Photochemical & Photobiological Sciences

Accepted Manuscript

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View Article Online
DOI: 10.1039/C6PP00097E

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Perspective

Heat Shock Proteins in the context of photodynamic therapy: autophagy, apoptosis and immunogenic cell death

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Abstract

Photodynamic therapy (PDT) is an anti-tumor treatment administered for the elimination of early-stage malignancies and the palliation of symptoms in patients with late -stage tumors, which involves the activation of a photosensitizer (PS) using light of a specific wavelength, which also generates singlet oxygen and other reactive oxygen species (ROS) that cause tumor cell death. Several mechanisms are involved in the protective responses to PDT including the expression of the chaperone/heat shock proteins (HSPs). The HSPs are a family of proteins that are induced by cells in response to exposure to stressful conditions. In the last few decades, it has been discovered that HSPs can play an important role in cell survival, due to the fact that they are responsible for many cytoprotective mechanisms. These proteins have different functions depending on their intracellular or extracellular location. In general, intracellular HSPs havebeen related to an anti-apoptotic function and recently, HSPs-induced autophagy has shown to have a protective role in these chaperones. In contrast, extracellular HSPs or membrane-bound HSPs mediate immunological functions. In the present article, we attempt to review the current knowledge concerning the role of HSPs in the outcome of PDT in relation to autophagy and apoptosis mediated-resistance to photodynamic treatment. We will also discuss how certain PDT protocols optimally stimulate the immune system through HSPs.

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DOI: 10.1039/C6PP00097E

Keywords: photodynamic therapy; heat shock proteins; autophagy; apoptosis; immunogenic cell death.

Background

PDT is an anti-tumor treatment administered for the elimination of early-stage malignancies and the palliation of symptoms in patients with late-stage tumors (1)(2) which consists in the administration of a drug called photosensitizer, which can be topically or systemically given to the patient , followed by light irradiation in the visible range of electromagnetic wave. This combination, along with the presence of molecular oxygen, is used in order to cause the photodynamic reaction needed to generate reactive oxygen species, (ROS) which kills nearby cells (3)(4).

The greatest advantages of PDT over the conventional cancer treatments, are minimal systemic toxicity and high selectivity to the tumor, due to the fact that PSs tend to build up in tumors and irradiation is focused on the tumor tissue. As a result, damage to healthy tissue is minimal (5). In addition, PDT offers the possibility of repetitive cycles of these treatments and the combination of PDT/chemotherapy or PDT/radiotherapy. However, one of the problems of PDT, as well as other therapies, is the existence of resistant cells (4,6,7).The inability of these cells to suffer from death after several treatments brings a selective advantage in the tumor progression and resistance to therapies. The major obstacle to improving the overall response of the treatment and ensuring the survival of cancer patients is to attack the problem of cancer cells resistance (8).

PDT appears to stimulate several different signaling pathways, some of which lead to cell death, whereas others mediate cell survival. Therefore, the ultimate outcome after PDT comes from the combined action or interaction (or both) of these different pathways (9–12). PDT induces cancer cell death by apoptosis or necrosis (13), and these mechanisms can operate concurrently. Moreover, when the apoptotic pathway is unavailable, PDT can cause cancer cell death through induction of autophagy -related cell death (14). However, it has been shown that PDT can induce autophagy as a death or as a survival mechanism, depending on a variety of parameters including the nature of the photosensitizer, PDT dose, and cell type (14).

Several mechanisms are involved in the protective responses to PDT which include: activation of transcription factors, antioxidant enzymes, anti-apoptotic pathways, multidrug resistance family proteins (MDRs), and overexpression of the chaperone/heat shock proteins (HSPs) (15–18).

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HSPs were first characterized as intracellular molecular chaperones of nascent proteins: they help the nascent polypeptide chain attain a functional conformation through the facilitation of protein folding, assembly, stabilization and transport, and the proteolytic turnover necessary for protein intracellular localization and function. Also, they lead to degradation of naïve, aberrantly folded, damaged or mutated proteins (19). HSPs have been classified according to their size into: HSP90, HSP70, HSP60, HSP40 and small HSPs including HSP27 (20). Moreover, in the last decades it has also been discovered that HSPs can play an important role in cell survival because they are responsible for many cytoprotective mechanisms, especially under stress conditions (21). The cytoprotective function of HSPs is not only fulfilled due to their role in repairing the damaged proteins by different agents, but also for their anti-apoptotic properties (19). In addition, these molecules are now recognized to be participants in signal transduction pathways, autophagy modulation and important regulators of inflammatory and immune response (16,22,23). Therefore, an integrated response to cellular stress could be mediated by HSPs following PDT.

In this article, we provide a comprehensive review on the current status of the global response that could be triggered by HSPs after photodynamic treatment. We have focused on the survival role of HSPs as modulators of PDT-induced autophagy and inhibition of apoptosis as well as their implications in PDT-induced immunogenic cell death.

HSPs and autophagy: an integrated protective response following PDT

The ROS production following PDT leads to oxidative damage of cellular macromolecules, including numerous proteins that undergo multiple modifications such as fragmentation, cross-linking, unfolding and aggregation (24). In this situation, the cells normally handle damage through Chaperones Heat Shock-mediated response (25). The chaperones (e.g. HSP90, HSP70, HSP60 and small HSPs) identify unfolding proteins and help to refold them (26). If the refold is not possible, the chaperones will target this protein for destruction and delivery on the proteolytic system (27)(26). However, after exacerbated accumulation of unfolded proteins in PDT treated cells, the incapacity of chaperone/proteolytic system to repair or to clean the damage results in accumulations of abnormal proteins, leading to the formation of aggregates that are toxic for the cells (28) (Figure 1 A-E).

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In mammalian cells, the autophagic-lisosomal system represents a major proteolytic system for clearance of irreversibly oxidized cytosolic aggregated proteins and ROS-damage organelles (28). There are three primary forms of autophagy: chaperone-mediated autophagy, microautophagy and macroautophagy. Chaperone mediated autophagy (CMA) involves direct translocation of the targeted proteins across the lysosomal membrane (29).

During CMA, the cytosolic chaperone heat shock cognate (Hsc)70 binds proteins targeted for degradation and translocates them into the lysosomes (30) by interacting with lysosome-associated membrane protein type 2A (LAMP-2A) (31). Microautophagy is the least-characterized process, but it is used to sequester cytoplasm by invagination and/or septation of the lysosomal/vacuolar membrane. Finally, macroautophagy involves the formation of cytosolic double-membrane vesicles that sequester portions of the cytoplasm (32). In the review, we refer to “macroautophagy” as “autophagy”. During autophagy, the cytoplasmic material is engulfed into double membrane structures called autophagosomes. The autophagosomes fuse with lysosomes where the content is degraded and recycled (33) (Figure 1 F-I).

In the past, it was believed that the main function of autophagy was to supply energy to the cells because, under starved conditions, the cells activated autophagy to degrade part of its cytoplasm in order to obtain the energy necessary to maintain cell homeostasis (34)(35). In more recent studies, it has been shown that autophagy plays a protective role against ROS-induced stress (36), cell death, pathogen infection, neurodegenerative diseases and tumorigenesis (37). Thus, autophagy serves as an adaptive response that protects cells during periods of prolonged stress (33). Nowadays, it has been reported that more than thirty-five Autophagy-Related Genes (ATG) participate in autophagy processes including those that express microtubule-associated protein light chain 3 (LC3), Beclin-1, and other autophagy-related proteins (38).

Protein aggregates can be resolved after becoming ubiquitinated on multiple sites following interaction with the ubiquitin ligase parkin (39,40).

Aggregates marked by polyubiquitination form aggresomes and these are retro-transported to the microtubule organizing centre (MTOC) and become enclosed in autophagosomes. It is thought that protein p62/SQSTM1 may play a key role in this process, based on its ability to bind both UBL domain protein LC-3 and the polyubiquitinated proteins in the aggresome through its UBA domain (39,41) (Figure 1E).

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It has been seen that autophagy could increase cell survival through the resolution of intracellular protein aggregates within the autophagosomes after PDT treatment. In cells pre-treated with a proteosomal inhibitor bortezomib, PDT induced robust vacuolization of the cytoplasm, with frequent lysosomal/autophagosomal vesicles and extended ER (24).

Autophagy appears to be a common outcome in photodynamic therapy protocols, and its role as cell survival or cell death mechanism in relation to PDT was well summarized by Reiners and colleagues, as well as by Milla and colleagues (14,42). However, the molecular mechanism involved in autophagy induction after PDT protocols remains unclear.

On the other hand, HSPs can help the cells recover from PDT damage. PDT, with several photosensitizers, induces a wide panel of different HSPs, such as HSP27 (43), HSP70 (44) and HSP60 (45).

In the last years, it has also been shown that molecular chaperones can mediate the formation of the autophagosomes. Therefore, we propose that HSPs proteins could promote resistance to PDT, and this involves not only their properties as chaperones and transporters to proteolytic systems, but also the induction of autophagy.

In this sense, the small HSPs family appears to play a key role in macroautophagy. HSP22 forms a complex with HSP70 co-chaperone BAG3 that can prevent protein aggregates formation, increase the levels of LC3-II and stimulate autophagy-mediated degradation of aggregates in an eIF2 alpha-dependent manner (46) (Figure 1E,H). BAG3 was reported to inhibit proteasomal degradation of Hsp90 client proteins (47) and to enhance degradation of polyQ aggregates by autophagy (46). The role of the other small HSPs in autophagy is largely poor. Chen *et al*. have shown that HSP27 induces resistance to cisplatin-induced apoptosis in hepatocellular carcinoma cells through activation of autophagy (48). Moreover, Matsumoto and colleagues showed that HSP27 induces autophagic flux and inhibits apoptosis in renal tubular cells (23). It is possible that the presence of aggregated proteins may be detected by small HSPs family molecules through their molecular chaperone properties, bound and delivered to the autophagosome (49) (Figure 1H).

Furthermore, it has been reported that HSP70 could induce autophagy. Overexpression of HSP72 augmented autophagy through c-Jun N-terminal kinase (JNK) phosphorylation and Beclin-1 up-regulation. Up-regulation of HSP72 by geranylgeranylacetone increased autophagy and inhibited apoptosis (50). Bhalla and colleagues demonstrated that stress increases intracellular levels of acetylated inducible HSP70, which binds to the Beclin-1–Vps34 complex (essential to induce autophagy). Acetylated HSP70 also recruits E3 ligase

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for SUMOylation, KRAB–ZFP-associated protein 1 (KAP1), inducing Lys840 SUMOylation and increasing Vps34 activity bound to Beclin 1. Knockdown of HSP70 abolished the Beclin-1–Vps34 complex formation, as well as inhibiting KAP1 binding to Vps34 and autophagosomes formation (51) (Figure 1G).

The role of HSP90 in autophagy is controversial. Joo JH. *et al*. show that the interaction of Ulk1 and HSP90-Cdc37 stabilizes and activates Ulk1, which in turn is required for the phosphorylation and release of Atg13 from Ulk1. It is also a requirement of the recruitment of Atg13 to damaged mitochondria and subsequent elimination by autophagy. Hsp90-Cdc37, Ulk1, and Atg13 phosphorylation are all required for efficient mitochondrial elimination mediated by autophagy (52) (Figure 1 F,H). Additionally, HSP90 forms a complex with Beclin 1 through an evolutionarily conserved domain to maintain the stability of Beclin 1 (Figure 1G). In monocytic cells, geldanamycin (GA) (an Hsp90 inhibitor) effectively promoted proteasomal degradation of Beclin 1 (53). In contrast, the specific inhibition of HSP90 by some chemicals can lead to degradation of its clients via either, the ubiquitin proteasome system, or autophagy (54). Besides, HSP90 inhibits the activities of the IκB kinase/nuclear factor-κB (IKK/NF-κB) signaling pathway, leading to less nuclear translocation and inactivation of NF-κB and the subsequent weak binding of the beclin1 promoter, which facilitates the transition from autophagy to apoptosis (55).

Finally, the autophagy pathway could be regulated directly by Heat Shock Factor (HSF1). HSF1 is a master regulator of heat shock response, and it has been revealed that HSF1 regulates autophagy by directly binding to ATG7 promoter and transcriptionally up-regulating its expression (56) (Figure 1J).

The family of HSPs 27, 60, 70 and 90, have been strongly linked to resistance to PDT (57). In our laboratory, we observed an augmented level of these chaperones in squamous carcinoma PDT-resistant cells. Moreover, we discovered that PDT-induced autophagy was controlled by HSP27, and resistance to ALA-Met/PDT in colon and skin carcinoma cells was induced by this chaperone (unpublished results). These HSPs have been implicated in resistance to PDT through the binding to denatured proteins or protein translocation and they are components of signal transduction pathways or anti-apoptotic activity (15–17) . Therefore, autophagy could be an important HSPs-induced mechanism of resistance to PDT.

Accordingly, an integrated response can be induced after PDT in an attempt to eliminate unfolded and aggregated proteins as well as whole ROS-damage organelles in order to protect the cellular integrity.

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Further investigations are needed to increase our understanding of the molecular interaction between HSPs and autophagy machinery as well ashow could PDT modulate autophagy through heat shock proteins.

HSPs and apoptosis: dual role in the PDT-effect

Apoptosis has been reported as the main mode of PDT-mediated cell death, through excessive ROS levels. Apoptotic pathways are triggered by PDT according to the target cells, the photosensitizer and the irradiation doses used (58–60). However, several studies have demonstrated direct evidence of altered apoptosis pathways in cells which rendered resistance to PDT. HSPs are powerful anti-apoptotic proteins and they have the capacity to block the cell death process at different levels. It has been shown that PDT induces transcription and translation of HSPs (57). The role of these chaperones in regulating the apoptotic death way triggered by PDT has been seldom studied. There were few publications on this topic, which employed different treatment conditions and cell lines. Due to the fact that challenges have been met at the time of reaching general conclusions, we strongly believe that the field needs to be updated. Some research has been focused on the response to PDT in cells with different HSPs expression levels while others have laid emphasis on the study of induction of HSPs after PDT.

HSP27 is thought to regulate apoptosis by maintaining the redox equilibrium of the cell and it has been demonstrated to inhibit apoptosis by increasing the intracellular level of antioxidant glutathione (GSH) (61). HSP27 can also inhibit apoptosis by either inhibiting the release of mitochondrial cytochrome *c* or by binding directly to cytochrome *c* (Figure 2) (62).

The effects of HSP27 on PDT response are controversial and unclear. The death response (both apoptosis and necrosis) to aminolevulinic acid-PDT is lower in a breast cancer cell line that expresses higher constitutive levels of HSP27, than in a cell line that expresses normal lower levels of HSP27 (63). On the contrary, in human oral cancer cells, the silencing of HSP27 attenuated apoptosis through the caspase-mediated pathway and regulated Bax, Bcl-2 and PARP protein expression in PDT-treated cells employing hematoporphyrin (64).

The HSP27 effects in inducing or attenuating cell death after PDT would depend of the photodynamic treatment conditions, the photosensitizer and/or the cancer cell type.

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Interestingly, the HSP27 inhibitor Quercetin (3,3',4',5,7-pentahydroxy flavone) has been studied in clinical trials. It is one of the most widely distributed bioflavonoids in the plant kingdom and it is known to have antitumor activity by triggering apoptosis (65,66). For this reason, we can speculate that Quercetin would be included in some PDT regimens with the aim to enhance the response to the treatment.

For many years, HSP60 was considered a typical intraorganellar chaperone. However, it has lately been demonstrated that HSP60 is also found in the cytosol, not only after mitochondrial release, but also independently of such release, and the evidence also indicates that both, the mitochondrial and the cytosolic forms of HSP60 can function in pro-survival or pro-apoptotic pathways, depending on the cellular situation (65).

Inhibition of apoptosis by HSP60 is associated with up-regulation of the anti-apoptotic molecules Bcl-2, Bcl-xL and survivin, maintenance of the mitochondrial transmembrane potential, and inhibition of caspase 3 activation (66). In addition, HSP60 interacts with mitochondrial HSP70 (67),survivin and p53, inhibiting the process of apoptosis (68). HSP60 is a regulator of mitochondrial permeability transition, contributing to a cytoprotective chaperone network that antagonizes cancer cell death dependent from cyclophilin D (CypD), a component of the mitochondrial permeability transition pore (Figure 2). The molecular chaperone HSP60, is directly associated with CypD. This interaction occurs in a multichaperone complex comprising HSP60, HSP90 and tumor necrosis factor receptor-associated protein-1 (TNFRP1), selectively assembled in tumor but not in normal mitochondria. The inhibition of HSP60 by siRNA triggers CypD–dependent mitochondrial permeability transition, caspase-dependent apoptosis and suppression of intracranial glioblastoma growth *in vivo* (69). Opposite to the anti-apoptotic effects, it is also known that HSP60 can enhance caspase activation. Thus, HSP60 can have opposite effects with regard to tumor cell survival (70).

The photodynamic treatment induces HSP60 and this may contribute to cell resistance. Two resistant induced Photofrin-PDT human cell populations have increased basal levels of HSP60, relative to the parental populations. This increase is caused by Photofrin alone or photosensitization. Besides, HSP60 induction was found to be greater in the two resistant variants, compared with parental populations (45). This investigation does not directly study the relation between HSP60 and the reduction of apoptosis after PDT, but it suggests the correlation between the two.

The levels of HSP60 can be reduced with therapeutic purposes. Flavonoids can lower the levels of HSP60 in a number of human tumor cell lines. On the contrary, cadmium induces

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HSP60 expression (67). In the PDT applications, it should be analysed whereas it is convenient to inhibit or increase HSP60 expression, due to its dual role in apoptosis.

The HSP GRP78 (glucose-regulated protein 78) is upregulated under several reticulum stress-inducing stimulus, including antitumor PDT (71). GRP78 resides in reticulum and regulates the unfolded protein response promoting cell survival (72). However, the role of GRP78 in response to PDT is controversial and it can induce cellular resistance or cellular sensitivity in a PDT dose-dependent manner or type of PSs employed (73). In a recent study, Firczuk and collaborators indicated that GRP78 mRNA and protein expression were upregulated after PDT in different cancer cells and it promoted resistance to cell death. Moreover, the specific inhibition of GRP78 employing the cytotoxin catalytic A subunit (SubA) fused with epidermal growth factor (EGF) sensitizes cancer cells to Photofrin-mediated PDT. However, the inhibition of GRP78 increased the atypical non apoptotic cell death, either in apoptosis competent cells or apoptosis incompetent cells, suggesting that the combination of PDT and GRP78 inhibitor could be employed to kill apoptosis resistant cells (74).

It has been demonstrated that HSP70 overexpression can inhibit multiple cell death pathways including intrinsic apoptosis, and this is due to the ability of HSP70 to bind directly to pro-apoptotic protein BAX and prevent its translocation to mitochondria (75,76). Furthermore, HSP70 blocks apoptosome formation via association and inhibition of APAF-1 and procaspase 9 recruitment (77). On the other hand, extrinsic apoptosis can be also hindered by HSP70. In this context, Guo and co-workers have revealed that HSP70 binds to death receptors DR4 and DR5; consequently, the death-inducing signaling complex (DISC) cannot be assembled (78). Additionally, HSP70 suppresses the cleavage of proapoptotic protein Bid and cytochrome c release from mitochondria through inhibition of JNK activity (79). HSP70 can also bind to apoptosis-inducing factor (AIF) leading to inhibition of caspase-independent apoptosis (80) (Figure 2).

It has been proposed that HSP70 contributes to resistance to PDT via apoptosis inhibition. In this sense, the overexpression of HSP70 through heat treatment induces a significant reduction of apoptosis after PDT (74). Also, Helbig and collaborators manifested that HSP70 blocks the caspase recruitment domain (CARD) of APAF-1, which in turn inhibits the association between APAF- 1 and procaspase 9 necessary to form the apoptosome (75).

HSP70 is present in almost all intracellular compartments. In PDT context, it has been demonstrated that under apoptosis-inducing PDT conditions, HSP70 can be translocated

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from cytoplasm to cell surface, where it can be released into the medium. Furthermore, the translocation of HSP70 depends on PDT dose and it is related to either mitochondrial disruption or direct surface stress, and its main function is to stabilize the plasma membrane integrity. However, under lethal PDT treatment, membrane HSP70 fails to prevent apoptosis and contrarily promotes immunogenic cell death (76,78).

As it was previously mentioned, HSP70 can induce autophagy under stress conditions and it has been shown that autophagy can promote cell survival by inhibiting apoptosis (81–83). Therefore, the role of HSPs in apoptosis resistance could be more complex.

Several types of HPS70 inhibitor compounds have been developed and tested as anticancer agents in pre-clinical or clinical trials, such as derivatives of flavonoids (Epigallocatechin Gallate, Myricetin), sulfoglycolipids (Sulfogalactoglycerolipid, Sulfogalactosylceramide (SGC), AdamantylSGC), dihydropyrimidines (NSC 630668-R/I, MAL3-I0I, MAL 2-IIB, SW02) and others (15-DSG, Dibenzyl-8-aminoadenosine analog, MKT-077, Pyrrhocoricin, Geranylgeranylacetone, Fatty acid acyl benzamides, Pifithrin-μ, Apoptozole) (19). A combinatorial treatment of PDT and HSP70 inhibitors would be designed. However, as mentioned later in this review, it would be preferred not to inhibit this chaperone, but to promote its immunogenic cell death effect regulating the PDT doses.

HSP90 enhances the survival pathway regulated by Akt and reduces the intrinsic apoptotic pathway. Anna Rodina et al. have demonstrated that apoptosis following HSP90 inhibition in small-cell lung cancer is triggered by inactivation of Akt. This leads to reduction in BAD phosphorylation, releasing the protein from 14-3-3 so that it is free to heterodimerize with antiapoptotic members of the Bcl-2 family of proteins and/or to activate the proapoptotic proteins Bax and Bak in the mitochondrial membrane. Abolition of Bcl-2 antiapoptotic role leads to mitochondrial depolarization and cytochrome c release from the mitochondria. Inhibition of HSP90 also releases Apaf-1 from the HSP90 complex, freeing it to interact with caspase-9, and induces the apoptotic cascade by activation of procaspase-3 (86).

HSP90 inhibitors are being developed as anticancer agents, and they have shown promising results in solid tumours and some haematological malignancies. HSP90 inhibitors are the most numerous of the HSPs inhibitors in clinical development. They include geldanamycin derivatives (Tanespimycin (17-AAG), Alvespimycin (17-DMAG), Retaspimycin (IPI-504), IPI-493), resorcinol derivatives (Ganetespib (STA-9090), NVP-AUY922 (VER52296), AT-13387, KW-2478), purine analogues (BIIB021 (CNF 2024), MPC-3100, Debio 0932 (CUDC-305), PU-H71), and other synthetic inhibitors (SNX-5422,

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DS-2248, XL-888) (87). The optimal use of HSP90-targeted therapeutics will depend on understanding the complexity of HSP90 regulation (88).

Ferrario A. et al. have demonstrated that targeting HSP90 with the geldanamycin derivative 17-AAG enhances the therapeutic efficacy of PDT. PDT increases the expression of the anti-apoptotic and pro-angiogenic proteins survivin, Akt, HIF-1α, MMP-2 and VEGF in mouse mammary carcinoma cells and tumors. This expression decreases significantly when 17-AAG is included in the treatment regimen (89).

Role of HSPs in the immunostimulatory effect of PDT: the other side of the coin?

The direct cytotoxic effect of a treatment on tumor cells, which allows the recognition of molecular immunogenic determinants in dying cells by immune cells makes this an ideal therapy treatment. In the past few years, the concept of immunogenic cell death (ICD) has emerged and it has not been associated with any specific cell death pathway. In particular, ICD stimulates an immune response against dead-cell antigens and especially when they derive from cancer cells (84).

The importance of a cancer treatment to cause an ICD is clinically relevant because it is associated with an immune response against the cancer cells that emphasizes the effect of therapy (85). This means that patient's dying cancer cells act as a vaccine that stimulates a tumor-specific immune response, which will result in the control or eradication of residual cancer cells. These damaged/dying cells acquired inmmunostimulatory properties upon exposure or secretion of intracellular molecules known as Damage-Associated Molecular Patterns (DAMPs) most of which are recognized by pattern recognition receptors (86).

It is well-known that PDT-killed tumor cells tend to stimulate an anti-tumor immunity (87). Particularly, this response is fully explained by PDT-mediated cytotoxicity, which takes place due to ROS production and it has also been found that ICD is stressor-dependent (88).

In the last years, PDT has been associated with certain DAMPs and it has been observed that photosensitizers localization at subcellular level was important in ICD triggering upon PDT, especially endoplasmic reticulum (ER) (71) . The principals DAMPs associated to PDT are adenosine triphosphate (ATP), high-mobility group protein B1 (HMBG1), and exposed molecules on the outer membrane of dying cells such as CRT (ecto-CRT), heat-shock proteins and ER sessile proteins (86). However, HSPs proteins, especially HSP70,

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are the best characterized DAMPs involved in PDT-triggered cell death which are able to confer immunogenicity (89) (Table 1).

Initially, HSPs were thought to be exclusively intracellular proteins that only access the extracellular compartments after severe damage. Now it is known that a fraction of these proteins, normally localized in the cytoplasm or nucleus, can be released from cells and function as intercellular-signaling ligands, even when these cells are completely viable(90,91). At least two members of HSPs, HSP70 and 90 move from the intracellular side to the plasma membrane after stress conditions (92). These characteristics allow categorizing HSPs as prototypic “danger” associated molecular patterns or DAMPs.

It has been shown that Photofrin-PDT treated SCCVII cancer cells can expose molecules superficially such as, ecto-HSP70, ecto-HSP60, and ecto-GRP94 (GRP -glucose-regulated protein) more strongly in apoptotic state rather than in healthy conditions (16). Furthermore, these PDT-treated cells were also found to release HSP70 and it is captured by macrophages triggering aToll-like receptor (TLR)–based signal transduction activity resulting in the production of inflammatory cytokine tumor necrosis factor α (TNFα) (16). When these processes were extended to in vivo settings, it was found that the spectrum of DAMPs exposed to the PDT treated SCCVII tumor cells was different. SCCVII tumor cells still engaged with ecto-HSP70, but they no longer exposed ecto-HSP60 and ecto-GRP94, instead exposed GRP78 on their surface (16).

Furthermore, these authors implicated ecto-HSP70 in the opsonisation of cancer cells by C3 complement protein (98). Likewise, Zhou et al. (99), demonstrated that Photofrin-PDT induced HSP70 secretion and release in murine mammary tumor cells and this orchestrated an immunological regulatory mechanism towards murine macrophages. In fact, macrophages incubated with PDT-treated cells showed a high level of TNFα secretion (99). What is more, Mitra et al. (100) also observed intracellular activation of HSP70 and its extracellular release in EMT6 cells during meso-tetrahydroxyphenyl chlorin (mTHPS, Foscan)-PDT, they also observed a strong correlation between high levels of surface exposed or extracellular released HSP70s with mTHPC-PDT doses that resulted in long-term tumor cure (100). Additionally, Etminan et al. (101) observed upregulation of HSP70 surface expression for glioblastoma cell lines U87 and U251 during 5-ALA-PDT. Moreover, when Etminan et al. blocked HSP70 by the addition of antibodies, they observed that the tumor antigens as well as DC maturation induced by the ALA/PDT treated cells was almost completely inhibited (101). Another photosensitizer associated with HSP70 exposure is Hypericin. Human bladder carcinoma cells T24 treated with

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hypericin-based PDT (Hyp-PDT), present surface-exposure of HSP70 in absence of HSP90 (102).

Recently, Panzarini and co-workers showed that apoptotic and autophagic cells treated with RBAc-PDT increased surface exposition and release of HSP70 and HSP90. Interestingly, however, this event was always higher in cells dying by apoptosis than autophagy (103). Accordingly, Garg et al. express in their revision that cancer cell-associated autophagy (specifically macroautophagy) controlled the emission of DAMPs, therefore this suppress key mechanisms that trigger anticancer immune responses as elicited by immunogenic cell death. Particularly, Gar et al. have detected the increase of phenotypic maturation of Dendritic Cells and clonal expansion of CD4+/CD8+ T cells after autophagy knock-down in Hyp-PDT treated cancer cells (104).

Consequently, PDT-triggered cell death is associated with DAMPs expression and particularly HSP70 (89). Extracellular HSP70 is a powerful agent for tumor immunotherapy,which can break tolerance to tumor-associated antigens and origin specific tumor cell killing by cytotoxic CD8+ T cells (90). The pro-immune effects of extracellular HSP70 are, to some level, extensions of its molecular properties as an intracellular stress protein (105). The HSP70 family are induced massively after stress, preventing cell death by inhibiting aggregation of cell proteins and directly antagonizing multiple cell death pathways (106). HSP70 family members possess a domain in the C terminus that chaperones unfolded proteins and peptides, and an N-terminal ATPase domain that controls the opening and closing of the peptide binding domain. These properties not only enable intracellular HSP70 to inhibit tumor apoptosis, but also promote formation of stable complexes with cytoplasmic tumor antigens that can then escape intact from dying cells to interact with APC and stimulate anti-tumor immunity (107).

Pre-clinical and clinical studies have demonstrated that PDT eliminates tumors directly by tumor cell death and indirectly by enhancing anti-tumor immunity. PDT can trigger not only innate immunity but also the adaptive one (42).

Conclusion

HSPs and autophagy have been proposed as resistance mechanisms to PDT, possibly through apoptosis inhibition. However, how HSPs and autophagy could work together to avoid cells death after phototherapy needs to be studied in detail.

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Autophagy and HSP response share common features, since both represent inducible response to stress, particularly those that induce accumulation of abnormal proteins (e.g. PDT). Recent studies suggest a molecular interplay between HSPs and autophagy. In this review, we propose that after PDT protocol, the heat shock chaperones could inhibit apoptosis and induce autophagy as a resistance mechanism (Figure 3). Moreover, the autophagy induction could inhibit the PDT- induced immunogenic cell death, emphasizing the role as a resistance mechanism (Figure 3). In this sense, the use of combined PDT protocols that produce HSPs expression, such as HSP70, with an HSP27 inhibitor, which in turn blocks autophagy, could improve the PDT efficiency and increase the cure rate (Figure 3). Nevertheless, we believe that it is crucial to discover other DAMPs involved in cell death induced by PDT and this would help to establishing better therapeutic design for cancer patients.

However, much is still unknown and additional preclinical and clinical experiments need to be done. Further experiments need to join a PDT provoking immunologic response, and the inhibition of autophagy to potentially optimize photodynamic treatment.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

CONICET, Agencia PICT, SeCyT-UNRC and Anca-Mihaela Nichifor.

References

1. Brown SB, Brown EA, Walker I. The present and future role of photodynamic therapy in cancer treatment. Lancet Oncol [Internet]. 2004 Aug [cited 2012 Mar 21];5(8):497–508. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15288239
2. Dougherty TJ. An update on photodynamic therapy applications. J Clin Laser Med Surg [Internet]. 2002 Feb [cited 2012 Mar 21];20(1):3–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11902352
3. Macdonald IJ, Dougherty TJ. Basic principles of photodynamic therapy. J Porphyr Phthalocyanines [Internet]. 2001 Feb [cited 2012 Mar 29];5(2):105–29. Available from: http://www.worldscinet.com/jpp/05/0502/S1088424601000159.html
4. Pinton C. Repetitive photodynamic therapy with topical delta-aminolaevulinic acid as an appropriate approach to the routine treatment of superficial non-melanoma skin tumors. J Photochem Photobiol. 1995;29:53–7.
5. Vrouenraets MB, Visser GWM, Snow GB, van Dongen GAMS. Basic principles, applications in oncology and improved selectivity of photodynamic therapy.

View Article Online
DOI: 10.1039/C6PP00097E

Anticancer Res. Jan;23(1B):505–22.

6. Hirschberg H, Sørensen DR, Angell-Petersen E, Peng Q, Tromberg B, Sun C-H, et al. Repetitive photodynamic therapy of malignant brain tumors. J Env Pathol Toxicol Oncol [Internet]. 2006 Jan [cited 2012 Jun 6];25(1-2):261–79. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16566723

7. Steen J. Madsen, Chung-Ho Sun BJT and HH. Repetitive 5-aminolevulinic acid-mediated photodynamic therapy on human glioma spheroids. J Neurooncol. 2003;62(3):243–50.

8. Solyanik GI. Multifactorial nature of tumor drug resistance. Exp Oncol [Internet]. 2010 Sep [cited 2015 Nov 10];32(3):181–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21403614

9. Tong Z, Singh G, Rainbow AJ. Sustained activation of the extracellular signal-regulated kinase pathway protects cells from photofrin-mediated photodynamic therapy. Cancer Res [Internet]. 2002 Oct 1 [cited 2014 Apr 30];62(19):5528–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12359764

10. Tong Z, Singh G, Valerie K, Rainbow AJ. Activation of the stress-activated JNK and p38 MAP kinases in human cells by Photofrin-mediated photodynamic therapy. J Photochem Photobiol B [Internet]. 2003 Oct 15 [cited 2014 Apr 30];71(1-3):77–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14705642

11. Vittar N, Awruch J, Azizuddin K, Rivarola V. Caspase-independent apoptosis, in human MCF-7c3 breast cancer cells, following photodynamic therapy, with a novel water-soluble phthalocyanine. Int J Biochem Cell Biol. 2010;42(7):1123–31.

12. Garg AD, Maes H, Romano E, Agostinis P. Autophagy, a major adaptation pathway shaping cancer cell death and anticancer immunity responses following photodynamic therapy. Photochem Photobiol Sci [Internet]. 2015 Aug [cited 2015 Nov 4];14(8):1410–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25666525

13. Agostinis P, Berg K, Cengel KA, Foster TH, Girotti AW, Gollnick SO, et al. Photodynamic Therapy of Cancer : An Update. CA Cancer J Clin. 2011;

14. Reiners JJ, Agostinis P, Berg K, Oleinick NL, Kessel D. Assessing autophagy in the context of photodynamic therapy. Autophagy. 2010 Jan;6(1):7–18.

15. Carvalho P, Duarte CB, Almeida RD, Manadas BJ. Intracellular signaling mechanisms in photodynamic therapy. Rev Lit Arts Am. 2004;1704:59–86.

16. Korbelik M, Sun J, Cecic I. Photodynamic therapy-induced cell surface expression and release of heat shock proteins: relevance for tumor response. Cancer Res [Internet]. 2005 Feb 1 [cited 2012 Mar 26];65(3):1018–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15705903

17. Cheung CHA, Chen H-H, Cheng L-T, Lyu KW, Kanwar JR, Chang J-Y. Targeting Hsp90 with small molecule inhibitors induces the over-expression of the anti-apoptotic molecule, survivin, in human A549, HONE-1 and HT-29 cancer cells. Mol Cancer [Internet]. 2010 Jan [cited 2014 May 13];9:77. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2873435&tool=pmcentrez&rendertype=abstract

18. Casas A, Venosa G Di, Hasan T, Batlle A. Mechanisms of Resistance to

View Article Online
DOI: 10.1039/C6PP00097E

Photodynamic Therapy. Current. 2011;(Ii):2486–515.

19. Jego G, Hazoumé A, Seigneuric R, Garrido C. Targeting heat shock proteins in cancer. Cancer Lett. Elsevier; 2013 May;332(2):275–85.

20. Seigneuric R, Mjahed H, Gobbo J, Joly A-L, Berthenet K, Shirley S, et al. Heat shock proteins as danger signals for cancer detection. Front Oncol. 2011 Jan;1:37.

21. Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, et al. Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones. 2009 Jan;14(1):105–11.

22. Chen C-Y, Chen H-F, Gi S-J, Chi T-H, Cheng C-K, Hsu C-F, et al. Decreased heat shock protein 27 expression and altered autophagy in human cells harboring A8344G mitochondrial DNA mutation. Mitochondrion. 2011 Sep;11(5):739–49.

23. Matsumoto T, Urushido M, Ide H, Ishihara M, Hamada-Ode K, Shimamura Y, et al. Small Heat Shock Protein Beta-1 (HSPB1) Is Upregulated and Regulates Autophagy and Apoptosis of Renal Tubular Cells in Acute Kidney Injury. Abe H, editor. PLoS One. Public Library of Science; 2015 May;10(5):e0126229.

24. Szokalska A, Makowski M, Nowis D, Wilczynski GM, Kujawa M, Wójcik C, et al. Proteasome inhibition potentiates antitumor effects of photodynamic therapy in mice through induction of endoplasmic reticulum stress and unfolded protein response. Cancer Res. 2009 May;69(10):4235–43.

25. Vabulas RM, Raychaudhuri S, Hayer-Hartl M, Hartl FU. Protein folding in the cytoplasm and the heat shock response. Cold Spring Harb Perspect Biol. 2010 Dec;2(12):a004390.

26. Mathew A, Morimoto RI. Role of the heat-shock response in the life and death of proteins. Ann N Y Acad Sci. 1998 Jun;851:99–111.

27. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev. 2002 Apr;82(2):373–428.

28. Nyström T. Role of oxidative carbonylation in protein quality control and senescence. EMBO J. 2005 Apr;24(7):1311–7.

29. Kaushik S, Bandyopadhyay U, Sridhar S, Kiffin R, Martinez-Vicente M, Kon M, et al. Chaperone-mediated autophagy at a glance. J Cell Sci. 2011 Feb;124(Pt 4):495–9.

30. Agarraberes FA, Dice JF. A molecular chaperone complex at the lysosomal membrane is required for protein translocation. J Cell Sci. 2001 Jul;114(Pt 13):2491–9.

31. Bandyopadhyay U, Kaushik S, Varticovski L, Cuervo AM. The chaperone-mediated autophagy receptor organizes in dynamic protein complexes at the lysosomal membrane. Mol Cell Biol. 2008 Sep;28(18):5747–63.

32. Klionsky DJ, Ohsumi Y. Vacuolar import of proteins and organelles from the cytoplasm. Annu Rev Cell Dev Biol. 1999 Jan;15:1–32.

33. Kang R, Zeh HJ, Lotze MT, Tang D. The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differ. 2011 Apr;18(4):571–80.

34. Li L, Chen Y, Gibson SB. Starvation-induced autophagy is regulated by mitochondrial reactive oxygen species leading to AMPK activation. Cell Signal. 2013

View Article Online
DOI: 10.1039/C6PP00097E

Jan;25(1):50–65.

35. Mizushima N. The pleiotropic role of autophagy: from protein metabolism to bactericide. Cell Death Differ. 2005 Nov;12:1535–41.

36. Xie Y, Xiao F, Luo L, Zhong C. Activation of autophagy protects against ROS-mediated mitochondria-dependent apoptosis in L-02 hepatocytes induced by Cr(VI). Cell Physiol Biochem. 2014 Jan;33(3):705–16.

37. Nowak J, Archange C, Tardivel-Lacombe J, Pontarotti P, Pébusque M-J, Vaccaro MI, et al. The TP53INP2 protein is required for autophagy in mammalian cells. Mol Biol Cell. 2009 Feb;20(3):870–81.

38. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol Cell Biol. 2001 Mar;2(3):211–6.

39. Kirkin V, McEwan DG, Novak I, Dikic I. A role for ubiquitin in selective autophagy. Mol Cell. 2009 May;34(3):259–69.

40. Chin L-S, Olzmann JA, Li L. Parkin-mediated ubiquitin signalling in aggresome formation and autophagy. Biochem Soc Trans. 2010 Feb;38(Pt 1):144–9.

41. Simms-Waldrip T, Rodriguez-Gonzalez A, Lin T, Ikeda AK, Fu C, Sakamoto KM. The aggresome pathway as a target for therapy in hematologic malignancies. Mol Genet Metab. 2008 Jul;94(3):283–6.

42. Milla Sanabria L, Rodríguez ME, Cogno IS, Rumie Vittar NB, Pansa MF, Lamberti MJ, et al. Direct and indirect photodynamic therapy effects on the cellular and molecular components of the tumor microenvironment. Biochim Biophys Acta [Internet]. 2013 Jan [cited 2014 Jun 13];1835(1):36–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23046998

43. Wang HP, Hanlon JG, Rainbow AJ, Espiritu M, Singh G. Up-regulation of Hsp27 plays a role in the resistance of human colon carcinoma HT29 cells to photooxidative stress. Photochem Photobiol. 2002 Jul;76(1):98–104.

44. Gomer CJ, Ryter SW, Ferrario A, Rucker N, Wong S, Fisher AM. Photodynamic therapy-mediated oxidative stress can induce expression of heat shock proteins. Cancer Res [Internet]. 1996 May 15 [cited 2013 Jan 19];56(10):2355–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8625311

45. Hanlon JG, Adams K, Rainbow AJ, Gupta RS, Singh G. Induction of Hsp60 by Photofrin-mediated photodynamic therapy. J Photochem Photobiol B [Internet]. 2001 Nov 1 [cited 2013 Jan 19];64(1):55–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11705730

46. Carra S, Seguin SJ, Lambert H, Landry J. HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. J Biol Chem. 2008 Jan;283(3):1437–44.

47. Doong H, Rizzo K, Fang S, Kulpa V, Weissman AM, Kohn EC. CAIR-1/BAG-3 abrogates heat shock protein-70 chaperone complex-mediated protein degradation: accumulation of poly-ubiquitinated Hsp90 client proteins. J Biol Chem. 2003 Aug;278(31):28490–500.

48. Chen R, Dai RY, Duan CY, Liu YP, Chen SK, Yan DM, et al. Unfolded protein response suppresses cisplatin-induced apoptosis via autophagy regulation in

View Article Online
DOI: 10.1039/C6PP00097E

human hepatocellular carcinoma cells. Folia Biol (Praha). 2011 Jan;57(3):87–95.

49. Di Napol M. The Ubiquitin Proteasome System in the Central Nervous System: From Physiology to Pathology. Nova Science Pub Inc; 2008. 1154 p.

50. Li S, Zhou Y, Fan J, Cao S, Cao T, Huang F, et al. Heat shock protein 72 enhances autophagy as a protective mechanism in lipopolysaccharide-induced peritonitis in rats. Am J Pathol. 2011 Dec;179(6):2822–34.

51. Yang Y, Fiskus W, Yong B, Atadja P, Takahashi Y, Pandita TK, et al. Acetylated hsp70 and KAP1-mediated Vps34 SUMOylation is required for autophagosome creation in autophagy. Proc Natl Acad Sci U S A. 2013 Apr;110(17):6841–6.

52. Joo JH, Dorsey FC, Joshi A, Hennessy-Walters KM, Rose KL, McCastlain K, et al. Hsp90-Cdc37 chaperone complex regulates Ulk1- and Atg13-mediated mitophagy. Mol Cell. 2011 Aug;43(4):572–85.

53. Xu C, Liu J, Hsu L-C, Luo Y, Xiang R, Chuang T-H. Functional interaction of heat shock protein 90 and Beclin 1 modulates Toll-like receptor-mediated autophagy. FASEB J. 2011 Aug;25(8):2700–10.

54. Liu K-S, Liu H, Qi J-H, Liu Q-Y, Liu Z, Xia M, et al. SNX-2112, an Hsp90 inhibitor, induces apoptosis and autophagy via degradation of Hsp90 client proteins in human melanoma A-375 cells. Cancer Lett. 2012 May;318(2):180–8.

55. Jiang Q, Wang Y, Li T, Shi K, Li Z, Ma Y, et al. Heat shock protein 90-mediated inactivation of nuclear factor-κB switches autophagy to apoptosis through becn1 transcriptional inhibition in selenite-induced NB4 cells. Mol Biol Cell. 2011 Apr;22(8):1167–80.

56. Desai S, Liu Z, Yao J, Patel N, Chen J, Wu Y, et al. Heat shock factor 1 (HSF1) controls chemoresistance and autophagy through transcriptional regulation of autophagy-related protein 7 (ATG7). J Biol Chem. 2013 Mar;288(13):9165–76.

57. Casas A, Di Venosa G, Hasan T, Al Batlle. Mechanisms of resistance to photodynamic therapy. Curr Med Chem. 2011 Jan;18(16):2486–515.

58. Oleinick NL, Morris RL, Belichenko I. The role of apoptosis in response to photodynamic therapy: what, where, why, and how. Photochem Photobiol Sci [Internet]. 2002 Jan [cited 2015 Oct 23];1(1):1–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12659143

59. Kessel D. Apoptosis and associated phenomena as a determinants of the efficacy of photodynamic therapy. Photochem Photobiol Sci [Internet]. 2015 Aug [cited 2015 Nov 18];14(8):1397–402. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25559971

60. Rangasamy S, Ju H, Um S, Oh D-C, Song JM. Mitochondria and DNA Targeting of 5,10,15,20-Tetrakis(7-sulfonatobenzo[b]thiophene) Porphyrin-Induced Photodynamic Therapy via Intrinsic and Extrinsic Apoptotic Cell Death. J Med Chem [Internet]. 2015 Sep 10 [cited 2015 Nov 18];58(17):6864–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26295496

61. Creagh EM, Sheehan D, Cotter TG. Heat shock proteins--modulators of apoptosis in tumour cells. Leukemia [Internet]. 2000 Jul [cited 2015 Nov 11];14(7):1161–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10914538

View Article Online
DOI: 10.1039/C6PP00097E

62. Kalmar B, Greensmith L. Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev [Internet]. 2009 Apr 28 [cited 2015 Oct 14];61(4):310–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19248813

63. Rocchi P, So A, Kojima S, Signaevsky M, Beraldi E, Fazli L, et al. Heat shock protein 27 increases after androgen ablation and plays a cytoprotective role in hormone-refractory prostate cancer. Cancer Res [Internet]. 2004 Sep 15 [cited 2013 Feb 1];64(18):6595–602. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15374973

64. Kim J, Jung H, Lim W, Kim S, Ko Y, Karna S, et al. Down-regulation of heat-shock protein 27-induced resistance to photodynamic therapy in oral cancer cells. J Oral Pathol Med [Internet]. 2013 Jan [cited 2015 Nov 11];42(1):9–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22563860

65. Cappello F, Conway de Macario E, Marasà L, Zummo G, Macario AJL. Hsp60 expression, new locations, functions and perspectives for cancer diagnosis and therapy. Cancer Biol Ther [Internet]. 2008 Jun [cited 2015 Nov 11];7(6):801–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18497565

66. Cohen-Sfady M, Pevsner-Fischer M, Margalit R, Cohen IR. Heat shock protein 60, via MyD88 innate signaling, protects B cells from apoptosis, spontaneous and induced. J Immunol [Internet]. 2009 Jul 15 [cited 2015 Nov 11];183(2):890–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19561102

67. Deocaris CC, Kaul SC, Wadhwa R. On the brotherhood of the mitochondrial chaperones mortalin and heat shock protein 60. Cell Stress Chaperones [Internet]. 2006 Jan [cited 2015 Nov 11];11(2):116–28. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1484513&tool=pmcentrez&rendertype=abstract

68. Ghosh JC, Dohi T, Kang BH, Altieri DC. Hsp60 regulation of tumor cell apoptosis. J Biol Chem [Internet]. 2008 Feb 22 [cited 2012 Dec 26];283(8):5188–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18086682

69. Ghosh JC, Siegelin MD, Dohi T, Altieri DC. Heat shock protein 60 regulation of the mitochondrial permeability transition pore in tumor cells. Cancer Res [Internet]. 2010 Nov 15 [cited 2015 Sep 28];70(22):8988–93. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2982903&tool=pmcentrez&rendertype=abstract

70. Xanthoudakis S, Roy S, Rasper D, Hennessey T, Aubin Y, Cassady R, et al. Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. EMBO J [Internet]. 1999 Apr 15 [cited 2015 Nov 11];18(8):2049–56. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1171289&tool=pmcentrez&rendertype=abstract

71. Garg AD, Krysko D V, Vandenabeele P, Agostinis P. DAMPs and PDT-mediated photo-oxidative stress: exploring the unknown. Photochem Photobiol Sci [Internet]. 2011 May [cited 2014 Jul 23];10(5):670–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21258717

72. Zhu G, Lee AS. Role of the unfolded protein response, GRP78 and GRP94 in organ homeostasis. J Cell Physiol [Internet]. 2015 Jul [cited 2016 Jan 28];230(7):1413–20.

View Article Online
DOI: 10.1039/C6PP00097E

Available from: http://www.ncbi.nlm.nih.gov/pubmed/25546813

73. Gomer CJ, Ferrario A, Rucker N, Wong S, Lee AS. Glucose regulated protein induction and cellular resistance to oxidative stress mediated by porphyrin photosensitization. Cancer Res [Internet]. 1991 Dec 15 [cited 2014 Apr 30];51(24):6574–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1835901

74. Firczuk M, Gabrysiak M, Barankiewicz J, Domagala A, Nowis D, Kujawa M, et al. GRP78-targeting subtilase cytotoxin sensitizes cancer cells to photodynamic therapy. Cell Death Dis [Internet]. 2013 Jan [cited 2015 Nov 11];4:e741. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3730435&tool=pmcentrez&rendertype=abstract

75. Stankiewicz AR, Lachapelle G, Foo CPZ, Radicioni SM, Mosser DD. Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation. J Biol Chem [Internet]. 2005 Nov 18 [cited 2016 Jan 27];280(46):38729–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16172114

76. Gotoh T, Terada K, Oyadomari S, Mori M. hsp70-DnaJ chaperone pair prevents nitric oxide- and CHOP-induced apoptosis by inhibiting translocation of Bax to mitochondria. Cell Death Differ [Internet]. 2004 Apr [cited 2016 Jan 27];11(4):390–402. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14752510

77. Saleh A, Srinivasula SM, Balkir L, Robbins PD, Alnemri ES. Negative regulation of the Apaf-1 apoptosome by Hsp70. Nat Cell Biol [Internet]. Macmillan Magazines Ltd.; 2000 Aug [cited 2016 Jan 28];2(8):476–83. Available from: http://dx.doi.org/10.1038/35019510

78. Guo F, Sigua C, Bali P, George P, Fiskus W, Scuto A, et al. Mechanistic role of heat shock protein 70 in Bcr-Abl-mediated resistance to apoptosis in human acute leukemia cells. Blood [Internet]. 2005 Feb 1 [cited 2016 Jan 27];105(3):1246–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15388581

79. Gabai VL, Mabuchi K, Mosser DD, Sherman MY. Hsp72 and stress kinase c-jun N-terminal kinase regulate the bid-dependent pathway in tumor necrosis factor-induced apoptosis. Mol Cell Biol [Internet]. 2002 May [cited 2016 Jan 28];22(10):3415–24. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=133785&tool=pmcentrez&rendertype=abstract

80. Evans CG, Chang L, Gestwicki JE. Heat shock protein 70 (hsp70) as an emerging drug target. J Med Chem [Internet]. 2010 Jun 24 [cited 2013 Nov 13];53(12):4585–602. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2895966&tool=pmcentrez&rendertype=abstract

81. Boya P, González-Polo R-A, Casares N, Perfettini J-L, Dessen P, Larochette N, et al. Inhibition of macroautophagy triggers apoptosis. Mol Cell Biol. 2005 Feb;25(3):1025–40.

82. Ishida Y, Nagata K. Autophagy eliminates a specific species of misfolded procollagen and plays a protective role in cell survival against ER stress. Autophagy. 2009 Nov;5(8):1217–9.

83. Scherz-Shouval R, Weidberg H, Gonen C, Wilder S, Elazar Z, Oren M. p53-

View Article Online
DOI: 10.1039/C6PP00097E

dependent regulation of autophagy protein LC3 supports cancer cell survival under prolonged starvation. Proc Natl Acad Sci U S A. 2010 Oct;107(43):18511–6.

84. Green DR, Ferguson T, Zitvogel L, Kroemer G. Immunogenic and tolerogenic cell death. Nat Rev Immunol [Internet]. 2009 May [cited 2014 Jul 18];9(5):353–63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2818721&tool=pmcentrez&rendertype=abstract

85. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol [Internet]. 2013 Jan [cited 2014 Jul 21];31:51–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23157435

86. Panzarini E, Inguscio V, Dini L. Immunogenic cell death: can it be exploited in PhotoDynamic Therapy for cancer? Biomed Res Int [Internet]. 2013 Jan;2013:482160. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3591131&tool=pmcentrez&rendertype=abstract

87. Gollnick SO. Stimulation of the host immune response by photodynamic therapy (PDT). Proceedings of SPIE [Internet]. SPIE; 2004 [cited 2012 Mar 26]. p. 60–70. Available from: http://link.aip.org/link/?PSISDG/5319/60/1

88. Dudek AM, Garg AD, Krysko D V, De Ruysscher D, Agostinis P. Inducers of immunogenic cancer cell death. Cytokine Growth Factor Rev [Internet]. 2013 Aug [cited 2016 May 25];24(4):319–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23391812

89. Helbig D, Simon JC, Paasch U. Photodynamic therapy and the role of heat shock protein 70. Int J Hyperthermia [Internet]. 2011 Jan [cited 2014 Jul 24];27(8):802–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21966972

90. Colaco C a, Bailey CR, Walker KB, Keeble J. Heat shock proteins: stimulators of innate and acquired immunity. Biomed Res Int [Internet]. 2013 Jan;2013:461230. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3677648&tool=pmcentrez&rendertype=abstract

91. Moseley P. Stress proteins and the immune response. Immunopharmacology [Internet]. 2000 Jul 25;48(3):299–302. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10960671

92. Krysko D V, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer [Internet]. Nature Publishing Group; 2012 Dec [cited 2014 Jul 15];12(12):860–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23151605

93. Suzue K, Zhou X, Eisen HN, Young RA. Heat shock fusion proteins as vehicles for antigen delivery into the major histocompatibility complex class I presentation pathway. Proc Natl Acad Sci U S A [Internet]. 1997 Nov 25 [cited 2014 Jul 23];94(24):13146–51. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=24277&tool=pmcentrez&rendertype=abstract

94. Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Annu

View Article Online
DOI: 10.1039/C6PP00097E

Rev Immunol [Internet]. 2002 Jan [cited 2014 Jul 11];20:395–425. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11861608

95. van Eden W, Spiering R, Broere F, van der Zee R. A case of mistaken identity: HSPs are no DAMPs but DAMPERs. Cell Stress Chaperones [Internet]. 2012 May [cited 2014 Jul 17];17(3):281–92. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3312964&tool=pmcentrez&rendertype=abstract

96. Kono H, Rock KL. How dying cells alert the immune system to danger. Nat Rev Immunol [Internet]. 2008 Apr [cited 2014 Jul 10];8(4):279–89. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2763408&tool=pmcentrez&rendertype=abstract

97. Mosser DD, Morimoto RI. Molecular chaperones and the stress of oncogenesis. Oncogene [Internet]. 2004 Apr 12 [cited 2013 Jan 30];23(16):2907–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15077153

98. Korbelik M, Stott B, Sun J. Photodynamic therapy-generated vaccines: relevance of tumour cell death expression. Br J Cancer [Internet]. 2007 Nov 19 [cited 2012 Mar 1];97(10):1381–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2360230&tool=pmcentrez&rendertype=abstract

99. Zhou F, Xing D, Chen WR. Regulation of HSP70 on activating macrophages using PDT-induced apoptotic cells. Int J Cancer [Internet]. 2009 Sep 15 [cited 2014 Jul 23];125(6):1380–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2756092&tool=pmcentrez&rendertype=abstract

100. Mitra S, Giesselman BR, De Jesús-Andino FJ, Foster TH. Tumor response to mTHPC-mediated photodynamic therapy exhibits strong correlation with extracellular release of HSP70. Lasers Surg Med [Internet]. 2011 Sep [cited 2013 Aug 20];43(7):632–43. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3226748&tool=pmcentrez&rendertype=abstract

101. Etminan N, Peters C, Lakbir D, Bünemann E, Börger V, Sabel MC, et al. Heat-shock protein 70-dependent dendritic cell activation by 5-aminolevulinic acid-mediated photodynamic treatment of human glioblastoma spheroids in vitro. Br J Cancer [Internet]. 2011 Sep 27 [cited 2014 Jul 23];105(7):961–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3185943&tool=pmcentrez&rendertype=abstract

102. Garg AD, Krysko D V, Vandenabeele P, Agostinis P. Hypericin-based photodynamic therapy induces surface exposure of damage-associated molecular patterns like HSP70 and calreticulin. Cancer Immunol Immunother [Internet]. 2012 Feb [cited 2012 Mar 11];61(2):215–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22193987

103. Panzarini E, Inguscio V, Fimia GM, Dini L. Rose Bengal acetate photodynamic therapy (RBAc-PDT) induces exposure and release of Damage-Associated Molecular Patterns (DAMPs) in human HeLa cells. PLoS One [Internet]. 2014 Jan [cited 2015 Nov 3];9(8):e105778. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4139382&tool=pmcentrez

View Article Online
DOI: 10.1039/C6PP00097E

&rendertype=abstract

104. Garg AD, Dudek AM, Ferreira GB, Verfaillie T, Vandenabeele P, Krysko D V, et al. ROS-induced autophagy in cancer cells assists in evasion from determinants of immunogenic cell death. Autophagy [Internet]. 2013 Sep [cited 2015 Nov 4];9(9):1292–307. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23800749

105. Calderwood SK, Theriault JR, Gong J. Message in a bottle: role of the 70-kDa heat shock protein family in anti-tumor immunity. Eur J Immunol [Internet]. 2005 Sep [cited 2014 Jul 23];35(9):2518–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16144035

106. Anderson KM, Srivastava PK. Heat, heat shock, heat shock proteins and death: a central link in innate and adaptive immune responses. Immunol Lett [Internet]. 2000 Sep 15;74(1):35–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10996625

107. Mosser DD, Caron AW, Bourget L, Mosser DD, Caron AW, Bourget L, et al. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis . Role of the Human Heat Shock Protein hsp70 in Protection against Stress-Induced Apoptosis. 1997;17(9).

View Article Online
DOI: 10.1039/C6PP00097E

Table 1. HSPs expression associated with different photosensitizers used in PDT

Cell Type	Photosensitizer used for PDT	HSPs expression	Ref.
Squamous cells carcinoma SCCVII	Photofrin	HSP70, HSP60 and GRP94 (*in vitro*) HSP70 and GRP78 (*in vivo*)	(16)
Murine mammary tumor cells C127	Photofrin	HSP70	(99)
EMT6 cells	Foscan	HSP70	(100)
Glioblastoma cell lines U87 and U251	5-ALA	HSP70	(101)
Human bladder carcinoma cells T24	Hypericin	HSP70	(102)
HeLa cells	Rose Bengal Acetate(RBAc)	HSP70 and HSP90	(103)

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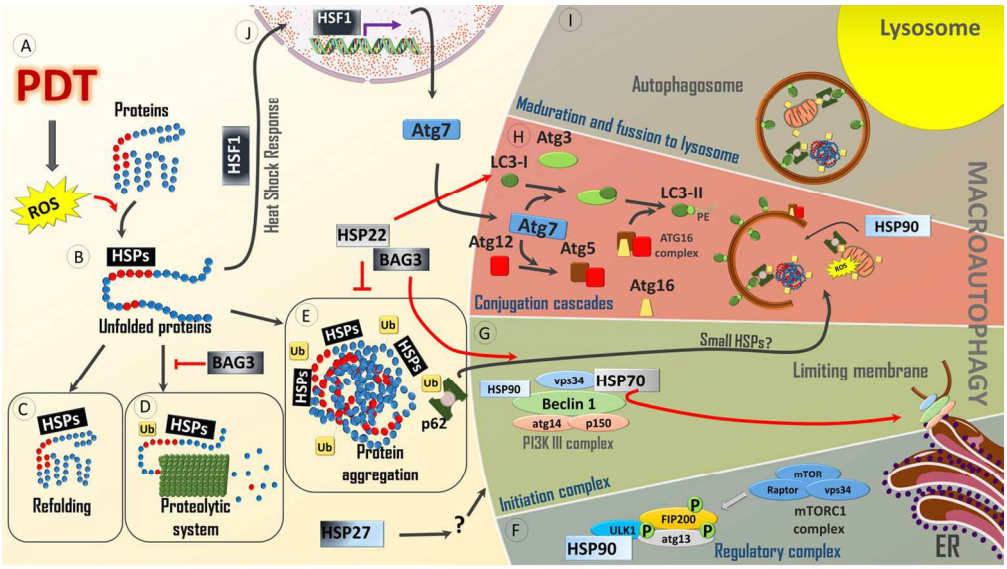

Integrative HSPs/autophagy response after photodynamic therapy. The ROS produced following PDT induce proteins denaturalization (A,B). In response to unfolded protein, the chaperones HSPs identify this proteins and help to refold them (C). If the refold is not possible, the HSPs will target this protein for destruction on the proteolytic system for recycling and synthesis of new proteins (D). If the ability of chaperones to refold or target unfolded proteins to proteolytic system is compromised, the accumulation of abnormal proteins will result in protein aggregates formation (E). The HSPs can also modulate the degradation of this aggregated into the lysosomes through autophagic process. The heat shock chaperones can modulate the formation of autophagosomes. The autophagy (macroautophagy) is initiated by the regulatory complex (ULK1, atg13, FIP200) which receives stress signals from mTORC1 complex and can be stabilized by HSP90 which stabilizes and activates ULK1 (F). Then, the initiation complex (PI3KIII) which relocates to the area of formation of autophagosomes (formation of limiting membrane) is stabilized and activated by HSP70 which binds to the Beclin-1–Vps34 complex inducing Vps34 activity. The stability of Beclin-1 also depends of the HSP90 binding (G). Autophagosome formation also require Atg12 and LC3 conjugation systems, LC3 system is important for transport, cargo selection, and maturation of autophagosomes and the levels of LC3 can be induced by HSP22. On the other hand, the autophagic degradation of protein aggregates can be stimulated by HSP22/BAG3 complex. Moreover, this aggregates could be transported to the autophagosomes by small HSPs and be recognized by the LC3 complex through interaction with p62 (H). The aggregates and other cellular components (e.g. damaged mitochondrion) are degraded by lysosomal enzymes after autophagosome/lysosome fusion (I). Finally, in presence of denatured proteins, the HSF1 transcription factor translocate to the nucleus and induce expression of genes involved in the heat shock response. Interestingly, HSF1 also induce Atg7 expression contributing to the autophagosome formation (J).

Figure 1
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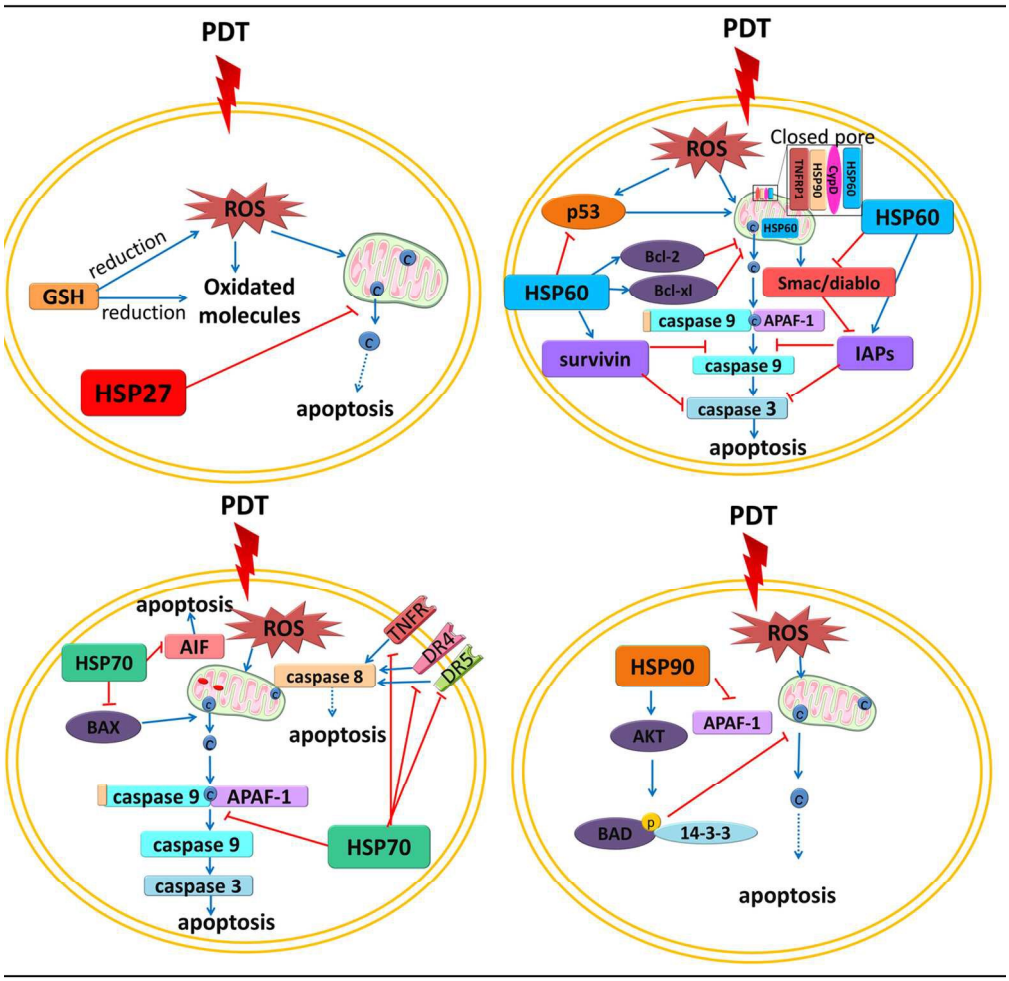

HSPs in PDT-induced apoptosis. PDT induces transcription and translation of HSPs. HSP27 inhibits apoptosis by increasing the intracellular level of antioxidant glutathione (GSH). Also, HSP27 prevents the release of mitochondrial cytochrome c or binds directly to cytochrome c. Inhibition of apoptosis by HSP60 is associated with up-regulation of the anti-apoptotic molecules Bcl-2, Bcl-xL and survivin, maintenance of the mitochondrial transmembrane potential, and inhibition of caspase 3 activation. Also, HSP60 interacts with mitochondrial HSP70, with survivin and p53, inhibiting the process of apoptosis. HSP60 is a regulator of mitochondrial permeability transition, contributing to a cytoprotective chaperone network that antagonizes cancer cell death dependent from cyclophilin D (CypD). HSP70 can bind directly to the pro-apoptotic Bcl-2 family member BAX and prevent it from translocation to mitochondria. Additionally, HSP70 prevents the recruitment of APAF-1 and procaspase-9 to the apoptosome. HSP70 binds to the death receptors DR4 and DR5, inhibiting the assembly of the death-inducing signaling complex (DISC). HSP70 also prevents the tumor necrosis factor-α (TNF-α) pathway. Moreover, HSP70 inhibits caspase-independent cell death, by binding directly to apoptosis-inducing factor (AIF). HSP90 enhances the survival pathway regulated by Akt and reduces the intrinsic apoptotic pathway. Akt leads to BAD phosphorylation retaining 14-3-3, so that it is not free to heterodimerize with antiapoptotic members of the Bcl-2 family of proteins and/or to activate the proapoptotic proteins Bax and Bak in the mitochondrial membrane. HSP90 also retains Apaf-1 in the HSP90 complex.

Figure 2
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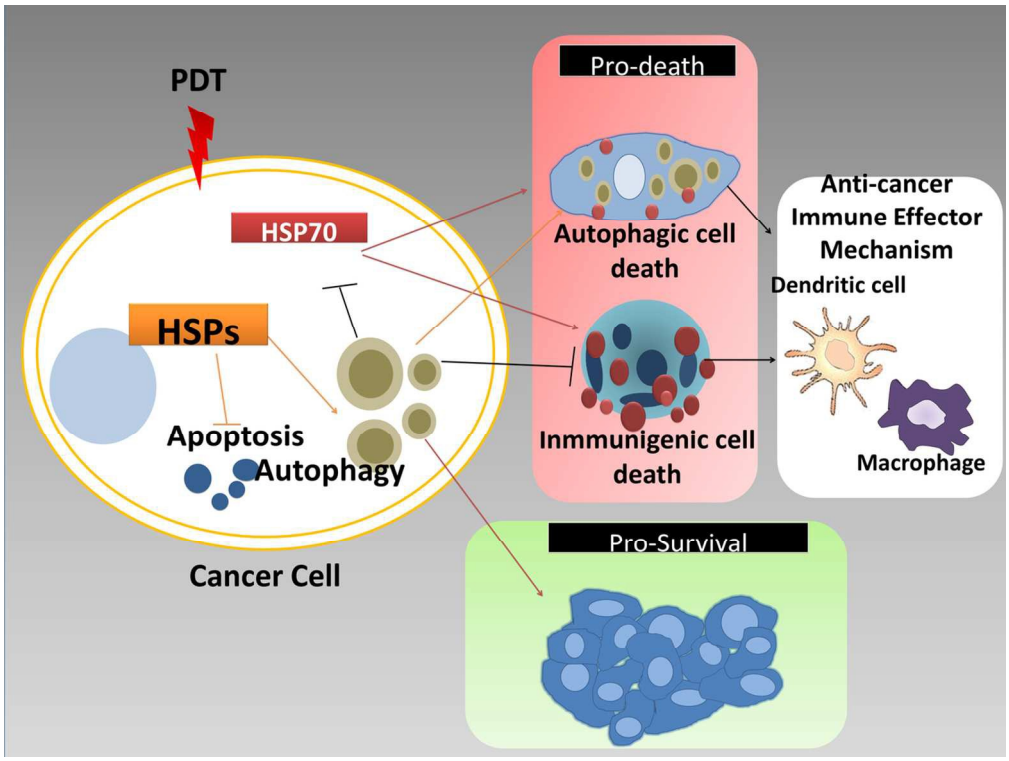
DOI: 10.1039/C6PP00097E



Pro-survival and Pro-death role of PDT-induced HSPs. HSPs induced by PDT control autophagy and inhibit apoptosis through the binding to denatured proteins or protein translocation and they are components of signal transduction pathways or antiapoptotic activity, and hence provoke the survival of the cancer cell. Moreover, it incites the reduction of the immunogenicity cell death and therefore prevents the elicitation of anticancer immune responses. Although, HSPs can induce autophagy as a death mechanism depending on a variety of parameters including the nature of the photosensitizer, PDT dose, and cell type. Beside, HSP70 is the best characterized DAMPs involved in PDT-triggered cell death able to confer immunogenicity. This HSP70 is exposed and released by apoptotic and autophagic cells death after PDT provoking an elicitation of anticancer immune responses.

Figure 3
117x87mm (300 x 300 DPI)