*Journal of Biotechnology* **16**(4): 595-601, 2018

# VITAMIN C INHIBITED FASL-INDUCED APOPTOTIC DEATH OF MOUSE DENDRITIC CELLS THROUGH C-FLIP EXPRESSION

**Nguyen Thi Xuan**[✉,*], **Le Thi Thu Hien**[✉,*]

*Institute of Genome Research, Vietnam Academy of Science and Technology*

[*] Shared corresponding authorship
[✉] To whom correspondence should be addressed. E-mail: xuannt@igr.ac.vn and hienlethu@igr.ac.vn

Received: 19.10.2018
Accepted: 28.12.2018

SUMMARY

Vitamin C (VitC) is a potent antioxidant and contributes as an apoptosis inhibitor by preventing death receptor-triggered caspase 8 activity. Fas ligand (FasL) induces the apoptotic cell death via activation of Fas signaling, which is dependent on the expression level of anti-apoptotic molecule c-FLIP (FADD-like IL-1beta-converting enzyme-inhibitory proteins). The present study addressed the effects of VitC on survival of dendritic cells (DCs), a regulator of innate and adaptive immunity. To this end, mouse bone marrow cells were isolated and cultured to attain bone marrow-derived DCs (BMDCs). The cells were treated with FasL in the presence or absence of VitC. Real time RT-PCR, Western blotting and FACS analysis were performed to determine different hallmarks of DC apoptosis. As a result, FasL treatment resulted in activation of caspase 8 and stimulation of cell membrane scrambling, the effects were supressed when VitC was present in the cell culture or the cells were transfected with FLIP siRNA. In conclusion, VitC prevented FasL-triggered DC apoptosis mediated through the expression of c-FLIP.

**Keywords:** *c-FLIP; dendritic cell; Fas ligand; phosphatidylserine; vitamin C*

## INTRODUCTION

Dendritic cells (DCs) are the most potent antigen-presenting cells in the immune system. After capture of foreign antigens, they migrate to the lymphoid organs where DCs become mature and present antigens to naïve T cells to elicit the effective adaptive immune response (Llanos *et al.,* 2011; Xuan et al., 2016). These mature DCs are then induced to undergo apoptosis and disappear from the lymph nodes (LNs) (Llanos et al., 2011; Xuan et al., 2016). The apoptosis is initiated through two major apoptotic pathways: the death receptor-mediated extrinsic pathway and the mitochondrial-involved intrinsic pathway (Martino, 2007).

The death receptor-mediated DC apoptosis is triggered by a binding with respective ligand, leading to the programed cell-death (Xuan et al., 2010). A death receptor named Fas is abundantly expressed on immature DCs and significantly increased when the cells mature (Ashany et al., 1999). The interaction of Fas and Fas ligand (FasL) induces differentiation of naïve T cells by activation of intracellular signalling pathways in DCs (Suss et al., 1996) and is followed by activation of cysteinyl aspartate-specific protease (caspase) cascade and suicidal cell death (Buonocore et al., 2002). The sensitivity levels of Fas-triggered apoptosis is dependent on the expression of c-FLIP (FADD-like IL-1beta-converting enzyme-inhibitory proteins) (Golan-Gerstl et al., 2012), whose level is regulated by TRAIL (TNF-related apoptosis-inducing ligand (Sun et al., 2015), BIN1 (bridging integrator 1) (Esmailzadeh et al., 2015) and estradiol (Jaita et al., 2016). The c-FLIP elicits anti-inflammatory effects by supressing transcription of inflammation-related genes such as tumor necrosis factor (TNF)-α and interleukin (IL)-2, which are secreted by mature DCs, therefore this protein contributes as an inhibitor of mouse DC maturation and activation (Wu et al., 2015).

The effect of c-FLIP on the apoptotic death is different from other cell types. This protein participates in inhibiting extrinsic apoptotic pathways by preventing the activation of caspase 8 in mouse embryonic fibroblasts (Conti et al., 2016),

whereas its effect on caspase-8 activity in mouse DCs is not observed (Wu et al., 2015). In addition to its effects on the death receptor-dependent pathway, the expression of c-FLIP is also involved in protection against the apoptotic intrinsic pathway, a mitochondrion-dependent apoptosis regulated by Bcl-2 family proteins in human melanoma cells (Hamai et al., 2006). Increased expression of anti-apoptotic molecules and decreased expression of pro-apoptotic molecule blocks the mitochondrial depolarization (Lucken-Ardjomande et al., 2005) to promote cell survival.

Vitamin C (VitC) is well known as natural antioxidant and plays a role in counteracting oxidative stress and cellular damage by scavenging oxygen-derived free radicals including ROS (reactive oxygen species) production (Habu et al., 2015). Thus, VitC has been described as an inhibitor of the programed cell death. VitC supplementation blocks Fas-mediated apoptosis through reduced activity of caspase cascade and diminished level of ROS in cancer cells as well as in human monocytes (Jeong et al., 2016; Perez-Cruz et al., 2003). Moreover, VitC functions as a stimulator of immune response by eliciting some anti-inflammatory activity in DCs (Jeong et al., 2014; Kim et al., 2012). By the presence of VitC mature DCs produce enhanced level of IL-12p70 to promote the differentiation of $CD8^+$ memory T cell (Jeong et al., 2014) and VitC upregulates expression of costimulatory and antigen-presenting molecules in DC line (Kim et al., 2012).

Although the anti-apoptotic effect of VitC on other cells is well documented, the effect of VitC on apoptosis of DCs has not been reported. Thus, the present study explored whether VitC influences survival of DCs. To this end, bone marrow derived mouse DCs (BMDCs) were treated with FasL in the presence or absence of VitC and different hallmarks of apoptosis were determined.

## MATERIALS AND METHODS

### Mice

Wild type pathogen-free BALB/c mice at the age of 6 to 8 weeks were purchased from Taconic Farms (Hudson, NY, USA) and housed in a specific pathogen-free facility at Institute of Genome Research. The animals had free access to food and drinking water. Animal care and experimental procedures were performed according to the Vietnamese law for the welfare of animals and were approved by the ethical committee of Institute of Genome Research.

### Bone marrow-derived DCs

BALB/c mice were anesthetized with isoflurane gas and bone marrow cells were flushed out of the cavities from the femur and tibia with sterile PBS (pH=7.4). Cells were washed twice with RPMI-1640 and seeded out at a density of $4 \times 10^6$ cells per 60-mm dish. Cells were cultured for 8 days in RPMI-1640 (GIBCO, Grand Island, NY, USA) containing: 10% FCS, 1% penicillin/streptomycin, 1% glutamine, 1% non-essential amino acids (NEAA) and 50 µm β-mercaptoethanol. Cultures were supplemented with GM-CSF (35 ng/mL, Sigma Aldrich, St. Louis, MO, USA) and fed with fresh medium containing GM-CSF on days 3 and 6. Nonadherent and loosely adherent cells were harvested after 8 days of culture. Experiments were performed on days 8-10. BMDCs were treated with FasL (500 ng/ml, Sigma Aldrich, St. Louis, MO, USA) in the presence or absence of VitC (100 ng/ml, Sigma Aldrich).

### FLIP small interfering RNA (siRNA)

FLIP-targeted siRNA (Santa Cruz) was transfected into DCs ($10^5$ cells/ 1ml) at a final concentration of 100 nM using Nucleofector technology (Lonza, GA, USA) according to the manufacturer's recommendations. After electroporation, cells were incubated for 24 h at 37°C, 5% $CO_2$. After washing three times with PBS the cells were treated for 24 h with FasL in the presence or absence of VitC.

### RNA extraction and real-time RT-PCR

Total RNA was isolated from mouse DCs by using the Qiashredder and RNeasy Mini Kit from Qiagen. For cDNA first strand synthesis, 1 µg of total RNA in 12.5 µl DEPC-$H_2O$ was mixed with 1 µl of oligo-dT primer (500 µg/ml, Invitrogen, Carlsbad, CA, USA) and heated for 2 min at 70°C. To determine c-FLIP transcript levels, quantitative real-time PCR with the LightCycler System (Roche, Basel, Switzerland) was applied. The following primers were used: FLIP primers (sc-35389-PR, Santa Cruz, Dallas, Texas, USA) and actin primers: 5'-CATTGCTGACAGGATGCAGAA-3' (forward) and 5'-ATGGTGCTAGGAGCCAGAGC-3' (reverse). PCR reactions were performed in a final volume of 20 µl containing 2 µl cDNA, 2.4 µl $MgCl_2$ (3 µM), 1 µl primer mix (0.5 µM of both

primers), 2 µl cDNA Master SybrGreen I mix (Roche), and 12.6 µl DEPC-treated water. The target DNA was amplified during 40 cycles of 95ºC for 10 s, 60ºC for 10 s, and 72ºC for 16 s, each with a temperature transition rate of 20ºC/s, a secondary target temperature of 50ºC, and a step size of 0.5ºC. Melting curve analysis was performed at 95ºC, 0 s; 60ºC, 10 s; 95ºC, 0 s to determine the melting temperature of primer dimers and the specific PCR products. The ratio between the respective gene and corresponding β-actin was calculated per sample according to the ΔΔ cycle threshold method (Livak et al., 2001).

**Immunoblotting**

DCs ($2*10^6$ cells) were washed twice in PBS, then solubilized in lysis buffer (Thermo Fisher, Waltham, MA, USA) containing protease inhibitor cocktail (Sigma-Aldrich). Samples were stored at -80°C until use for western blotting. Cell lysates were separated by 10% SDS-PAGE and blotted on nitrocellulose membranes. The blots were blocked with 5% nonfat-milk in triethanolamine-buffered saline (TBS) and 0.1% Tween-20. Then the blots were probed overnight with monoclonal antibodies directed against either c-$FLIP_L$ or GAPDH (Cell signalling, Danvers, MA, USA) diluted 1:1000 in blocking buffer, washed 5 times, probed with secondary antibodies (anti mouse or anti-rabbit, GE healthcare) diluted 1:5000 for 1 h at room temperature and washed final 5 times. Antibody binding was detected with the enhanced chemiluminescence (ECL) kit (GE Healthcare, Chicago, Illinois, USA). Densitometer scans of the blots were performed using Quantity One (BioRad, Hercules, California, USA).

**Caspase 8 activity assay**

Caspase 8 activity was determined using a Caspase-8 fluorometric assay kit from Biovision (Milpitas, CA, USA) according to the manufacturer's instructions. Briefly $1x10^6$ cells were washed twice with cold PBS, fixed and permeabilized with 'Cytofix/Cytoperm' solution and then washed twice with 'Perm/ Wash' buffer. Then cells were stained with FITC conjugated anti-active Caspase 8 antibody in 'Perm/ Wash' buffer for 60 minutes. After 2 washing steps, the cells were analyzed by flow cytometry (FACS Aria Fusion, BD Biosciences, San Jose, CA, USA).

**Phosphatidylserine translocation**

Apoptotic cell membrane scrambling was evidenced from annexin V binding to phosphatidylserine (PS) at the cell surface. The percentage of PS-translocating cells was evaluated by staining with fluorescein isothiocyanate (FITC)-conjugated Annexin V. In brief $5\ x10^5$ cells were harvested and washed twice with Annexin washing buffer (AWB). The cell pellet was resuspended in 100 µl of Annexin-V-Fluos labelling solution (Roche) (20µl Annexin-V-Fluos labelling reagent in 1 ml AWB) and incubated for 15 min at room temperature. After washing with AWB, the cells were analyzed by flow cytometry.

**Statistics**

Data are provided as means ± standard error of the mean (SEM). All experiments were performed at least three times. Statistical significance was determined using ANOVA. For all statistical analysis, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ were considered statistically significant.

RESULTS

**Effect of VitC on the expression of c-FLIP in DCs**

The expression level of c-FLIP has been determined to involve in resistance to Fas-induced cell death (Golan-Gerstl et al., 2012; Wajant, 2003). To explore the modulation effect of VitC on c-FLIP expression, BMDCs were cultured with GM-CSF for 8 days and subsequently treated with FasL in the presence or absence of VitC. The results are in accordance to data of Wajant et al. (Wajant, 2003) that treatment of the cells with FasL down-regulated the c-FLIP transcript level (Figure 1A) and protein (Figure 1BC), the effects were suppressed by the presence of VitC (Figure 1A-C), indicating the promoting role of VitC on c-FLIP expression in DCs.

**Effect of VitC on caspase 8 activity in DCs**

VitC participates in regulating the transcription of apoptosis-related genes (Jeong et al., 2014; Kim et al., 2012). Our study showed the inhibitory effect of VitC on the activation of caspase 8. Accordingly, treatment of cells with FasL in the absence of VitC for 24h was followed by activation of caspase 8 and the activity of this caspase was dramatically reduced when the cells were exposed with VitC (Figure 2A-B). In addition, to ask whether the regulation of the

caspase 8 activation is mediated by c-FLIP expression, BMDCs were transfected with FLIP siRNA and followed by treatment with FasL in the presence or absence of VitC for 24h. The expression c-FLIP was dramatically reduced in FLIP-silenced DCs (Figure 2C). The downregulation of c-FLIP expression abolished FasL-induced caspase 8 activity (Figure 2A-B), suggesting that caspase 8 activity induced by FasL in the absence of VitC was dependent on the expression of c-FLIP in BMDCs.

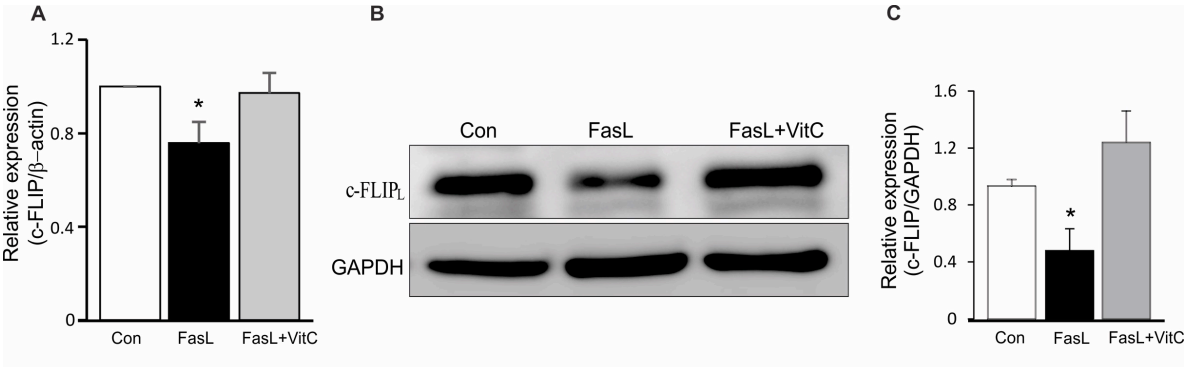


**Figure 1.** Effect of VitC on the expression of c-FLIP in DCs. (A) Arithmetic means ± SEM (n = 5) of c-FLIP transcript level detected by real-time PCR analysis in DCs were treated either without (white bar) or with FasL in the absence (black bar) or in the presence (grey bar) of VitC using β-actin as a reference gene. (B) Original Western blot of DCs, which were either treated with FasL in the absence or presence of VitC or left untreated (control). Protein extracts were analyzed by Western blotting using antibody directed to c-FLIP$_L$. Protein loading was controlled by GAPDH antibody. (C) Arithmetic mean ±SEM (n = 4) of the abundance of c-FLIP protein as the ratio of c-FLIP /GAPDH. *($p<0.05$) represent significant difference from control condition, ANOVA.

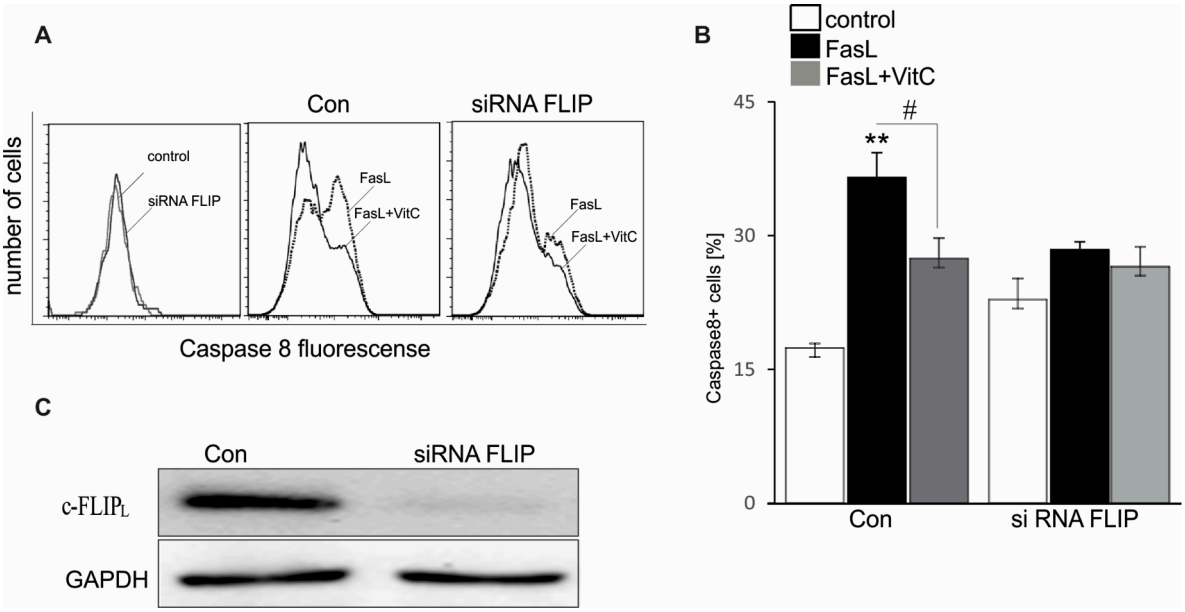


**Figure 2.** Effect of VitC on caspase 8 activity in DCs. (A) Histograms of caspase 8 activity as obtained by FACS analysis in a representative experiment on control- (1$^{st}$ and 2$^{nd}$ panels) and FLIP-silenced (1$^{st}$ and 3$^{rd}$ panels) DCs were either treated without or with FasL in the absence or presence of VitC. (B) Arithmetic means ± SEM (n = 4-6) of the percentage of control- (left bars) and FLIP-silenced (right bars) DCs with activated caspase 8. (C) Original Western blot of DCs, which were either treated with c-FLIP siRNA or left untreated (control). Protein extracts were analyzed by Western blotting using antibody directed to c-FLIP$_L$. Protein loading was controlled by GAPDH antibody. ** ($p<0.01$) represent significant difference from control condition and $^{\#}$ ($p<0.05$) represent significant difference from FasL-treated DCs, ANOVA.

**Effect of VitC on cell membrane scrambling in DCs**

The activation of caspases is expected to trigger DC apoptosis. Thus, next experiments were performed to ask, whether FasL triggered the cell membrane scrambling reflecting PS exposure at the cell surface. The PS exposure was determined from annexin V binding. As illustrated in Figure 3A-B, exposure of DCs to FasL was followed by stimulation of annexin V binding, the effect was reversed when VitC was present in the cell culture. Similarly, the increased annexin V binding in FasL-treated DCs was also blocked when the cells were transfected with FLIP siRNA (Figure 3A-B). Therefore, the annexin V binding stimulated by FasL in the absence of VitC was sensitive to the appearance of c-FLIP.

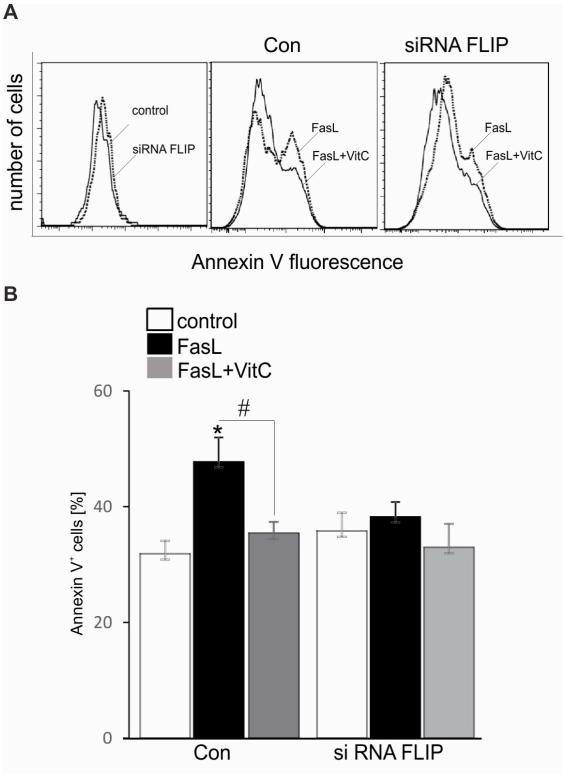


**Figure 3.** Effect of VitC on cell membrane scrambling in DCs. (A) Histograms of annexin V binding as obtained by FACS analysis in a representative experiment on control- (1st and 2nd panels) and FLIP-silenced (1st and 3rd panels) DCs were either treated without or with FasL in the absence or presence of VitC. (B) Arithmetic means ± SEM (n = 4-6) of the percentage of control- (left bars) and FLIP-silenced (right bars) DCs with annexin V binding. * ($p<0.05$) represent significant difference from control condition and # ($p<0.05$) represent significant difference from FasL-treated DCs, ANOVA.

DISCUSSION

The present study reveals that treatment of DCs with FasL in the absence of VitC caused the apoptotic cell death as was evidenced by activation of caspase 8 and PS exposure. VitC has been reported to enhance maturation and differentiation of leukocytes including DCs (Jeong et al., 2014; Kim et al., 2012) through the modulation of mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways (Kim et al., 2012). Besides, the role of VitC in preventing the ROS-mediated apoptosis are determined in many cell types including cancer cells and human monocytes (Jeong et al., 2016; Jin et al., 2014; Perez-Cruz et al., 2003). However, its effects on the survival of DC have not been reported yet. Similarly to VitC, two members of the TNF family including CD40L and tumor necrosis factor (TNF)-related activation-induced cytokine (TRANCE) have been shown to stimulate the activation of FasL-dependent NF-κB pathway leading to upregulation of anti-apoptotic molecules such as Bcl-2 and Bcl-xL (Ouaaz et al., 2002) and c-FLIPs (Tschopp et al., 1998), facilitating DC survival.

Several studies have indicated the inhibitory role of c-FLIP from the pro-apoptotic effects in many cell types including human DCs (Hamai et al., 2006; Wu et al., 2015), whereas our study indicated the mediating role of c-FLIP on the modulation of FasL-triggered DC apoptosis. Clearly, the activation of intracellular signalling pathways in human DCs and mouse DCs results in different expression levels in the transcriptional productions. The PI3K pathway is considered as an inhibitor of TLR4-mediated inflammatory response in mouse DCs (Shumilina et al., 2007), whereas activation of this pathway results in increased production of inflammatory cytokines in human DCs (Liu et al., 2011). In addition, c-FLIP is described as a stimulator of NF-κB-dependent intracellular signalling pathways (Micheau et al., 2001; Tschopp et al., 1998) allowing the cell maturation and differentiation in immune response (Haverkamp et al., 2014). Therefore, it would be expected to be a stimulator of cell activation leading to mature DCs, which are induced to undergo the cell death. Similar to our observation, treatment of cells with FasL suppresses the expression of c-FLIP (Wajant, 2003), the effect was abolished when VitC was present in the cell culture. Accordingly, the promoting effect of VitC on the expression level of c-FLIP in this finding would turn out to contribute to

DC activation and maturation rather than DC apoptosis.

Besides, the molecular mechanism underlying the regulation of Fas-mediated apoptosis is still unclear. The induction of the apoptosis by ligation of Fas with anti-Fas antibody in B cell and macrophages has been determined (Ashany et al., 1995; Richardson et al., 1994), whereas other studies have indicated that the interaction between Fas and FasL results in activation of caspase cascade and suicidal cell death (Buonocore et al., 2002; Tschopp et al., 1998). Consistently, FasL was observed to stimulate caspase 8-dependent PS exposure and VitC supressed these effects (Figure 2-3). Similarly, several researches indicated that VitC participates in regulating the expression of some genes involved in apoptosis (Levy et al., 1996; Perez-Cruz et al., 2003; Vissers et al., 2004).

## CONCLUSION

VitC inhibited FasL-mediated DC apoptosis is expected to exert some anti-inflammatory action and promote the immune response, an effect may apply its use in the treatment of inflammatory disorders and cancer. In any case, the inhibition of caspase activation and apoptotic death of DCs by VitC is expected to promote the immune response.

**Acknowlegements:** *This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 108.06-2017.16.*

## REFERENCES

Ashany D, Savir A, Bhardwaj N, Elkon KB (1999) Dendritic cells are resistant to apoptosis through the Fas (CD95/APO-1) pathway. *J Immunol* 163: 5303–11.

Ashany D, Song X, Lacy E, Nikolic-Zugic J, Friedman SM, Elkon KB (1995) Th1 CD4+ lymphocytes delete activated macrophages through the Fas/APO-1 antigen pathway. *Proc Natl Acad Sci USA* 92: 11225–9.

Buonocore S, Van Meirvenne S, Demoor FX, Paulart F, Thielemans K, Goldman M, *et al* (2002) Dendritic cells transduced with viral interleukin 10 or Fas ligand: no evidence for induction of allotolerance in vivo. *Transplantation* 73: S27–30.

Conti S, Petrungaro S, Marini ES, Masciarelli S, Tomaipitinca L, Filippini A, *et al* (2016) A novel role of c-FLIP protein in regulation of ER stress response. *Cell Signal* 28: 1262–1269.

Esmailzadeh S, Huang Y, Su MW, Zhou Y, Jiang X (2015) BIN1 tumor suppressor regulates Fas/Fas ligand-mediated apoptosis through c-FLIP in cutaneous T-cell lymphoma. *Leukemia* 29: 1402–13.

Golan-Gerstl R, Wallach-Dayan SB, Zisman P, Cardoso WV, Goldstein RH, Breuer R (2012) Cellular FLICE-like inhibitory protein deviates myofibroblast fas-induced apoptosis toward proliferation during lung fibrosis. *Am J Respir Cell Mol Biol* 47: 271–9.

Hamai A, Richon C, Meslin F, Faure F, Kauffmann A, Lecluse Y, *et al* (2006) Imatinib enhances human melanoma cell susceptibility to TRAIL-induced cell death: Relationship to Bcl-2 family and caspase activation. *Oncogene* 25: 7618–34.

Haverkamp JM, Smith AM, Weinlich R, Dillon CP, Qualls JE, Neale G, *et al* (2014) Myeloid-derived suppressor activity is mediated by monocytic lineages maintained by continuous inhibition of extrinsic and intrinsic death pathways. *Immunity* 41: 947–59.

Jaita G, Zarate S, Ferraris J, Gottardo MF, Eijo G, Magri ML, *et al* (2016) Estradiol upregulates c-FLIPlong expression in anterior pituitary cells. *Horm Metab Res* 48: 275–9.

Jeong CH, Joo SH 2016 Downregulation of reactive oxygen species in apoptosis. *J Cancer Prev* 21: 13–20.

Jeong YJ, Kim JH, Hong JM, Kang JS, Kim HR, Lee WJ, *et al* (2014) Vitamin C treatment of mouse bone marrow-derived dendritic cells enhanced CD8(+) memory T cell production capacity of these cells in vivo. *Immunobiology* 219: 554–64.

Kim HW, Cho SI, Bae S, Kim H, Kim Y, Hwang YI, *et al* (2012) Vitamin C up-regulates expression of CD80, CD86 and MHC class II on dendritic cell line, DC-1 via the activation of p38 MAPK. *Immune Netw* 12: 277–83.

Levy R, Shriker O, Porath A, Riesenberg K, Schlaeffer F (1996) Vitamin C for the treatment of recurrent furunculosis in patients with imparied neutrophil functions. *J Infect Dis* 173: 1502–5.

Liu KJ, Lee YL, Yang YY, Shih NY, Ho CC, Wu YC, *et al* (2011) Modulation of the development of human monocyte-derived dendritic cells by lithium chloride. *J Cell Physiol* 226: 424–33.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) *Method. Methods* 25: 402–8.

Llanos C, Carreno LJ, Kalergis AM (2011) Contribution of dendritic cell/T cell interactions to triggering and maintaining autoimmunity. *Biol Res* 44: 53–61.

Lucken-Ardjomande S, Martinou JC (2005) Regulation of Bcl-2 proteins and of the permeability of the outer mitochondrial membrane. *C R Biol* 328: 616–31.

Martino A (2007) Sphingosine 1-phosphate as a novel immune regulator of dendritic cells. *J Biosci* 32: 1207–12.

Micheau O, Lens S, Gaide O, Alevizopoulos K, Tschopp J (2001) NF-kappaB signals induce the expression of c-FLIP. *Mol Cell Biol* 21: 5299–305.

Ouaaz F, Arron J, Zheng Y, Choi Y, Beg AA (2002) Dendritic cell development and survival require distinct NF-kappaB subunits. *Immunity* 16: 257–70.

Perez-Cruz I, Carcamo JM, Golde DW (2003) Vitamin C inhibits FAS-induced apoptosis in monocytes and U937 cells. *Blood* 102: 336–43.

Richardson BC, Lalwani ND, Johnson KJ, Marks RM (1994) Fas ligation triggers apoptosis in macrophages but not endothelial cells. *Eur J Immunol* 24: 2640–5.

Shumilina E, Zahir N, Xuan NT, Lang F (2007) Phosphoinositide 3-kinase dependent regulation of Kv channels in dendritic cells. *Cell Physiol Biochem* 20: 801–8.

Sun J, Luo H, Nie W, Xu X, Miao X, Huang F, *et al* (2015) Protective effect of RIP and c-FLIP in preventing liver cancer cell apoptosis induced by TRAIL. *Int J Clin Exp Pathol* 8: 6519–25.

Suss G, Shortman K (1996) A subclass of dendritic cells kills CD4 T cells via Fas/Fas-ligand-induced apoptosis. *J Exp Med* 183: 1789–96.

Tschopp J, Irmler M, Thome M (1998) Inhibition of fas death signals by FLIPs. *Curr Opin Immunol* 10: 552–8.

Vissers MC, Hampton MB (2004) The role of oxidants and vitamin C on neutrophil apoptosis and clearance. *Biochem Soc Trans* 32: 499–501.

Wajant H (2003) Targeting the FLICE Inhibitory Protein (FLIP) in cancer therapy. *Mol Interv* 3: 124–7.

Wu YJ, Wu YH, Mo ST, Hsiao HW, He YW, Lai MZ (2015) Cellular FLIP inhibits myeloid cell activation by suppressing selective innate signaling. *J Immunol* 195: 2612–23.

Xuan NT, Shumilina E, Schmid E, Bhavsar SK, Rexhepaj R, Gotz F, *et al* (2010) Role of acidic sphingomyelinase in thymol-mediated dendritic cell death. *Mol Nutr Food Res* 54: 1833–41.

# VITAMIN C ỨC CHẾ QUÁ TRÌNH APOPTOSIS GÂY RA BỞI FASL TRONG TẾ BÀO TUA THÔNG QUA BIỂU HIỆN GEN C-FLIP

**Nguyễn Thị Xuân, Lê Thị Thu Hiền**

*Viện Nghiên cứu hệ gen, Viện Hàn lâm Khoa học và Công nghệ Việt Nam*

TÓM TẮT

Vitamin C (VitC) là chất chống oxy hóa mạnh và có vai trò như một chất ức chế quá trình apoptosis bằng cách ngăn chặn hoạt động của caspase 8. Fas ligand (FasL) gây ra sự chết tế bào theo chương trình thông qua hoạt động của tín hiệu Fas và phụ thuộc vào mức độ biểu hiện của gen c-FLIP. Trong nghiên cứu này, chúng tôi đã tiến hành xác định ảnh hưởng của VitC đến với sự sống sót của tế bào tua (TBT), tế bào này có vai trò điều hòa miễn dịch bẩm sinh và miễn dịch thu được. Vật liệu sử dụng là tế bào tủy xương chuột được nuôi cấy để thu được các tế bào tua có nguồn gốc từ tủy xương và được xử lý với FasL và VitC. Kỹ thuật nghiên cứu gồm real time RT-PCR, western blotting và phân tích tế bào theo dòng chảy được tiến hành để xác định các marker khác nhau của quá trình apoptosis. Kết quả nhận được cho thấy, xử lý tế bào với FasL làm hoạt hóa caspase 8 và màng tế bào bị co rút lại. Các tác động này được ngăn chặn khi VitC được thêm vào môi trường nuôi cấy hoặc các tế bào bị bất hoạt gen cFLIP. Kết quả nghiên cứu cho thấy VitC đã ngăn chặn quá trình apoptosis của tế bào tua gây ra bởi FasL thông qua biểu hiện của gen c-FLIP.

**Từ khóa:** *c-FLIP; tế bào tua; Fas ligand; phosphatidylserine; vitamin C*