ROS signaling during granzyme B-mediated apoptosis

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Abbreviations: Dy_m, mitochondrial transmembrane potential; AIF, apoptosis-inducing factor; Apaf1, apoptotic protease-activating factor 1; Bax, BCL2-associated X protein; Bcl-2, B-cell CLL/lymphoma 2; Bid, BH3 interacting domain death agonist; Cyt c, cytochrome c; DNA-PK, DNA-dependent protein kinase; EndoG, endonuclease G; ETC, electron transport chain; GA, granzyme A; GB, granzyme B; HtrA2, high temperature requirement protein A2; IAP, inhibitor of apoptosis; ICAD, Inhibitor of Caspase-activated DNase; MOMP, mitochondrial outer membrane permeabilization; NADH, Nicotinamide adenine dinucleotide; NDUFA9, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9; NDUFS1/2, NADH dehydrogenase (ubiquinone) Fe-S protein 1/2; NDUFV1, NADH dehydrogenase (ubiquinone) flavoprotein 1; NOX, nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase; NuMa, Nuclear mitotic apparatus protein 1; P12/17/20, 12, 17 and 20 kDa cleavage fragments of the maturation process of caspase 3; P, perforin; PARP, Poly-(ADP-ribose) polymerase; ROS, reactive oxygen species.

Reactive oxygen species (ROS) are involved in cell signaling, aging, and death and play a role in carcinogenesis. However, whether ROS are bystanders or active effectors of apoptosis was unclear until recently. New evidence shows that the killer lymphocyte protease granzyme B activates a conserved biochemical pathway centered on respiratory chain disruption to trigger mitocentric ROS-dependent apoptosis.

Human cytotoxic lymphocytes (CTLs) eradicate pathogen-infected or transformed cells primarily through the cytotoxic granule pathway, which relies on perforin (PFN)- and granulysin-mediated delivery of the granzyme serine proteases into eukaryotic target cells or bacteria, respectively. Among the 5 human granzymes (A, B, H, K, and M) and 10 mouse homologues (A, B, C, G, E, F, G, K, M, and N), granzymes B and A (GB and GA) are the most abundantly expressed, and the best characterized.[1] Like caspases, GB cleaves its substrates after aspartic acid residues to induce cell death. In fact, GB behaves as a hybrid between initiator and effector caspases—like initiator caspases, GB activates the effector caspases 3 and 7 and the proapoptotic BH3-only protein Bid; however, GB can also directly cleave important effector caspase substrates such as ICAD, PARP-1, lamin B, NuMa, DNA-PKc and tubulin for cell death induction.[1] Mitochondria adjust both their morphology and function, acting as hubs that integrate cues for the appropriate cellular response. In response to activation of death receptors, DNA damage, or ER stress, activation of BH3-only proteins of the Bcl2 family triggers the oligomerization of Bax and Bak leading to mitochondrial outer membrane permeabilization (MOMP).[2] This critical step of many cell death pathways results in release of the apoptogenic factors cytochrome C (Cyt C), EndoG, Smac/Diablo, HtrA2 and AIF from the mitochondria into the cytosol.[2] As a consequence, cytosolic Cyt C triggers activation of Apaf1 and caspase 9 apoptosome platform, and Smac removes the repression of the inhibitor of apoptosis (IAP) proteins. Together, these events amplify effector caspase activation.[2] For a long time, MOMP was considered sufficient for apoptogenic factor release; however, recent findings suggest that the story is more complex. In fact, reactive oxygen species (ROS) are necessary for Cyt C release.[3] Cyt C engages in both electrostatic and hydrophobic interactions with inner mitochondrial membrane cardiolipins that are disrupted by ROS for optimal release through MOMP.[3] GB triggers optimal Cyt C, Endo G, and Smac release in a ROS-dependent manner (Fig. 1).[4] It is likely that, similar to Cyt C, ROS-mediated untethering of Endo G and Smac is required for their release through the Bid/Bax/Bak-mediated MOMP in the GB pathway.[4] ROS are also necessary for GB-induced DNA fragmentation since ROS scavengers significantly reduce and delay oligonucleosomal DNA fragmentation without affecting ICAD cleavage and activation of CAD, the endonuclease involved in this process (Fig. 1). This result could be predicted by the compromised release of EndoG from
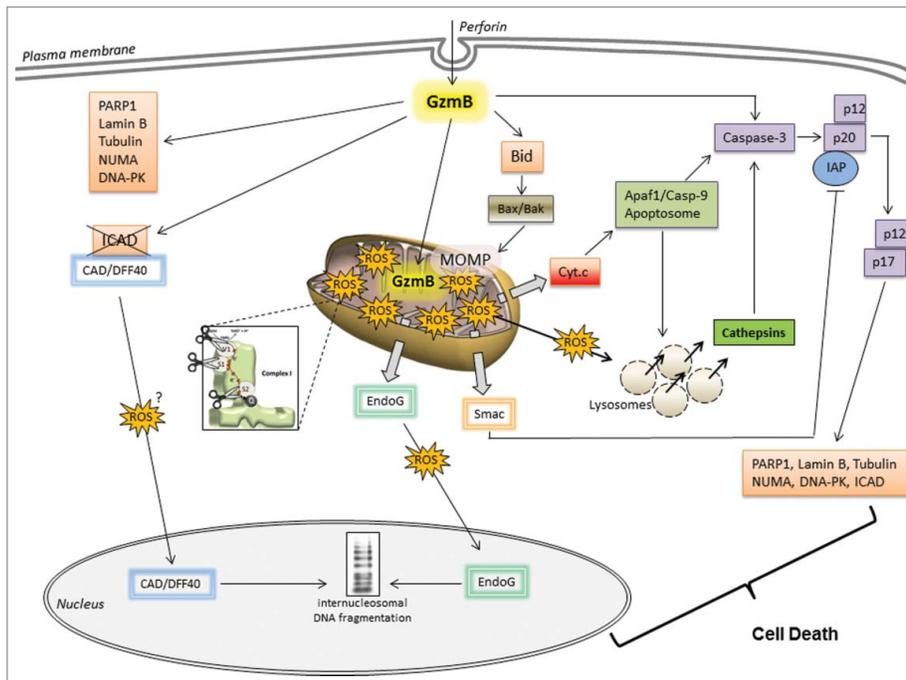
Figure 1. Contribution of ROS to granzyme B-induced apoptosis. Perforin mediates the delivery of GB into the target cell cytosol. GB can directly cleave PARP1, lamin B, tubulin, NUMA, DNA-PK, and ICAD to induce caspase-independent cell death. GB also enters the mitochondria by an unknown mechanism and cleaves NDUFS1, NDUFS2, and NDUFV1 from complex I to trigger ROS production. GB cleaves Bid, and truncated Bid activates Bax/Bak to induce MOMP. Together with ROS, MOMP allows the release of Cyt C, EndoG, Smac, HtrA2, and AIF (the latter 2 are not represented here). Cytosolic Cyt C promotes activation of the Apaf1/caspase 9 apoptosome that activates caspase 3. Smac removes IAP repression for full caspase activation. GB releases CAD from ICAD inhibition. Both CAD and EndoG shuttle to the nucleus probably in a ROS-dependent mechanism to induce oligonucleosomal DNA fragmentation. ROS and caspase 9 cooperate to trigger lysosomal membrane permeabilization and cathepsin release, thus amplifying caspase 3 activation. Among other substrates, activated caspase 3 cleaves PARP1, lamin B, tubulin, NUMA, DNA-PK, and ICAD, which together contribute to cell death.

mitochondria in the absence of ROS. Furthermore, ROS were found to be necessary for the nuclear shuttling of EndoG during cisplatin-induced death of head and neck squamous carcinoma cells,[5] suggesting that ROS may also be required for EndoG and CAD nuclear translocation following GB and PFN treatment (**Fig. 1**). An additional contribution of ROS in apoptosis progression was revealed by the work of Christoph Borner's group, who showed that during TNFα apoptotic signaling, caspase 9 induced lysosomal membrane permeabilization (LMP) in a ROS-dependent manner, leading to cathepsin release and further caspase activation and cell death.[6] ROS-induced LMP is also active in the GB pathway (**Fig. 1**).[4] Overall, these findings are consistent with the inhibitory effect of ROS scavengers on CTL-

mediated cell death, implying that ROS are active effectors of cell death.

The production of ROS during cell death has long been recognized; however, a molecular mechanism for their production has only recently begun to be unraveled. Although one report suggested an extra-mitochondrial origin of ROS during apoptosis,[7] Douglas Green's group first showed that staurosporine-mediated ROS-dependent apoptosis requires caspase 3 cleavage of NDUFS1, the 75-kDa subunit of the mitochondrial electron transport chain (ETC) complex I.[8] Later, Judy Lieberman's group reported that GA triggered a caspase- and MOMP-independent cell death that also required the disruption of complex I through the cleavage of NDUFS3 to induce ROS-dependent apoptosis.[9] Interestingly, GB cleaves NDUFS1, NDUFS2, and NDUFV1

subunits of this same complex to trigger ROS-dependent apoptosis (**Fig. 1**).[4] Actually, GB also alters complex II and complex III function, disrupts the supercomplex organization of the respiratory chain, and triggers a loss of the mitochondria cristae junctions.[4] In this pathway, electron flow is required for ROS production since ρ0 cells that carry a non-functional respiratory chain are resistant to granzyme-mediated ROS production and cell death. It is possible that cleavage of these complex I subunits exposes the electrons within the Fe-S cluster to ambient oxygen for ROS production. Additionally, the mitochondria targeted antioxidant MitoQ completely abrogates GB-induced ROS and cell death, further supporting a mitochondrial origin of ROS in this context. Integration of these data indicates that 3 cell death pathways—caspase 3, GA, and GB—converge on complex I for ROS production during apoptosis. Moreover, disruption of complex I by GA and GB following human killer cell attack has been implicated in ROS-dependent bacterial death, suggesting that cleavage of complex I subunits is an evolutionarily conserved mechanism of ROS-dependent death.[10] Our results indicate that GA and GB, which lack a mitochondrial targeting signal, still find their way into this double-membrane organelle to disrupt the respiratory chain and trigger an increase in ROS. We have also found that granzyme mitochondrial entry is MOMP-independent but requires an intact membrane potential. Since MOMP disrupts the mitochondrial membrane potential, GB mitochondrial import may precede MOMP. More studies will be necessary to delineate the hierarchy of these events.

Collectively, ROS contribute to many aspects of the apoptotic process. However, a full understanding of their mode of action requires much more work. Do ROS act through non-specific systemic redox-modification of macromolecules or through a discrete set of targets? How many ROS are needed? Which radical species are most effective for the various contributions of ROS to cell death? Putting emphasis on redox signaling during apoptosis will undoubtedly open new windows

of investigation and allow the characterization of useful biomarkers of cell death.

Disclosure of Potential Conflicts of Interest

References

1. Chowdhury D, Lieberman J. Death by a thousand cuts: granzyme pathways of programmed cell death. Annu Rev Immunol (2008) 26: 389-420; PMID:18304003; http://dx.doi.org/10.1146/annurev.immunol.26.021607.090404
2. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. Physiol Rev (2007) 87: 99-163; PMID:17237344; http://dx.doi.org/10.1152/physrev.00013.2006
3. Petrosillo G, Ruggiero FM, Paradies G. Role of reactive oxygen species and cardiolipin in the release of cytochrome c from mitochondria. FASEB J (2003) 17: 2202-8; PMID:14656982; http://dx.doi.org/10.1096/fj.03-0012com
4. Jacquemin G, Margiotta D, Kasahara A, Bassoy EY, Walch M, Walch M2, Thiery J, Lieberman J, Martinvalet D. Granzyme B-induced mitochondrial ROS are required for apoptosis. Cell Death and Differentiation (2014); PMID:25361078; http://dx.doi.org/10.1038/cdd.2014.180
5. Kim JS, Lee JH, Jeong WW, Choi DH, Cha HJ, Kim do H, Kwon JK, Park SE, Park JH, et al. Reactive oxygen species-dependent EndoG release mediates cisplatin-induced caspase-independent apoptosis in human head and neck squamous carcinoma cells. Int J Cancer (2008) 122: 672-80; http://dx.doi.org/10.1002/ijc.23158
6. Huai J, Vogtle FN, Jockel L, Li Y, Kiefer T, Ricci JE, Borner C. TNFalpha-induced lysosomal membrane permeability is downstream of MOMP and triggered by caspase-mediated NDUFS1 cleavage and ROS formation. J Cell Sci (2013) 126: 4015-25; PMID:23788428; http://dx.doi.org/10.1242/jcs.129999
7. Aguilo JI, Anel A, Catalan E, Sebastian A, Acin-Perez R, Naval J, Wallich R, Simon MM, Pardo J. Granzyme B of cytotoxic T cells induces extramitochondrial reactive oxygen species production via caspase-dependent NADPH oxidase activation. Immunol Cell Biol (2010) 88: 545-54; PMID:20125115; http://dx.doi.org/10.1038/icb.2010.5
8. Ricci JE, Munoz-Pinedo C, Fitzgerald P, Bailly-Maitre B, Perkins GA, Yadava N, Scheffler IE, Ellisman MH, Green DR. Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain. Cell (2004) 117: 773-86; PMID:15186778; http://dx.doi.org/10.1016/j.cell.2004.05.008
9. Martinvalet D, Dykxhoorn DM, Ferrini R, Lieberman J. Granzyme A cleaves a mitochondrial complex I protein to initiate caspase-independent cell death. Cell (2008) 133: 681-92; PMID:18485875; http://dx.doi.org/10.1016/j.cell.2008.03.032
10. Walch M, Dotiwala F, Mulik S, Thiery J, Kirchhausen T, Clayberger C, Krensky AM, Martinvalet D, Lieberman J. Cytotoxic cells kill intracellular bacteria through granulysin-mediated delivery of granzymes. Cell (2014) 157: 1309-23; PMID:24906149; http://dx.doi.org/10.1016/j.cell.2014.03.062