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Review Article

Harnessing Natural Products of Marine Origin for Induction of Immunogenic Cell Death

Kaylee Chen and Manikanda Raja Keerthi Raja*

Department of Biological Sciences, University of South Carolina, Columbia, South Carolina, USA

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ABSTRACT

Conventional cancer chemotherapy aims to kill highly proliferating tumor cells and is often immunosuppressive due to its off-target side effects. However, certain cytotoxic cancer chemotherapeutic drugs can kill tumor cells by triggering immunogenic cell death (ICD). Cells undergoing ICD release damage-associated molecular patterns (DAMPs) to activate robust innate and adaptive anti-tumor immune responses. Despite many compounds being able to trigger one or two hallmarks of ICD, very few *bona fide* ICD inducers are available. Identification of bioactive natural ICD inducers with low side effects and high tolerability represents a priority in biomedical research. In this review, we discuss the various strategies to regulate the hallmarks of ICD and enhance immunogenic potentials. We focus on evaluating the potential of natural compounds of marine origin to amplify the effects of ICD and therefore serve as novel therapeutic anti-cancer agents alone or in combination with existent chemo- or immune-therapies.

Introduction

Cancer is a major burden of a disease worldwide and ranks as a leading cause of death in many countries. There were an estimated 19.3 million new cases and 10 million cancer deaths worldwide in 2020. In America, about 1.9 million new cancer cases and approximately 0.6 million cancer deaths were estimated to occur in 2021 [1]. Historically, cancer was defined as a cell autonomous disease with an imbalance between oncogene activation and/or inactivation of tumor suppressor genes, leading to uncontrolled cell proliferation and resistance to cell death. Therefore, cytotoxic chemotherapy was developed with the major aim of killing proliferative cells. In the past decade, a striking development has been made in the field of immunotherapy. However, to date, many immunotherapy treatments have demonstrated efficacy in only a select group of cancers and usually in a minority of patients with those cancers [2]. Recently, combination of immunotherapy with chemotherapy has been clinically tested and yielded very promising results [3]. The biologic and immunological rationale to explain the efficacy of this combination is based on the ability of chemotherapy to restore an immune response through several complementary mechanisms. Indeed, there is an increasing body of evidence showing that chemotherapies can cause so-called 'immunogenic' cancer cell death (ICD), which can stimulate host anti-tumor immunity [4, 5]. However, most chemotherapeutic drugs were designed to kill the proliferating cells, highly toxic to immune cells, and therefore commonly immune suppressive. By screening the current chemotherapeutical drugs, very few of them are capable to induce ICD. Hence, there is an urgent need to develop novel drugs used alone or in combination with other existent anti-cancer therapies.

Natural compounds have long been a source of anti-cancer compounds. Natural products are generally low in cost, plentiful, and show less toxicity or side effects in clinical practice [6]. Some of the natural compounds such as paclitaxel and anthracycline have been used for cancer treatment for decades. Most of these FDA approved anti-cancer natural drugs have been acquired from terrestrial sources [6]. The ocean, accounting for around 70% of Earth, contains many organisms, which makes it a valuable source of biological compounds for biomedical

**Correspondence to: Manikanda Raja Keerthi Raja, Department of Biological Sciences, University of South Carolina, 715 Sumter Street, PSC621, Columbia, 29205, South Carolina, USA; Tel: 8037772928; Fax: 8037774002; E-mail: hchen@biol.sc.edu, keerthim@email.sc.edu*

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research [7, 8]. It has been recently estimated that between 700,000 and one million species live in the world's oceans; thus, the species so far investigated represent only a small percentage of the total number of the marine existing organisms. Many commercial marine-derived compounds have demonstrated anti-cancer capabilities [9]. The first commercial anti-cancer marine drug was cytarabine, which has been applied in the clinical therapy of leukemia since 1969 [10, 11]. After that, many compounds derived from marine organisms were introduced for cancer therapy. Currently, most marine-derived anti-cancer drugs or potential anti-cancer drugs are used as chemotherapeutic drugs for combinational treatment [9]. Numerous articles have illustrated the *in vitro* or *in vivo* anti-cancer capabilities of marine-derived compounds [12-14]. This review focuses on overviewing marine-derived compounds with the ability to regulate the hallmarks of ICD and the potential to be used alone or in combination to enhance the efficacy of immunotherapies.

Hallmarks of Immunogenic Cell Death

Chemotherapeutic drugs are well-known to induce apoptosis, which is a physiological programmed cell death pathway characterized by chromatin condensation, membrane blebbing and the activation of caspase cascade signaling pathways. Apoptosis has been classified as tolerogenic, as the phagocytosis that results from this type of cell death is non-immunogenic [15]. Nevertheless, several chemotherapeutic drugs have been shown to induce immunogenic cell death (ICD) [4, 16, 17]. According to accepted models, ICD relies on the establishment of adaptive stress responses that promote the spatiotemporally coordinated emission of endogenous danger signals from dying cells [4, 18]. The endogenous molecules known as "damage associated molecular patterns" (DAMPs) have immune-stimulatory effects like the promotion of dendritic cell (DC) maturation and tumor antigen presentation, activation of cytotoxic T cells, and chemotactic effects on innate immune cells (Figure 1) [19, 20]. DAMPs can be broadly categorized into 3 types depending on their stage and localization/release place: i) DAMPs appear on the cell surface e.g., calreticulin (CRT), 70-kDa heat shock protein (HSP70) and 90-kDa heat shock protein (HSP90); ii) DAMPs appear extracellularly e.g., ATP, interferons, and other pro-inflammatory cytokines; and iii) DAMPs appear as end-stage degradation factors e.g., high mobility group box 1 (HMGB1), annexin A1 (ANXA1), DNA and RNA [15-17, 21-25].

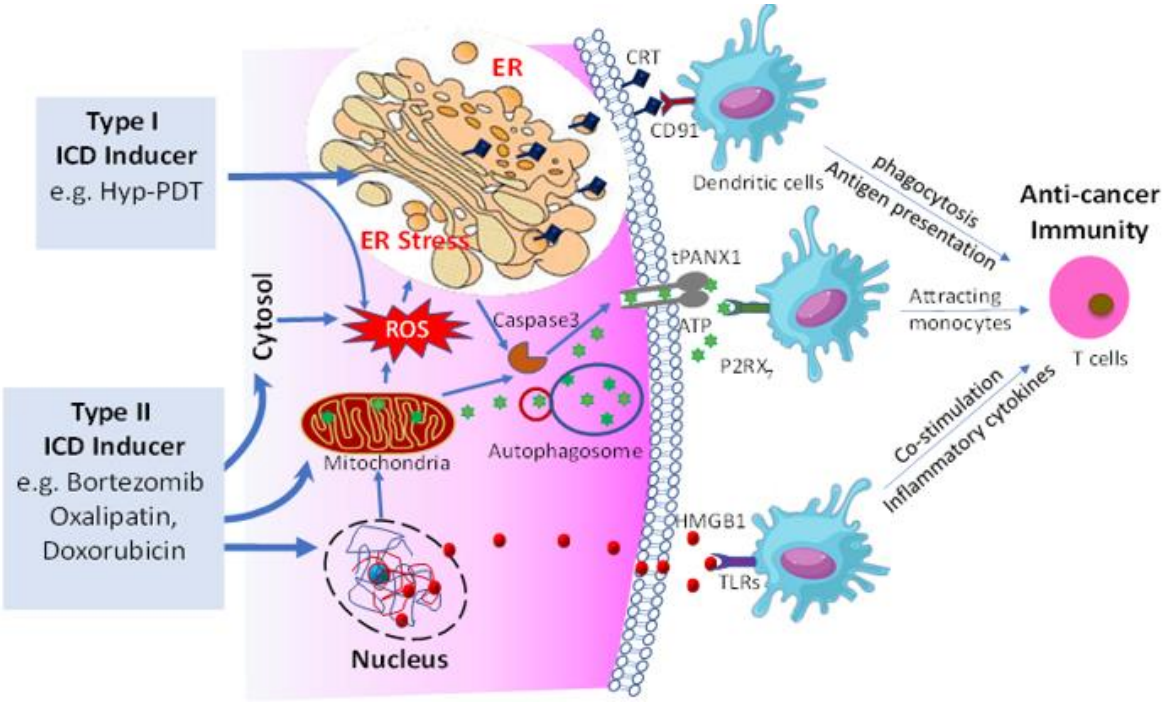

Figure 1: Hallmarks of Immunogenic Cell Death (ICD). Type I and Type II ICD inducers are defined based on their side or focused effect on the ER, respectively. Cancer cells dying due to ICD induce CRT exposure on the outer surface of plasma membrane and secrete ATP and HMGB1. These DAMPs have respective receptors on various immune cells. Most commonly, Antigen Presenting Cells (APCs) such as dendritic cells (DCs) are the ones that are first recruited to the site of ICD and pick up signals to start an immune response. They contain CD91, P2X7R, and TLR receptors on their surface which recognize CRT, ATP and HMGB1 respectively. ATP helps in the recruitment of DC to tumor bed, CRT helps in the uptake of tumor antigens by DCs and HMGB1 helps in optimal antigen presentation to T cells. Cytotoxic T lymphocytes (CTLs) activated by these mature DCs directly kill cancer cells.

I Pre-Apoptotic DAMP Exposure on Cell Surface

The initiation stage of ICD depends largely on the stress developed in the endoplasmic reticulum (ER) and the almost simultaneous induction of reactive oxygen species (ROS) in response to ICD inducers [26, 27]. ER stress is essentially triggered by the accumulation of misfolded or unfolded proteins in the ER. To cope with ER stress, cells initiate the unfolded protein response (UPR) mechanism, which stalls translation, thereby limiting protein synthesis and relieving the cell of the ER stress. Further increase in the amount of ER stress leads to exposure of DAMPs on the cell surface to alert the immune system, which is an active process preceding the morphological signs of apoptosis [28].

Early translocation of endoplasmic calreticulin to the tumor cell surface is one of the hallmarks of ICD and generates an "eat-me" signal for DC phagocytosis and tumor antigen uptake [16]. CRT binds to CD91 receptors on DCs, enabling phagocytosis of dying cancer cells as well as antigen cross-presentation to cytotoxic T cells (CTLs) [29]. CRT-CD91 interaction triggers the NF-κB signaling pathway in DCs and helps release a series of pro-inflammatory cytokines in the extracellular matrix, leading to immune response [29]. Other DAMPs which reside in the ER lumen or cytoplasm and are translocated to the cell surface during ER stress condition include HSP70 and HSP90. HSP70 and HSP90 can interact with the APCs like CD91, LOX1, and CD40, leading to the generation of anti-tumor T cells [30].

II Pre-Mortem DAMP Release

Excessive ER stress eventually leads cell to apoptosis, autophagy, or ICD [31]. Both apoptotic and autophagic pathways are involved in the induction of ATP release which is another hallmark of ICD. Early studies showed that pre-mortem autophagy is required for ICD associated secretion of ATP [25]. Upon induction of autophagy, ATP is found to co-localize with the microtubule-associated proteins 1A/1B light chain 3 (MAP1LC3) protein, which is critical for the biogenesis of autophagosomes [17]. At a later stage in apoptosis, LAMP1 and phosphatidylserine are expressed on the cell surface, confirming the fusion of autolysosomes with the plasma membrane and the subsequent release of the cargo, which includes ATP, in the extracellular microenvironment. However, pharmacological activation of autophagy alone is not sufficient to induce an immunogenic response. Studies demonstrated that a precise balance between caspase mediated apoptotic cell death and autophagy must be taken into account for sufficient ATP secretion during the course of ICD. For example, mitoxantrone-treated ATP secretion was inhibited when tumor cells were treated with broad spectrum caspase inhibitors. It was discovered that cleavage of PANX1 by activated caspase 3 plays a critical role in the release of ATP from apoptotic cells [23].

The ATP released from dying cells acts as a prominent "find me" signal for immature macrophages and DCs upon binding to the $P2Y_2$ receptors of myeloid cells [25]. Apart from that, the ATP released from dying cells also activates $P2RX_7$ receptors on dendritic cells (DCs) and stimulates the NLRP3 inflammasomes to activate and release IL-1β, which is most important for priming IFN-γ–producing, tumor antigen-specific $CD8^+$ T cells. ICD-based secreted ATP has also been reported to mediate intratumoral recruitment and differentiation of antigen presenting cells and reduce T cell mobility, thereby allowing better tissue scanning by T cells [32]. Therefore, sufficient release of ATP in the extracellular environment is critical for an immunogenic response.

Besides secreting DAMPs like ATP, cancer cells also secrete cytokines to activate and modulate immune responses during and after ICD induction. For example, cancer cells responding to anthracycline results in the secretion of type I interferons (IFNs) and chemokine C–X–C motif ligand 10 (CXCL10) [33, 34]. Type I IFNs typically function as an alarming signal to stimulate the activation of macrophages, DCs, and natural killer (NK) cells. CXCL10 is known to mediate chemotactic effects on T cells [34].

III Post-Mortem DAMP Release

All chemotherapeutic agents that efficiently kill malignant cells will increase plasma and/or nuclear membrane permeabilization, which results in post-mortem release of cellular components, including DAMPs. Among them, HMGB1 release represents one of the important hallmarks of ICD. Extracellular HMGB1 mediates robust adjuvant-like effects by binding to various distinct pattern recognition receptors (PRRs), including toll-like receptors (TLRs) and advanced glycosylation end-product-specific receptor (AGER) [15]. Upon binding to the toll-like receptor (TLR)-4 on DCs, HMGB1 can activate the production of pro-inflammatory cytokines through MYD88-dependent signaling pathways and assist in proper antigen-presentation [35]. Interestingly, extracellular HMGB1 has also been found to suppress the activity of immunosuppressive Treg cells [36].

HMGB1 is not the only endogenous TLR-agonist that can be released by cancer cells undergoing ICD. For instance, anthracycline treated breast and colon cancer cells undergoing ICD were observed to release ANXA1 [24]. ANXA1 belongs to a superfamily of proteins that bind acidic phospholipids in a Ca^{2+}-dependent manner. Secreted or surface-exposed ANXA1 can elicit autocrine, paracrine or juxtracrine signaling via formyl peptide receptor 1 (FPR1) to induce anti-cancer immune response [24]. Passive release of nucleic acids, including double-stranded DNA, that signal via TLR-7/8/9 on innate immune cells (like neutrophils), thereby regulating their activation and anti-cancer activity [37].

Strategies to Induce ICD in Cancer Cells

Many clinical trials have begun to evaluate the clinical relevance of ICD in the last ten years [38-43]. Some of these trials have generated very promising results by combining ICD inducers with immune checkpoint blockers (ICBs) or monoclonal antibodies. For example, Federico *et al*. combined ICDs with a monoclonal antibody, resulting in a very impressive response rate of 61.5% in children with recurrent neuroblastoma and very strong indications of increased immune activation [44]. These clinical trials have made it clear that ICD-inducing compounds will play a very important role in the future of cancer therapeutics.

In general, there are two major types of ICD inducers: type I and II inducers [45]. Type I ICD inducers trigger ICD-associated immunogenicity through secondary 'off-target' (mostly mild) ER stress in parallel with the main 'on-target' effect that drives apoptosis via non-ER targets. Most clinically employed ICD inducers such as the anthracyclines, oxaliplatin and bortezomib, are considered type I ICD inducers. Type II ICD inducers, such as photodynamic therapy (PDT), directly target the ER and orchestrate both danger and apoptotic signaling through ROS-based ER stress. Some ICD inducers cannot be easily categorized into type I or type II because they may have multiple effects. Currently, very few *bona fide* ICD inducers are available for cancer treatment. The intrinsic features of the therapeutic agent under consideration or defective cancer cells that fail to express certain types of DAMPs can prohibit the induction of ICD. Different strategies are developed to boost the immunogenic effects, such as i) enhancing emission of one or more DAMP(s); ii) complementing the missing

DAMP(s); or iii) boosting the DAMP downstream signaling pathways [46].

Marine Drugs as Potential ICD Inducers for Cancer Treatment

Since the first approved marine-derived compound, cytarabine (Ara-C, Cytosar-U), was authorized by the FDA in 1969 to act as a first-line drug against leukemia, many chemical mixes of marine origin have been isolated and manufactured for cancer therapy [11, 13, 14]. Currently, there are a total of 9 marine-derived compounds available on the market as anti-cancer drugs and an additional 19 compounds in different phases of clinical trials, most of them having been developed in the past decade or so [13, 47, 48]. All current drugs are used as cytotoxic agents for combinational chemotherapy or targeted therapy or as delivery agents to reduce toxicities, and the immunomodulatory effects have not been evaluated and studied systematically. Interest in finding novel potential modulators of tumor immunotherapy from natural products has been increasing worldwide. As ICD represents an important target in directing and developing new pharmacological interventions, we focus on discussing the anti-cancer effects and mechanism of several drugs with the potential to cause DAMP release and induce ICD (Table 1).

Table 1: Anti-cancer marine products with the potential to induce ICD.

Compound	Marine Organism	Targeting ICD pathways	Potential DAMP Targets	Clinical Status	Studies in Cancer Type	Ref.
Type I ICD inducer candidates						
Plitidepsin	Tunicate	Inhibition of eEF1A2, induction of ER stress	CRT and other cell surface DAMPs	Approved In Australia	Hematological cancer	[52]
Type II ICD inducer candidates						
Marizomib	Bacterium	ER stress, ROS induction, and caspase 3 activation	CRT cell surface DAMP, ATP secretion	Phase III	Multi-cancers	[57, 62]
Fucoidan	Algae, Seagrasses, Echinoderm	ER stress, ROS induction, Activation of TLRs	All DAMPs	Phase II (Hepatic cancer)	Multi-cancers	[72, 74, 75, 77]
Coibamide	Cyanophyceae	Autophagy, Caspase 3	ATP secretion	Pre-clinical	Glioma	[79, 81]
Polyunsaturated Aldehydes	Diatoms	Necroptosis	ATP secretion, HMGB1 and other end-stage DAMPs	Pre-clinical	Colon and lung cancer	[85, 86]

I Plitidepsin

The cyclic depsipeptides plitidepsin (dehydrodidemnin B) and didemnin B are chemically related compounds. Didemnin B was isolated from the Caribbean tunicate *Trididemnum solidum* back in 1981, which is the first marine natural product to enter clinical trials in patients with advanced hematological cancer [49, 50]. However, it failed to pass the clinical trials because of its inefficiency and toxicity. Plitidepsin was isolated from the Mediterranean tunicate *Aplidium albicans* in 1996 [51]. Compared to Didemin B, plitidepsin was found to be more potent and less toxic. It was approved by the Australian regulatory authorities to treat myeloma, leukemia and lymphoma in December 2018.

Plitidepsin appears to lead to cell cycle arrest, growth inhibition, and apoptosis induction via regulating multiple pathways. The main target of plitidepsin, eukaryotic elongation factor 1A2 (eEF1A2), is usually overexpressed in many tumors, such as multiple myeloma, breast cancer and lung cancer [52]. The interaction of plitidepsin with eEF1A inhibits the transportation of misfolded proteins to the proteasome, leading to an accumulation of toxic proteins in the tumor cells and subsequently a disruption of joining protein complexes with binding partners such as peroxiredoxin 1 (PRDX-1), serine-protein kinase 1 (SPK1) and protein kinase R (PKR) [52]. This further triggers the sustained activation of JNK and p38/MAPK, finally inducing apoptosis [53]. Recently, plitidepsin was also found to induce oxidative stress and trigger an ER stress-induced unfolded protein response (UPR) in Hela and multiple myeloma cells [54]. As ER stress and simultaneous ROS induction are well known to induce CRT and other DAMP exposure, it is reasonable to predict that Plitidepsin can function as a type I ICD inducer [21, 27, 28]. However, Plitidepsin simultaneously inhibits autophagic flux while inducing apoptosis [54]. Both caspase 3 activation and induction of autophagy can promote ATP secretion during ICD [23]. Further study is needed to determine how Plitidepsin may affect ATP secretion.

II Marizomib

Marizomib (salinosporamide A; NPI-0052), is a cytotoxic β-lactone-γ-lactam produced by a strictly marine bacterium and isolated in 2003 [55]. Used as a single agent or in combination with clinically used drugs, marizomib has shown strong anti-cancer activities in both solid tumors and hematological malignancies [56, 57]. It is now in phase III clinical trials treating non-small-cell lung cancer (NSCLC), pancreatic cancer, melanoma, lymphoma and multiple myeloma [58]. Mechanistic studies showed that Marizomib is a strong proteasome inhibitor. The ubiquitin-26S proteasome complex contains a proteolytic 20S core comprising of three pairs of catalytic subunits, which have caspase-, trypsin, and chymotrypsin-like (CT-L) activities, where protein degradation occurs [59]. Marizomib irreversibly inhibited all three catalytic functions of the 20S proteasome [57, 60, 61]. Inhibition of proteasome results in the accumulation of abnormal proteins, leading to ER stress and eventually apoptosis of cancer cells.

Marizomib is also capable of decreasing the membrane potential of mitochondria as well as increasing production of superoxide [62]. ER

stress and ROS production will likely cause DAMP exposure for ICD induction [26, 27]. However, Marizomib is also found to inhibit oxidative phosphorylation in triple negative breast cancer, leading to the reduction of ATP synthesis [62]. It is not known whether Marizomib affects ATP secretion. In addition, at high concentrations, Marizomib suppresses T cell activation by regulating T cell proliferation, and reducing the production of pro-inflammatory cytokines, including IL-2 and IFNγ [63]. The activity of the proteasome is known to be involved in the differentiation of CD8+ T cells into effector and memory T cells [64]. Further studies should be conducted to determine the optimized concentration of Marizomib at which it is sufficient for inducing ICD with minimal suppressive effects on the proliferation and functions of T cells.

III Fucoidan

Fucoidan, a fucose-rich polysaccharide, is isolated from brown seaweed such as *Cladosiphon okamuranus* and *Fucus evanescens* [65, 66]. Fucoidan shows a high efficiency in the treatment of a variety of cancers, including breast cancer, prostate cancer, lung cancer, hepatoma, and leukemia [67-69]. Its anti-tumor activity is exerted by regulating multiple signaling pathways in cancer cells, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), extracellular signal-regulated kinase mitogen-activated protein kinase (ERK1/2 MAPK), p38MAPK, phosphatidylinositol 3- kinase (PI3K)/protein kinase B (AKT) and Wnt/β-catenin pathways [68, 69]. The *in vivo* studies demonstrated that fucoidan suppresses tumor growth and significantly diminishes lung metastasis of breast cancer cells. Clinical trial evaluating the efficacy of fucoidan as a supplemental therapy in the management of patients with metastatic colorectal cancer demonstrated that fucoidan combined with chemo-target agents significantly improved the disease control rate [70, 71].

Mechanistic research indicates that fucoidan may induce apoptosis in breast and colon cancer cells via modulation of the endoplasmic reticulum stress cascades. ER stress triggers several processes necessary for apoptosis. One of them is the release of ER Ca^{2+} stores into the cytosol to activate the Ca^{2+}-signal transducer CaMKII, and the other is the activation of the p-eIF2a/CHOP pathway. Fucoidan treatment induces the activation of p-CaMKII in MDA-MB-231 cells rather than in HCT116 cells but activates phosphorylation of eIF2a in both types of cells [72]. Therefore, fucoidan triggers ER stress in a cell context dependent manner [72]. Recent studies revealed the function of fucoidan in inducing ER stress-related autophagy and cell apoptosis in gastric adenocarcinoma cells [73]. Based on these studies, it is reasonable to predict that fucoidan can induce CRT and other DAMPs' exposure in breast cancer and colon cancer cells but enhance ATP release in gastric cancer cells.

DAMPs released from ICD cells bind to PRRs to help the recruitment and activation of DCs, which in turn present tumor antigens to cytotoxic T cells [20]. Besides inducing DAMP releases from cancer cells, fucoidan can directly enhance the function of spleen dendritic cells (DCs) and has shown an adjuvant effect *in vivo* [74, 75]. Systemic administration of fucoidan induces up-regulation of CD40, CD80, and CD86 expression and production of IL-6, IL-12, and TNF-α in spleen cDCs [74]. Fucoidan also promotes the generation of IFN-γ-producing Th1 and Tc1 cells in an IL-12-dependent manner [74]. Therefore, fucoidan can function as an adjuvant to boost Th1 immune response and CTL activation. Although the exact mechanism is still not known, some evidence suggests that fucoidan stimulates macrophage and DC activation via scavenger receptor-A (SR-A) in *in vitro* studies [74, 76]. In addition, fucoidans have been shown to directly interact with TLR-2 and TLR-4 to stimulate immune defenses [77]. Future studies are warranted to examine whether the adjuvant function of fucoidan will be critical for the vaccination effects of fucoidan-induced ICD.

IV Coibamide

Coibamide A is a potent antiproliferative depsipeptide that was first isolated from a marine *Leptolyngbya cyanobacterium* in 2008 [78]. The anti-cancer activities of Coibamide A were evaluated against the NCI's *in vitro* panel of 60 cancer cell lines. It showed selectively cytotoxic potency for breast, glioma, colon, and ovarian cancer cells [78]. Later detailed analysis of the mechanisms showed that in response to coibamide A, glioblastoma cells undergo apoptosis with caspase 3 activation and an alternate form of cell death that is exacerbated by the presence of the caspase inhibitor and characterized by extensive cytoplasmic vacuolization and a lack of apoptotic features [79, 80]. In line with this, coibamide A can trigger significant cell death even in the absence of the cytochrome c-mediated apoptotic pathway. Another study showed that coibamide A can simultaneously induce early autophagosome accumulation through a ULK-phosphorylation and apoptosis [79]. Coibamide A appears to promote cross-signaling between ATG5-dependent autophagy and caspase-dependent apoptosis [81]. Caspase 3 activation and autophagic cell death are required for ATP secretion, with a consequential activation of both inflammatory response and immune system cells for the elimination of cell debris [17, 23]. However, one recent study showed that coibamide A treatment of breast cancer cells also result in severe lysosome defects, which was ascribed to the impaired glycosylation of lysosome membrane protein LAMP1 and LAMP2 [82]. The lysosome defects may result in the blockage of autophagosome-lysosome fusion, which could negatively affect ATP secretion for ICD induction.

V Polyunsaturated Aldehyde

In 1999, Miralto *et al.* isolated three polyunsaturated aldehydes from the marine diatoms *Thalassiosira rotula*, *S. costatum*, and *P. delicatissima*, and found that polyunsaturated aldehydes had anti-proliferative activity on the human colon adenocarcinoma cell line (Caco-2) by inducing apoptosis [83]. For a while, the research on PUAs focused on their effects on the reproduction of marine organisms and their ability to function as infochemicals to mediate plankton interaction [84]. Until 2014, Sansone *et al.* studied the anti-cancer effect of the commercially available polyunsaturated aldehydes on the adenocarcinoma cell lines [85]. The authors found that polyunsaturated aldehydes are able to induce both extrinsic apoptosis and necroptosis in lung and colon adenocarcinoma cell lines [85, 86]. Necroptosis can be initiated by immune ligands, such as Fas, TNF superfamily receptors, and CD40, which activate the receptor-interacting protein kinase 3 (RIPK3) [87, 88]. In turn, RIPK3 (and MLKL) causes the release of ATP and HMGB1, which are known as ICD inducers [89].

Conclusion and Future Perspective

Natural compounds acting as ICD inducers on tumor cells could represent a new frontier in cancer interception and therapy. Considering that the biochemical diversity of the ocean is higher than that of the land, the discovery of novel ICD inducers from marine samples is an intriguing prospect. Among 9 marine-derived anti-cancer compounds available on the market and a handful used in late clinical studies of cancer treatment, a very limited number of them have the potential to induce ICD [13, 47, 48]. One possible reason for this enormous disparity in the distribution and implementation of anti-cancer drugs of marine origin is past screening strategies biased toward a cell-killing effect. Inducing ICD could turn "immunogenically dying tumor cells into a powerful platform for cancer vaccination," providing a new directive in screening anti-cancer drugs [4, 18]. Further research must focus on identifying compounds that trigger atypical apoptotic cell death modality with the appearance of ICD hallmarks.

We have reviewed several anti-cancer marine compounds with the potential to induce ICD. However, none of them can be classified as legitimate ICD inducers at current stages because of a lack of full analysis of ICD hallmark release and *in vivo* vaccination or anti-tumor immunity analysis. Based on the available information, these compounds have shown the potential to expose one or more ICD-associated DAMPs. In most cases, not all ICD-associated DAMPs are checked or induced by one compound. Given that not all danger signals may be universally required for the engagement of adaptive immunity in all scenarios, it will be essential to validate the potential of these compounds to induce ICD alone or in combination with other existent anti-cancer drugs.

Lastly, most natural products consisting of marine compounds possess extensive pharmacologic effects, but their targets and molecular mechanisms have not been fully elucidated, especially those related to tumor immunity. The stability and efficacy of some compounds can be further enhanced through chemical modification. For instance, cisplatin is intrinsically unable to trigger ICD while its derivative oxaliplatin can induce ICD [90]. Further understanding the molecular mechanisms of natural products will enable the modification of these compounds to maximize their ICD-inducing potential or even the conversion of a non-immunogenic compound to an ICD inducer. Overall, marine natural products of vast biochemical diversity will constitute a most promising innovative avenue for the discovery of novel anti-cancer drugs and demonstrate potential strategies in tumor immunotherapy in the future.

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