Class A scavenger receptor activation inhibits endoplasmic reticulum stress-induced autophagy in macrophage

Hanpeng Huang, Xiaoyu Li, Yan Zhuang, Nan Li, Xudong Zhu, Jin Hu, Jingjing Ben, Qing Yang, Hui Bai, Qi Chen

Atherosclerosis Research Centre, Laboratory of Molecular Intervention for Cardiovascular Diseases, Nanjing Medical University, Nanjing, Jiangsu 210029, China; State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, Jiangsu 210029, China.

Received 10 July 2013, Revised 21 August 2013, Accepted 20 October 2013, Epub 12 December 2013

Abstract

Macrophage death in advanced atherosclerosis promotes plaque necrosis and destabilization. Involvement of autophagy in bulk degradation of cellular components has been recognized recently as an important mechanism for cell survival under endoplasmic reticulum (ER) stress. We previously found that the engagement of class A scavenger receptor (SR-A) triggered JNK-dependent apoptosis in ER-stressed macrophages. However, pro-apoptotic mechanisms mediated by SR-A are not fully understood. Therefore, we sought to see if SR-A mediated apoptosis was associated with autophagy in macrophages. Here, we showed that fucoidan inhibited microtubule-associated protein light chain 3-phospholipid conjugates (LC3-II) formation as well as the number of autophagosomes under ER stress. The inhibition of LC3-II formation was paralleled by the activation of the mTOR pathway, and the inhibition of mTOR allowed LC3-II induction in macrophages treated with thapsigargin plus fucoidan. Furthermore, apoptosis induced by fucoidan was prevented under ER stress by the mTOR inhibitor. We propose that fucoidan, a SR-A agonist, may contribute to macrophage apoptosis during ER stress by inhibiting autophagy.

Keywords: SR-A, autophagy, ER stress, apoptosis, macrophage

INTRODUCTION

Autophagy, or “self eating”, serves as a dynamic recycling system that produces new building blocks and energy for cellular renovation and homeostasis. The process of autophagy is characterized by the formation of double-membrane vesicles known as autophagosomes, which is mediated by the Atg12-Atg5-Atg16 complex and microtubule-associated protein light chain 3-phospholipid conjugates (LC3-II). The outer membrane of the autophagosome fuses with the lysosome, and cytoplasm-derived materials are degraded in the autolysosome. To date, autophagy has been implicated in various physio-pathological processes, including cell death, cell survival and tumorigenesis. Accumulating evidence indicated that autophagy may serve as a cell death mechanism under certain cellular scenarios and this autophagy-dependent cell death has been defined as “autophagic cell death” or “type II programmed cell death”. Intriguingly, autophagy has also been shown as a pro-survival mechanism against cell death in response to a variety of stimuli including oxidative stress, metabolic stress and endoplasmic reticulum (ER) stress.
In cardiomyocytes, low baseline levels of regulated autophagy are beneficial to maintaining cardiac structure and function, but uncontrolled or excessive autophagy can cause extensive self-destruction and cell death\[^9\]. Macrophage apoptosis is a critical process in the formation of necrotic cores in vulnerable atherosclerotic plaques\[^10\]. In vitro and in vivo studies indicated that macrophage apoptosis in advanced atheromata is triggered by a combination of ER stress and the engagement of class A scavenger receptor (SR-A)\[^11\], which together induce death through a rise in cytosolic calcium and the activation of toll-like receptor-4 (TLR4)\[^12,13\]. We previously found that the engagement of SR-A triggered JNK-dependent apoptosis in ER-stressed macrophages\[^14\]. However, whether autophagy is involved in SR-A-mediated apoptosis in macrophage has not been defined.

In this study, we demonstrated that fucoidan, a ligand for SR-A\[^15\], could inhibit ER stress induced autophagy by activating the mammalian target of rapamycin (mTOR) pathway. Our observations suggest that the suppression of ER stress induced autophagy may contribute to a mechanism of SRA-engaged macrophage apoptosis.

**MATERIALS AND METHODS**

**Reagents and plasmid constructs**

Polyclonal antibodies against phospho-Akt (Ser 473), phospho-mTOR, phospho-p70 S6 kinase and caspase-3 were obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-LC3 polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Thapsigargin (Tg), 3-methyladenine (3-MA) and rapamycin were supplied by Sigma (St. Louis, MO, USA). Roswell Park Memorial Institute medium 1640 (RPMI 1640), pcDNA3.1-EGFP vector, fetal calf serum (FCS), glutamine, penicillin, streptomycin, G418 and Lipofectamine\[^2\] 2000 were obtained from Invitrogen (Carlsbad, CA, USA). The construction of pcDNA3.1-EGFP-LC3 plasmid was described previously\[^16\]. MLC3 sequence from pCMV.SPORT6.1 vector was subcloned to pcDNA3.1-EGFP vector.

**Cell culture**

RAW264.7 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), and cultured in RPMI-1640 containing 10% (v/v) foetal calf serum (FCS), supplemented with 2 mmol/L glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. RAW264.7 cells were transfected with plasmid pcDNA3.1-GFP-LC3. pcDNA3.1-GFP-LC3 recombinant eukaryotic expression vector was gifted by professor Zhigang He at Harvard University. RAW264.7 cells were transfected by pcDNA3.1-GFP-LC3 plasmid using Effectene Transfection Reagent. GFP-LC3 stably expressing cells were selected and maintained in G418 (600 mg/mL).

SR-A\(^{-/-}\) mice, congenic to 129/ICR strain, were used in this study. The characterization of SR-A\(^{-/-}\) mice was described previously\[^17\]. All aspects of the animal care and experimental protocols were in accordance with the guidelines for the ‘‘Principles of Laboratory Animal Care’’ and approved by the Experimental Animal Care and Use Committee of the authors’ affiliated institution. Peritoneal macrophages (PM) were harvested 4 days after thioglycollate was injected into the mouse peritoneal cavity. Cells were washed with chilled phosphate-buffered saline (PBS) (pH 7.4), and macrophages were resuspended in RPMI 1640 containing 10% FCS, 100 U/mL penicillin and 100 µg/mL streptomycin, and plated on 60-mm round Petri dishes. After a 2 hour incubation at 37°C, 5% CO\(_2\) non-adherent cells were removed, and the remaining adherent cells were cultured in RPMI 1640 containing 10% FCS, 100 U/mL penicillin and 100 µg/mL streptomycin.

**Western blotting assays**

Cells were washed twice with PBS and lysed in lysis buffer (50 mmol/L Tris-HCl, pH 7.6, 0.15 mol/L NaCl, 1% Triton X-100, 5 mmol/L EDTA, 1 mmol/L sodium orthovanadate, 10 µg/mL leupeptin and 10 µg/mL aprotinin). Cell lysates were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to polyvinylidene difluoride (PVDF) membrane and blocked for 30 minutes in blocking buffer (Tris-buffered saline, pH 7.6, 0.05% Tween and 3% non-fat dry milk). After incubation with primary antibody diluted in blocking buffer for 60 minutes and washing, blot was incubated for 30 minutes with appropriate secondary anti-IgG-horseradish peroxidase conjugate. The membrane was washed 3 times for 10 minutes each and developed with SuperSignal chemiluminescent substrate (Pierce, Rockford, IL, USA).

**Immunofluorescence analysis**

RAW264.7 cells were grown on coverslips for 24 hours at 37°C. After fixation with 3% paraformaldehyde in PBS for 15 minutes at room temperature, cells were permeabilized with 0.1% Nonidet P-40, PBS for 5 minutes, and blocked with 2% BSA, 0.01% Tween 20 and PBS (PBST-BSA) for 30 minutes. The antibody against nuclei (DAPI, Sigma) in
PBST-BSA was incubated with cells for 1 minute and each coverslip was then washed 3 times for 10 minutes. Morphologic observation was performed with a Zeiss LSM 710 META confocal microscope. Two-channel optical images (DAPI and GFP) were collected with sequential scanning mode (405- and 488-nm excitation, respectively and 450- and 522-nm emission, respectively) of the Zeiss LSM 710 META confocal system. Cells containing 3 or more GFP-LC3 dots were defined as autophagy-positive cells. Pictures were obtained using sequential scanning, and the exposure settings and gain of laser were kept the same for each condition.

Apoptosis assay

After treatment, RAW264.7 cells were washed, resuspended in the staining buffer, and examined with the Annexin V-FITC and propidium iodide (PI) apoptosis kit (Biouniquer Technology Co., Ltd, Shanghai, China) according to the manufacturer’s instructions. Stained cells were detected by FACS (FACSCalibur; BD Biosciences, CA, USA). Annexin V-positive and PI-negative cells were regarded as apoptotic cells.

Statistical analysis

Results were expressed as mean ± S.D. Statistical significance between groups was assessed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls (SNK) post-hoc test. The effect of autophagy on apoptosis was a one-way ANOVA design; however, the variances were unequal, and the Welch test was performed followed by a Tamhane post-hoc test. *P < 0.05 was considered statistically significant.

RESULTS

Fucoidan inhibits ER stress-induced autophagy in macrophages

ER stress results in autophagy in cells[7]. Tg was used to induce ER stress in RAW264.7 cell and autophagosome formation was assessed by following

![Fig. 1 Fucoidan inhibits thapsigargin (Tg)-induced autophagy.](image-url)
the phospholipid conjugation of protein LC3-I (cytosolic form) to LC3-II (autophagosomal membrane-bound form)\(^{[2,18]}\). Time-course experiments revealed that LC3 type II, an indicator of autophagosome formation, was increased after 2-hour treatment by Tg in RAW264.7 cells. When fucoidan was simultaneously added to cells, LC3 II accumulation was reduced at 8 hours and maximal inhibition was seen at 12 hours after treatment (Fig. 1A). As shown in Fig. 1B, Tg-induced LC3 II accumulation was decreased by 48.5% ± 13.7% by treatment with fucoidan for 12 hours, whereas single fucoidan treatment did not change LC3 II expression in macrophages. Moreover, Tg treatment led to a redistribution of GFP-LC3 from a diffuse distribution to a punctuate distribution in GFP-LC3-expressing RAW264.7 cells, which was inhibited by 52.2% ± 8.2% by co-incubation of fucoidan (Fig. 1C). These data suggested that Tg-induced autophagic response in macrophages could be inhibited by simultaneous treatment with fucoidan.

**Inhibition of autophagy promotes apoptosis in macrophages**

To identify the role of autophagy in apoptosis, we treated RAW264.7 cell with Tg alone or Tg plus 3-MA, an inhibitor of autophagy. As shown in Fig. 2, treatment with Tg alone insignificantly increased apoptosis in macrophages. However, simultaneous treatment with 3-MA caused a dramatic increase in apoptotic cells, indicating that the inhibition of autophagy may promote apoptosis in macrophages.

**Fucoidan activates the mTOR pathway through SR-A**

Autophagy is negatively regulated by the mTOR pathway in response to stress signals\(^{[19,20]}\). Akt, a serine/threonine kinase, can activate mTOR and p70 S6 kinase (S6K). To understand the molecular mechanism whereby fucoidan inhibited autophagy in macrophages, we analyzed these signaling molecules activities. It was found that fucoidan or fucoidan plus Tg treatment induced AKT, mTOR and p70S6K phosphorylation in macrophages at 2 and 4 hours while single Tg treatment had no effect on this pathway. Phosphorylation of AKT, mTOR and p70S6K was obviously activated by fucoidan or Tg plus fucoidan at 4 hours (Fig. 3A). These results revealed that fucoidan may activate the AKT/mTOR/p70S6K pathway in macrophages.

To determine whether the activation of the AKT/mTOR/p70S6K pathway by fucoidan was mediated through SR-A, PMs from SR-A knockout and wildtype mice were used for the experiments. In wildtype macrophages, AKT, mTOR and p70S6K were markedly

**Fig. 2** The inhibition of autophagy increases apoptosis in RAW264.7 cells. RAW264.7 cells were incubated with 10 mmol/L 3-MA, 0.5 μmol/L Tg, or 10 mmol/L 3-MA plus 0.5 μmol/L Tg for 12 hours, respectively. Non-treated cells were used as a control. Cells were stained with annexin V and propidium iodide (PI) and analyzed by FACS. Results were expressed as mean ± SD \(N = 5\), \(*P < 0.01\) compared with control.

**Fig. 3** SR-A is required for fucoidan-induced activation of the mTOR pathway. A: RAW264.7 cells were incubated with the indicated reagents, alone or in combination: 25 μg/mL fucoidan and 0.5 μmol/L Tg for 2 and 4 hours. Cell lysates were applied to Western blotting and detected by antibodies against p-AKT, p-mTOR, p-p70S6K and β-actin. B: Wildtype (WT), and SRA\(^{-/-}\) macrophages were left untreated or treated with 0.5 μmol/L Tg or 0.5 μmol/L Tg plus 25 μg/mL fucoidan for 12 hours. Cell lysates were subjected to Western blotting and detected by antibodies against p-AKT, p-mTOR, p-p70S6K and β-actin.
activated by treatment with Tg plus fucoidan compared with Tg treatment. However, these effects were abolished in the SR-A deficient macrophages (Fig. 3B), indicating that SR-A would be requisite for fucoidan-induced activation of the mTOR pathway in macrophages.

**Blockage of the mTOR pathway restores ER stress-induced autophagy**

To observe the role of mTOR in Tg-induced autophagy, we further used rapamycin, a pharmacologic inhibitor of mTOR, to treat macrophages. We found that rapamycin had a potent inhibitory effect on the activation of the mTOR pathway induced by Tg plus fucoidan treatment (Fig. 4A). Moreover, rapamycin restored LC3-II to the level similar to treatment with Tg alone (Fig. 4B). Consistently, the blockage of the mTOR pathway by rapamycin also restored the occurrence of autophagosomes in GFP-LC3 cells that was significantly inhibited by treatment with Tg plus fucoidan. These results suggested that rapamycin antagonized the inhibitory effect of fucoidan on Tg induced autophagy in macrophages.

**Blockage of the mTOR pathway inhibits fucoidan-Tg induced macrophage apoptosis**

We further examined whether the blockage of the mTOR pathway was able to mitigate macrophage apoptosis induced by the co-addition of Tg and fucoidan. As shown in Fig. 5A, treatment with Tg or fucoidan alone did not dramatically impact on macrophage apoptosis. However, co-treatment with Tg and fucoidan significantly increased the number of TUNEL-positive cells, indicating Tg-induced apoptosis. Conversely, treatment with rapamycin mitigated Tg plus fucoidan-induced apoptosis in a concentration-dependent manner (Fig. 5B). Co-treatment with rapamycin restored the number of TUNEL-positive cells to levels similar to Tg treatment alone. These results suggested that rapamycin antagonized the pro-apoptotic effect of fucoidan on Tg-induced macrophage apoptosis.
apoptosis. It caused a low production of cleaved caspase-3, a pivotal asparagine protease in the apoptotic response (Fig. 5B). However, the combined application of these two reagents led to a marked increase in macrophage apoptosis and the production of cleaved caspase-3, consistent with our previous study [14]. Accordingly, additional rapamycin treatment significantly inhibited cell apoptosis and caspase-3 cleavage induced by Tg plus fucoidan, which indicated that the inhibition of the mTOR pathway may prevent ER stress-dependent apoptosis.

**DISCUSSION**

Fucoidan shows antitumor activity by inducing apoptosis in cultured human cancer cells [21-23]. Moreover, long time treatment with fucoidan at high concentration can induce autophagy and suppress cell proliferation in AGS human gastric cancer cells [24]. However, it appears that the roles of autophagy in regulating cell death are highly dependent on cell type and stimulus. For instance, fucoidan itself cannot induce autophagy or apoptosis in macrophage [14]. Macrophage apoptosis is triggered by ER stress in combination with the engagement of SR-A, but not either stimulus alone [11]. We found that fucoidan could inhibit ER stress triggered autophagy mediated by SR-A in macrophage.

This is consistent with the finding that pattern recognition receptors (PRRs) such as nucleotide-binding oligomerization domain-like receptor (NLR) C4 NLRC4 and NLRP4 have an inhibitory effect on autophagy [25]. In contrast, the activation of quintessential PRRs like toll such as toll receptor 4 (TLR4) and TLR7 can induce autophagy in RAW264.7 cells to defend against pathogen invasion [26].

Three distinct forms of autophagy have been identified, including macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy is thought to be the major type of autophagy. Autophagy, by means of self-cannibalization, may contribute to cell survival or death depending on the threshold level [27-29]. Autophagy can protect against cell death during nutrient starvation and other stressors [30]. Mild ER stress inhibits neuronal death by promoting autophagy in drosophila and mouse models of Parkinson’s disease [31]. The induction of autophagy by ER stress under therapeutic dosage before ischemia can be cardio-protective for rats [32], whereas high levels of autophagy promote cell death [33]. Apoptosis and autophagy have been shown to act in synergy and also to counter each other, since they are not mutually exclusive pathways at all [34]. The inhibition of differentiation-induced autophagy leads to monocyte apoptosis [34]. In the present...
study, we showed that thapasigargin induced a significant increase in the formation of autophagosomes but little apoptosis. Moreover, the co-addition of thapasigargin and fucoidan compromised this process and markedly induced apoptosis. In this case, autophagy does not lead to cell death, but instead acts to reduce apoptosis by creating a cellular milieu in which survival is favored. Autophagy might serve as a cell survival mechanism by maintaining ER function through the consumption of protein aggregates and misfolded proteins, thus limiting ER stress response and subsequent apoptosis. Accordingly, the suppression of autophagy promotes cell apoptosis. Interestingly, Liao et al. have demonstrated that blocking autophagy rendered macrophages more susceptible to cell death and promoted plaque necrosis in mice. It is interesting that we have also proved that the internalization of SR-A and its ligand complex into cells is negatively regulated by the interaction of SR-A with glucose-regulated protein 78 (GRP78), a chaperon of ER stress. Thus, the anti-ER stress property of fucoidan/SR-A may involve binding of SR-A to GRP78 in macrophages.

Several cell-signaling pathways contribute to the regulation of autophagy, which are cell type-specific and signal-dependent. The mTOR pathway is one of the most important autophagy regulators, which negatively control autophagy. In addition, mTOR has been found to have a pleiotropic function in the regulation of cell apoptosis. It works as an apoptosis inhibitor or an apoptosis inducer under different conditions. Therefore, mTOR may play an important role in regulating the cross-talk between autophagy and apoptosis. By activating the mTOR pathway via SR-A, fucoidan alone or in combination with Tg could consequently inhibit Tg-induced autophagy. It is worth noting that the inhibition of the mTOR pathway by rapamycin treatment could rescue autophagy response and reduce apoptosis in macrophage even in the presence of Tg plus fucoidan. Our observations support the notion that SR-A and the mTOR pathway are key elements in regulating a balance between autophagy and the apoptotic responses in macrophage.

Macrophage apoptosis plays an important role in the pathogenesis of many diseases. Macrophage apoptosis in early atherosclerotic lesions would limit plaque development through a negative regulation of inflammation. In advanced atherosclerosis, macrophage apoptosis coupled with defective phagocytic clearance of dead cells leads to plaque necrosis. SR-A functions in mediating macrophage apoptosis and cleaning up of these apoptotic cells. We showed that fucoidan, a well-defined nonlipoprotein ligand for SR-A, could promote macrophage apoptosis by repressing ER stressor triggered autophagy. Multiple ER stressors
and SR-A ligands are known to exist in atheromata. Athero-relevant ER stressors include oxidant stress, peroxinitrite, insulin resistance, glucosamine, saturated fatty acids, hypoxia, homocysteine, oxidized phospholipids, oxysterols and serum starvation. SR-A ligands that trigger macrophage apoptosis during ER stress include modified forms of LDL, advanced glycation end products (AGES), β-amyloid and anionic phospholipids, as well as pathogens and pathogen-associated molecules. In vitro results imply that the ligands of SR-A may have an antagonistic effect on ER stress triggered by ER stressors. Whether it takes place in vivo pathophysiological situations needs to be validated. Our previous studies revealed an unique signal motif in the cytoplasm domain that mediates the internalization of SR-A and demonstrated that SR-A-induced apoptosis is mainly through the caveolae route, which is linked to p38 kinase and JNK signaling. It is possible that SR-A engaged macrophage apoptosis is regulated by the p38 and JNK pathways directly. The impact of SR-A on autophagy may constitute a supplementary regulatory mechanism for macrophage apoptosis. In atherosclerosis, once macrophage apoptosis is triggered, the consequence of apoptosis, whether beneficial by suppressing cellularity in early lesions, or detrimental by contributing to necrotic core formation in advanced lesion, is likely dependent on the efficiency of phagocytes. The apoptotic macrophages that failed to be cleared by defective efferocytosis accumulate eventually and build up necrotic debris promotes inflammation, plaque instability, and acute thrombosis.

In summary, we report that fucoidan is essential for the activation of the mTOR pathway and the inhibition of autophagic response under conditions of ER stress in macrophages, which results in apoptosis. A postulated model for fucoidan regulation of autophagy is shown in Fig. 6. The inhibitory effect of fucoidan on autophagy could be abrogated by blocking the mTOR pathway, and thus, Tg-fucoidan induced apoptosis was prevented. Fucoidan/SR-A may contribute to macrophage apoptosis during ER stress by suppressing autophagy through its regulation of the mTOR pathway.

Acknowledgements

This work was supported by the National Basic Research Program (973) Grant (No. 2012CB517503 and No. 2011CB503903) and National Natural Science Foundation of China (No. 81230070 and No. 81070120) to Qi Chen, the National Natural Science Foundation of China Grant (No. 81000118) to Jingjing Ben and the National Natural Science Foundation of China Grant (No. 81100857) to Xiaoyu Li.

References

[1] Mizushima N, Komatsu M. Autophagy: Renovation of cells and tissues. Cell 2011;147:728-41.
[2] KabeYA, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J 2000;19:5720-8.
[3] Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132:27-42.
[4] Klionsky DJ. Autophagy: From phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol 2007;8:931-7.
[5] Wu YT, Tan HL, Huang Q, Ong CN, Shen HM. Activation of the p38-akt-mTOR signaling pathway promotes necrotic cell death via suppression of autophagy. Autophagy 2009;5:324-34.
[6] Eisenberg-Lerner A, Bialik S, Simon HU, Kimchi A. Life and death partners: Apoptosis, autophagy and the cross-talk between them. Cell Death Differ 2009;16:966-75.
[7] Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, et al. Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol 2006;26:9220-31.
[8] Sakiyama T, Musch MW, Ropeleski MJ, Tsubouchi H, Chang EB. Glutamine increases autophagy under basal and stressed conditions in intestinal epithelial cells. Gastroenterology 2009;136:924-32.
[9] Takemura G, Miyata S, Kawase Y, Okada H, Maruyama R, Fujiwara H. Autophagic degeneration and death of cardiomyocytes in heart failure. Autophagy 2006;2:212-4.
[10] Feng B, Zhang D, Kuriakose G, Devlin CM, Kock M, Tabas I. Niemann-pick c heterozygosity confers resistance to lesional necrosis and macrophage apoptosis in murine atherosclerosis. Proc Natl Acad Sci U S A 2003;100:10423-8.
[11] Devries-Seimon T, Li Y, Yao PM, Stone E, Wang Y, Davis RJ, et al. Cholesterol-induced macrophage apoptosis requires er stress pathways and engagement of the type a scavenger receptor. J Cell Biol 2005;171:61-73.
[12] Seimon TA, Obstfeld A, Moore KJ, Golenbock DT, Tabas I. Combinatorial pattern recognition receptor signaling alters the balance of life and death in macrophages. Proc Natl Acad Sci U S A 2006;103:19794-9.
[13] Lim WS, Timmins JM, Seimon TA, Sadler A, Kolodgie FD, Virmani R, et al. Signal transducer and activator of transcription-1 is critical for apoptosis in macrophages subjected to endoplasmic reticulum stress in vitro and in advanced atherosclerotic lesions in vivo. Circulation 2008;117:940-51.
[14] Zhu XD, Zhuang Y, Ben JJ, Qian LL, Huang HP, Bai H, et al. Caveolae-dependent endocytosis is required for class a macrophage scavenger receptor-mediated apoptosis in macrophages. J Biol Chem 2011;286:8323-9.
[15] Liang CP, Han S, Okamoto H, Carnemolla R, Tabas I, Accili D, et al. Increased cd36 protein as a response to defective insulin signaling in macrophages. J Clin Invest 2004;113:764-73.
[16] Zhuang Yan BJ, Bai Hui, Chen Qi. Establishment of a stable gfp-lc3-expressed raw264.7 cell line. Acta Univ Med Nanjing 2009:296.
[17] Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K, et al. A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. *Nature* 1997;386:292–6.

[18] Tanida I, Minematsu-Ikeguchi N, Ueno T, Kominami E. Lyosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. *Autophagy* 2005;1:84–91.

[19] Jung CH, Ro SH, Cao J, Otto NM, Kim DH. mTOR regulation of autophagy. *FEBS Let* 2010;584:1287–1295.

[20] Sudarsanam S, Johnson DE. Functional consequences of mTOR inhibition. *Curr Opin Drug Discov Devel* 2010;13:31–40.

[21] Aisa Y, Miyakawa Y, Nakazato T, Shibata H, Saito K, Ikeda Y, Kizaki M. Fucoidan induces apoptosis of human sultan cells accompanied by activation of caspase-3 and down-regulation of erk pathways. *Am J Hematol* 2005;78:7–14.

[22] Nagamine T, Hayakawa K, Kusakabe T, Takada H, Nakazato K, Hisanaga E, Iha M. Inhibitory effect of fucoidan on huh7 hepatoma cells through downregulation of cxc12. *Nutr Cancer* 2009;61:340–7.

[23] Yamasaki-Miyamoto Y, Yamasaki M, Tachibana H, Yamada K. Fucoidan induces apoptosis through activation of caspase-9 on human breast cancer mcf-7 cells. *J Agric Food Chem* 2009;57:8677–82.

[24] Park HS, Kim GY, Nam TJ, Deuk Kim N, Hyun Choi Y. Antiproliferative activity of fucoidan was associated with the induction of apoptosis and autophagy in ags human cells. *Agric Food Chem* 2009;57:8677–82.

[25] Park HS, Kim GY, Nam TJ, Deuk Kim N, Hyun Choi Y. Antiproliferative activity of fucoidan was associated with the induction of apoptosis and autophagy in ags human gastric cancer cells. *J Food Sci* 2011;76:T77–83.

[26] Jouan N, Kobiyama K, Shina M, Ogata K, Ishii KJ, Takeshita F. Nlrp4 negatively regulates autophagic processes through an association with beclin1. *J Immunol* 2011;186:1646–55.

[27] Mihalache CC, Simon HU. Autophagy regulation in macrophages and neutrophils. *Exp Cell Res* 2012;318:1187–92.

[28] Kroemer G, Levine B. Autophagic cell death: The story of a misnomer. *Nat Rev Mol Cell Biol* 2008;9:1004–10.

[29] Kouris N, Tavermarakis N. Autophagy and cell death in model organisms. *Cell Death Differ* 2009;16:21–30.

[30] Levine B, Yuan J. Autophagy in cell death: An innocent convict? *J Clin Invest* 2005;115:2679–88.

[31] Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 2008;451:1069–75.

[32] Fouillet A, Levet C, Virgone A, Robin M, Douren P, Rieusset J, et al. ER stress inhibits neuronal death by promoting autophagy. *Autophagy* 2012;8:915–26.

[33] Petrovski G, Das S, Juhasz B, Kertesz A, Tosaki A, Das DK. Cardioprotection by endoplasmic reticulum stress-induced autophagy. *Antioxid Redox Signal* 2011;14:2191–200.

[34] Mairi MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: Crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 2007;8:741–52.