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Free Fatty Acids Induce Pyroptosis in Pancreatic β-Cells

Dear Editor,

Pyroptosis as a new mechanism of programmed cell death is different from the other two important programmed cell death pathways, apoptosis and necrosis. Apoptosis is a prototype of programmed cell death mediated by activation of the apoptotic caspases, which include caspase-2, -3, -6, -7, -8 and -9[1]. In the early process of apoptosis, cell exhibits shrinkage and pyknosis. Pyknosis is the result of chromatin condensation and this is the most characteristic feature of apoptosis. Finally, cells are fragmented into apoptotic bodies that are usually engulfed by surrounding macrophages, leading to the noninflammatory nature of the cell death[2]. In contrast to apoptosis, necrosis is triggered by activation of receptor-interacting protein kinase-1 (RIPK1) and RIPK3. RIPK3 phosphorylates the pseudokinase mixed lineage kinase domain-like (MLKL), causing it to translocate to the plasma membrane to induce cell permeabilization, swelling and lysis[3].

Pyroptosis is a form of necrotic and inflammatory programmed cell death induced by inflammatory caspases, namely caspase-1, -3, -4, -5 and -11[4-5]. The gasdermin family proteins, which include the GSDMA, GSDMB, GSDMC, GSDMD and DFNA5, are the key pyroptosis substrate of inflammatory caspases[6]. The gasdermin family proteins could divide into two domains, the N-terminal gasdermin-N domain and the C-terminal gasdermin-C domain, which are linked by a long loop. Activated caspases can efficiently cleave gasdermin proteins within the linking loop to release the gasdermin-N domain. Gasdermin-N domain can directly target the plasma membrane and form pores with an inner diameter of 10–14 nm, which then raptures the plasma membrane and release the cytosol contents[7].

The apoptosis of pancreatic β-cell induced by free fatty acid (FFA) is widely studied *in vivo* and *in vitro* [8-9]. However, the pyroptosis of pancreatic β-cell induced by FFA has not been reported. In this paper, we firstly identified pyroptosis as a novel pathway that partially contributes to the pancreatic β-cell death induced by FFA and revealed that the mechanism of pyroptosis could involve DFNA5.

In this study, we firstly confirmed the phenomenon of apoptosis in rat pancreatic β-cell line INS1 treatment with 0.5 mmol/L saturated free fatty acid, palmitate (PA), for 24 h using three different apoptotic assays. The results showed that after PA treatment a portion of INS1 cells exhibited a brightly nucleus, positive Annexin-V and TUNEL signals, which is consistent with characteristics of apoptotic cells (Figure 1). However, the pyroptotic cells were also exhibited the same characteristics of nucleus condensation, positive Annexin-V and TUNEL signals [10], therefore these results could not confirm whether there is pyroptosis occurred in the INS1 cells induced by PA. Since the difference between the apoptosis and pyroptosis in the plasma membrane integrity, we thereby employ fluorescent protein EGFP to indicate the plasma membrane integrity and distinguish pyroptosis from apoptosis. INS1 cells were transfected with EGFP plasmid for 24 h and then cultured with hoechst 33258 which is used to detect the death cells. After treatment with PA, INS1 cells were monitored by real-time lapse microscopy to detect the fluorescence intensity of EGFP and hoechst 33258. The results showed that after the stimulation of PA, the fluorescence intensity of Hoechst 33258 is getting stronger in a portion of cells, indicating the pyknosis and cell is going to death (Figure 1a, b). Interestingly, these cells could be divided into two types according to the trend of pyknosis and membrane integrity, one of which exhibits complete cell membrane and slow nuclear shrinkage, namely apoptosis (Figure 2a)[2]. The other type presents sudden breakage of cell membrane and rapid nuclear shrinkage, consistent with characteristics of pyroptosis

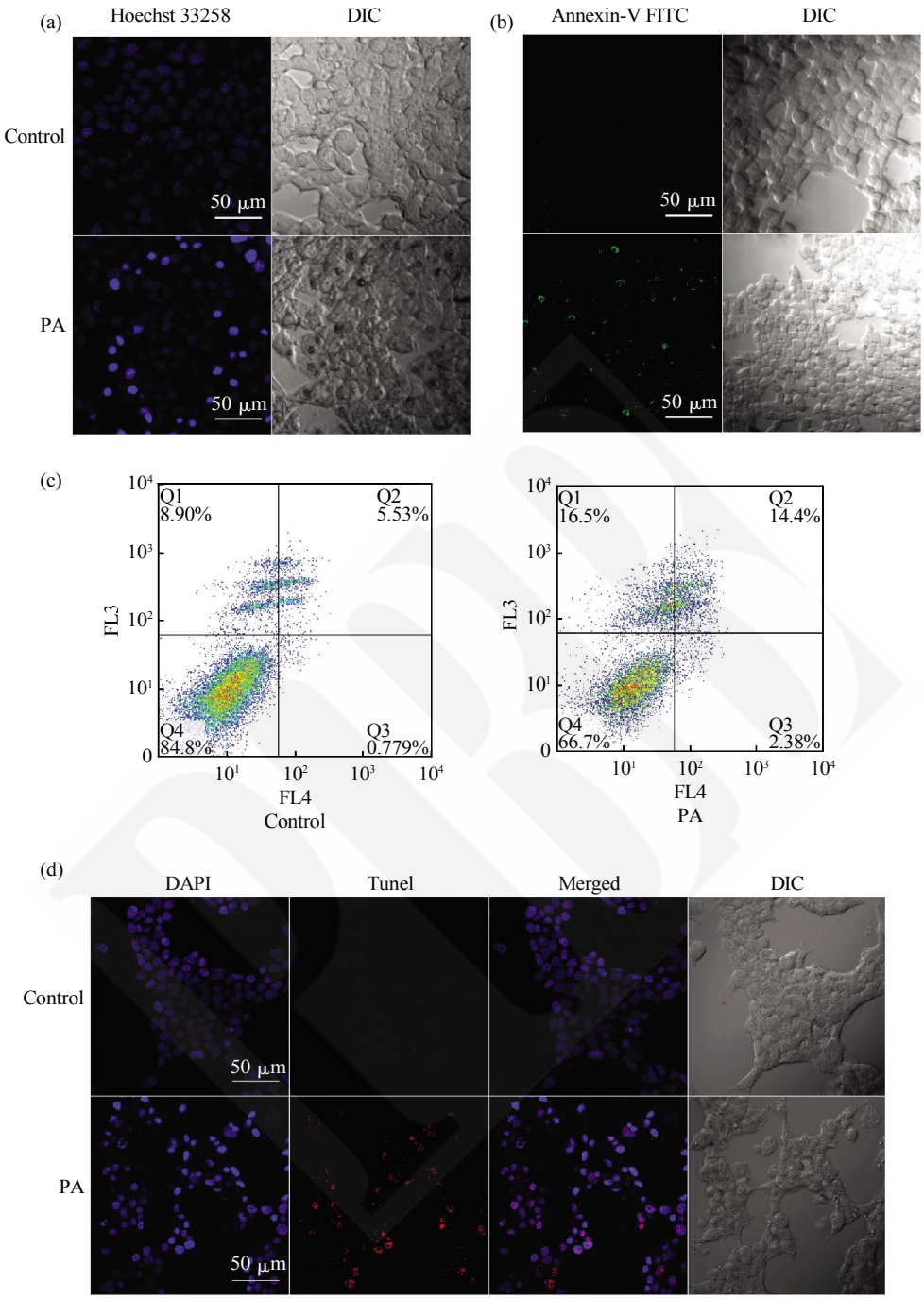

Fig. 1 The cell death of INS1 cell induced by PA

The cell death of INS1 cell was detected with Hoechst 33258 (a), Annexin-V-FITC (b, c) and TUNEL (d). (a), (b) and (d) were detected by confocal microscopy, and (c) was detected by flow cytometry.

(Figure 2b) [11]. Further quantification revealed that pyroptotic cells accounted for about ten percent of total cell count, while apoptotic cell accounted for less than twenty percent of total cell count (Figure 2c).

To further verify the pyroptosis in the INS1 cells induced by PA, we examined if the gasdermin family proteins translocate to the plasma membrane to induce cell membrane rupture after PA treatment. Firstly, we determined the expression profile of the gasdermin family proteins. Genome-wide transcriptome analysis of INS1 cells revealed that GSDMA and DNFA5 are highly expressed in the INS1 cells, while there is almost no expression for GSDMC and GSDMD (Figure 2d). Western-blot result showed that the expression of GSDMA and DNFA5 was not changed (Figure 2e). To detect whether GSDMA and DNFA5

can be targeted to the plasma membrane after PA treatment, we expressed EGFP fusion proteins to the N-terminal of GSDMA and DNFA5 in INS1 cell to address the subcellular location. The confocal image showed that EGFP-GSDMA/DNFA5 were diffused uniformly in the cytosol of INS1 cells (Figure 2f, g).

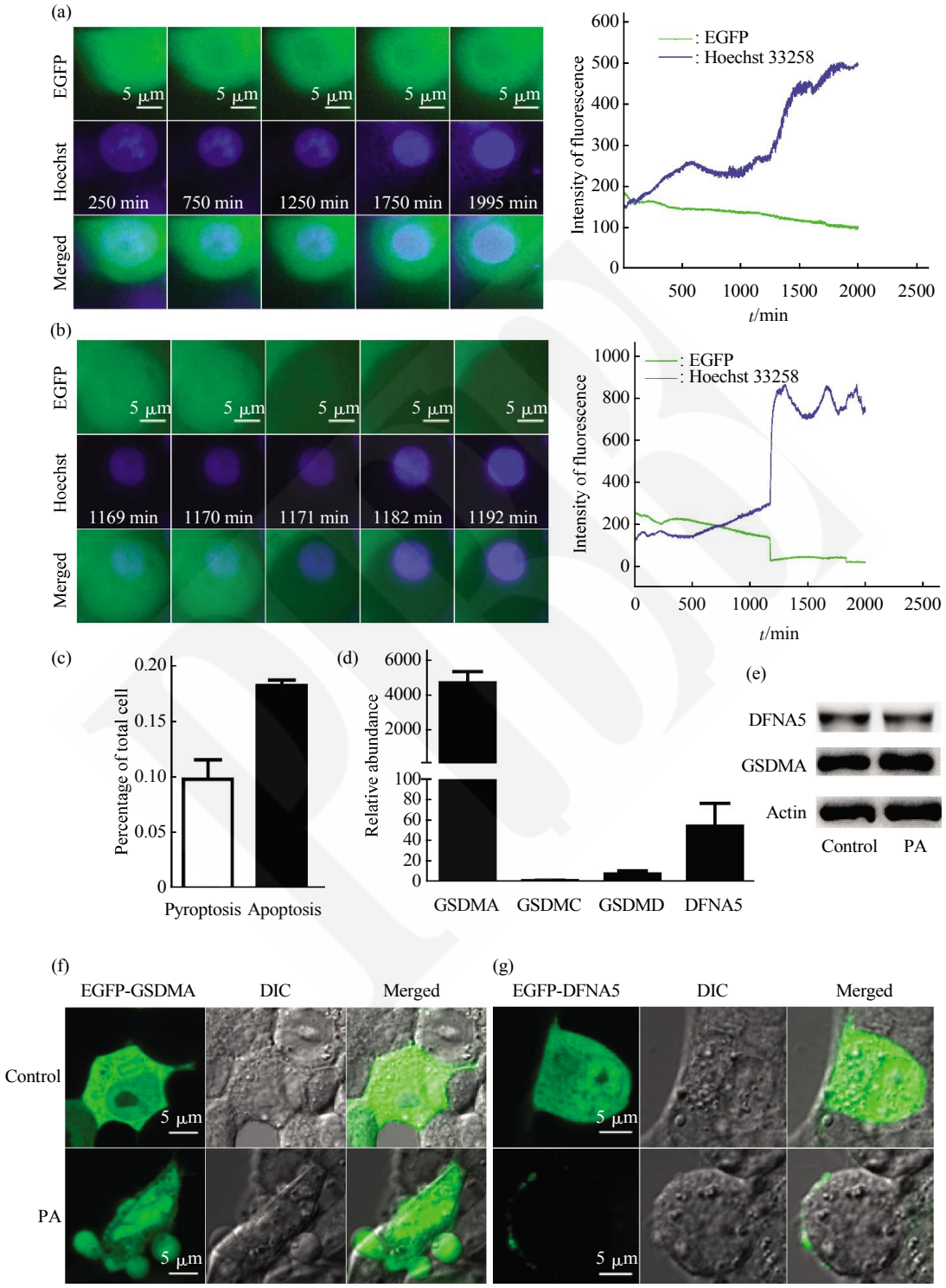

Fig. 2 The pyroptosis of INS1 cell induced by PA

(a, b) Real-time lapse images of INS1 cells treated with palmitate. Cell membrane integrity was monitored by EGFP, and nucleus condensation was monitored by hoechst 33258. Representative real-time lapse images was show in the left panel and relative intensity change of EGFP and hoechst 33258 fluorescence in one representative cell was graphed over time in the right pannel. Apoptotic cell is represented with (a) and pyroptotic cell is represented with (b). (c) Quantification of apoptotic and pyroptotic cells in total cells. Results shown are mean±SEM from three independent experiments. (d) The gasdermin family proteins expression profile in INS1 cell is dertermined by RNA-seq. Results shown are mean ±SEM from three independent experiments. (e) Western-blot detects the expression of intrinsic DFNA5 and GSDMA after PA treatment. (f, g) Confocal images of EGFP-GSDMA and EGFP-DFNA5 expression INS1 cells treated with or without palmitate for 24 h.

After PA stimulation, a portion of cells displayed multiple bubble-like protrusions namely pyroptotic bodies [11], further verified the pyroptosis in the INS1 cells. While EGFP-GSDMA in the pyroptotic cell still diffused uniformly in the cytosol (Figure 2e), EGFP-DNFA5 translocated to the plasma membrane after PA treatment (Figure 2f). These results suggested that the pyroptosis in INS1 cell induced by PA could involve DNFA5.

ER stress and oxidative stress are the main mechanism of FFA-induced apoptosis[12]. There is now more evidence that the inflammation can induced pancreatic β-cell death *in vivo* and *in vitro* [13]. Pyroptosis is a form of inflammatory programmed cell death pathway activated by caspase-1, -4, -5 and -11[6]. Now emerging evidence indicate that the caspase-3 can also trigger pyroptosis through specific cleavage of the DFNA5 [14]. In our study, we found that PA can induce the translocation of DFNA5 to the plasma membrane, suggesting that the pyroptosis induced by PA could be triggered by caspase-3 not caspase-1, consistent with the previous report that lack of caspase-1 can not provide any protection of islets in response to glucolipotoxicity [15]. Pyroptotic cells can release large amounts cytokines such as IL-1β [4]. In addition, there are ample evidences indicating that IL-1β release from the islets can attract macrophages invasion and cause dysfunction of islets [13]. So we speculate that pyroptosis plays a vital role in this process. It is thus intriguing to test whether inhibition of pyroptosis or konckout of DFNA5 could protect the islets from macrophage attacks or not in the diabetic animal models. If so, pyroptosis or DFNA5 will emerge as a promising target for developing anti-diabetic therapy.

Overall, our studies reported that the pyroptosis is one of the cell death pathways of pancreatic β-cell induced by PA and accounts for more than one third of total death cells.

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References

[1] Taylor R C, Cullen S P, Martin S J. Apoptosis: Controlled demolition at the cellular level. Nat Rev Mol Cell Biol, 2008, **9**(5): 231−241

[2] Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol, 2007, **35**(4): 495−516

[3] Linkermann A, Green D R. Necroptosis. N Engl J Med, 2014, **370**(5): 455−465

[4] Man S M, Karki R, Kanneganti T D. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. Immunol Rev, 2017, **277**(1): 61−75

[5] Man S M, Kanneganti T D. Converging roles of caspases in inflammasome activation, cell death and innate immunity. Nat Rev Immunol, 2016, **16**(1): 7−21

[6] Shi J, Gao W, Shao F. Pyroptosis: Gasdermin-mediated programmed necrotic cell death. Trends Biochem Sci, 2017, **42**(4): 245−254

[7] Ding J, Wang K, Liu W, *et al*. Pore-forming activity and structural autoinhibition of the gasdermin family. Nature, 2016, **535**(7610): 111−116

[8] Poitout V, Robertson R P. Glucolipotoxicity: Fuel excess and beta-cell dysfunction. Endocrine Reviews, 2008, **29**(3): 351−366

[9] Giacca A, Xiao C, Oprescu A I, *et al*. Lipid-induced pancreatic beta-cell dysfunction: Focus on *in vivo* studies. Am J Physiol Endocrinol Metab, 2011, **300**(2): E255-262

[10] Wang Y, Gao W, Shi X, *et al*. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. Nature, 2017, **547**(7661): 99−103

[11] Chen X, He W T, Hu L, *et al*. Pyroptosis is driven by non-selective gasdermin-d pore and its morphology is different from mlkl channel-mediated necroptosis. Cell Research, 2016, **26**(9): 1007−1020

[12] Biden T J, Boslem E, Chu K Y, *et al*. Lipotoxic endoplasmic reticulum stress, beta cell failure, and type 2 diabetes mellitus. Trends Endocrinol Metab, 2014, **25**(8): 389−398

[13] Donath M Y, Boni-Schnetzler M, Ellingsgaard H, *et al*. Islet inflammation impairs the pancreatic β-cell in type 2 diabetes. Physiology, 2009, **24**(6): 325-331

[14] Rogers C, Fernandes-Alnemri T, Mayes L, *et al*. Cleavage of dfna5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. Nat Commun, 2017, **8**: 14128

[15] Wali J A, Gurzov E N, Fynch S, *et al*. Activation of the nlrp3 inflammasome complex is not required for stress-induced death of pancreatic islets. Plos One, 2014, **9**(11): e113128