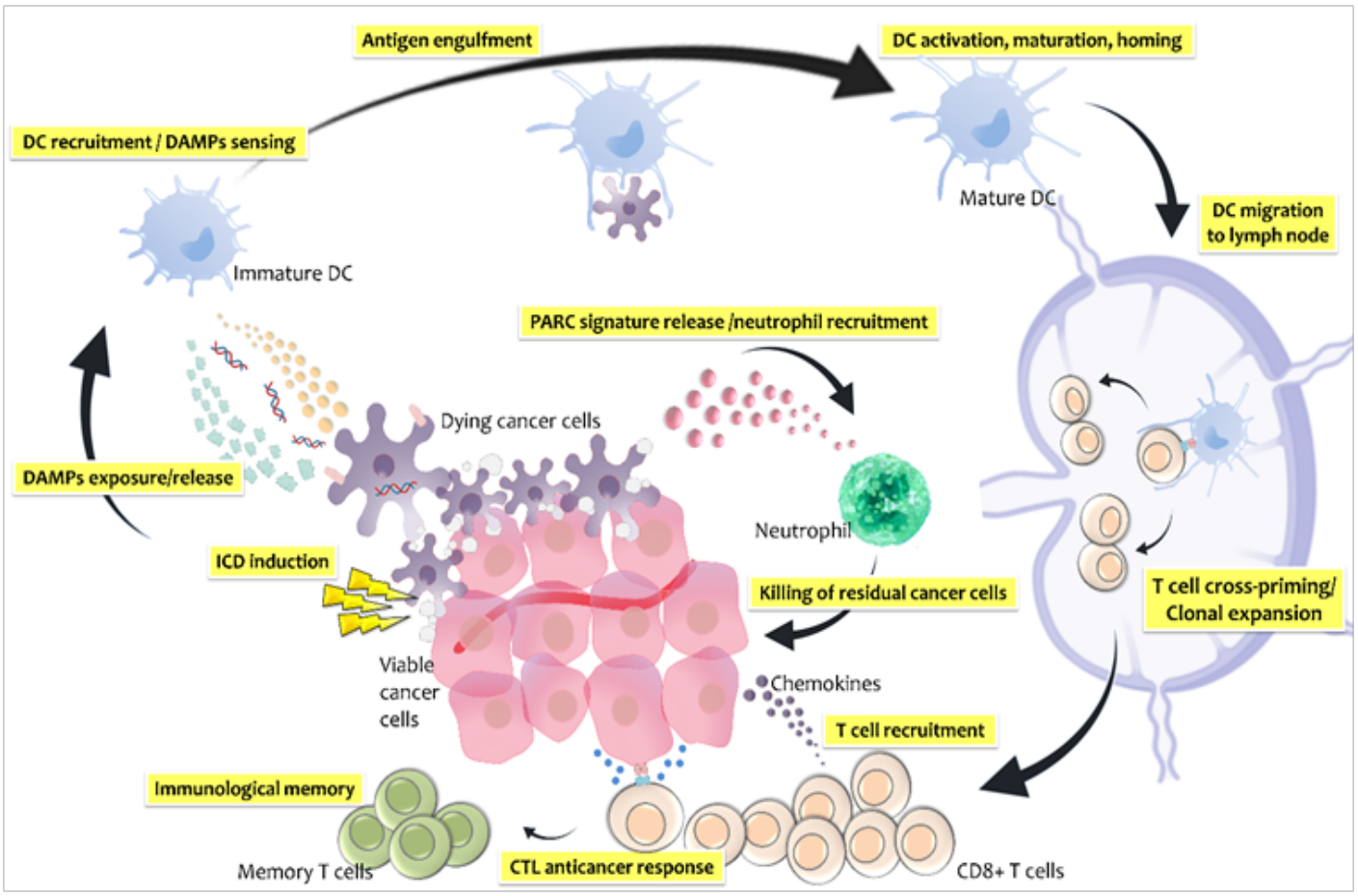

Figure 1. Main events during Immunogenic cell death (IDC). During ICD induction, cancer cells expose DAMPs such as calreticulin (CRT) and other chaperones on their surface, release high-mobility group box 1 (HMGB1) and annexin A1 (ANXA1), secrete ATP, and prompt a type I interferon (IFN) response on cancer cells that drives the production of T cell chemoattractant-cytokines. This spatiotemporal coordinated release pattern allows the recruitment, phagocytic activity and maturation of Dendritic Cells (DCs), favoring the uptake of cancer cell corpses and debris thereof. This promotes DCs to migrate to lymph nodes and prime a cytotoxic T lymphocyte (CTL)-dependent immune response involving αβ and γδ T cells. Such strong tumor-specific T cell response is often able to eradicate residual cancer cells that were not killed by the initial cytotoxic stimulus. In addition, cancer cells subject to ICD have been shown to present an 'altered-self mimicry', with a pathogen response-like chemokine (PARC) signature consisting of concurrent release of CXCL1, CCL2 and CXCL10. This signature preferentially attracts neutrophils as first innate immune responders, which exert cytotoxicity against residual cancer cells via respiratory burst/reactive oxygen species (ROS) or nitric oxide (NOS).

cell death [5, 22, 40]. Thus, as pathogens and cancer cells usually display an increased antigenicity, they have had to survive under selective pressures that made them subvert Microbe Associated Molecular Patterns (MAMPs) and DAMPS emission/sensing. This lowers their adjuvanticity and therefore provides pathogens and cancer cells with tools for cell death to be overlooked by the immune system [8].

It is noticeable that antigenicity, adjuvanticity, and a 'suitable microenvironment', that can sustain recruitment and activation of APCs, cytotoxic lymphocyte (CTL) functions, and establishment of immunological memory, are three major parameters on which adaptive immunity elicited by ICD relies on. Importantly, none of them are completely inherent to dying cells, but also determined by the host, which highlights the need for *in vivo* assessments of any instance of ICD [41]. The specific role of immunogenic signals such as: Calreticulin, ATP, type I interferons, endogenous nucleic acids, AnexinA1 and HMGB1 during ICD will be discussed here in further detail as well as the relevance of the ER stress to enable the release of DAMPs and consequently inducing ICD.

Calreticulin exposure

Calreticulin (CRT) is a protein located mainly in the endoplasmic reticulum (ER), where it functions as a chaperon and participates in ER Ca^{2+} homeostasis and signaling [42]. This protein constitutes one of the main and specific DAMPs related with ICD activation. During ICD, CRT translocates from the ER lumen to the outer side of dying cell's plasma membrane (PM) [5]. The CRT translocation pathway is usually connected to –and dependent on– ER stress and ROS production [18]. In the case of anthracyclines or oxaliplatin-induced ICD, CRT exposure involves a series of signaling events comprising response to ER stress mediated by eIF2α phosphorylation, proteolysis of the ER protein BAP31, activation of pro-apoptotic proteins BAX and BAK, the secretory pathway involving transport of CRT-containing vesicles from the ER to Golgi apparatus, and their exocytosis

18. Panaretakis T, Kepp O, Brockmeier U, Tesniere A, Bjorklund AC, Chapman DC, *et al*. Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. EMBO J. 2009;28:578-90.

19. Elliott M, Chekeni F, Trampont P, Lazarowski E, Kadl A, Walk SF, et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature. 2009;461:282-6.

20. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, *et al*. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1β-dependent adaptive immunity against tumors. Nat Med. 2009;15:1170-8.

21. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature. 2002;418:191-5.

mediated by interaction of proteins from the exocytic vesicles and the cell membrane [18]. Genetic or pharmacological interventions that impair any step of such pathway abrogate CRT exposure and therefore dramatically attenuate immunogenicity of cell death induced by chemotherapeutics [18]. .

Surfaced-exposed CRT binds to immune cells expressing the CD91 receptor, such as macrophages and DCs [43]. This interaction promotes DCs production of proinflammatory cytokines such as IL-6 and TNF-α, which in turn modulate the activity of immunostimulatory type-1 polarized (Th1) cells and IL17-producing T cells (Th17) [44]. Importantly, CRT acts as a potent phagocytosis-promoting signal in the surface of dying cells, leading to recruitment of APCs (e.g., DCs) to the tumor bed, and engulfment of cell corpses and debris, efficient processing of dead cell's antigens, optimal presentation to T cells, and priming a cognate immune response [5]. It is worth mentioning that the immunostimulatory effects of cell surface-CRT are strongly inhibited by the co-expression of CD47, which is a phagocytosis inhibition signal expressed by a variety of solid and hematopoietic tumors. The expression levels of CRT and/or CD47 have been correlated to the disease outcome [45-47]. For instance, in bladder cancer, neuroblastoma and mantle cell lymphoma patients, the correlation between surface-CRT exposure with that of CD47 has been associated to a negative prognosis [48]. Consistently, in the case of acute myeloid leukemia (AML) patients, exposed CRT and lack of CD47 in AML cells was associated with a higher survival rate [49]. All this clinical evidence suggests the crucial role played by surface translocation of CRT for the establishment of long term antitumor immune response triggered by ICD succumbing cells.

ATP release

Besides being the most abundant intracellular metabolite involved in energy-requiring process and signaling pathways, ATP can be released from cells subjected to physical or chemical stress, such as plasma membrane disruption or exposure to cytotoxic agents [50]. In the context of ICD, ATP is released from dying cells in a process that relies on the autophagic machinery as it depends on the accumulation of ATP within autolysosomes and its exocytosis. This is accompanied by the translocation to the plasma membrane of lysosomal-associated membrane protein 1 (LAMP1), cellular blebbing, and opening of Pannexin 1 channels [40, 51, 52]. Autophagy-deficient tumor cells fail to elicit tumor antigen-specific immune responses in mouse models [53, 54]. Moreover, compounds that induce autophagy by reducing cytoplasmic protein acetylation, called 'caloric restriction mimetics' (CRMs), have a positive impact on ICD-inducing therapies, as they promote ATP release from dying cancer cells. *In vivo* experiments have confirmed that thiostrepton, a natural antibiotic with CRM properties, supports tumor growth control by oxaliplatin (an ICD-inducing agent). This effect was lost with the knockdown of pro-autophagic transcription factors, corroborating the importance of pre-mortem autophagy and ATP release for ICD [55].

On the other hand, ectonucleotidases such as CD39 (which converts ATP into ADP and AMP) or CD73 (converting AMP into adenosine, an immunosuppressive metabolite) also depress the levels of extracellular ATP, and hence affect the perception of ATP by the immune cells [56]. Tumor infiltrating lymphocytes (TILs) such as immunosuppressive regulatory T (Tregs) cells have been shown to express CD39 on their surface, and this contributes to tumor growth and progression [57].

Extracellular ATP released during the course of ICD is a crucial chemotactic targeting signal for the recruitment of macrophages and DC precursors, and is sensed by purinergic receptor P2RY2. When P2RY2 is absent from the myeloid compartment of the host, ATP sensing is abrogated and recruitment of myeloid cells is impaired [19]. Secreted ATP has another prominent function during ICD: its sensing through purinergic receptor P2RX7 on DCs leads to the activation of the NLRP3 inflammasome. This event initiates the proteolytic maturation and secretion of IL-1β and IL-18 [20, 58]. IL-1β secreted from DCs is essential for triggering a cascade of vital events for ICD, including the recruitment of IL-17-producing γδ T cells and IFN-γ-producing CD8+ αβ T cells into the tumor bed [20, 59]. Consequently, *Nlrp3*$^{-/-}$, *Il17a*$^{-/-}$ or *Il17ra*$^{-/-}$ mice, as well as mice receiving IL-1β neutralizing antibodies, fail to mount an adaptive immune response in vaccination experiments with syngeneic cancer cells treated with ICD inducing agents [20, 59].

Type I IFN response and cancer cells' endogenous nucleic acids

Type I IFNs are a family of cytokines involved mainly with antiviral responses, secreted by virtually all cells [60]. They stimulate the activation of DCs, macrophages and Natural Killer (NK) cells, alerting the organism of a possible pathogen infection [61]. The mimicry of pathogen defense response has been described as a phenomenon evoked by ICD-undergoing cancer cells. It is clear now that cancer cells succumbing to ICD are able to autonomously produce and release type I IFNs, upon the detection of endogenous dsRNA by TLR3 [23] or dsDNA by cGAS protein, a cytosolic DNA sensor involved in Type I IFN responses [24, 62]. Once secreted, type I IFNs mediate immunostimulatory effects when recognized by immune cells expressing Interferon alpha/beta receptor (IFNAR). This occurs by i) promoting DCs maturation and migration to lymph nodes [63]; ii) increasing survival and cytotoxicity of CD8$^+$ CTL [64, 65], and iii) eliciting pro-inflammatory cytokine production from macrophages, such as IL-1β and IL-18 [66].

Furthermore, type I IFN also acts in an autocrine/paracrine manner that activates the expression of IFN-stimulated genes, that include the chemoattractant for T cells C-X-C motif chemokine ligand 10 (CXCL10) [23]. Notably, ICD has been associated with a cancer cell-autonomous, pathogen response-like chemokine (PARC)-signature. This PARC signature involves the release of CXCL1, CCL2, and CXCL10 chemokines, which recruit neutrophils as first innate immune responders [26]. Notably, cancer cell-derived nucleic acids can be released during ICD, and be efficiently

22. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, *et al*. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med. 2007;13:1050-9.

23. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot D, Adam J, *et al*. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med. 2014;20:1301-9.

24. Vanpouille-Box C, Alard A, Aryankalayil MJ, Sarfraz Y, Diamond J, Schneider R, *et al*. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. Nat Commun. 2017;8:15618.

25. Chiba S, Baghdadi M, Akiba H, Yoshiyama H, Kinoshita I, Dosaka-Akita H, *et al*. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. Nat Immunol. 2012;13(9):832-42.

26. Garg AD, Vandenberk L, Fang S, Fasche T, Van Eygen S, Maes J, *et al*. Pathogen response-like recruitment and activation of neutrophils by sterile immunogenic dying cells drives neutrophil-mediated residual cell killing. Cell Death Differ. 2017;24:832-43.

27. Vacchelli E, Ma Y, Baracco EE, Sistigu A, Enot DP, Pietrocola F, *et al*. Chemotherapy-Induced antitumor immunity requires formyl peptide receptor 1. Science. 2015;350:972-8.

28. Green DR, Ferguson T, Zitvogel L, Kroemer G. Immunogenic and tolerogenic cell death. Nat Rev Immunol. 2009;9:353-63.

29. Grivennikov S, Greten F, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883-99.

30. Garg AD, Vandenberk L, Koks C, Verschuere T, Boon L, Van Gool SW, *et al*. Dendritic cell vaccines based on immunogenic cell death elicit danger signals and T cell- driven rejection of high- grade glioma. Sci Transl Med. 2016;8:328ra327.

31. Kepp O, Senovilla L, Vitale I, Vacchelli E, Adjemian S, Agostinis P, *et al*. Consensus guidelines for the detection of immunogenic cell death. Oncoimmunology. 2014;3:e955691.

32. Sukkurwala AQ, Adjemian S, Senovilla L, Michaud M, Spaggiari S, Vacchelli E, *et al*. Screening of novel immunogenic cell death inducers within the NCI mechanistic diversity set. Oncoimmunology. 2014;3:e28473.

33. Dunn G, Bruce A, Ikeda H, Old L, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991-8.

34. Vesely M, Kershaw M, Schreiber R, Smyth M. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011;29:235-71.

35. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol. 2006;6:715-27.

36. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646e674.

taken up by DCs, macrophages and neutrophils, leading to a strong type I IFN response that translates into a potent immunostimulatory effect [26, 67, 68].

Released nucleic acids also act on neutrophils by signaling through the TLR7/8/9-MyD88 axis. The ATP-P2Rs and nucleic acids-TLR7/8/9-MyD88 axes together regulate the neutrophil phenotypic maturation ($CD86^{high}$/MHC-II^{high}) and its pro-inflammatory profile ($IL1\beta^{high}IL6^{high}$), that in turn trigger hydrogen peroxide and nitric oxide-based respiratory burst, thereby killing residual cancer cells [26]. Supporting all the above-mentioned notions, studies demonstrate that the use of IFNAR1-neutralizing antibodies, CXCL10 receptor (CXCR3)-neutralizing antibodies, or tumor cells lacking *ifnar1*, *ifnar2* or *tlr3* genes, all have a negative impact on immunogenicity induced by anticancer treatments [23]. Similarly, studies in breast carcinoma patients have shown that reduced levels of interferon regulatory factor 7 (IRF7), one of the transducers involved in type I IFN signaling, correlated with decreased metastasis-free survival rates [69].

ANXA1 release

Another ICD-related DAMP is the cytosolic, ubiquitous protein AnnexinA1 (ANXA1) [6]. ANXA1 facilitates resolution of inflammation, as it promotes recruitment of phagocytic cells and so, disposal of apoptotic bodies. Such events occur upon binding to Formyl peptide receptor 2 (FPR2), a G protein-coupled receptor expressed in human monocytes, macrophages, neutrophils, among other immune cells involved in resolving inflammation [70].

Mitoxantrone and Doxorubicin are two FDA-approved anticancer drugs with ICD-inducing abilities that release ANXA1 when used to treat tumor cells. In this context, ANXA1 released from dying tumor cells reportedly interacts with FPR1, expressed by DCs. Such interaction was proven to be crucial, not for recruitment of inflammatory DCs, but to bring them into close proximity to cancer cells, establishing contact and promoting the take-up and processing of their TAAs to be presented to T cells [27]. Despite the extensive knowledge about the relationship of ANXA1 and its receptor with tumor prognosis, the exact intracellular mechanism of ANXA1 release during ICD remains to be elucidated. Studies in breast cancer patients showed that cancer cells may use FPR1 single nucleotide polymorphism to evade danger signaling through ANXA1. This type of mutation was associated with shortened time-to-metastasis and decreased overall survival [27].

HMGB1 release

HMGB1 is a non-histone chromatin-binding protein, ubiquitously expressed by almost all eukaryotic cells [71]. In the nucleus, HMGB1 binds the minor groove of DNA and modulates its accessibility to regulatory elements including transcription factors and nucleosomes [72]. Hyperacetylation of HMGB1 lysine residues is a key post-translational modification that regulates the shuttling of HMGB1 between nuclear and cytoplasmatic location. It also promotes its relocation to the cytosol, where it acts as an autophagy regulator [73, 74]. HMGB1 can be passively released from necrotic cells and act as a danger signal for the immune system. It can also be actively secreted during pyroptotic cell death. This type of cell death is activated in response to homeostasis perturbations [75] and mediated by the activation of the inflammasome, which recruits and activates caspase 1 [76]. This caspase mediates the proteolytic processing of pro-IL1β and pro-IL18 into mature IL1β and IL18, respectively. Caspase 1 provokes the activation of Gasdermin, a pore-forming protein [77]. After pore formation, cellular swelling, osmotic lysis [78] as well as proinflammatory cytokine (*e.g.* IL1 β) [79] and DAMPs release [80] take place.

Extracellular HMGB1 has been found to play a key role during ICD; however, the mechanism of externalization during this type of cell death still needs to be completely understood. Once released, HMGB1 can signal through various PRRs, such as TLR2, TLR4 and the receptor for advanced glycosylation end products (RAGE) [81, 82]. Notably, only TLR4 seems to be indispensable for HMGB1-mediated adaptive immune responses against mouse cancer cells succumbing to ICD. Knockout mice for Tlr4 or Myd88 were found to respond worse to anticancer chemotherapeutics than their wildtype immunocompetent counterparts. In vitro administration of HMGB1 to TLR4-expressing DCs prevents accelerated lysosomal degradation of tumor antigens and so enhances antigen processing and cross-presentation [22]. In co-culture experiments, tumor antigens cross-presentation was abolished in the presence of HMGB1 neutralizing antibodies or when DCs were depleted of TLR4. Moreover, HMGB1-TLR4 signaling on DCs promotes the expression of pro-IL1β, which is processed by the inflammasome into IL-1β [22]. Furthermore, HMGB1 stimulates the production of other pro-inflammatory cytokines such as TNF, IL-1, IL-6 and IL-8 by neutrophils, monocytes and macrophages [81, 83]. Despite such evidence, the role of released HMGB1 during ICD is still a matter of debate. Studies have found that it can shift from a chemoattractant DAMP, to a pro-inflammatory cytokines-inductor DAMP, and even to an inactive DAMP, depending on multiple variants that include the context of the extracellular space and HMGB1 oxidation state [84-86].

ER stress and DAMPs release

Myriads of anti-cancer agents have been studied to unveil their immunogenic potential. Interestingly, all those surveys converge on a common denominator: endoplasmic reticulum stress and the production of reactive oxygen species (ROS) [32, 87]. ICD-inducers elicit a ROS-based proteotoxicity at ER, which eventually results in activation of danger signaling pathways that traffic DAMPs toward the extracellular space [88]. Notably, silencing molecular effectors of the ER stress pathway triggered by ICD inducers reduces DAMPs (i.e., CRT and ATP) emission by dying cancer cells and reduces their immunogenicity *in vivo*. These data reinforce the concept that a robust ER stress response preferably accompanied or induced by ROS production is a relevant biochemical prerequisite for danger signaling and ICD [89].

Activation of the ER stress pathways also known as the unfolded protein response (UPR), and specially,

37. Meije C, Swart G, Lepoole C, Das P, van den Oord JJ. Antigenic profiles of individual-matched pairs of primary and melanoma metastases. Hum Pathol. 2009;40:1399-407.

38. Gubin M, Artyomov M, Mardis E, Schreiber R. Tumor neoantigens: building a framework for personalized cancer immunotherapy. J Clin Invest. 2015;125: 3413-21.

39. van Kempen T, Wenink M, Leijten E, Radstake T, Boes M. Perception of self: distinguishing autoimmunity from autoinflammation. Nat Rev Rheumatol. 2015;11:483-92.

40. Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, Lazarowski ER, *et al*. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature. 2010;467(7317):863-7.

41. Yamazaki T, Buqué A, Rybstein M, Chen J, Sato A, Galluzzi L. Methods to Detect Immunogenic Cell Death In Vivo. In: Thurin M, Cesano A, Marincola F (eds). Biomarkers for Immunotherapy of Cancer. Methods in Molecular Biology, vol 2055. Humana, New York, NY; 2020.

42. Gelebart P, Opas M, Michalak M. Calreticulin, a Ca^{2+}-binding chaperone of the endoplasmic reticulum. Int J Biochem Cell Biol. 2005;37:260-6.

43. Gardai SJ, McPhillips KA, Frasch SC, Janssen WJ, Starefeldt A, Murphy-Ullrich JE, *et al*. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. Cell. 2005;123:321-3.

44. Pawaria S, Binder R. CD91-dependent programming of T-helper cell responses following heat shock protein immunization. Nat Commun. 2011;2:521.

45. Majeti R, Chao M, Alizadeh A, Pang W, Jaiswal S, Gibbs Jr KD, *et al*. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell. 2009;138: 286-99.

46. Suzuki S, Yokobori T, Tanaka N, Sakai M, Sano A, Inose T, *et al*. CD47 expression regulated by the miR-133a tumor suppressor is a novel prognostic marker in esophageal squamous cell carcinoma. Oncol Rep. 2012;28:465-72.

47. Barclay AN, van den Berg TK. The interaction between signal regulatory protein alpha (SIRPalpha) and CD47: structure, function, and therapeutic target. Annu Rev Immunol. 2014;32:25-50.

48. Chao MP, Jaiswal S, Weissman-Tsukamoto R, Alizadeh A, Gentles AJ, Volkmer J, *et al*. Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. Sci Transl Med. 2010;2:63ra94.

49. Wemeau M, Kepp O, Tesniere A, Panaretakis T, Flament C, De Botton S, *et al*. Calreticulin exposure on malignant blasts predicts a cellular anticancer immune response in patients with acute myeloid leukemia. Cell Death Dis. 2010;1:e104.

50. Selzner N, Selzner M, Graf R, Ungethuem U, Fitz JG, Clavien PA. Water induces autocrine stimulation of tumor cell killing through ATP release and P2 receptor binding. Cell Death Differ. 2004;11:172-80.

the protein kinase R-like endoplasmic reticulum kinase (PERK)-mediated arm of the UPR is vital for the vast majority, if not all, the scenarios where ICD occurs [87]. Tunicamycin and thapsigargin, two potent chemical ER stressors, both of which induce strong UPR responses [90-92], have been shown to efficiently restore CRT relocation and/or in vivo immunogenicity of cisplatin or mytomicin C [10]. Michaud *et al*. propose that the incapacity of cisplatin to trigger ICD is at least partially due to its failure to stimulate the premortem ER-stress response required for the externalization of the phagocytic signal CRT on the surface of dying cancer cells. They developed a synthetic system for inducing ER stress, namely by the tetracycline-inducible expression of the ER-restricted protein reticulon-1c (Rtn-1c), in a murine cancer cell line genetically modified. Enforced Rtn-1c expression combined with cisplatin treatment promoted CRT externalization to the surface of cancer cells.

In contrast to single agent treatments, the tetracycline-mediated Rtn-1c induction combined with cisplatin chemotherapy stimulated ICD. More importantly, established tumors into syngeneic immunocompetent mice, forced to constitutively express Rtn-1c *in vivo* by continuous treatment with tetracycline, became responsive to cisplatin and exhibited a corresponding reduction in the rate of tumor growth. Altogether, these results indicated that the artificial induction of ICD by genetic manipulation of the ER-stress response could improve the efficacy of chemotherapy with cisplatin by stimulating anticancer immunity [93].

PERK is at the 'core' of ICD and the upstream coordinator of DAMP trafficking mechanisms. Intriguingly, the function of PERK seems to differ between Type I and Type II ICD inducers [87]. Type I ICD inducers encompass all the drugs that trigger ICD-associated immunogenicity through secondary or collateral, mostly mild, ER stress (off-target). This effect goes in parallel with the main 'on-target' effect driving apoptosis via non-ER targets. Most clinically employed ICD inducers fall within this category [94]. Type II ICD inducers, instead, selectively target the ER and orchestrate both danger and apoptotic signaling through 'focused/on-target' (ROS-based) ER stress [87, 88]. When ICD is induced by anthracyclines or oxaliplatin (Type I ICD inducers), CRT exposure is reliant on the sequential activation of three main modules: i) a ROS-modulated ER-stress arm regulated by intracellular Ca^{2+} elevation and dependent on PERK-mediated phosphorylation of eIF2α; ii) a caspase-module requiring Bax/Bak and B-cell receptor associated protein 31 (BAP31) cleavage by ER-associated caspase-8 and iii) an ER-to-Golgi anterograde transport-module ultimately eliciting N-ethylmaleimide-sensitive fusion protein-attachment protein receptor (SNARE)-dependent exocytosis [18].

As abovementioned, the interruption of this complex pathway at any level (with pharmacological or genetic interventions) abolishes CRT exposure, dampens the immunogenicity of apoptosis and reduces the immune response elicited by anticancer chemotherapies [7, 18]. Alternatively, CRT-exposure mechanisms elicited by hypericin-mediated photodynamic therapy (Hyp-PDT) (Type II ICD inducer), rely on a more simplified danger signaling pathway consisting of: i) a focused ROS-ER-stress module; ii) a PERK-mediated proximal secretory pathway independent of eIF2α phosphorylation; iii) a similar ER-to-Golgi anterograde transport and, iv) phosphoinositide 3-kinase (PI3K)-regulated exocytosis [12, 88, 95]. Remarkably, following Hyp-PDT, the PERK-regulated danger signaling pathway coordinates the concomitant pre-apoptotic export of both surface-CRT and secreted ATP [12]. Depending on the trigger stimuli, PERK could be involved only in CRT emission or both in ATP and CRT emission [87, 96, 97].

Although danger signaling during ICD exhibits a certain level of plasticity depending on the type of ICD inducer under consideration [30], ablation of PERK in cancer cells compromises DAMPs exposure and suppresses the tumor-rejecting anticancer vaccination effect of ICD *in vivo* for both Type I and Type II ICD inducers [18, 94]. The reason behind the reliance of the danger signaling on PERK rather than on other UPR sensors (*e.g*., IRE1a) remains enigmatic. Likewise, the mechanism, shared by Type I and Type II ICD inducers, linking PERK to intracellular Ca^{2+} elevation, induced by ER stress which stimulates the efflux of Ca^{2+} from the ER lumen to cytosol, and to the actin cytoskeleton in the path to mobilize DAMPs, remains unclear. The relevance of PERK over other UPR sensors could be explained by the newly discovered UPR-independent function of PERK in modulating the dynamics of the actin cytoskeleton, through its interaction with the actin-binding protein filamin A (FLNA) [87, 98].

A recent study from Van Vliet *et al*, provided compelling evidence showing that PERK is able to sense and rapidly respond to cytosolic Ca^{2+} elevations through its cytosolic domain, by enabling the formation of ER-plasma membrane appositions [98]. The interface between the ER and the plasma membrane becomes essential to regulate Ca^{2+} fluxes and the refilling of the ER Ca^{2+} store through the evolutionary conserved process of store operated Ca^{2+} entry (SOCE). SOCE is stimulated in response to depletion of the ER Ca^{2+} store and is the main pathway regenerating ER Ca^{2+} levels and maintaining Ca^{2+} signaling. The molecular entities mediating SOCE are the ER Ca^{2+} sensors stromal interacting protein 1 (STIM1) and the PM Ca^{2+} release-activated calcium channel protein 1 (Orai1) [99]. Upon ER-Ca^{2+} depletion, STIM1 senses a decrease in the luminal Ca^{2+} levels through its ER luminal EF hand domain, a motif found in a large family of Ca^{2+}-binding proteins, which triggers STIM1 oligomers and their association with the PM [100, 101] where they bind Orai1. Binding of STIM1 to Orai1 allows its clustering and generation of the PM channel, that allows extracellular Ca^{2+} influx into the cytosol through its interaction with FLNA [102].

This newly identified function of PERK in forming ER-PM contact sites could be relevant for the mechanisms of trafficking of DAMPs via the secretory pathway and SNAP (soluble N-ethylmaleimide-sensitive factor attachment protein) receptor (SNARE)-mediated exocytosis. These housekeeping processes have been shown to be required for Type I and Type II ICD inducers [18, 88]. A PERK-FLNA axis could in fact sustain, through the rapid formation of ER-PM junctions, intracellular Ca^{2+} levels and Ca^{2+} modulated actin cytoskeleton

51. Martins I, Wang Y, Michaud M, Ma Y, Sukkurwala AQ, Shen S, *et al*. Molecular mechanisms of ATP secretion during immunogenic cell death. Cell Death Differ. 2014;21:79-91.

52. Wang Y, Martins I, Ma Y, Kepp O, Galluzzi L, Kroemer G. Autophagy-dependent ATP release from dying cells via lysosomal exocytosis. Autophagy. 2013;9(10):1624-5.

53. Michaud M, Martins I, Sukkurwala A, Adjemianet S, Ma Y, Pellegatti P, *et al*. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science. 2011;334: 1573-7.

54. Garg AD, Dudek AM, Agostinis P. Autophagy-dependent suppression of cancer immunogenicity and effector mechanisms of innate and adaptive immunity. Oncoimmunology. 2013;2:10.

55. Kepp O, Kroemer G. Autophagy induction by thiostrepton for the improvement of anticancer therapy. Autophagy. 2020;16:1166-7.

56. Antonioli L, Blandizzi C, Pacher P, Haskó G. Immunity, inflammation and cancer: a leading role for adenosine. Nat Rev Cancer. 2013;13:842-57.

57. Sun X, Wu Y, Gao W, Enjyoji K, Csizmadia E, Müller CE, *et al*. CD39/ENTPD1 expression by $CD4^{+}Foxp3^{+}$ regulatory T cells promotes hepatic metastatic tumor growth in mice. Gastroenterology. 2010;139:1030-40.

58. Zitvogel L, Kepp O, Galluzzi L, Kroemer G. Inflammasomes in carcinogenesis and anticancer immune responses. Nat Immunol. 2012;13:343-51.

59. Ma Y, Aymeric L, Locher C, Mattarollo SR, Delahaye NF, Pereira P, *et al*. Contribution of IL-17-producing γδT cells to the efficacy of anticancer chemotherapy. J Exp Med. 2011;208:491-503.

60. Isaacs A, Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci. 1957;147:258-67.

61. McNab F, Mayer-Barber K, Sher A, Wack A, O'garra A. Type I interferons in infectious disease. Nat Rev Immunol. 2015;15:87-103.

62. Mackenzie KJ, Carroll P, Martin CA, Murina O, Fluteau A, Simpson DJ, *et al*. cGAS surveillance of micronuclei links genome instability to innate immunity. Nature. 2017;548(7668):461-5.

63. Papewalis C, Jacobs B, Wuttke M, Ullrich E, Baehring T, Fenk R, *et al*. IFN-α skews monocytes into $CD56^{+}$-expressing dendritic cells with potent functional activities *in vitro* and *in vivo*. J Immunol. 2008;180:1462-70.

64. Crouse J, Bedenikovic G, Wiesel M, Ibberson M, Xenarios I, Von Laer D, *et al*. Type I interferons protect T cells against NK cell attack mediated by the activating receptor NCR1. Immunity. 2014;40(6): 961-73.

65. Xu H, Grusdat M, Pandyra A, Polz R, Huang J, Sharma P, *et al*. Type I interferon protects antiviral CD8+ T cells from NK cell cytotoxicity. Immunity. 2014;40(6):949-60.

66. Novikov A, Cardone M, Thompson R, Shenderov K, Kirschman KD, Mayer-Barber KD, *et al*. Mycobacterium tuberculosis triggers host type I IFN signaling to regulate IL-1beta production in human macrophages. J Immunol. 2011;187:2540-7.

remodeling, which are key mediators of ER-to-Golgi trafficking and vesicle exocytosis [87, 103].

In some, but not all, experimental settings [18, 88, 104, 105], an increase in the cytosolic concentrations of Ca^{2+} appears to be required for the ICD-associated exposure of CRT on the outer side of the plasma membrane. The evidence that sustains this statement is based on: i) the Ca^{2+} ionophore A23187, but neither the K^{+} ionophore nigericin or the protonophore carbonyl cyanide m-chlorophenylhydrazone, was able to induce CRT exposure, ATP secretion, and HMGB 1 release [105]; ii) intracellular and extracellular Ca^{2+} chelators prevent CRT exposure as promoted by cardiac glycosides [105]; iii) the overexpression of Reticulon-1C, a manipulation that led to a decrease in the Ca^{2+} concentration within the endoplasmic reticulum lumen, in a neuroblastoma cell line, allowed the ICD-associated CRT exposure [104]; iv) the inhibition of the sarco-endoplasmic reticulum Ca^{2+}-ATPase pump and its subsequent reduction of endoplasmic reticulum Ca^{2+} load, promoted pre-apoptotic CRT exposure on the cell surface [104]; v) anthracyclines, the first bona fide ICD inducers characterized [3], have a well-documented effect on Ca^{2+} homeostasis [18]; vi) although thapsigargin and tunicamycin are commonly used to induce the UPR pathway, only the first one incites calcium depletion and promotes cell surface expression and secretion of CRT [106]. Alternatively, Hypericin-based PDT induces a rapid increase in cytosolic Ca^{2+}, however, its chelation of cytosolic Ca^{2+} with BAPTA-AM, a cell-permeable Ca^{2+} specific chelator, did not affect the surface mobilization of CRT induced by Hyp-PDT [88]. Therefore, alterations in Ca^{2+} homeostasis could not be a general condition for the ICD-associated translocation of CRT to the plasma membrane [96].

Conclusions

In recent years, it has been witnessed a burst in immunotherapy-based strategies to take advantage of the host immune system-cancer cells interaction. Many efforts are made nowadays to restore the immunogenicity of cancer cells, and ICD induction stands out as a clinically relevant goal. Despite the accumulating evidence highlighting the role of DAMPs signaling and sensing for ICD, several aspects on this matter remain to be further investigated. For instance: the controversial roles of some of them, balancing between immunostimulatory and immunosuppressive effects depending on the context [85, 86]; the release of immunosuppressive DAMPs, including adenosine or prostaglandin E2 [107, 108]; or the metabolic control of cell death and its impact on DAMPs release [109]. Similarly, most ICD scenarios occur with at least some degree of ER stress, revealing the ability of ICD inducers to disturb cellular homeostasis and evoke stress responses associated with DAMPs signaling.

Some answers remain to be answered, regarding ER stress-ICD-related events, including the role of PERK in ER–plasma membrane appositions during ICD, or its requirement for other processes involving association between various membranes [98, 110]. In this review we have covered the main events associated with the immunogenicity of ICD and the close association of this type of cell death with ER stress responses. Notably, many new ICD inducers have emerged in the recent years, widening the range of stimuli able to drive regulated cell death with immunogenic properties. In this regard, it has been studied recently the use of nano-systems aimed to selectively accumulate and stress the ER under light irradiation in the context of photodynamic therapy. Such direct ROS-mediated ER stress has proven to be effective in inducing a strong ICD-associated antitumor efficacy, and even eradication of distant tumors through an abscopal effect [111, 112].

Moreover, combinatorial regimens aiming for the most effective outcomes have emerged as the leading strategies in cancer. In the specific case of ICD induced by chemotherapeutics, efforts are being made to toss out the Maximum Tolerated Dose approach (which ensures maximum cytotoxicity with limited side effects) but which is usually not accompanied by immunomonitoring and, hence, does not weight the contribution of the immune system to the host's response [113, 114]. Thus, the most representative known strategy involves the use of cyclophosphamide, oxaliplatin, doxorubicin, epirubicin or bortezomib as the ICD-inducing drug, in combination with immune checkpoint blockers. Moreover, immunostimulatory antibodies, adoptive T cell therapies including engineered CAR T cells, immunostimulatory cytokines and DC-based vaccines, are among the most trending combinatorial partners used to expand clinical efficacy of said ICD-inducing treatments [114].

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

67. Deng L, Liang H, Xu M, Yang X, Burnette B, Arina A, *et al*. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity. 2014;41:843-52.

68. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MYK *et al*. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity. 2014;41:830-42.

69. Bidwell B, Slaney C, Withana N, Forster S, Cao Y, Loi S, *et al*. Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. Nat Med. 2012;18:1224-31.

70. Perretti M, D'Acquisto F. Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. Nat Rev Immunol. 2009;9:62-70.

71. Goodwin GH, Johns EW. Isolation and characterisation of two calf-thymus chromatin non-histone proteins with high contents of acidic and basic amino acids. Eur J Biochem. 1973;40(1):215-9.

72. Bustin M. Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol Cell Biol. 1999;19(8):5237-46.

73. Bonaldi T, Talamo F, Scaffidi P, Ferrera D, Porto A, Bachi A, *et al*. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J. 2003;22:5551-60.

74. Evankovich J, Cho S, Zhang R, Cardinal J, Dhupar R, Zhang L, *et al*. High mobility group box 1 release from hepatocytes during ischemia and reperfusion injury is mediated by decreased histone deacetylase activity. J Biol Chem. 2010;285(51):39888-97.

75. Jorgensen I, Miao EA. Pyroptotic cell death defends against intracellular pathogens. Immunol Rev. 2015;265(1):130-42.

76. Lu A, Magupalli VG, Ruan J, Yin Q, Atianand MK, Vos MR, *et al*. Unified Polymerization Mechanism for the Assembly

of ASC-Dependent Inflammasomes. Cell. 2014;156:1193-1206.

77. Liu X, Lieberman J. Knocking 'em Dead: Pore-forming proteins in immune defense. Annu Rev Immunol. 2020;38:455-85.

78. Van Opdenbosch N, Lamkanfi M. Caspases in cell death, inflammation, and disease. Immunity. 2019;50:1352-64.

79. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, *et al*. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature. 2016;535:153-8.

80. de Vasconcelos NM, Van Opdenbosch N, Van Gorp H, Parthoens E, Lamkanfi M. Single-cell analysis of pyroptosis dynamics reveals conserved GSDMD-mediated subcellular events that precede plasma membrane rupture. Cell Death Differ. 2018;26:146-61.

81. Chen G, Ward MF, Sama AE, Wang H. Extracellular HMGB1 as a proinflammatory cytokine. J Interferon Cytokine Res. 2004;24:329-33

82. Park JS, Gamboni-Robertson F, He Q, Svetkauskaiteet D, Kim JY, Strassheim D, *et al*. High mobility group BOX 1 protein interacts with multiple Toll-like receptors. Am J Physiol Cell Physiol. 2006;290:C917-24.

83. Andersson U, Wang H, Palmblad K, Aveberger AC, Bloom O, Erlandsson-Harris H, *et al*. High mobility group 1 protein (Hmg-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Exp Med. 2000;192(4):565-70.

84. Tang D, Billiar TR, Lotze MT. A Janus Tale of Two Active High Mobility Group Box 1 (HMGB1) redox states. Mol Med. 2012;18:1360-2.

85. Venereau E, Casalgrandi M, Schiraldi M, Antoine DJ, Cattaneo A, De Marchis F, *et al*. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. J Exp Med. 2012;209(9):1519-28.

86. Yang H, Lundbäck P, Ottosson L, Erlandsson-Harriset H, Venereau E, Bianchi ME, *et al*. Redox modification of cysteine residues regulates the cytokine activity of High Mobility Group Box-1 (HMGB1). Mol Med. 2012;18:250-9.

87. Rufo N, Garg AD, Agostinis P. The Unfolded Protein Response in Immunogenic Cell Death and Cancer Immunotherapy. Trends in Cancer. 2017;3(9):643-58.

88. Garg AD, Krysko DV, Verfaillie T, Kaczmarek A, Ferreira GB, Marysael T, *et al*. A novel pathway combining calreticulin exposure and ATP secretion in immunogenic cancer cell death. EMBO J. 2012;31: 1062-79.

89. Garg AD, Martin S, Golab J, Agostinis P. Danger signalling during cancer cell death: origins, plasticity and regulation. Cell Death Differ. 2014;21:26-38.

90. Obeng EA, Carlson LM, Gutman DM, Harrington WJ, Lee KP, Boise LH. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. Blood. 2006;107:4907-17.

91. Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, Leuzzi B, *et al*. Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications. FEBS J. 2019;286:241-78.

92. Shen K, Johnson DW, Vesey DA, McGuckin MA, Gobe GC. Role of the unfolded protein response in determining the fate of tumor cells and the promise of multi-targeted therapies. Cell Stress Chaperones. 2018;23:317-34.

93. Michaud M, Sukkurwala AQ, Di Sano F, Zitvogel L, Kepp O, Kroemer G. Synthetic induction of immunogenic cell death by genetic stimulation of endoplasmic reticulum stress. Oncoimmunology. 2014;3:28276.

94. Garg AD, Galluzzi L, Apetoh L, Baert T, Birge RB, Bravo-San Pedro JM, *et al*. Molecular and translational classifications of DAMPs in immunogenic cell death. Front Immunol. 2015;6:588.

95. van Vliet AR, Garg AD, Agostinis P. Coordination of stress, Ca^{2+} and immunogenic signaling pathways by PERK at the endoplasmic reticulum. Biol Chem. 2016;397(7):649-56.

96. Kepp O, Menger L, Vacchelli E, Locher C, Adjemian S, Yamazaki T, *et al*. Crosstalk between ER stress and immunogenic cell death. Cytokine Growth Factor Rev. 2013;24:311-8.

97. Serrano-del Valle A, Anel A, Naval J, Marzo I. Immunogenic cell death and immunotherapy of multiple myeloma. Front Cell Dev Biol. 2019;7:50.

98. van Vliet AR, Giordano F, Gerlo S, Segura I, Van Eygen S, Molenberghs G, *et al*. The ER stress sensor PERK coordinates ER-plasma membrane contact site formation through interaction with Filamin-A and F-actin remodeling. Mol Cell. 2017;65:1-15.

99. Varnai P, Hunyady L, Balla T. STIM and Orai: the long-awaited constituents of store-operated calcium entry. Trends Pharmacol Sci. 2009;30:118-28.

100. Luik R, Wu M, Buchanan J, Lewis R. The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. J Cell Biol. 2006;174:815-25.

101. Wu M, Buchanan J, Luik R, Lewis R. Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. J Cell Biol. 2006;174:803-13.

102. Orci L, Ravazzola M, Le Coadic M, Shen W, Demaurex N, Cosson P. From the Cover: STIM1-induced precortical and cortical subdomains of the endoplasmic reticulum. Proc Natl Acad Sci USA. 2009;106: 19358-62.

103. Porat-Shliom N, Milberg O, Masedunskas A, Weigert R. Multiple roles for the actin cytoskeleton during regulated exocytosis. Cell Mol Life Sci. 2013;70:2099-121.

104. Tufi R, Panaretakis T, Bianchi K, Criollo A, Fazi B, Di Sano F, *et al*. Reduction of endoplasmic reticulum Ca^{2+} levels favors plasma membrane surface exposure of calreticulin. Cell Death Differ. 2008;15:274-82.

105. Menger L, Vacchelli E, Adjemian S, Martins I, Ma Y, Shen S, *et al*. Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. Sci Transl Med. 2012;4(143):ra99.

106. Peters L, Raghavan M. Endoplasmic reticulum calcium depletion impacts chaperone secretion, innate immunity, and phagocytic uptake of cells. J Immunol. 2011;187:919-31.

107. Cekic C, Linden J. Purinergic regulation of the immune system. Nat Rev Immunol. 2016;16:177-92.

108. Hangai S, Ao T, Kimura Y, Matsuki K, Kawamura T, Negishi H, *et al*. Pge2 induced in and released by dying cells functions as an inhibitory DAMP. Proc Natl Acad Sci USA. 2016;113:3844-9.

109. Green DR, Galluzzi L, Kroemer G. Metabolic control of cell death. Science. 2014;345:1250256

110. Verfaillie T, Rubio N, Garg AD, Bultynck G, Rizzuto R, Decuypere JP, *et al*. PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. Cell Death Differ. 2012;19:1880-91.

111. Deng H, Zhou Z, Yang W, Lin L, Wang S, Niu G *et al*. Endoplasmic reticulum targeting to amplify immunogenic cell death for cancer immunotherapy. Nano Lett. 2020;20:1928-33.

112. Li W, Yang J, Luo L, Jiang M, Qin B, Yin H, *et al*. Targeting photodynamic and photothermal therapy to the endoplasmic reticulum enhances immunogenic cancer cell death. Nat Commun. 2019;10:3349.

113. Pol J, Vacchelli E, Aranda F, Castoldi F, Eggermont A, Cremer I, *et al*. Trial watch: immunogenic cell death inducers for anticancer chemotherapy. Oncoimmunology. 2015;4:e1008866.

114. Vanmeerbeek I, Sprooten J, De Ruysscher D, Tejpar S, Vandenberghe P, Fucikova J, *et al*. Trial watch: chemotherapy-induced immunogenic cell death in immuno-oncology. Oncoimmunology. 2020;9(1):1703449.

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