Molecular Pathways

Clinical Cancer Research

Immunogenic Tumor Cell Death for Optimal Anticancer Therapy: The Calreticulin Exposure Pathway

Laurence Zitvogel[1,2,3], Oliver Kepp[2,3,4], Laura Senovilla[2,3,4], Laurie Menger[2,3,4], Nathalie Chaput[1,2,3], and Guido Kroemer[2,3,4]

Abstract

In response to some chemotherapeutic agents such as anthracyclines and oxaliplatin, cancer cells undergo immunogenic apoptosis, meaning that their corpses are engulfed by dendritic cells and that tumor cell antigens are presented to tumor-specific $CD8^+$ T cells, which then control residual tumor cells. One of the peculiarities of immunogenic apoptosis is the early cell surface exposure of calreticulin (CRT), a protein that usually resides in the lumen of the endoplasmic reticulum (ER). When elicited by anthracyclines or oxaliplatin, the CRT exposure pathway is activated by pre-apoptotic ER stress and the phosphorylation of the eukaryotic translation initiation factor eIF2α by the kinase PERK, followed by caspase-8-mediated proteolysis of the ER-sessile protein BAP31, activation of the pro-apoptotic proteins Bax and Bak, anterograde transport of CRT from the ER to the Golgi apparatus and exocytosis of CRT-containing vesicles, finally resulting in CRT translocation onto the plasma membrane surface. Interruption of this complex pathway abolishes CRT exposure, annihilates the immunogenicity of apoptosis, and reduces the immune response elicited by anticancer chemotherapies. We speculate that human cancers that are incapable of activating the CRT exposure pathway are refractory to the immune-mediated component of anticancer therapies. *Clin Cancer Res; 16(12); 3100–4. ©2010 AACR.*

Background

The preclinical development of anticancer agents has been based on their evaluation in cell-free assays (in the case of targeted therapies) and the assessment of their cytotoxic potential on human cancer cell lines, first *in vitro* and then *in vivo*, after their inoculation into immunodeficient mice (1). For this reason, the possible contribution of therapy-elicited anticancer immune responses to clinical responses has been grossly neglected. Large studies involving thousands of patients have now shown that the infiltration of cancers by positive and negative immune effectors has a major prognostic and predictive impact (2, 3). Moreover, there is growing suspicion that, at least in some cases, treatment-elicited immunogenic cell death can elicit an anticancer immune response that reinforces the therapeutic effect of conventional anticancer chemotherapies and radiotherapies (4).

Developmental and homeostatic cell death is mostly mediated by apoptosis, which fails to induce inflammatory or (auto)immune responses and even may mediate antiphlogistic, immunosuppressive, and tolerogenic effects (5, 6). Although chemotherapy mostly triggers cell death with an apoptotic morphology, the therapy-induced (and hence by definition nonphysiological) cell death of cancer cells can be accompanied by a series of subtle changes in the composition of the cell surface and the secretion of soluble molecules that allow innate immune effectors, in the first place dendritic cells (DC), to "sense" immunogenicity (6). In this scenario, DCs engulf portions of the stressed and dying cell, incorporate tumor-derived antigenic peptides into major histocompatibility complex (MHC) molecules, and present them with adequate costimuli to T cells, thus stimulating the generation of tumor-specific $CD8^+$ T cells (5, 7).

In response to some chemotherapeutic agents (such as anthracyclines and oxaliplatin) and ionizing irradiation, tumor cells undergo immunogenic apoptosis, meaning that they trigger a protective immune response when they are injected subcutaneously in the absence of any adjuvant into immunocompetent mice (8–14). In contrast, cells succumbing in response to other anticancer drugs (such as alkylating agents and cisplatin) fail to trigger such an immune reaction (8). A systematic comparison of the plasma membrane surface proteome of cells that succumb to immunogenic as opposed to nonimmunogenic apoptosis has revealed one major difference. Only cells that undergo immunogenic apoptosis ectopically expose a protein that is normally found in the lumen of the endoplasmic reticulum (ER), namely the Ca^{2+}-binding chaperone calreticulin (CRT; ref. 15).

Authors' Affiliations: [1]INSERM, U1015, and CICBT507, [2]Institut Gustave Roussy, [3]Université Paris-Sud, and [4]INSERM, U848, Villejuif, France

Corresponding Author: Guido Kroemer, INSERM U848, Institut Gustave Roussy, PR1, 39 rue Camille Desmoulins, F-94805 Villejuif, France. Phone: 33-1-42-11-60-46; Fax: 33-1-42-11-60-47; E-mail: kroemer@orange.fr.

doi: 10.1158/1078-0432.CCR-09-2891

Subsequent experiments showed that the immunogenicity of apoptosis critically relies on CRT exposure. Thus, the knockdown of CRT abolishes the immunogenicity of cell death in multiple tumor models (13–16), whereas the absorbance of recombinant CRT protein to cells that die of nonimmunogenic cell death restores their immunogenicity (17, 18). In addition, CRT exposure determines the engulfment of dying tumor cells by specific DC subsets (15), in line with previous studies showing that surface CRT can serve as an "eat me" signal (19). Thus, CRT exposure marks a major checkpoint of immunogenic cell death, and the molecular mechanisms that determine the translocation of CRT to the cell surface have been studied in some detail.

The Calreticulin Exposure Pathway

In immunogenic cell death, selective CRT exposure (without exposure of other ER proteins such as calnexin) occurs at a relatively early stage when the cells still have a normal morphology and lack phosphatidylserine exposure (9). This early CRT-exposure contrasts with a later phase of advanced apoptosis (be it immunogenic or nonimmunogenic) when intracellular membranes, mostly from the ER, are recruited to the surface giving rise to a general alteration in the glycocalix as well as to the nonspecific recruitment of ER membrane proteins such as calnexin to the cell surface (20). The CRT exposure that precedes and accompanies immunogenic apoptosis is the result of a complex, stimulus-dependent process, which occurs in several steps (Fig. 1). CRT exposure is potently triggered by pharmacological cell death inducers that provoke the production of reactive oxygen species (ROS) as well as an ER stress response. Quenching of chemotherapy-elicited ROS with antioxidants or, at least in the case of anthracyclines, inhibition of the inducible NO synthase, suffices to abolish CRT exposure (18, 21). However, the addition of ROS or ROS-generating agents alone is not sufficient to induce CRT exposure (18).

One common feature of chemotherapeutic agents that trigger CRT exposure (such as oxaliplatin or the anthracyclines doxorubicine and mitoxantrone) is their capacity to induce an ER stress response (18). This response is accompanied by the activating phosphorylation of the ER stress kinase PERK, as well as by the phosphorylation of the PERK substrate eukaryotic translation initiation factor eIF2α on serine 51. Both the knockout-knockdown of PERK and the replacement of eIF2α by a nonphosphorylatable mutant (S51A) abolish CRT exposure induced by anthracyclines or oxaliplatin. In contrast, the removal of other elements of the ER stress response, ATF6 or IRE1, does not affect CRT exposure (18). Anthracyclines also cause the dissociation of the eIF2α-specific phosphatase PP1 from its obligate cofactor GADD34, implying that PP1 is inhibited (22). Pharmacological agents that directly or indirectly inhibit PP1/GADD34 (such as tautomycin and salubrinal) as well as cell-permeable peptides that competitively dissociate the PP1/GADD34 complex are highly efficient in stimulating CRT exposure even in conditions that do not lead to cell death (9, 22). Altogether these results indicate that the S51 phosphorylation of eIF2α, which is required for other stress responses including translational arrest by ER stress (23), formation of stress granules (24), and autophagy (25), is also critical for CRT exposure. However, ER stress alone is not sufficient to cause pre-apoptotic CRT exposure. Thus, ER stress-inducing agents like tunicamycin or thapsigargin fail to stimulate the translocation of CRT to the cell surface when used alone (9, 26). However, thapsigargin (an inhibitor of the SERCA Ca^{2+} pump), overexpression of reticulon 1C, as well as targeted expression of the ligand-binding domain of the IP_3 receptor, which all reduce the intraluminal ER Ca^{2+} concentration, improved CRT exposure in neuroblastoma cells that normally failed to expose CRT in response to anthracylins (26). These results suggest that ER Ca^{2+} levels contribute to the control of CRT exposure, in line with the direct effects of Ca^{2+} on the conformation of the CRT protein (27).

The inhibition of caspases with two broad-spectrum inhibitors, the synthetic peptide Z-VAD-fmk or p35 (a baculovirus-derived protein that can be transfected into mammalian cells), completely abolishes CRT exposure (9) and subverts the immunogenicity of cell death induced by anthracyclines or oxaliplatin (8). Further analyses revealed that caspase-8 is the apical caspase that is activated downstream of ER stress by anthracyclines (18). This result is reminiscent of prior studies showing that caspase-8 can be activated by ER stress and catalyzes the cleavage of Bap31, an ER-sessile protein (28). Selective deletion, depletion, or inhibition of caspase-8 abolished CRT exposure, and similarly, depletion of Bap31 or its replacement by a noncleavable mutant inhibits CRT exposure induced by anthracyclines or oxaliplatin (18). Thus, caspase-8-mediated cleavage of Bap31 is crucial for CRT exposure. Activation of caspase-8 by ligation of death receptors (for instance by addition of TRAIL or an agonistic CD95 antibody) also induces CRT exposure. Antioxidants fail to prevent TRAIL-induced apoptosis, yet they abolish CRT exposure (18), underscoring the contribution of ROS to this process. It is important to note that caspase-8 activation leading to CRT exposure occurs relatively early after addition of anthracyclines, several hours before the apoptosis-associated activation of caspase-3 (18). Bap31 is a Bcl-2 interacting protein, and overexpression of Bcl-2 inhibits CRT exposure. Moreover, the knockout or knockdown of either Bax or Bak prevents CRT translocation, indicating that this process is under the control of the multidomain proteins from the Bcl-2 family (18). Whether this relays to effects of Bcl-2, Bax, and Bak on ER Ca^{2+} fluxes (29) and/or on the ER stress response (30) has to be clarified in the future.

Once the ER stress response has triggered the "apoptotic module" of the ER exposure pathway (with caspase-8 activation, Bap31 cleavage, and conformational activation of Bax and Bak), CRT is translocated from the ER lumen to the plasma membrane surface. This process involves the actin cytoskeleton-mediated anterograde transport of

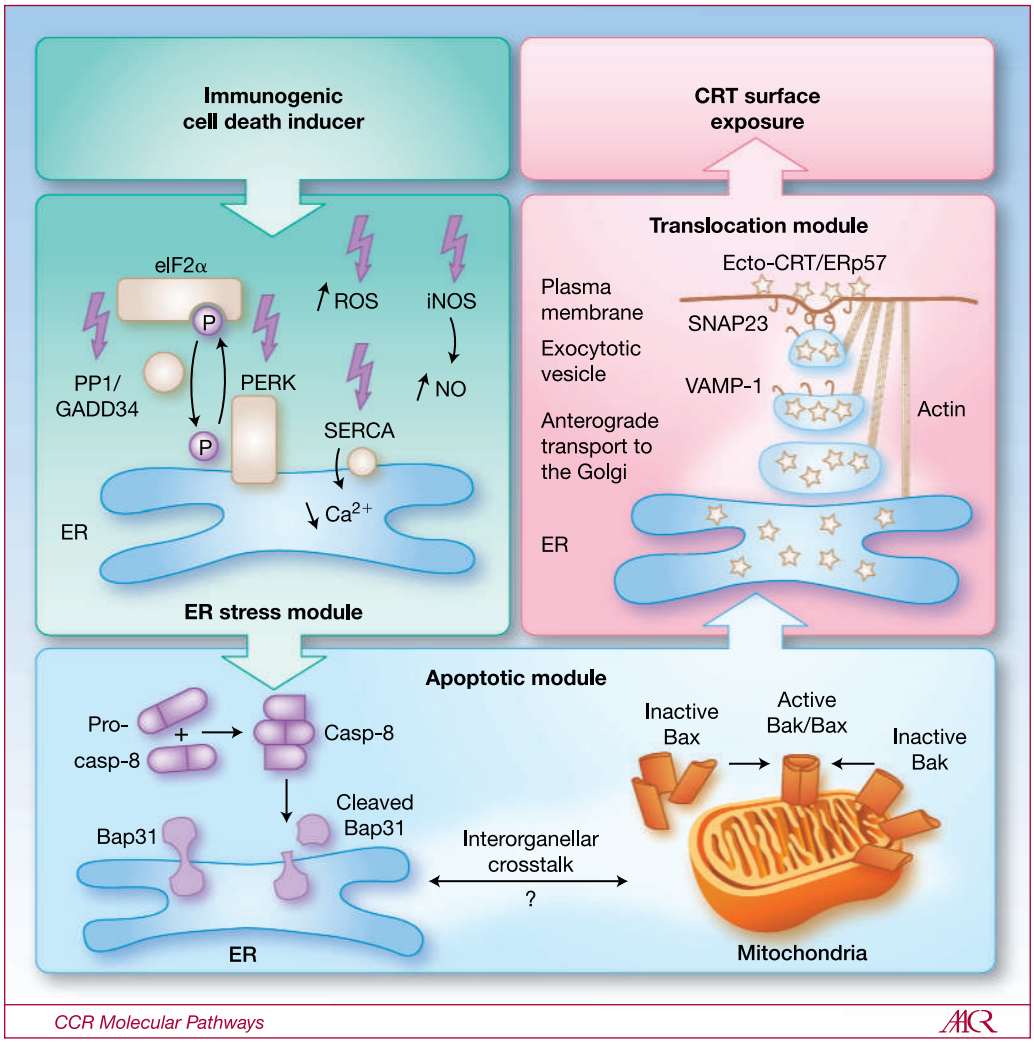

Fig. 1. The CRT exposure pathway. Three different hierarchical modules are involved in the exposure of CRT in response to immunogenic cell death inducers. The phosphorylation of eiF2α due to the activation of PERK (the kinase) or the inhibition of PP1/GADD34 (the phosphatase), the production of ROS and/or NO, and the possible inhibition of SERCA are implicated in the "ER stress module." The "apoptotic module" comprises the apical caspase-8 dependent cleavage of Bap31 and the activation of the Bcl-2-family proteins Bax and Bak, which might mediate an interorganellar crosstalk between the ER and mitochondria. However, it is not know at which subcellular location Bcl-2, Bax, and Bak influence the CRT exposure pathway. Within the "translocation module" CRT is anterogradely transported from the ER lumen to the Golgi apparatus, facilitated by rearrangements of the actin cytoskeleton. Then the vesicle-associated SNARE, VAMP1, mediates membrane fusion by interaction with the plasma membrane-associated SNARE, SNAP23, allowing luminal CRT to reach its final destination, the surface of the cell.

CRT from the ER to the Golgi apparatus, followed by the active exocytosis of CRT-containing vesicles based on the interaction between vesicle-associated SNAREs (such as VAMP1) and plasma membrane-associated SNAREs (such as SNAP23/25; ref. 18). During its voyage from the ER lumen to the cell surface, CRT continues to bind to one of its major interactors, the disulfide isomerase ERp57. This interaction is obligatory for CRT translocation because the deletion and/or depletion of ERp57 abrogates CRT exposure, and *vice versa* the deletion and/or depletion of CRT precludes ERp57 exposure (17). It has not yet been clarified how endogenous CRT is anchored to the plasma membrane surface. Recombinant CRT protein readily binds to a saturable surface receptor that is present on both healthy and dying tumor cells of any kind and whose molecular identity is elusive. Similarly, the molecular identity of the CRT receptor on DCs is not clearly established. It is still unknown whether the candidate CRT receptors CD91 (19) and scavenger receptor class-A (31) are required for the DC-specific engulfment of CRT-exposing tumor cells.

Clinical-Translational Advances

When CT26 colon cancer cells, B16F1 melanoma cells, or MCA205 fibrosarcoma cells are treated with anthracyclines and then are injected subcutaneously into histocompatible mice, they elicit an anticancer immune response

that precludes the growth of live tumor cells that are inoculated 1 week later into the opposite flank. The immunogenicity of cell death is suppressed when CRT exposure is blocked, for instance by knocking down CRT (13–16) and can be reestablished by absorbing recombinant CRT to the cell surface (9, 10). Similarly, any manipulation that interrupts the CRT exposure pathway abolishes the immunogenicity of anthracycline- and oxaliplatin-induced cell death, although they have no major effect on the lethal effects of the chemotherapeutics on tumor cells *in vitro*. This anti-immunogenic effect is caused by the knockdown of PERK, caspase-8, Bap31, Bax, Bak, VAMP, SNAP31, or ERp57 and to the use of chemical inhibitors of ROS, caspases, ER-Golgi trafficking, or the actin cytoskeleton (18). Stable knockdown of ERp57 in CT26 tumors had no effect on the sensitivity of the cells to anthracyclines *in vitro*, as measured by the induction of apoptosis and the loss of clonogenic survival. However, ERp57-deficient tumors failed to respond to anthracycline-based chemotherapy *in vivo* unless recombinant CRT protein was injected into the tumor bed (17). These results underscore the contribution of immunogenic tumor cell death and the ensuing anticancer immune response to the therapeutic efficacy of chemotherapy.

It is noteworthy that the CRT exposure pathway is phylogenetically conserved-even yeast cells translocate CRT toward the cell surface when they are treated with anthracyclines and this pathway involves the yeast orthologs of PERK, Bap31, SNARE, and ERp57 (32, 33)-and that multiple viruses including oncoviruses encode proteins that interrupt this pathway. For example, the E5 protein encoded by human papilloma virus (HPV16 and HPV31) binds to Bap31 (34), and the E2 protein encoded by hepatitis C virus (HCV) inhibits PERK and other kinases that can phosphorylate eIF2α (35). HCV also upregulates cellular FLIP (to inhibit caspase-8; ref. 36), and stimulates the generation of CRT fragments that become detectable in the serum of infected patients (37) and that might act as competitive antagonists of CRT receptors on DCs. These findings point to the possibility, that viruses might have developed specific strategies to subvert the CRT exposure pathway and hence to avoid the immune recognition of dying virus-infected cells.

On a similar note, it seems plausible that the immune system selects tumor cell variants that lose the ability to expose CRT and then escape from anticancer immune responses, a possibility of "immunoediting" (38) that has not yet been investigated in detail. Profiling of the expression levels (either at the mRNA or protein levels) of the essential elements of the CRT exposure pathway does not inform on the functional state of this system. For example, the presence of PERK does not show whether it is activated, and the mere expression levels of CRT or ERp57 do not reveal their subcellular localization. In spite of this limitation, some studies show that low protein levels of CRT and ERp57 negatively influence the prognosis of bladder and gastric carcinoma, respectively (39, 40). However, because CRT and ERp57 are also involved in loading antigenic peptides into MHC class I molecules (41), these results are difficult to be interpreted with respect to the CRT exposure pathway. There are multiple studies showing that the loss of caspase-8 expression or altered expression levels in multidomain Bcl-2 family proteins (Bcl-2, Bcl-X_L, Mcl-1, Bax, Bak, etc.) have a profound impact on tumor prognosis. Again, the multifunctional nature of these proteins, and notably their role in the regulation of apoptosis and autophagy, makes it difficult to weigh the relative contribution of defective CRT exposure to the observed effects.

As a result, it will be important to precisely determine post-translational modifications in the essential actors of the CRT exposure pathway (such as the phosphorylation of PERK and eIF2α, the proteolytic cleavage of caspase-8 and Bap31, as well as the subcellular redistribution of ERp57 and CRT), as they occur after chemotherapy or radiotherapy in patients, and to study the impact of these modifications on the efficacy of anticancer treatments. We anticipate that exploring such tumor cell-intrinsic parameters that determine the immunogenicity of cell death will yield clinically useful information.

It has already been shown that loss-of-function mutations in two receptors that detect immunogenic cell death signals, namely toll-like receptor 4 (TLR4, which detects HMGB1 released from dying cells) and purinergic receptor P2RX7 (which detects ATP secreted by dying cells), reduce the efficacy of adjuvant chemotherapies with anthracyclines (in breast cancer) and oxaliplatin (in colorectal cancer; refs. 11, 12, 42). These results reinvigorate our quest for the CRT receptor present on dendritic cells and its contribution to the anticancer immune response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

INSERM, Ligue contre la Cancer, Fondation de France, Fondation pour la Recherche Médicale, Institut National du Cancer, Cancéropôle Ile-de-France, AICR, Apoptrain and Fondation Gustave Roussy.

Received 03/08/2010; revised 03/31/2010; accepted 04/06/2010; published OnlineFirst 04/26/2010.

References

1. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008;8:59–73.
2. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene 2010;29:1093–102.

3. Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105–13.
4. Zitvogel L, Apetoh L, Ghiringhelli F, Andre F, Tesniere A, Kroemer G. The anticancer immune response: indispensable for therapeutic success? J Clin Invest 2008;118:1991–2001.
5. Green DR, Ferguson T, Zitvogel L, Kroemer G. Immunogenic and tolerogenic cell death. Nat Rev Immunol 2009;9:353–63.
6. Zitvogel L, Kepp O, Kroemer G. Decoding cell death signals in inflammation and immunity. Cell 2010;140:798–804.
7. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010;140:883–99.
8. Casares N, Pequignot MO, Tesniere A, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. J Exp Med 2005;202:1691–701.
9. Obeid M, Tesniere A, Ghiringhelli F, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 2007;13:54–61.
10. Obeid M, Tesniere A, Panaretakis T, et al. Ecto-calreticulin in immunogenic chemotherapy. Immunol Rev 2007;220:22–34.
11. Apetoh L, Ghiringhelli F, Tesniere A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007;13:1050–9.
12. Ghiringhelli F, Apetoh L, Tesniere A, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1β-dependent adaptive immunity against tumors. Nat Med 2009;15:1170–8.
13. Cao C, Han Y, Ren Y, Wang Y. Mitoxantrone-mediated apoptotic B16–1 cells induce specific anti-tumor immune response. Cell Mol Immunol 2009;6:469–75.
14. Tongu M, Harashima N, Yamada T, Harada T, Harada M. Immunogenic chemotherapy with cyclophosphamide and doxorubicin against established murine carcinoma. Cancer Immunol Immunother 2010;59:769–77.
15. Obeid M, Panaretakis T, Joza N, et al. Calreticulin exposure is required for the immunogenicity of γ-irradiation and UVC light-induced apoptosis. Cell Death Differ 2007;14:1848–50.
16. Perez CA, Fu A, Onishko H, Hallahan DE, Geng L. Radiation induces an antitumour immune response to mouse melanoma. Int J Radiat Biol 2009;85:1126–36.
17. Panaretakis T, Joza N, Modjtahedi N, et al. The co-translocation of ERp57 and calreticulin determines the immunogenicity of cell death. Cell Death Differ 2008;15:1499–509.
18. Panaretakis T, Kepp O, Brockmeier U, et al. Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. EMBO J 2009;28:578–90.
19. Gardai SJ, McPhillips KA, Frasch SC, et al. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. Cell 2005;123:321–34.
20. Franz S, Herrmann K, Furnrohr BG, et al. After shrinkage apoptotic cells expose internal membrane-derived epitopes on their plasma membranes. Cell Death Differ 2007;14:733–42.
21. De Boo S, Kopecka J, Brusa D, et al. iNOS activity is necessary for the cytotoxic and immunogenic effects of doxorubicin in human colon cancer cells. Mol Cancer 2009;8:108.
22. Kepp O, Galluzzi L, Giordanetto F, et al. Disruption of the PP1/GADD34 complex induces calreticulin exposure. Cell Cycle 2009;8:3971–7.
23. Scheuner D, Song B, McEwen E, et al. Translational control is required for the unfolded protein response and *in vivo* glucose homeostasis. Mol Cell 2001;7:1165–76.
24. Yamasaki S, Anderson P. Reprogramming mRNA translation during stress. Curr Opin Cell Biol 2008;20:222–6.
25. Talloczy Z, Jiang W, Virgin HW IV, et al. Regulation of starvation- and virus-induced autophagy by the eIF2α kinase signaling pathway. Proc Natl Acad Sci U S A 2002;99:190–5.
26. Tufi R, Panaretakis T, Bianchi K, et al. Reduction of endoplasmic reticulum Ca2+ levels favors plasma membrane surface exposure of calreticulin. Cell Death Differ 2008;15:274–82.
27. Villamil Giraldo AM, Lopez Medus M, Gonzalez Lebrero M, et al. The structure of calreticulin C-terminal domain is modulated by physiological variations of calcium concentration. J Biol Chem 2010;285:4544–53.
28. Breckenridge DG, Stojanovic M, Marcellus RC, Shore GC. Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome c release to the cytosol. J Cell Biol 2003;160:1115–27.
29. Rong YP, Aromolaran AS, Bultynck G, et al. Targeting Bcl-2-IP3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals. Mol Cell 2008;31:255–65.
30. Hetz C, Bernasconi P, Fisher J, et al. Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1α. Science 2006;312:572–6.
31. Berwin B, Hart JP, Rice S, et al. Scavenger receptor-A mediates gp96/GRP94 and calreticulin internalization by antigen-presenting cells. EMBO J 2003;22:6127–36.
32. Madeo F, Durchschlag M, Kepp O, et al. Phylogenetic conservation of the preapoptotic calreticulin exposure pathway from yeast to mammals. Cell Cycle 2009;8:639–42.
33. Kepp O, Senovilla L, Galluzzi L, et al. Viral subversion of immunogenic cell death. Cell Cycle 2009;8:860–9.
34. Regan JA, Laimins LA. Bap31 is a novel target of the human papillomavirus E5 protein. J Virol 2008;82:10042–51.
35. Pavio N, Romano PR, Graczyk TM, Feinstone SM, Taylor DR. Protein synthesis and endoplasmic reticulum stress can be modulated by the hepatitis C virus envelope protein E2 through the eukaryotic initiation factor 2α kinase PERK. J Virol 2003;77:3578–85.
36. Saito K, Meyer K, Warner R, Basu A, Ray RB, Ray R. Hepatitis C virus core protein inhibits tumor necrosis factor α-mediated apoptosis by a protective effect involving cellular FLICE inhibitory protein. J Virol 2006;80:4372–9.
37. Chignard N, Shang S, Wang H, et al. Cleavage of endoplasmic reticulum proteins in hepatocellular carcinoma: Detection of generated fragments in patient sera. Gastroenterology 2006;130:2010–22.
38. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol 2006;90:1–50.
39. Cathro HP, Smolkin ME, Theodorescu D, Jo VY, Ferrone S, Frierson HF, Jr. Relationship between HLA class I antigen processing machinery component expression and the clinicopathologic characteristics of bladder carcinomas. Cancer Immunol Immunother 2010;59:465–72.
40. Leys CM, Nomura S, LaFleur BJ, et al. Expression and prognostic significance of prothymosin-α and ERp57 in human gastric cancer. Surgery 2007;141:41–50.
41. Gold LI, Eggleton P, Sweetwyne MT, et al. Calreticulin: non-endoplasmic reticulum functions in physiology and disease. FASEB J 2010;24:665–83.
42. Tesniere A, Schlemmer F, Boige V, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene 2010;29:482–91.