OPEN

Citation: Cell Death and Disease (2013) 4, e826; doi:10.1038/cddis.2013.372

www.nature.com/cddis

Correspondence

# Calreticulin surface exposure is abrogated in cells lacking, chaperone-mediated autophagy-essential gene, LAMP2A

AD Garg[1], AM Dudek[1] and P Agostinis*,[1]

*Cell Death and Disease* (2013) **4,** e826; doi:10.1038/cddis.2013.372; published online 3 October 2013
**Subject Category:** Experimental Medicine

*Dear Editor*,

Surface-exposed calreticulin (ecto-CRT) and secreted ATP have emerged as incontrovertible danger signals.[1–3] A surge in studies describing the impact of these danger signals has also spurred a tremendous interest in the discovery of signaling pathways regulating their 'emission'. Recent research has shown that the pathways regulating the emission of these danger signals exhibit a certain degree of 'plasticity' and context-dependency.[1–4] This applies mainly to the pathways regulating ecto-CRT emission and ATP secretion induced by mitoxantrone (MTX)[2] and hypericin-based photodynamic therapy (Hyp-PDT).[3–5]

Irrespective of the inducers though, molecular components regulating endoplasmic reticulum (ER)-stress and autophagy have been found to form the 'core' of pathways behind ecto-CRT and secreted ATP.[1,3,5] While ER-stress tends to regulate these pathways positively,[1,3] macroautophagy has recently emerged to play a more contextual role.[1,6] While on one hand, macroautophagy was found to positively regulate MTX-induced ATP secretion,[2] on the other we found that macroautophagy negatively regulates Hyp-PDT-induced ecto-CRT[6] (without affecting ATP secretion[6]). This further increased our curiosity regarding the role of autophagy pathways behind ecto-CRT/secreted ATP. Interestingly, it was recently shown that the lysosomal protein LAMP1 can regulate MTX-induced ATP secretion.[2] We have previously shown that oxidative stress generated by Hyp-PDT along with macroautophagy can stimulate chaperone-mediated autophagy (CMA), a process mediated by the lysosomal protein, LAMP2A[7,8] Cells lacking LAMP2A were found to be extremely sensitized toward Hyp-PDT-induced apoptotic cell death, which is mediated by severe reactive oxygen species-induced ER stress.[7] On the back of these exciting results, we became curious about the impact this CMA-essential gene can have on cell-surface exposure of CRT and secretion of ATP.

To this end, we tested the ability of LAMP2A$^{+/+}$ and LAMP2A$^{-/-}$ cells to emit secreted ATP/ecto-CRT following MTX/Hyp-PDT treatments. MTX and Hyp-PDT induced significant secretion of ATP in LAMP2A$^{+/+}$ cells as compared with the untreated cells (Supplementary Figure S1A). Similarly, the LAMP2A$^{-/-}$ cells also retained their capacity to secrete ATP following MTX/Hyp-PDT treatment, to the same extent as the LAMP2A$^{+/+}$ cells (Supplementary Figure S1A).

Next, we analyzed the induction of ecto-CRT and found both MTX and Hyp-PDT to be potent inducers of ecto-CRT in the LAMP2A$^{+/+}$ cells (Supplementary Figure S1B), as expected.[4,5] Interestingly, we found that the LAMP2A$^{-/-}$ cells exhibited a strong inability to emit ecto-CRT, not only following MTX/Hyp-PDT treatments but also in basal conditions (Supplementary Figure S1B). This complete abrogation of ecto-CRT in the absence of LAMP2A indicated an 'innate' dysfunction of ecto-CRT trafficking pathway in these cells. It has been well established that cells lacking LAMP2A exhibit a compensatory rise in macroautophagy, under basal conditions, to counter the absence of CMA.[7,8] We had recently observed that ablation of macroautophagy (via ATG5-siRNA) increased Hyp-PDT-induced ecto-CRT.[6] Thus, we wondered whether we can rescue ecto-CRT emission in LAMP2A$^{-/-}$ cells if we knockdown ATG5 (ATG5$^{KD}$). ATG5$^{KD}$ in LAMP2A$^{+/+}$ cells (Supplementary Figure S1C, left) further increased the induction of ecto-CRT after treatment (as compared with LAMP2A$^{+/+}$ cells transfected with Scr-siRNA) (Supplementary Figure S1C, right), especially after Hyp-PDT, as expected.[6] Contrary to expectations though, ATG5$^{KD}$ in LAMP2A$^{-/-}$ cells ($\sim$50%$^{KD}$; Supplementary Figure S1C, left) failed to re-establish ecto-CRT, both under basal and treated conditions (Supplementary Figure S1C, right). These results indicate that possibly the absence of CMA in LAMP2A$^{-/-}$ cells underlies their inability to emit ecto-CRT (rather than compensatory rise in ATG5-regulated macroautophagy) – a premise that needs to be comprehensively investigated.

In conclusion, we found that LAMP2A is dispensable for both MTX and Hyp-PDT-induced ATP secretion. However interestingly, we observed that the absence of LAMP2A abrogates ecto-CRT emission (in basal and MTX/Hyp-PDT treatment scenarios) – a dysfunction that cannot be rescued by siRNA-based knock-down of ATG5. In future, it would be imperative to investigate whether there is a direct link between ecto-CRT emission and CMA induction.

[1]Cell Death Research and Therapy Unit, Department for Cellular and Molecular Medicine, University of Leuven (KULeuven), Leuven, Belgium
*Corresponding author: P Agostinis, Cell Death Research and Therapy Unit, Department for Cellular and Molecular Medicine, Campus Gasthuisberg, O&N1, Herestraat 49, Box 901, 3000 Leuven, Belgium. Tel: +32 16 345715; Fax: +32 16 345995; E-mail: patrizia.agostinis@med.kuleuven.be

## Conflict of Interest

The authors declare no conflict of interest.

**Acknowledgements**. This work was supported by grants from the Fund for Scientific Research Flanders (FWO-Vlaanderen; G.0661.09, G.0728.10 and G.0584.12N) and KU Leuven (GOA/11/009) to PA; ADG is a postdoctoral fellow supported by the BOF Postdoctoral Mandate (PDM) from KU Leuven (PDMK/12/146). This paper presents research results of the IAP7/32, funded by the Interuniversity Attraction Poles Programme, initiated by the Belgian State, Science Policy Office. We thank Dr. AM Cuervo (Albert Einstein College of Medicine, NY, USA) for the LAMP2A$^{+/+}$ and LAMP2A$^{-/-}$ cells.

1. Garg AD *et al. Cell Death Differ* 2013; e-pub ahead of print 17 May 2013; doi:10.1038/cdd.2013.48.
2. Martins I *et al. Cell Death Differ* 2013; e-pub ahead of print 12 July 2013; doi:10.1038/cdd.2013.75.
3. Dudek AM *et al. Cytokine Growth Factor Rev* 2013; **24**: 319–333.
4. Garg AD *et al. Cancer Immunol Immunother* 2012; **61**: 215–221.
5. Garg AD *et al. EMBO J* 2012; **31**: 1062–1079.
6. Garg AD *et al. Autophagy* 2013; **9**: 1292–1307.
7. Dewaele M *et al. J Cell Mol Med* 2011; **15**: 1402–1414.
8. Massey AC *et al. Proc Natl Acad Sci USA* 2006; **103**: 5805–5810.



Supplementary Information accompanies this paper on Cell Death and Disease website (http://www.nature.com/cddis)