Autophagy Plays a Protective Role in Advanced Glycation End Product-Induced Apoptosis in Cardiomyocytes

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Key Words
Advanced glycation end products • Cardiomyocytes • Autophagy • Apoptosis • Signalling pathways

Abstract
Background/Aims: To investigate the effect of advanced glycation endproduct-induced autophagy in rat cardiomyocytes and to identify the role of autophagy in advanced glycation end product-induced cell apoptosis. Methods: After cultured rat cardiomyocytes were treated with advanced glycation end products (AGEs), protein expression was detected by western blotting, autophagosomes were observed by electron microscopy, the cell apoptotic rate was determined by flow cytometry, and cell variability was quantified by the MTT assay. Results: After cultured cardiomyocytes were treated with AGEs, the level of autophagy-associated protein LC3-II was up-regulated and SQSTM1/p62 was down-regulated; the number of autophagosomes was increased. Compared with the control group, the apoptotic rate of cardiomyocytes increased, and the cardiomyocyte viability was decreased in the AGE-treated group. Furthermore, pretreating cells with 3-MA, an autophagy inhibitor, could enhance these effects. Treatment with AGEs activated phospho-ERK, phospho-JNK, and phospho-p38/MAPK but inhibited phospho-Akt and phospho-mTOR. Pretreatment with an ERK inhibitor and an Akt activator could inhibit AGE-induced autophagy, demonstrating that AGEs induce autophagy in cardiomyocytes through the ERK and Akt signalling pathways. Conclusion: AGEs can induce autophagy through the PI3K/AKT/mTOR and ERK signalling pathways and induce apoptosis through the PI3K/AKT/mTOR and p38/MAPK signalling pathways in rat cardiomyocytes. Autophagy plays a protective role in AGE-induced apoptosis in cardiomyocytes.

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Introduction

Advanced glycation end products (AGEs) arise from a non-enzymatically glycated, irreversible process called the Maillard reaction [1]. The accumulation of AGEs has been implicated in the accelerated process of diabetic cardiovascular complications through interactions with the receptor for advanced glycation end products (RAGE) [2]. AGEs play an important role in the development and progression of diabetic cardiomyopathy through endothelial dysfunction, myocardial apoptosis, oxidative stress, increased inflammation, and enhanced extracellular matrix accumulation in diabetes [3-5].

Autophagy is a degradation process that delivers intracellular components to lysosomes through autophagosomes in all eukaryotic cells [6]. There are multiple stages, structures, and proteins involved in the process of autophagy. During autophagy, to begin with, LC3-I is recruited to the autophagosome in the cytoplasmic form [7]. Moreover, by the activating enzyme Atg7 and the conjugating enzyme Atg3, phosphatidylethanolamine-conjugated LC3-II is localized in the membrane of autophagosome [8]. Finally, LC3-II is degraded by autophagy. Another autophagy marker, p62, also called sequestosome 1 (SQSTM1), binds LC3 and recruits proteins into autophagosomes for degradation [9]. Thus, LC3-II and SQSTM1 levels indicate autophagic activity. Autophagic flux is a method that is used to measure autophagy, which is always assessed using MAP1LC3B/LC3 and SQSTM1/p62 western blotting [10]. PI3K/AKT/mTOR is the most important intracellular signal pathway to negatively regulate autophagy [11-13]. In addition, MAPKs, a family of serine/threonine protein kinases, are also involved in the process of autophagy [14, 15].

The crosstalk between autophagy and apoptosis is complex [16]. Autophagy and apoptosis are under the control of multiple common upstream signals, including the PI3K/AKT/mTOR signalling pathway. Generally, autophagy can promote cell survival by blocking apoptosis [17]. However, under certain special conditions, autophagy or autophagy-related proteins can culminate in apoptosis or autophagic cell death. Our previous research shows that AGEs can induce autophagy in a time- and dose-dependent manner, which is involved in AGE-induced proliferation of rat vascular smooth muscle cells [18]. It is known that AGEs increase the rate of apoptosis and ROS generation of cardiomyocytes through the ERK1/2 and p38 MAPK signalling pathways but do not affect the distribution of the cell cycle [19, 20]. Another recent study indicates that advanced glycation end products trigger autophagy via the PI3K/AKT/mTOR signalling pathway in cardiomyocytes [21]. However, the relationship between AGE-RAGE mediated autophagy and apoptosis in cardiomyocytes has not been elucidated. In this study, we sought to explore the signalling pathway involved in AGE-induced autophagy in cardiomyocytes and the crosstalk between AGE-induced autophagy and apoptosis in cardiomyocytes.

Materials and Methods

Materials

A polyclonal rabbit anti-LC3B antibody, MTT, BSA, 3-MA, and MAPK inhibitors, including PD98059, SP600125 and SB203580, were obtained from Sigma (St. Louis, MO, USA). Monoclonal rabbit antibodies, including anti-SQSTM1/p62, anti-P38, anti-phospho-P38, anti-JNK, anti-phospho-JNK, anti-ERK, anti-phospho-ERK, anti-AKT, anti-mTOR, anti-phospho-mTOR and anti-phospho-AKT, were obtained from Cell Signalling Technology (MA, USA). The Vybrant apoptosis assay kit (488) was obtained from Invitrogen (CA, USA). An HRP-marked anti-GAPDH antibody was purchased from Kangchen (Shanghai, China). Rat IGF-1 was obtained from R&D (Minneapolis, MN, USA).

Rat neonatal cardiomyocyte culture

Cardiomyocytes were obtained from 1-2 day old Sprague-Dawley rats (provided by the experimental animal centre of Zhejiang Chinese Medicine University). Heart tissue was cut into small pieces of ~1 mm3 and digested with PBS containing 0.1% trypsin and 0.1% type II collagenase five to sixtimes. The suspension
was centrifuged and the supernatant was collected, centrifuged and resuspended in DMEM cell culture medium with 10% FBS. Fibroblasts in the cell suspension were reduced by pre-plating for 1 h according to differential cell adhesion [22]. 5´-BrdU (0.01 mM) was also added to inhibit the growth of fibroblast during the first three days. The medium was replaced every 48 h until the cardiomyocytes reached >80% confluence for use.

Preparation of AGEs
AGEs were prepared as described previously [23]. Briefly, BSA was incubated with 0.5 M glucose in phosphate-buffered saline (PBS) in dark and sterile conditions for 16 weeks at 37°C. Unincorporated sugars were removed by dialysis with PBS (pH 7.4). Control non-glycated BSA was incubated in the absence of glucose under the same conditions. Preparations were tested for endotoxins using an endotoxin testing kit (Chromogenic TAL Endpoint Assay Kit, China), and the AGE-BSA solutions were confirmed to be endotoxin free (<2.5U/ml of endotoxin).

Western blot analysis
Cells were solubilized in a lysis buffer containing RIPA and a mixture of protease and phosphatase inhibitors. The total protein concentrations were determined by a BCA Protein Assay Kit (Applygen Technologies, Inc., China). After the samples were heat-denatured, they were analysed on a 10 or 15% tris-glycine gradient gel, transferred to nitrocellulose membranes and blocked with 5% non-fat dry milk in tris-buffered solution (TBS) for 1 h at room temperature. The membranes were incubated with primary antibody overnight at 4°C. After being washed three times, the membranes were incubated with horseradish peroxidase conjugated secondary antibodies for 1 h at room temperature. The immune complexes were visualized by ultrachemiluminescence (ECL) reagents and imaged using an Image Quant LAS-4000 (Fujifilm, Tokyo, Japan). The band densities were determined using Multi-Gauge Software (Fujifilm, Tokyo, Japan).

Electron microscopy
The ultrastructural analysis was performed to examine autophagy. Rat neonatal cardiomyocytes were grown in 6-well plates treated with 100 μg/ml AGEs for 6 h, fixed with a solution containing 3% glutaraldehyde and then sent to Zhejiang University for electron microscopic analysis.

Cell viability MTT assay
Cardiomyocytes were plated in a 96-well plate. After 48 hours, the medium was changed, and the cells were incubated with fresh medium containing AGEs (100 μg/ml) or 3-MA (2 mM) for another 48 hours. Then, 20 μl of MTT was added to each well for a final concentration of 0.5 mg/mL for 4 hours at 37°C. The medium was then discarded, and 100μl of DMSO was added to each well. The absorbance was measured at 490 nm.

Flow cytometric (FCM) analysis of apoptosis
After treatment, cells (5 × 105 cells/ml) were collected and suspended in 100μl of 1× annexin-binding buffer, and then, 5μl of Alexa Fluor 488 and 5μl of propidium iodide (PI) were added and then incubated for 15 min at room temperature in the dark. After incubation, 400 μl of 1× binding buffer was added for FCM detection. Flow cytometry was performed using a Zeiss fluorescence microscope (Carl Zeiss, Oberkochen, Germany).

Statistical analysis
All data were obtained from at least 3 individual experiments. The values are expressed as the mean ± SEM. Statistical analysis between groups was performed by one-way ANOVA. The statistical significance was set at p<0.05.

Results
AGE-induced autophagy in cardiomyocytes
To determine whether AGEs can affect the level of autophagy in cardiomyocytes, we treated cells with AGEs at various concentrations (0, 4, 20, 100 and 500 μg/ml) for 6 h.
The expression of LC3-II and the ratio of LC3-II to LC3-I were notably increased, whereas SQSTM1/p62 was decreased in a dose-dependent manner in AGE-treated cells (Fig. 1A). Cells were also treated with AGEs (100μg/ml) for various times (0, 1, 2, 6, 12, and 24 h). The expression of LC3-II and the ratio of LC3-II to LC3-I were significantly increased after treatment with AGEs, peaking at 6 h. However, the expression of SQSTM1/p62 was decreased under the same conditions (Fig. 1B).

To directly visualize autophagy, we used transmission electron microscopy to examine autophagic vacuoles (autophagosomes). We treated cells with 100μg/ml AGEs for 6 h. In the control group, autophagic vacuoles were rarely detected. However, we found that autophagic vacuoles containing cellular material or membranous structures were increased in cardiomyocytes (bold arrows in Fig. 1C).

**AGE-induced apoptosis in cardiomyocytes**

To determine whether AGEs induced apoptosis, we used MTT and FCM analysis with Alexa Fluor 488 Annexin V/PI. Cardiomyocytes were treated with AGEs at various concentrations (0, 20, 100 and 500 μg/ml) for 48 h. As shown in Fig. 2, the apoptotic rate of cardiomyocytes increased and the cardiomyocyte viability was decreased in a dose-dependent manner in the AGE-treated group.

The ERK1/2 signalling pathway is involved in AGE-induced autophagy, whereas the p38 MAPK signalling pathway is involved in AGE-induced apoptosis in cardiomyocytes.

It has been well documented that the MAPK signalling pathway is important for cells to respond to many extracellular signals. To investigate the mechanisms involved in

**Fig. 1.** AGE-induced autophagy in cardiomyocytes. (A) Western blot analysis of LC3-I/II and SQSTM1/p62 protein levels treated with AGEs. Cells were treated for 6 h with 0, 4, 20, 100 and 500μg/ml AGEs. (B) Cells were treated with 100μg/ml AGEs for 0, 1, 2, 6, 12 and 24 h. (C) Representative electron micrographs of cardiomyocytes treated with 100μg/ml AGEs for 6 h.
AGE-induced autophagy and apoptosis, we examined the phosphorylation of the MAPKs (ERK1/2, p38, and JNK) cascade in cardiomyocytes by western blotting. Cells were treated with 100 μg/ml AGEs for 0, 7.5, 15, 30, 60 and 120 min. As is shown in Fig. 3, AGEs stimulated the phosphorylation of ERK1/2 and p38 in cardiomyocytes in a time-dependent manner, but not activated the phosphorylation of JNK (Fig. 3A). ERK1/2 and p38 Phosphorylation peaked at 15-30 min and then declined.

Then, to delineate the pathways involved in AGE-induced autophagy and apoptosis, we assessed the effects of a panel of pharmacologic inhibitors, including those of ERK1/2 (PD98059, 20μM), JNK (SP600125, 20μM) and p38 (SB203580, 10μM). In our experiments, the ERK1/2 inhibitor PD98059, but not the p38 inhibitor SB203580 or JNK inhibitor SP600125, suppressed the expression of LC3-II (Fig. 3B). The results indicate that the ERK1/2 signal transduction pathway is involved in AGE-induced autophagy. By contrast, the p38 inhibitor SB203580, but not the ERK1/2 inhibitor PD98059 or JNK inhibitor SP600125, suppressed AGE-induced apoptosis (Fig. 3C). These results demonstrate that AGEs induced apoptosis via the p38 MAPK signalling pathway in cardiomyocytes.

The PI3K/Akt/mTOR signalling pathway is involved in AGE-induced autophagy and apoptosis

The PI3K/Akt/mTOR signalling pathway is a major pathway that negatively regulates autophagy. In cardiomyocytes, phosphorylation of Akt and mTOR decreased 30 min to 2 h after treatment with AGEs (100μg/ml) (Fig. 4A). To examine the role of the PI3K/Akt/mTOR pathway in AGE-induced autophagy, we used insulin-like growth factor 1 (IGF-1) to activate this signalling pathway [24].

In addition, pretreatment with IGF-1 (200ng/ml) suppressed AGE-induced LC3-II expression in cardiomyocytes. This result suggests that the PI3K/Akt/mTOR signalling pathway is involved in AGE-induced autophagy in cardiomyocytes (Fig. 4B). Similarly, compared with the control group, pretreatment with IGF-1 (200ng/ml) also reduced the
apoptotic rate in the AGE group in cardiomyocytes (Fig. 4C). These results demonstrate that the PI3K/Akt/mTOR signalling pathway is involved in AGE-induced apoptosis (Fig. 4B).

The role of autophagy in AGE-induced apoptosis in cardiomyocytes

To understand the role of autophagy in AGE-induced apoptosis in cardiomyocytes, we used 3-MA, an inhibitor that blocks early stages of the autophagy pathway. Compared with the control group, cells treated with 100μg/ml AGEs for 48 h showed an increased apoptotic rate and decreased viability of cardiomyocytes by FCM analysis and MTT assays (Fig. 5). These results indicate that autophagy plays a protective role in AGE-induced apoptosis in cardiomyocytes.
Discussion

Diabetic cardiomyopathy is characterized by cardiac dysfunction that develops in many cases of type 2 diabetes mellitus in the absence of coronary artery disease or hypertension [25]. Advanced glycation end products (AGEs) play an important role in the development and progression of diabetic cardiomyopathy [3]. However, the underlying mechanisms are not completely understood. It has been reported that increased oxidative injury in diabetic hearts may trigger the activation of stress signalling pathways, facilitating cardiomyocyte cell death [26, 27]. However, recent evidence also suggests that, in addition to apoptosis, other processes, such as autophagy, may also be involved in controlling cell death in the pathogenesis of diabetic cardiomyopathy [28].

Autophagy is a cellular housekeeping process that is important for the removal of misfolded proteins, damaged organelles and intracellular pathogens [29]. In general, the decreased levels of SQSTM1/p62 and conversion of LC3-I to LC3-II are commonly used as markers for autophagic flux [30]. In our study, we found that the level of LC3-II was up-regulated and SQSTM1/p62 was down-regulated after cultured cardiomyocytes were treated with AGEs. The number of autophagosomes was also detected under the same condition by transmission electron microscopy. These demonstrated that autophagic flux was enhanced by AGEs in cardiomyocytes.

There is a complex crosstalk between autophagy and apoptosis. Nevertheless, studies have yielded conflicting results. Under certain cellular conditions, autophagy may be utilized as an adaptive response for survival and to avert apoptosis [31]. Under other conditions, autophagy appears to promote apoptosis or apoptotic programmed cell death. Autophagy

![Fig. 4. The PI3K/Akt/mTOR signalling pathways involved in AGE-induced autophagy and apoptosis. (A) Western blot analysis of the PI3K/Akt/mTOR pathway in cardiomyocytes treated with AGEs. (B) Western-blot analysis of LC3I/II expression in AGE- (100 μg/ml, 6h) and IGF-1-treated cells. Cells pre-treated with IGF-1 (200ng/ml) suppressed AGE-induced expression of LC3-II in cardiomyocytes. (C) Flow cytometry analysis of cell apoptosis in cardiomyocytes treated with AGEs (100μg/ml, 48 h) in the presence of IGF-1 (200ng/ml). Statistical analysis was performed with one-way ANOVA. *p < 0.05 relative to the control; #p < 0.05 relative to AGEs; values = mean ± SEM (n = 4).](image)
is a double-edged sword in cardiomyocyte demise and survival. Yutaka et al. previously reported that autophagy plays a protective role during ischemia, whereas it may be detrimental during reperfusion [32]. In this study, the autophagy inhibitor 3-MA could attenuate the effects of AGE-induced apoptotic rate and increase the viability of cardiomyocytes. This result indicates that AGE-induced autophagy plays a protective role in the AGE-stimulated apoptosis of cardiomyocytes. This may provide a new therapeutic strategy for preventing diabetic cardiomyopathy in diabetic patients.

In our study, we found that AGEs increased the phosphorylation of ERK and p38 and decreased the phosphorylation of AKT and mTOR in a time-dependent manner. Furthermore, autophagy in cardiomyocytes treated with AGEs was reduced when cells were pretreated with the ERK inhibitor PD98059 but not the p38 inhibitor SB203580. Interestingly, the apoptotic rate of cardiomyocytes was reduced when cells were pretreated with the p38 inhibitor SB203580 but not the ERK inhibitor PD98059. Activation of the AKT pathway using IGF-1 inhibited AGE-induced autophagy and apoptosis. These results imply that the ERK and AKT pathways are involved in AGE-induced autophagy, whereas the P38 and AKT pathways are involved in AGE-induced apoptosis. Depending on the experimental conditions, the members of the MAPK and AKT signalling pathways may have different roles in the regulation of autophagy and apoptosis. Zhang et al. reported that the PI3K/AKT/mTOR and JNK signalling pathways were involved in cathepsin S inhibition-induced autophagy and apoptosis in human glioblastoma cells [14]. However, because we could not exclude autophagy-regulating proteins in the modulation of apoptosis, further investigation is needed.

In summary, our studies demonstrate that AGEs induced autophagy through the PI3K/AKT/mTOR and ERK signalling pathways and induced apoptosis through the PI3K/AKT/mTOR and P38 signalling pathways. Autophagy plays a protective role in AGE-induced apoptosis.
apoptosis in cardiomyocytes. This suggests that regulating the AGE-autophagy pathway can attenuate apoptosis of cardiomyocytes and therefore may reduce the development of diabetic cardiomyopathy in diabetic patients. Further studies are needed to dissect the relationship between autophagy and apoptosis in animal models and to explore possible drug-targeting methods to regulate this pathway.

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Disclosure Statement

The authors have no conflict of interest.

References

1. Yamagishi S, Takeuchi M, Inagaki Y, Nakamura K, Imai T: Role of advanced glycation end products (AGES) and their receptor (RAGE) in the pathogenesis of diabetic microangiopathy. Int J Clin Pharmacol Res 2003;23:129-134.
2. Chen JW, Ni BB, Li B, Yang YH, Jiang SD, Jiang LS: The responses of autophagy and apoptosis to oxidative stress in nucleus pulposus cells: Implications for disc degeneration. Cell Physiol Biochem 2014;34:1175-1189.
3. Bodiga VL, Eda SR, Bodiga S: Advanced glycation end products: Role in pathology of diabetic cardiomyopathy. Heart Fail Rev 2014;19:49-63.
4. Yuan Q, Zhou QY, Liu D, Yu L, Zhan L, Li XJ, Peng HY, Zhang XL, Yuan XC: Advanced glycation end-products impair Na+/K+-ATPase activity in diabetic cardiomyopathy: Role of the adenosine monophosphate-activated protein kinase/sirtuin 1 pathway. Clin Exp Pharmacol Physiol 2014;41:127-133.
5. Nożyński J, Zakliczynski M, Konecka-Mrowka D, Zakliczynska H, Pięt M, Zembala-Nożyńska E, Lange D, Zembala M: Advanced glycation end products and lipofuscin deposits share the same location in cardiocytes of the failing heart. Exp Gerontol 2013;48:223-228.
6. Glick D, Barth S, Macleod KF: Autophagy: Cellular and molecular mechanisms. J Pathol 2010;221:3-12.
7. Kim JH, Hong SK, Wu PK, Richards AL, Jackson WT, Park JI: Raf/mek/erl can regulate cellular levels of LC3b and sqstm1/p62 at expression levels. Exp Cell Res 2014;327:340-352.
8. Zhao M, Sun L, Yu XJ, Miao Y, Liu JJ, Wang H, Ren J, Zang WJ: Acetylcholine mediates AMPK-dependent autophagic cytoprotection in h9c2 cells during hypoxia/reoxygenation injury. Cell Physiol Biochem 2013;32:601-613.
9. Komatsu M, Waguri S, Koike M, Sou S, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, Hamazaki J, Nishito Y, Iemura S, Natsume T, Yanagawa T, Uwayama J, Warabi E, Yoshida H, Ishii T, Kobayashi A, Yamamoto M, Yue Z, Uchiumi Y, Kominami E, Tanaka K: Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 2007;131:1149-1163.
10. Gump JM, Thorburn A: Sorting cells for basal and induced autophagic flux by quantitative ratiometric flow cytometry. Autophagy 2014;10:1327-1334.
11. Makarov P, Golovin K, Teper E, Kutikov A, Mehrizin R, Corcoran A, Tulin A, Uzzo RG, Kolenko VM: Piperlongumine promotes autophagy via inhibition of akt/mTOR signalling and mediates cancer cell death. Br J Cancer 2014;110:899-907.
12. Lee CH, Lin ST, Liu JJ, Chang WW, Hsieh JY, Wang WK: Salmonella induce autophagy in melanoma by the downregulation of akt/mTOR pathway. Gene Ther 2014;21:309-316.
13 Sun H, Wang Z, Yakisch JS: Natural products targeting autophagy via the pi3k/akt/mtor pathway as anticancer agents. Anticancer Agents Med Chem 2013;13:1048-1056.
14 Zhang L, Wang H, Xu J, Zhu J, Ding K: Inhibition of cathepsin s induces autophagy and apoptosis in human glioblastoma cell lines through ros-mediated pi3k/akt/mtor/p70s6k and jnk signaling pathways. Toxicol Lett 2014;228:248-259.
15 McClung JM, Judge AR, Powers SK, Yan Z: P38 mapk links oxidative stress to autophagy-related gene expression in cachectic muscle wasting. Am J Physiol Cell Physiol 2010;298:C542-549.
16 Marino O, Niso-Santano M, Baehrelce EH, Kroemer G: Self-consumption: The interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 2014;15:81-94.
17 Bhutia SK, Kegelman TP, Das SK, Azab B, Su ZZ, Lee SG, Sarkar D, Fisher PB: Astrocyte elevated gene-1 induces protective autophagy, Proc Natl Acad Sci U S A 2010;107:22243-22248.
18 Hu P, Lai D, Lu P, Gao J, He H: Erk and akt signaling pathways are involved in advanced glycation end product-induced autophagy in rat vascular smooth muscle cells. Int J Mol Med 2012;29:613-618.
19 Li SY, Sigmon VK, Babcock SA, Ren J: Advanced glycation endproduct induces ros accumulation, apoptosis, map kinase activation and nuclear o-glcnacylation in human cardiac myocytes. Life Sci 2007;80:1051-1056.
20 Xie Y, Xiao F, Luo L, Zhong C: Activation of autophagy protects against ros-mediated mitochondria-dependent apoptosis in l-02 hepatocytes induced by cr(vi). Cell Physiol Biochem 2014;33:705-716.
21 Hou X, Hu Z, Xu H, Xu J, Zhang S, Zhong Y, He X, Wang N: Advanced glycation endproducts trigger autophagy in cardiomyocyte via rage/pi3k/akt/mtor pathway. Cardiovasc Diabetol 2014;13:78.
22 Blondel B, Roijen I, Cheneval JP: Heart cells in culture: A simple method for increasing the proportion of myoblasts. Experientia 1971;27:356-358.
23 Hou FF, Chertow GM, Kay J, Boyce J, Lazarus JM, Braatz JA, Owen WF, Jr.: Interaction between beta 2-microglobulin and advanced glycation end products in the development of dialysis related-amyloidosis. Kidney Int 1997;51:1514-1519.
24 Mitsiades CS, Mitsiades N, Pouladi V, Schlossman R, Akiyama M, Chauhan D, Hideshima T, Treon SP, Munshi NC, Richardson PG, Anderson KC: Activation of nf-kappab and upregulation of intracellular anti-apoptotic proteins via the igf-1/akt signaling in human multiple myeloma cells: Therapeutic implications. Oncogene 2002;21:5673-5683.
25 Wei YM, Li X, Xu M, Abais JM, Chen Y, Kewalramani G, An D, Qi D, Abrahani A, Rodrigues B: Cardiomyocyte apoptosis induced by short-term diabetes requires mitochondrial gsh depletion. Am J Physiol Heart Circ Physiol 2005;289:H768-776.
26 Chen F, Chen B, Xiao FQ, Wu YT, Wang RH, Sun ZW, Fu GS, Mou Y, Tao W, Hu XS, Hu SJ: Autophagy protects against senescence and apoptosis via the ras-mitochondria in high-glucose-induced endothelial cells. Cell Physiol Biochem 2014;33:1058-1074.
27 Zhang H, Guo M, Chen JH, Wang Z, Du XJ, Liu S, Zhang Y, Li WH: Osteopontin knockdown inhibits alphav,beta3 integrin-induced cell migration and invasion and promotes apoptosis of breast cancer cells by inducing autophagy and inactivating the pi3k/akt/mtor pathway. Cell Physiol Biochem 2014;33:991-1002.
28 Guo B, Huang J, Wu W, Feng D, Wang X, Chen Y, Zhang H: The nascent polypeptide-associated complex is essential for autophagic flux. Autophagy 2014;10:1738-1748.
29 Zhao S, Luo H, Kan G, Zhao Y, Lin L, Tang Q, Yu C, Sun W, Cai L, Cui S: The protective role of autophagy in heterochrom and glabheptic stellar cells exposed to h2o2 or nutritional stress. Cell Physiol Biochem 2014;34:463-473.
30 Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, Sadoshima J: Distinct roles of autophagy in the heart during ischemia and reperfusion: Roles of amp-activated protein kinase and beclin 1 in mediating autophagy. Circ Res 2007;100:914-922.