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

Lena M de León-Esperón, Flavia Llorente Alvarez, Olivia Díaz Navarro, Carmen Soto Febles, Maria E Lanio, Carlos Álvarez
Centro de Estudio de Proteínas, Facultad de Biología, Universidad de La Habana (UH)
Calle 25 # 455 entre I y J. El Vedado, La Habana, Cuba
Laboratorio NaNo Cancer UH-Centro de Inmunología Molecular, La Habana, CP 11600, Cuba
calvarez@fibio.uh.cu

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.

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 promissoria 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 tumoriales 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 endoplasmático (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.

How to cite (Vancouver style):

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

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 above-mentioned 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 immune surveillance 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 antitumor 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 adjuvantivity [8]. On one hand, dying cells must display antigens that have not previously elicited a central or peripheral tolerance. Such neo-epitopes can emerge either during cell infection through pathogen-encoded genes, or during oncosenescence, 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, adjuvantivity 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


Biotecnología Aplicada 2021; Vol.38, No.3

de León-Esperón LM, et al. Immunogenic Cell Death, DAMPs and ER stress
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, AnnexinA1 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.

---

**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 (IPN) 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 α 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).
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].

Surface-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-I 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 expression 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 Panx1 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 over, compounds that induce autophagy by reducing immune responses in mouse models [53, 54]. More specifically, autophagy-deficient cancer cells, such as ovarian cancer cells [40, 51, 52], Autophagy-deficient cancer cells, such as ovarian cancer cells [40, 51, 52], exhibit increased immune evasion and reduced immunogenicity compared to wild-type cells [53, 54].

**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 invoked 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 chemotactrant 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 processed and presented as antigens, promoting the activation of CD8+ T-cells [31]. Additionally, type I IFN response can be enhanced by the presence of immune modulators, such as DNA sensor involved in Type I IFN responses [24, 36]. Type I IFN response and cancer cells’ endogenous nucleic acids

On the other hand, eukaryotic cell death, DAMPs and ER stress

**References**


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 cell 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 (CD66chigh/MHC-IIhigh) and its pro-inflammatory profile (IL1βhigh/IL18high), 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, CXCR1/2 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, and 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 TAA’s 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 localization. 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-IL-1β and pro-IL-18 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 pro-inflammatory cytokine (e.g. IL1β) [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 Tlr2 or Myd88 were found to respond worse to anticancer chemotherapy 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-IL-1β, 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 chemottractant 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 signal induced ICD [89].

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 mytomycin 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 reticulin-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] (103).

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 hyaluronan-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 expression or both in ATP and CRT expression [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., IRE1α) 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 [99]. 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-PMT 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 rapid formation of ER-PMT junctions, intracellular Ca\(^{2+}\) levels and Ca\(^{2+}\) modulated actin cytoskeleton

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 Ca²⁺ 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 Ca²⁺ ionophore A23187, but neither the K⁺ ionophore nigericin or the protonophore carbonyl cyanide m-chlorophenylhydrazone, was able to induce CRT exposure, ATP secretion, and HMGB1 release [105]; ii) intracellular and extracellular Ca²⁺ 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 Ca²⁺ 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 Ca²⁺-ATPase pump and its subsequent reduction of endoplasmic reticulum Ca²⁺ 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 Ca²⁺ 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 Ca²⁺, however, its chelation of cytosolic Ca²⁺ with BAPTA-AM, a cell-permeable Ca²⁺ specific chelator, did not affect the surface mobilization of CRT induced by Hyp-PDT [88]. Therefore, alterations in Ca²⁺ 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].

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.

Immunogenic Cell Death, DAMPs and ER stress

PERK is a stress sensor that coordinates ER-plasma membrane contact site formation through interaction with Filamin-A and F-actin remodeling. Mol Cell. 2017;65:1-15.


van Vliet AR, Garg AD, Agostinis P. Coordination of stress, Ca2+ and immunogenic signaling pathways by PERK at the endoplasmic reticulum. Biol Chem. 2016;397(7):649-56.


Orci L, Ravazzola M, Le Comid M, Shen W, Demaurex N, Cosson P. From the Cover: STIM1-induced preclinical and  


