Postprint: Effects of L-Theanine on Hydrogen Peroxide-Induced Apoptosis in Passaged Goat Rumen Epithelial Cells

Authors: Jie Hongdong, Han Qipeng, Tan Zhiliang, Zhou Chuanshe, Kong Zhiwei, Chen Liang, Ren Ao

Date: 2017-10-23T00:00:00+00:00

Abstract

This experiment established a hydrogen peroxide (H2O2)-induced apoptosis model in goat rumen epithelial passaged cells to investigate the effects of L-theanine on the apoptosis rate and expression levels of key genes in the apoptosis pathway. Rumen epithelial passaged cells from 42-day-old Xiangdong black goats were cultured in DMEM/F12 medium containing 5% fetal bovine serum (FBS). When cell density reached 60%–70%, they were randomly divided into five groups: a control group without additional supplements, Group I with 800 μmol/L H2O2, Group II with 800 μmol/L H2O2 + 4 mmol/L L-theanine, Group III with 800 μmol/L H2O2 + 8 mmol/L L-theanine, and Group IV with 800 μmol/L H2O2 + 16 mmol/L L-theanine, with three replicates per group. After 12 h of treatment, flow cytometry (FCM) was used to detect the apoptosis rate of goat rumen epithelial passaged cells, and real-time quantitative PCR (RT-qPCR) was employed to determine the expression levels of key apoptosis pathway genes including cysteine-aspartic acid protease-3 (Caspase-3), cysteine-aspartic acid protease-8 (Caspase-8), cysteine-aspartic acid protease-9 (Caspase-9), Fas-associated death domain protein (FADD), and apoptotic protease-activating factor (Apaf-1). The results showed: 1) According to annexin V/propidium iodide (PI) co-staining results, compared with Group I, the late apoptosis rate in each L-theanine supplemented group (Groups II, III, and IV) was significantly decreased ($P<0.05$), and the late apoptosis rate gradually decreased with increasing L-theanine concentration. 2) According to PCR detection results, compared with Group I, the expression levels of Caspase-3, Caspase-9, and Apaf-1 genes in each L-theanine supplemented group were significantly decreased ($P<0.05$). It was concluded that L-theanine exerts a protective effect against H2O2-induced apoptosis in goat rumen epithelial passaged cells, and these results may provide technical support and theoretical reference

for future research on the mechanism of oxidative stress injury in the rumen of ruminant livestock.

Full Text

Preamble

Effects of L-Theanine on Subculture Ruminal Epithelial Cells Apoptosis Induced by Hydrogen Peroxide in Goats

JIE Hongdong[1,2], HAN Qipeng[1], TAN Zhiliang[2,3], ZHOU Chuanshe[2,3]*, KONG Zhiwei[2], CHEN Liang[2], REN Ao[1]
(1. College of Horticulture & Landscape, Hunan Agricultural University, Changsha 410128, China;
2. Key Laboratory for Agro-ecological Processes in Subtropical Region, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Hunan Research Center of Livestock & Poultry Sciences, South-Central Experimental Station of Animal Nutrition and Feed Science of Ministry of Agriculture, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China;
3. Hunan Co-Innovation Center of Animal Production Safety, Changsha 410128, China)

Abstract: This study established a hydrogen peroxide (H_2O_2)-induced apoptosis model in subculture ruminal epithelial cells of goats to investigate the effects of L-theanine on apoptosis ratio and expression of key genes in the apoptosis pathway. Subculture ruminal epithelial cells from 42-day-old Xiangdong black goats were cultured in DMEM/F12 medium containing 5% fetal bovine serum (FBS). When cell density reached 60–70%, cells were randomly divided into five groups: a control group without supplementation, Group I with 800 μmol/L H_2O_2, Group II with 800 μmol/L H_2O_2 + 4 mmol/L L-theanine, Group III with 800 μmol/L H_2O_2 + 8 mmol/L L-theanine, and Group IV with 800 μmol/L H_2O_2 + 16 mmol/L L-theanine, with three replicates per group. After 12 hours of treatment, flow cytometry (FCM) was used to detect apoptosis ratio, and real-time quantitative PCR (RT-qPCR) was employed to measure expression of key apoptosis genes including cysteinyl aspartate-specific proteinase-3 (Caspase-3), cysteinyl aspartate-specific proteinase-8 (Caspase-8), cysteinyl aspartate-specific proteinase-9 (Caspase-9), Fas-associated death domain protein (FADD), and apoptotic protease activating factor-1 (Apaf-1). Results showed: (1) Annexin V/propidium iodide (PI) co-staining revealed that late apoptosis ratio in L-theanine supplementation groups (II, III, and IV) was significantly lower than Group I ($P < 0.05$), decreasing progressively with increasing L-theanine concentration. (2) PCR analysis demonstrated that Caspase-3, Caspase-9, and Apaf-1 gene expression levels were significantly reduced in all L-theanine supplementation groups compared to Group I ($P < 0.05$). These findings indicate that L-theanine exerts protective effects against H_2O_2-induced apoptosis in goat ruminal epithelial cells, providing technical support and the-

oretical reference for future research on oxidative stress damage mechanisms in the rumen of ruminant livestock.

Keywords: L-theanine; H_2O_2; goats; subculture ruminal epithelial cells; apoptosis

L-theanine, systematically named 5-N-ethyl-γ-glutamine or γ-glutamyl-L-ethylamide, does not participate in protein synthesis and exists in free form, accounting for 40–70% of total free amino acids in tea leaves [1]. Naturally occurring theanine is generally the L-form, which has been shown to protect against brain tissue injury [2] and liver tissue damage [3-6]. Additionally, L-theanine possesses multiple biological activities and pharmacological effects [7]. Studies have demonstrated that theanine (400 μg/mL) can cause 50% death in various cancer cell lines including HepG hepatoma cells, MCF-7 breast cancer cells, HT29 colon cancer cells, and PC-3 prostate cancer cells [8-9]. Theanine also effectively inhibits proliferation and induces apoptosis in Hela cells in a concentration- and time-dependent manner, suggesting its antitumor activity may involve suppression of glutamate metabolism in Hela cells [10]. Research on the effects of theanine on proliferation, apoptosis, and anti-angiogenesis of nasopharyngeal carcinoma CNE2 cells in vitro and in vivo indicated that theanine can inhibit tumor growth and angiogenesis while promoting tumor cell apoptosis, thereby suppressing CNE2 cell proliferation in a time- and dose-dependent manner [11]. Furthermore, Lei Mingsheng [12] found that theanine antagonizes bombesin-induced inhibition of dendritic cell (DC) surface molecule expression and interleukin-12 (IL-12) secretion, and studies on apoptosis rates and morphology suggest that theanine promotes maturation and function of suppressed DCs, indicating its potential to antagonize tumor environment-mediated DC inhibition.

Oxidative stress represents a significant health challenge in weaned young livestock, with symptoms such as lethargy, diarrhea, and reduced feed intake being associated with oxidative stress damage. Maintaining or enhancing normal physiological functions to mitigate oxidative stress injury is a primary approach to ensuring healthy growth in weaned livestock. Therefore, this study utilized hydrogen peroxide (H_2O_2) as an oxidative stress factor to establish an apoptosis model in subculture ruminal epithelial cells from weaned Xiangdong black goats, and investigated the effects of L-theanine on H_2O_2-induced apoptosis and expression of key apoptosis pathway genes [cysteinyl aspartate-specific proteinase-3 (Caspase-3), cysteinyl aspartate-specific proteinase-8 (Caspase-8), cysteinyl aspartate-specific proteinase-9 (Caspase-9), apoptotic protease activating factor-1 (Apaf-1), and Fas-associated death domain protein (FADD)]. The objective was to provide reference for understanding the relationship between nutritional metabolism and oxidative stress mechanisms in goat ruminal epithelial cells, thereby contributing theoretical foundations for sustainable livestock development.

1.1 Main Reagents

Cell culture reagents including fetal bovine serum (FBS), DMEM/F12 medium, trypsin-EDTA solution (0.25% trypsin + 0.02% EDTA), penicillin-streptomycin, epidermal growth factor (EGF), and insulin were purchased from Gibco (USA). Gentamicin/amphotericin B solution (R-015-10, 10$×$1 mL) was obtained from Thermo Fisher Scientific (USA), and L-theanine was purchased from Orgentec (Germany). For RT-qPCR, agarose was from Shanghai Macklin Biochemical Technology; the reverse transcription kit was from Beijing CoWin Biosciences; diethylpyrocarbonate (DEPC) and ethidium bromide (EB) solution were from Beijing SBS Genetech; TRIzol was from Invitrogen (USA); Taq polymerase, DL2000 DNA Marker, and dNTPs were from MBI Fermentas (USA); primers were synthesized by Nanjing GenScript Biotechnology; Sybgreen PCR Mix was from ABI (USA); and routine chemical reagents were from Beijing Chemical Reagents Company.

1.2.1 Grouping and Treatment of Subculture Ruminal Epithelial Cells

Three healthy 42-day-old Xiangdong black goats weighing (6.4 ± 0.8) kg were used in this study. After anesthesia, the goats were euthanized by jugular venous exsanguination, and rumen tissues were collected. The contents were removed, and tissues were rinsed repeatedly with physiological saline for sample collection [13]. Subculture ruminal epithelial cells were cultured in DMEM/F12 medium. When primary cells reached 80–90% confluence, the supernatant was discarded, and cells were washed 1–2 times with phosphate-buffered saline (PBS) before adding 1 mL of digestion solution containing 0.25% trypsin + 0.02% EDTA. After incubating in a 5% CO_2 incubator at 37 °C for 2–3 minutes, cells were observed under an inverted microscope. When cells began to brighten and round up, digestion was immediately terminated with DMEM/F12 medium containing 5% FBS. Adherent cells were pipetted into suspension, transferred to 15 mL centrifuge tubes, and centrifuged at 94 × g for 5 minutes at 4 °C. The supernatant was discarded, and cells were resuspended in 1 mL of complete DMEM/F12 medium (containing 5% FBS, 10 μg/mL insulin, 10 ng/mL EGF, 100 U/mL penicillin, 100 mg/mL streptomycin, 2.5 μg/mL gentamicin, and 2.5 μg/mL amphotericin B) for passaging at a 1:2 ratio. After 30 minutes of culture in a 5% CO_2 incubator at 37 °C, the cell-containing complete medium was transferred to culture dishes for continued culture, and this process was repeated once for further purification [14]. Purified subculture ruminal epithelial cells were maintained in DMEM/F12 medium with 5% FBS. When cell density reached 60–70%, cells were randomly divided into five groups: control group without supplementation, Group I with 800 μmol/L H_2O_2, Group II with 800 μmol/L H_2O_2 + 4 mmol/L L-theanine, Group III with 800 μmol/L H_2O_2 + 8 mmol/L L-theanine, and Group IV with 800 μmol/L H_2O_2 + 16 mmol/L L-theanine [4-5], with three replicates per group. After 12 hours of treatment, indicators were measured.

1.2.2 Detection of Apoptosis Ratio by Flow Cytometry

A BD FACSCalibur flow cytometer was used to detect apoptosis ratio via annexin V/propidium iodide (PI) co-staining in flow cytometry (FCM) [15].

1.2.3 PCR Detection of Apoptosis Gene Expression

Total RNA extraction was performed using the TRIzol one-step method [16-17], followed by DNase I treatment according to published protocols [18-19]. Reverse transcription was conducted following the M-MLV kit instructions [20-22].

1.2.4 Primer Design and Synthesis

1.2.4.1 Primer Synthesis

Based on sequencing results of new genes, primers were designed using molecular biology software including Primer Premier 5, Primer 3.0, and Oligo 6.71, with specificity verified using BLAST. PCR was used to detect tissue specificity, with primers listed in , synthesized by Shanghai Sangon Biotechnology.

1.2.4.2 RT-qPCR Reaction Conditions and Product Detection

The RT-PCR reaction system was established in a sterile 0.2 mL tube as shown in . The RT-qPCR program consisted of: 94 °C pre-denaturation for 5 min; 35 cycles of 94 °C denaturation for 30 s, 55 °C annealing for 45 s, and 72 °C extension for 2 min; followed by final extension at 72 °C for 10 min.

After RT-qPCR, 18 μL of PCR product was mixed with 2 μL of bromophenol blue for loading, with DL2000 DNA Marker as reference. Electrophoresis was performed on 2% agarose gel (containing 0.5 μg/mL EB) in 1×TAE buffer at 5 V/cm, and gels were photographed using a gel imaging system.

1.3 Statistical Analysis

Apoptosis data were collected using a BD FACSCalibur flow cytometer and analyzed with Flowjo software. Data were statistically analyzed using SAS 8.2 General Linear Model (GLM) [23-24], with contrast statements comparing the model group (Group I) versus control and among H_2O_2-treated groups (I, II, III, IV). LSMEANS was used to calculate mean values, and orthogonal polynomial contrasts were applied to examine linear or quadratic effects. SAS 8.2 IML procedure was used to correct coefficients before orthogonal polynomial analysis [25]. $P < 0.05$ was considered statistically significant, and $0.05 \leq P < 0.10$ indicated a significant trend.

2.1 Effects of L-Theanine on Apoptosis Ratio of H_2O_2-Induced Subculture Ruminal Epithelial Cells

As shown in [Figure 1: see original paper] and , late apoptosis ratio in Groups II, III, and IV was significantly lower than in Group I ($P < 0.05$), decreasing progressively with increasing L-theanine concentration, with significant differences among all groups ($P < 0.05$). These results demonstrate that L-theanine alleviates oxidative stress damage caused by H_2O_2 in goat ruminal epithelial cells.

[Figure 1: see original paper]

2.2.1 Total RNA Extraction Results

Electrophoretograms of partial total RNA samples are shown in [Figure 2: see original paper], where clear 28S and 18S bands indicate good RNA quality suitable for subsequent PCR analysis.

[Figure 2: see original paper]

2.2.2 Effects of L-Theanine on Expression of Key Genes in Apoptosis Pathway

As presented in , expression levels of Caspase-3, Caspase-9, and Apaf-1 were significantly reduced in Groups II, III, and IV compared to Group I ($P < 0.05$), while Caspase-8 expression was significantly decreased in Groups II and III ($P < 0.05$).

3.1 Effects of L-Theanine on Apoptosis Ratio of H_2O_2-Induced Subculture Ruminal Epithelial Cells

High oxygen concentrations directly damage ruminal epithelial cells, promoting apoptosis or exacerbating gastric diseases. Excessive oxidative free radicals represent a major cause of cellular injury, induced by ischemia-reperfusion, drug metabolism, and heavy metal poisoning [26]. H_2O_2, as a primary component of oxidative free radicals, is widely used in oxidative stress models [27-29]. Reducing H_2O_2-induced apoptosis ratio is crucial for alleviating oxidative stress damage and improving physiological function. Our apoptosis detection results demonstrate that L-theanine mitigates H_2O_2-induced oxidative stress injury in goat ruminal epithelial cells. The mechanism may involve L-theanine, as a glutamine derivative, being metabolized to glutamate and participating in glutathione (GSH) synthesis. Additionally, L-theanine may maintain intracellular antioxidant enzyme activity, reducing GSH consumption while increasing its production, thereby stabilizing cellular GSH content and maintaining redox balance to protect against oxidative stress damage [30].

3.2 Effects of L-Theanine on Expression of Key Genes in Apoptosis Pathway

This study investigated L-theanine effects on expression of apoptosis genes (FADD, Caspase-8, Apaf-1, Caspase-9, Caspase-3) in H_2O_2-induced goat ruminal epithelial cells. Results showed that compared to Group I, Caspase-3, Caspase-9, and Apaf-1 expression were significantly reduced in Groups II, III, and IV, while Caspase-8 expression was significantly decreased in Groups II and III. These findings indicate: (1) L-theanine inhibits apoptosis in subculture ruminal epithelial cells; (2) L-theanine reduces apoptosis ratio in a dose-dependent manner by downregulating mitochondrial pathway-related gene expression; and (3) L-theanine decreases apoptosis ratio through the extrinsic pathway within a certain concentration range, but not by directly stimulating extrinsic genes (FADD). The protective mechanisms involve both mitochondrial and extrinsic pathways: (a) Mitochondrial pathway: Reduced cytochrome C (CytC) binding to Apaf-1 decreases apoptosome formation, limiting pro-Caspase-9 recruitment and activation, thereby reducing active Caspase-9 and subsequent Caspase-3 activation. This attenuates PARP cleavage, decreases DNA fragmentation into n×(180-200) bp fragments, and preserves cytoskeletal, nuclear, and extracellular matrix protein integrity, reducing apoptosis [31]. (b) Extrinsic pathway: Death receptor-mediated apoptosis involves death ligand binding to receptors, which recruit adaptor proteins via death domains (DD) to form death-inducing signaling complexes (DISC). L-theanine reduces Fas/TRAIL-mediated FADD and pro-Caspase-8 recruitment, decreasing Caspase-8 activation. In Type I cells, reduced Caspase-8 activity limits Caspase-3 activation, while in Type II cells, insufficient Caspase-8 activation prevents adequate apoptosis induction. Additionally, reduced Caspase-8-mediated BID cleavage to truncated BID (tBID) diminishes mitochondrial apoptosis pathway activation, preventing death signal amplification. Consequently, decreased tBID translocation to the mitochondrial outer membrane reduces Bak oligomerization and mitochondrial apoptosis pathway activation [33-34]. All L-theanine concentrations significantly reduced late apoptosis ratio in a dose-dependent manner, demonstrating effective protection against H_2O_2-induced oxidative damage in goat ruminal epithelial cells.

References

[1] MUKAI T, HORIE H, GOTO T. Differences in free amino acids and total nitrogen contents among various prices of green tea[J]. Tea Research Journal, 1992, 1992(76): 45–50.
[2] PENG Bin, LIU Zhonghua, LIN Yong, et al. Study on the effect of L-theanine on improving depressive behavior in chronic stress rats[J]. Journal of Tea Science, 2014, 34(4): 355–363.
[3] GU Zuwei. Introduction to Modern Toxicology[M]. Beijing: Chemical Industry Press, 2005: 157.
[4] LI Guilan, KANG Jingjing, YIN Zhimin. Protective effect of L-theanine on H_2O_2-induced L02 cell injury and its mechanism[J]. Chinese Journal of Cell

Biology, 2014, 36(6): 785–790.
[5] JIANG Wei. Study on L-theanine inhibiting carbon tetrachloride-induced acute liver injury in mice[D]. Master' s Thesis. Nanjing: Nanjing University, 2012.
[6] BUKOWSKI J F, PERCIVAL S S. L-theanine intervention enhances human $\gamma\delta$ T lymphocyte function[J]. Nutrition Reviews, 2008, 66(2): 96–102.
[7] KHAN W, MUKHTAR H. Tea polyphenols health promotion[J]. Life Sciences, 2007, 81(7): 519–533.
[8] FRIEDMAN M, MACKRY B E, KIM H J, et al. Structure-activity relationships of tea compounds against human cancer cells[J]. Journal of Agricultural & Food Chemistry, 2007, 55(2): 243–253.
[9] BALEEIRO R B, ANSELMO L B, SOARES F A, et al. High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor-α in lung cancer[J]. Cancer Immunology, Immunotherapy, 2008, 57(9): 1335–1345.
[10] SUN Lingyan, YIN Cui, ZHANG Ling, et al. Effect of theanine on proliferation of human cervical cancer Hela cells[J]. Lishizhen Medicine and Materia Medica Research, 2012, 23(3): 643–645.
[11] LI Yandong, ZHENG Jianyun, LIU Bing, et al. Study on the effect of theanine on proliferation and apoptosis of nasopharyngeal carcinoma CNE2 cells[J]. Journal of Shanxi Medical University, 2011, 42(8): 633–635.
[12] LEI Mingsheng. Study on theanine stimulating dendritic cells to regulate T lymphocytes and inhibit lung adenocarcinoma cell growth[D]. Doctoral Dissertation. Changsha: Central South University, 2009.
[13] HAN Qipeng, LUO Ling, JIE Hongdong, et al. Characteristics of cell cycle distribution, proliferation and apoptosis of ruminal epithelial cells in Liuyang black goats[J]. Chinese Journal of Animal Nutrition, 2016, 28(7): 2269–2277.
[14] LIU Sile, KANG Jinhe, TAN Zhiliang, et al. Effects of different culture methods on growth and cytokeratin 18 expression of goat ruminal epithelial cells[J]. Chinese Journal of Animal Nutrition, 2016, 28(4): 1225–1232.
[15] HAN Qipeng, JIE Hongdong, LUO Ling, et al. Effects of glutamine and its dipeptide on H_2O_2-induced apoptosis and apoptosis-related gene expression in goat ruminal epithelial cells[J]. Chinese Journal of Animal Nutrition, 2016, 28(10): 3301–3310.
[16] FAN Shanji. Effect of tanshinone IIA on proliferation and apoptosis of SW480 cells[D]. Master' s Thesis. Hengyang: University of South China, 2014.
[17] TANG Lili. Changes in regulatory T cell numbers during early obesity in mice and their regulatory effect on macrophage subtypes in visceral adipose tissue[D]. Master' s Thesis. Changsha: Central South University, 2014.
[18] LEI Zhen. Construction of TLR4, MD2 and CD14 gene stably expressed cell lines for drug pyrogen detection platform[D]. Master' s Thesis. Yinchuan: Ningxia Medical University, 2014.
[19] XIE Tao. Effects of herbal cake-separated moxibustion on cholesterol reverse transport via TLR2/4-IRF3 signaling pathway in atherosclerotic rabbits[D]. Master' s Thesis. Changsha: Hunan University of Chinese Medicine, 2015.

[20] WANG Yaoyao, LI Ran, LI Yunsheng, et al. Effects of Nesfatin-1 on gonadotropins and their mRNA expression in pubertal female rats[J]. Journal of China Agricultural University, 2015, 20(3): 139-145.
[21] JIA Yu. Effects of Nucleostemin downregulation on PI3K/AKT/mTOR pathway-related genes in HL-60 leukemia cells[D]. Master' s Thesis. Zhengzhou: Zhengzhou University, 2014.
[22] HUANG Guoren. Study on the effect and mechanism of 1,3-dichloro-2-propanol on lipid metabolism in mice[D]. Master' s Thesis. Changjin: Jilin University, 2014.
[23] MOU Guoxue. Admissibility characteristics and optimality of linear estimation in general linear models[D]. Master' s Thesis. Beijing: Beijing Jiaotong University, 2008.
[24] GAO Shuxin, XU Shangzhong, LI Jinquan, et al. Analysis of factors affecting mastitis in dairy cows using general linear model[J]. Acta Agriculturae Boreali-Sinica, 2006, 21(4): 114-117.
[25] WANG Qinxiu. General linear regression model—parameter estimation, hypothesis testing, regression prediction[J]. Quantitative & Technical Economics, 1988(2): 39-44.
[26] YE Xuting, SHA Jihong, TANG Ying, et al. Exploration of oxidative stress models in biological cells[J]. Journal of Chinese Electron Microscopy Society, 2000, 19(3): 207-208.
[27] CAI Shanrong, ZHENG Shu, ZHANG Suzhan, et al. Establishment of DNA oxidative damage model in intestinal epithelial stem cells induced by hydrogen peroxide[J]. Journal of Zhejiang University: Medical Sciences, 2006, 35(4): 366-369.
[28] LIAO Derong, LIU Qigong, CHENG Yanzi, et al. Effect of vascular endothelial growth factor on H_2O_2-induced endothelial cell apoptosis and its mechanism[J]. Chinese Journal of Arteriosclerosis, 2006, 14(6): 483-485.
[29] ZHENG Yansong, LI Yuan, ZHANG Shanhong, et al. Establishment of myocardial cell oxidative injury model using low concentration hydrogen peroxide[J]. Journal of Fourth Military Medical University, 2001, 22(20): 1849-1851.
[30] YE Yin. Study on L-theanine inhibiting alcoholic liver injury by enhancing antioxidant activity in liver cells[D]. Master' s Thesis. Nanjing: Nanjing University, 2011.
[31] LONG S, WILSON M, BENGTÉN E, et al. Identification and characterization of a FasL-like protein cDNAs encoding channel catfish death-inducing signaling complex[J]. Immunogenetics, 2004, 56(7): 518-530.
[32] CHEN Yihua, ZHANG Huajie, NAN Fajun. Research progress on caspase inhibitors[J]. Chinese Bulletin of Life Sciences, 2006, 18(3): 247-254.
[33] YUAN Haibo, SHEN Zhongming. Molecular mechanism of cell apoptosis[J]. Journal of Shanghai University: Natural Science, 2001, 7(1): 77-80.
[34] TAIT S W G, GREEN D R. Mitochondrial regulation of cell death[J]. Cold Spring Harbor Perspectives Biology, 2013, 5(9): a008706.

Note: Figure translations are in progress. See original paper for figures.