The antimicrobial peptide PFR induces necroptosis mediated by ER stress and elevated cytoplasmic calcium and mitochondrial ROS levels: cooperation with Ara-C to act against acute myeloid leukemia

TO THE EDITOR:

Antimicrobial peptides (AMPs) are an ancient class of short polypeptides present in a large number of species in nature with a variety of functions. PFR (PFWRIRIRR-NH₂) is one kind of AMP identified among the derivatives of lactoferrin. Our previous results showed that PFR inhibited the proliferation of human acute myeloid leukemia (AML) HL60 cells potentially without toxicity against normal cells. In addition, PFR induced necrosis by membrane disruption detected using scanning electron microscopy. However, the underlying mechanisms of these effects are not clearly understood.

To investigate the mechanisms involved in necrosis induced by PFR in HL60 cells (Fig. S1a–d), we found that 5(6)-FAM was taken up by HL60 cells after PFR treatment in a time-dependent manner (Fig. 1a), indicating that PFR induced the formation of permeable pores with open diameters of at least the molecular size of 5(6)-FAM (=1 nm). In addition, levels of phosphorylated RIP1, RIP3, and MLKL were increased significantly after PFR treatment (Figs. 1b and S1e), indicating that necroptosis had occurred. Furthermore, necrostatin-1 (Nec-1), a specific inhibitor of necroptosis, significantly reduced propidium iodide (PI) uptake induced by PFR (Fig. 1c).

We further synthesized green fluorescent 5-FAM-PFR and traced its dynamic location for up to 6 h (Fig. S2a). The dynamic distribution of PFR on the cytomembrane (~3–10 min) and endoplasmic reticulum (ER) (after 30 min) was clearly indicated by green and bright yellow fluorescence, respectively (Figs. 1d and S2a). The unexpected localization of PFR on the ER prompted us to detect whether PFR induces ER stress because of the fact that ER stress is involved in cell death. The expression level of the classic ER stress marker GRP78 was increased significantly among the derivatives of lactoferrin. In addition, no serious side effects and no difference in weight gain (Fig. S5d) were observed in the combined group, and no toxicity was detected in the liver (Fig. S5e) and kidney (Fig. S5f).

In summary, we found a novel mechanism by which PFR induces necroptosis through ER stress, elevated cytoplasmic calcium, and mitochondrial ROS (Fig. 1n). Furthermore, PFR can also cooperate with Ara-C to enhance the efficacy of Ara-C in vitro and in vivo. The novel molecular mechanisms of PFR used to treat AML and the efficacy of cooperation between PFR and Ara-C may provide new insights into the molecular mechanisms of AMP and a new therapeutic option to treat human AML.
Fig. 1  PFR induces necroptosis through ER stress and elevated cytoplasmic calcium and mitochondrial ROS levels and cooperates with Ara-C to act against acute myeloid leukemia. Detailed explanations for all subfigures are given in the Supplementary Information.
ACKNOWLEDGEMENTS
This work was supported by grants from the National Natural Science Foundation of China (No. 81770176), the New Century 151 Talent Project of Zhejiang Province, the 521 Talent Foundation and the Fundamental Research Funds of Zhejiang Sci-Tech University (No. 2019Y1001), and the Science Technology Department of Zhejiang Province (No. LGC19H080001).

ADDITIONAL INFORMATION
The online version of this article (https://doi.org/10.1038/s41392-019-0073-6) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

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