Resveratrol, an activator of SIRT1, improves ER stress by increasing clusterin expression in HepG2 cells

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Abstract

Endoplasmic reticulum stress (ER stress) is involved in lipid metabolism and lipotoxicity and can lead to apoptosis. Resveratrol, a sirtuin 1 (SIRT1) agonist, prevents ER stress and improves ER stress-induced hepatic steatosis and cell death. Clusterin is a secreted chaperone and has roles in various physiological processes. However, changes in the expression of clusterin upon ER stress and the connection between SIRT1 and clusterin in protection against ER stress are not well known. In cells treated with tunicamycin, resveratrol increased the expression of clusterin mRNA and protein and the secreted clusterin protein level in conditioned medium. Resveratrol decreased protein expression of the ER stress markers, p-PERK, p-IRE1 α , and CHOP, and increased the expression of the ER-associated degradation (ERAD) factors, SEL1L and HRD1, in tunicamycin-treated cells. However, no changes in the expression of these genes were observed in clusterin siRNA-transfected cells. Moreover, increased LAMP2 and LC3 expression and decreased Rubicon expression were observed in cells treated with resveratrol or secreted clusterin. These data suggest that SIRT1 activation by resveratrol attenuates ER stress by promoting protective processes such as ERAD and autophagy pathways and that these protective effects are mediated by clusterin.

Keywords Clusterin · ER stress · SIRT1 · Chaperone · Autophagy · ERAD

Abbreviations

CHOP	C/EBP homologous protein
ER	Endoplasmic reticulum
NAFLD	Non-alcoholic fatty liver disease
ERAD	ER-associated degradation
HSP	Heat shock protein
IRE1 α	Inositol-requiring enzyme 1 α
PERK	Protein kinase RNA-like endoplasmic reticulum kinase

SERCA2b	Sarco(endo)plasmic reticulum Ca ²⁺ -ATPase 2b
sCLU	Secretory clusterin
SIRT1	Silent mating-type information regulation 2 homolog 1
UPR	Unfolded protein response

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Introduction

The endoplasmic reticulum (ER) is involved in regulating the synthesis, folding, and transport of proteins and calcium homeostasis. Alteration of ER function by environmental and genetic factors causes an accumulation of misfolded and unfolded proteins, termed ER stress. ER stress is associated with metabolic diseases, such as obesity, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD) (Lee and Ozcan 2014; Ozcan and Tabas 2012). Under ER stress conditions, the cell triggers a signaling pathway called the unfolded protein response (UPR), which is dependent on the UPR sensors (PERK, ATF6, and IRE1 α). The UPR initially aims to restore homeostasis for cell survival. However, if the stress is prolonged, the UPR can induce apoptosis.

Heat shock proteins (HSPs) are induced by heat and other stresses and function as molecular chaperones in cellular homeostasis. HSPs are responsible for protein folding, assembly, translocation, and degradation under normal cellular conditions (Wang et al. 2004). During cellular stress, HSPs assist in refolding damaged proteins and degrade aberrant proteins (Kelly 2002). The HSP-mediated degradation of unfolded proteins occurs via two main pathways—the ubiquitin-proteasome system (UPS) and autophagy pathways (Kriegenburg et al. 2012; Nikesitch and Ling 2016). The expression of HSPs is regulated by binding of the transcription factor, heat shock factor 1 (HSF1), to the heat shock element. Decreased HSF1 levels are involved in NAFLD progression by impairment of anti-inflammatory HSP70 pathways (Di Naso et al. 2015). Studies have also reported that HSP72, a member of the HSP70 family of proteins, reduces insulin resistance via blocking inflammation signaling in mice and humans (Chung et al. 2008) and inhibits ER stress-induced apoptosis through interaction with the UPR sensor, IRE1 α (Gupta et al. 2010).

Clusterin (also known as apolipoprotein J) is a heterodimer disulfide-linked glycoprotein that plays a role in various physiological processes, such as cell–cell interactions, aging, the regulation of apoptosis, and lipid transport (Wilson and Easterbrook-Smith 2000). Clusterin has secretory and nuclear isoforms, resulting from the translation of an alternatively spliced RNA (Leskov et al. 2003). The secretory isoform encodes a 75–80 kDa protein composed of two 40 kDa α - and β -chain subunits, while the nuclear isoform encodes a 49 kDa protein (de Silva et al. 1990; Leskov et al. 2003). The two clusterin isoforms are known to have opposing roles in cell death and survival (Criswell et al. 2005; Leskov et al. 2003; Pucci et al. 2004). Secretory/cytoplasmic clusterin (sCLU) inhibits mitochondria-mediated apoptosis by stabilizing the Ku70-Bax complex (Trogakos et al. 2009; Zhang et al. 2005). Wilson and Easterbrook-Smith (2000) reported that clusterin is a heat shock protein and functions as a chaperone. In addition, clusterin has functions similar to small HSPs that contribute to the stabilization of unfolded and misfolded proteins by preventing stress-induced protein aggregation (Humphreys et al. 1999; Wilson and Easterbrook-Smith 2000).

Resveratrol, a polyphenol found in grapes, has protective effects against oxidative damage, inflammation, and cancer through the activation of SIRT1 (Baur and Sinclair 2006; Zhu et al. 2011). Autophagy promotes cell survival by eliminating unneeded organelles and proteins aggregates, as well as facilitating bioenergetic homeostasis. Restoration of autophagy in mice fed a high fructose diet was shown to reduce ER stress-associated disruption of insulin signaling (Wang et al. 2015). Studies have reported that SIRT1 regulates aging (Salminen and Kaarniranta 2009), hepatic steatosis (Zhang et al. 2015), and inflammation responses via autophagy (Wang et al. 2017). However, the effects of resveratrol on ER stress are still controversial (Graham et al. 2016; Tabata

et al. 2007). In the present study, we examined whether resveratrol or inducers of ER stress could regulate clusterin expression, and whether amelioration of ER stress by resveratrol was mediated by clusterin.

Materials and methods

Cell culture and treatments

HepG2 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin at 37 °C in a humidified 5% CO₂ atmosphere. Tunicamycin (0.5–5 μ g/ml; Sigma–Aldrich, St. Louis, MO, USA) and thapsigargin (0.5–2 μ M; Sigma–Aldrich) were used to induce ER stress in cultured hepatocytes. To examine the effect of SIRT1 on the expression of clusterin, cells were incubated in culture media alone or media containing SIRT1 agonist, resveratrol (10–100 μ M), or antagonist EX-527 (10–30 μ M) (Sigma–Aldrich) for 24 h. For pharmacological treatments, cells were pretreated with 3 μ g/ml tunicamycin for 24 h, followed by treatment with or without 100 μ M resveratrol or 100 nM clusterin (human secretory form; Sigma–Aldrich) for 24 h.

Transient siRNA transfection

siRNA duplexes targeting clusterin and negative control siRNA were purchased from Bioneer (Daejeon, Korea). For siRNA-mediated gene silencing, HepG2 cells were transfected with synthetic siRNAs using Lipofectamine RNAi MAX transfection reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. After transfection, the culture medium was replaced with fresh medium and cells were treated with tunicamycin in the presence or absence of resveratrol, then harvested using a cell scraper and stored at –80 °C for further analysis.

Determination of cell viability

Cell viability was assessed using a Vybrant MTT cell proliferation assay kit (V13154, Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. Briefly, cells were seeded into 96-well culture plates at a density of 5×10^4 cells/well. After drug treatment, 10 μ l of 12 mM MTT stock solution was added to each well and incubated at 37 °C for 4 h, before addition of 100 μ l SDS-HCl solution to each well and further incubation at 37 °C for 12 h. Plates were gently shaken and absorbance was read at 570 nm using a microplate reader (Bio-Rad,

Hercules, CA, USA). The relative cell viability was expressed as a percentage of the control.

Quantitative RT-PCR

Total RNA from cells was isolated using Trizol reagent (Invitrogen), and reverse transcribed using a High-Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. Quantification of relative gene expression was performed using a Light-Cycler 480 (Roche, Lewis, UK) using SYBR Green (Roche). The primers used in qPCR are listed in supplementary Table 1. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative gene expression levels.

Western blotting

Cells were washed with phosphate-buffered saline (PBS) and lysed using radioimmunoprecipitation assay buffer (RIPA buffer; Cell Signaling Technology, Danvers, MA, USA) containing a protease inhibitor cocktail (Roche, Germany) and phosphatase inhibitors (Sigma–Aldrich) on ice. Protein concentration was determined using the Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories) and protein samples (15 μ g) were resolved on a 4–12% Bis-Tris NuPAGE gel (Invitrogen). Change in clusterin levels secreted by cells was determined SDS-PAGE analysis of cell culture supernatants using the same method as above. Following electrophoresis, gels were transferred onto a polyvinylidene difluoride (PVDF) membrane using an iBlot2 PVDF stack (Invitrogen). Membranes were blocked in 5% BSA TBST and incubated with the following primary antibodies: anti-Clusterin- α (sc-6419; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-SERCA2b (#4388; Cell Signaling Technology), anti-GRP78 (sc-13968; Santa Cruz Biotechnology, Inc.), anti-phosphorylated PERK (sc-3255; Santa Cruz Biotechnology, Inc.), anti-PERK (sc-13073; Santa Cruz Biotechnology, Inc.), anti-phosphorylated IRE1 α (ab104157; Abcam, Cambridge, MA, USA), anti-IRE1 α (#3294; Cell Signaling Technology), anti-ATF6 (sc-22799; Santa Cruz Biotechnology, Inc.), anti-CHOP (#2895; Cell Signaling Technology), anti-LC3 A/B (#12741; Cell Signaling Technology), anti-LAMP2 (ab18528; Abcam), anti-Rubicon (ab156052; Abcam), and anti- β -actin (#4967; Cell Signaling Technology). Membranes were incubated with horseradish peroxidase-conjugated secondary antibodies, and immunoreactive bands were visualized using an enhanced chemiluminescence kit (GE Healthcare, Piscataway, NJ, USA).

Statistical analysis

All results are expressed as mean $\pm$ SEM. Statistical analysis was performed using SPSS 12.0 software (SPSS Inc.,

Chicago, IL, USA). The data were analyzed using Student's *t* test or one-way analysis of variance followed by the Bonferroni/Dunn multiple range test. $p < 0.05$ was considered statistically significant.

Results

ER stress induced by tunicamycin and thapsigargin inhibits sCLU expression in cell culture media and lysates

We examined the effect of ER stress on the expression of clusterin. Treatment with ER stress inducers, tunicamycin and thapsigargin, dramatically decreased the expression of clusterin mRNA in HepG2 cells (Fig. 1a, d). Consistent with this, the expression of intracellular clusterin and secreted clusterin protein in cells treated with tunicamycin or thapsigargin decreased in a dose-dependent manner (Fig. 1b, c, e, f). However, the protein expression of ER stress marker, GRP78, was increased in cells treated with tunicamycin or thapsigargin (Fig. 1b, e). These data suggest that the expression and secretion of clusterin in hepatocytes are reduced during ER stress.

SIRT1 regulates the expression of clusterin in hepatocytes

To determine whether the expression of clusterin is regulated by SIRT1, HepG2 cells were exposed to different concentrations of resveratrol (a SIRT1 agonist) or Ex-527 (a SIRT1 antagonist). The expression of clusterin mRNA in resveratrol-treated cells was significantly increased in a dose-dependent manner (Fig. 2a), whereas clusterin expression in Ex-527-treated (20 and 30 μ M) cells was decreased (Fig. 2b).

Resveratrol reverses the decrease in ER stress-induced clusterin expression and reduces ER stress

We examined whether resveratrol restores the reduced expression of clusterin by tunicamycin. The expression of clusterin mRNA, intracellular clusterin, and secreted clusterin protein in cells treated with tunicamycin was significantly increased following resveratrol treatment (Fig. 3a–c). In addition, the expression of sarco(endo)plasmic reticulum Ca^{2+} -ATPase 2b (SERCA2b), which plays a role in the control of calcium-dependent cell growth, differentiation, and survival, was significantly increased in cells following resveratrol treatment. Conversely, the expression of ER stress marker proteins such as phospho-protein kinase RNA-like endoplasmic reticulum kinase (P-PERK), phospho-inositol-requiring enzyme 1 α (IRE1 α), and CHOP in tunicamycin-

Fig. 1 ER stress inhibits the expression of clusterin in HepG2 cell. Cells were treated with different doses of tunicamycin (0.05–3 μ g/ml) or thapsigargin (0.05–2 μ M) for 24 h. **a, d** mRNA expression of clusterin was measured by quantitative RT-PCR. Protein expression of GRP78 and

clusterin in cell lysates (**b, e**) and secreted clusterin in culture media (**c, f**) were analyzed by western blot. β -actin and Ponceau S served as loading controls. The experiments were repeated three times. * $p < 0.05$ and ** $p < 0.01$ compared with control (Con)

treated cells was decreased in cells following resveratrol treatment. However, no significant change on the expression of ATF6 was shown between in tunicamycin-treated

cells and in cells following resveratrol treatment (Fig. 3d). These data suggest that resveratrol increases the expression of clusterin and attenuates tunicamycin-induced ER stress.

Fig. 2 SIRT1 modulation of clusterin expression. HepG2 cells were treated with different dose of **a** SIRT1 agonist resveratrol (10–100 μ M) or **b** SIRT1 antagonist EX-527 (10–30 μ M) for 24 h. Clusterin mRNA expression was measured by quantitative RT-PCR normalized to β -actin expression. The experiments were repeated three times. * $p < 0.05$ and ** $p < 0.01$ compared with control (Con)

Fig. 3 Effect of resveratrol treatment on clusterin expression and ER stress in HepG2 cells treated with tunicamycin. HepG2 cells were pretreated with 3 μ g/ml tunicamycin, followed by incubation with or without resveratrol (Resv; 100 μ M) for 24 h. **a** Clusterin mRNA expression was analyzed by quantitative RT-PCR. Clusterin protein expression in **b** cell lysates, **c** culture media, and **d** SERCA2b, p-PERK, PERK, p-

IRE1 α , IRE1 α , ATF6 and CHOP proteins in cell lysates were analyzed by western blot. β -actin and Ponceau S served as loading controls. The experiments were repeated three times. * p < 0.05 and ** p < 0.01 compared with control (Con); # p < 0.05 and ## p < 0.01 compared with tunicamycin-treated cells

Resveratrol increases HSP72 chaperone and ERAD-associated gene expression in tunicamycin-treated cells

We next examined whether clusterin is involved in the protective effect of resveratrol against ER stress. Heat shock proteins, as molecular chaperones, are key components in recovery from stress through their roles in protein folding and degradation pathways (Duncan 2005). The expression of heat shock factor 1 (HSF1), which regulates the expression of the heat shock proteins, was increased in resveratrol-treated cells (Supplementary Fig. 1). In cells pretreated with tunicamycin, the expression of clusterin and HSP72 mRNAs was increased in cells following resveratrol treatment (Fig. 4a, b), as were mRNA expression levels of ERAD complex containing HRD1 (E3 ubiquitin-protein ligase) and SEL1L (the associated cargo receptor)

(Fig. 4c, d). However, inhibition of clusterin by siRNAs abolished the resveratrol-induced increase in HSP72, SEL1L, and HRD1 expressions in tunicamycin-treated cells. These data suggest that resveratrol promotes activation of the ERAD pathway, a mechanism for protecting cells against ER stress by degrading unfolded proteins, via promotion of clusterin expression.

Resveratrol stimulates autophagy by increasing clusterin expression

Autophagy functions to degrade abnormal proteins and is stimulated during ER stress to promote cell survival (Ogata et al. 2006; Xu et al. 2015). To determine whether resveratrol regulates autophagy during tunicamycin-induced ER stress, we examined the changes in the expression of autophagy-related proteins by western blot. The expression of LC3 and

Fig. 4 Effect of clusterin inhibition and resveratrol treatment on expression of HSP72 and ERAD-associated genes in tunicamycin-treated HepG2 cells. **a–d** HepG2 cells were transfected with 10 nM clusterin siRNA or scrambled siRNA (Scr) for 24 h and were pretreated with 3 μ g/ml tunicamycin, followed by incubation with or without resveratrol (Resv; 100 μ M) for 24 h. Clusterin, HSP72, SEL1L, and HRD1 mRNA expressions were analyzed by quantitative RT-PCR. The experiments were repeated three times. * p < 0.05 and ** p < 0.01 compared with control (Con); ## p < 0.01 compared with tunicamycin-treated cells; n.s., not significant

LAMP2 in cells treated with tunicamycin was increased by resveratrol, while the expression of autophagy inhibitor, Rubicon, was decreased (Fig. 5a). Interestingly, clusterin was shown to have a similar effect to resveratrol on the expression of autophagy-related proteins in cells treated with tunicamycin. The expression of LC3 and LAMP2 proteins was increased in cells treated with tunicamycin followed by clusterin treatment, and the expression of Rubicon was decreased, but no statistical significance (Fig. 5b). These data suggest that resveratrol and clusterin can stimulate autophagy pathways during tunicamycin-induced ER stress.

Discussion

In this study, we have demonstrated that SIRT1 attenuates tunicamycin-induced ER stress in hepatocytes. Moreover, we have shown for the first time that clusterin plays an important role in the protective effect of resveratrol against tunicamycin-induced ER stress. The expression of ER stress marker proteins such as p-PERK, p-IRE1 α , and CHOP by resveratrol was decreased, while the expression of SERCA2b, which is involved in the maintenance of ER calcium homeostasis, was increased, indicating a protective effect of SIRT1 during ER stress. Resveratrol treatment also

increased clusterin and HSP72 mRNA expressions and expression of ERAD and autophagy signaling molecules, which may be thought of as survival mechanisms, in tunicamycin-treated cells. However, no change in HSP72, SEL1L, and HRD1 expressions was observed in clusterin siRNA-transfected cells treated with tunicamycin followed by resveratrol treatment.

ER stress results from the accumulation of unfolded or misfolded proteins in the ER lumen and prolonged ER stress leads to cell death. Recently, there are many reports that ER stress plays a pivotal role in metabolic diseases (Han and Kaufman 2016; Hotamisligil 2010). Herein, we demonstrated that tunicamycin-induced ER stress can be reversed via resveratrol-induced activation of SIRT1.

Clusterin is a secreted glycoprotein and has a function similar to that of small heat shock proteins (sHSP), which protect cells from heat and other stresses (Humphreys et al. 1999). The clusterin promoter contains binding sites for heat shock factor 1 (HSF1), indicating that clusterin gene expression is regulated by HSF1. We observed that the expression of HSF1 was increased in cells following resveratrol treatment. Additionally, the expression of clusterin mRNA was increased with SIRT1 agonist resveratrol treatment and decreased with SIRT1 antagonist, EX-527, in a dose-dependent manner. Clusterin has chaperone-like activity that inhibits stress-

Fig. 5 Resveratrol and clusterin promote autophagy in tunicamycin-treated cells. HepG2 cells were pretreated with 3 μ g/ml tunicamycin, followed by treatment with or without **a** 100 μ M resveratrol (Resv) or **b** 100 nM clusterin (Clu) for 24 h. The expression of LC3, LAMP2, and

Rubicon proteins were analyzed by western blot. The experiments were repeated three times. * $p < 0.05$ and ** $p < 0.01$ compared with control (Con); # $p < 0.05$ and ## $p < 0.01$ compared with tunicamycin-treated cells

induced aggregation and precipitation of proteins (Poon et al. 2002). More specifically, Rohne et al. (2014) reported that glycosylation of clusterin is important for its chaperone activity. We found that the expression of secreted form of clusterin protein was increased in cells treated with ER-stress inducer, tunicamycin, following resveratrol treatment. These findings suggest that resveratrol regulates the expression of HSF1-dependent clusterin via SIRT1 and that resveratrol treatment can restore the chaperon activity of clusterin during tunicamycin-induced ER stress.

Heat shock responses and autophagy are highly conserved mechanisms that maintain protein homeostasis, through enhanced folding or clearance of misfolded or unfolded proteins during cellular stress (Dokladny et al. 2015; Ingemann and Kirkegaard 2014). These mechanisms are mediated by many molecular chaperones, in particular, the HSP70 chaperone machinery. HSP72 increases autophagy and protects cells from apoptosis (Buzzard et al. 1998). Chung et al. (2008) reported that HSP72 expression is reduced in human obesity and insulin resistance and that HSP72 overexpression in skeletal muscle prevents insulin resistance through interaction between HSP72 and UPR pathways. Consistent with this, our results show that resveratrol increased the expression of HSP72 and stimulated the clearance of abnormally folded

proteins through increased activation of the autophagy and ER-associated degradation (ERAD) pathways compared with that in tunicamycin-treated cells.

Autophagy maintains cellular homeostasis and promotes cell survival through clearance of damaged organelles and proteins aggregates. Autophagy is known to be activated for cellular protection during ER stress (Ogata et al. 2006), and this was also observed in the present study (Fig. 5). Recently, studies have suggested that autophagy may be a new therapeutic target for metabolic diseases such as obesity, diabetes, and fatty liver diseases (Czaja 2016; Mao et al. 2016; Rocchi and He 2015). Blockade of lysosome-associated membrane protein 2 (LAMP2) is known to dysregulate hepatic metabolism (Schneider et al. 2014), while overexpression of Rubicon inhibits autophagy and promotes hepatic lipid accumulation (Tanaka et al. 2016). In the complete process of autophagy for digestion, the fusion of autophagosomes with lysosomes is an important step. We observed that secreted clusterin, as well as resveratrol, decreased protein expression of Rubicon, which inhibits autophagosome-lysosome fusion, and increased the protein expression of LC3 and LAMP2 in cells treated with tunicamycin, indicating that clusterin has a protective effect against ER stress (Supplementary Fig. 2). These findings suggest that the cellular survival effects of resveratrol, via

autophagy-dependent pathways during ER stress, could be mediated by clusterin. However, further studies are needed to determine the mechanism of clusterin-activated autophagy.

In conclusion, we have shown that resveratrol treatment can improve tunicamycin-induced ER stress by inducing expression of molecular chaperones such as HSP72 and clusterin. Notably, we found that clusterin is involved in ERAD and autophagy pathways to protect cells against ER stress. Thus, the cellular protective effects of clusterin-mediated resveratrol may represent a new therapeutic approach for treatment ER stress-associated hepatic metabolic syndromes.

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References

- Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the in vivo evidence. *Nat Rev Drug Discov* 5:493–506
- Buzzard KA, Giaccia AJ, Killender M, Anderson RL (1998) Heat shock protein 72 modulates pathways of stress-induced apoptosis. *J Biol Chem* 273:17147–17153
- Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA, Vigh L, Hevener A, Febbraio MA (2008) HSP72 protects against obesity-induced insulin resistance. *Proc Natl Acad Sci U S A* 105:1739–1744
- Criswell T, Beman M, Araki S, Leskov K, Cataldo E, Mayo LD, Boothman DA (2005) Delayed activation of insulin-like growth factor-1 receptor/Src/MAPK/Egr-1 signaling regulates clusterin expression, a pro-survival factor. *J Biol Chem* 280:14212–14221
- Czaja MJ (2016) Function of autophagy in nonalcoholic fatty liver disease. *Dig Dis Sci* 61:1304–1313
- de Silva HV, Stuart WD, Duvic CR, Wetterau JR, Ray MJ, Ferguson DG, Albers HW, Smith WR, Harmony JA (1990) A 70-kDa apolipoprotein designated ApoJ is a marker for subclasses of human plasma high density lipoproteins. *J Biol Chem* 265:13240–13247
- Di Naso FC, Porto RR, Fillmann HS, Maggioni L, Padoin AV, Ramos RJ, Mottin CC, Bittencourt A, Marroni NA, de Bittencourt PI, Jr. (2015) Obesity depresses the anti-inflammatory HSP70 pathway, contributing to NAFLD progression. *Obesity* 23:120–129
- Dokladny K, Myers OB, Moseley PL (2015) Heat shock response and autophagy—cooperation and control. *Autophagy* 11:200–213
- Duncan RF (2005) Inhibition of Hsp90 function delays and impairs recovery from heat shock. *FEBS J* 272:5244–5256
- Graham RM, Hernandez F, Puerta N, De Angulo G, Webster KA, Vanni S (2016) Resveratrol augments ER stress and the cytotoxic effects of glycolytic inhibition in neuroblastoma by downregulating Akt in a mechanism independent of SIRT1. *Exp Mol Med* 48:e210
- Gupta S, Deepti A, Deegan S, Lisbona F, Hetz C, Samali A (2010) HSP72 protects cells from ER stress-induced apoptosis via enhancement of IRE1 α -XBP1 signaling through a physical interaction. *PLoS Biol* 8:e1000410
- Han J, Kaufman RJ (2016) The role of ER stress in lipid metabolism and lipotoxicity. *J Lipid Res* 57:1329–1338
- Hotamisligil GS (2010) Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 140:900–917
- Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR (1999) Clusterin has chaperone-like activity similar to that of small heat shock proteins. *J Biol Chem* 274:6875–6881
- Ingemann L, Kirkegaard T (2014) Lysosomal storage diseases and the heat shock response: convergences and therapeutic opportunities. *J Lipid Res* 55:2198–2210
- Kelly KJ (2002) Stress response proteins and renal ischemia. *Minerva urologica e nefrologica = The Italian journal of urology and nephrology* 54:81–91
- Kriegenburg F, Ellgaard L, Hartmann-Petersen R (2012) Molecular chaperones in targeting misfolded proteins for ubiquitin-dependent degradation. *FEBS J* 279:532–542
- Lee J, Ozcan U (2014) Unfolded protein response signaling and metabolic diseases. *J Biol Chem* 289:1203–1211
- Leskov KS, Klokoy DY, Li J, Kinsella TJ, Boothman DA (2003) Synthesis and functional analyses of nuclear clusterin, a cell death protein. *J Biol Chem* 278:11590–11600
- Mao Y, Yu F, Wang J, Guo C, Fan X (2016) Autophagy: a new target for nonalcoholic fatty liver disease therapy. *Hepatic medicine : evidence and research* 8:27–37
- Nikesitch N, Ling SC (2016) Molecular mechanisms in multiple myeloma drug resistance. *J Clin Pathol* 69:97–101
- Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, Murakami T, Taniguchi M, Tani I, Yoshinaga K, Shiosaka S, Hammarback JA, Urano F, Imaizumi K (2006) Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* 26:9220–9231
- Ozcan L, Tabas I (2012) Role of endoplasmic reticulum stress in metabolic disease and other disorders. *Annu Rev Med* 63:317–328
- Poon S, Treweek TM, Wilson MR, Easterbrook-Smith SB, Carver JA (2002) Clusterin is an extracellular chaperone that specifically interacts with slowly aggregating proteins on their off-folding pathway. *FEBS Lett* 513:259–266
- Pucci S, Bonanno E, Pichiorri F, Angeloni C, Spagnoli LG (2004) Modulation of different clusterin isoforms in human colon tumorigenesis. *Oncogene* 23:2298–2304
- Rocchi A, He C (2015) Emerging roles of autophagy in metabolism and metabolic disorders. *Front Biol* 10:154–164
- Rohne P, Prochnow H, Wolf S, Renner B, Koch-Brandt C (2014) The chaperone activity of clusterin is dependent on glycosylation and redox environment. *Cellular physiology and biochemistry : international journal of experimental cellular physiology. Biochem Pharmacol* 34:1626–1639
- Salminen A, Kaarniranta K (2009) SIRT1: regulation of longevity via autophagy. *Cell Signal* 21:1356–1360
- Schneider JL, Suh Y, Cuervo AM (2014) Deficient chaperone-mediated autophagy in liver leads to metabolic dysregulation. *Cell Metab* 20:417–432
- Tabata Y, Takano K, Ito T, Iinuma M, Yoshimoto T, Miura H, Kitao Y, Ogawa S, Hori O (2007) Vaticanol B, a resveratrol tetramer, regulates endoplasmic reticulum stress and inflammation. *Am J Physiol Cell Physiol* 293:C411–C418
- Tanaka S, Hikita H, Tatsumi T, Sakamori R, Nozaki Y, Sakane S, Shiode Y, Nakabori T, Saito Y, Hiramatsu N, Tabata K, Kawabata T, Hamasaki M, Eguchi H, Nagano H, Yoshimori T, Takehara T (2016) Rubicon inhibits autophagy and accelerates hepatocyte apoptosis and lipid accumulation in nonalcoholic fatty liver disease in mice. *Hepatology* 64:1994–2014
- Trougakos IP, Lourda M, Antonelou MH, Kletsas D, Gorgoulis VG, Papassideri IS, Zou Y, Margaritis LH, Boothman DA, Gonos ES (2009) Intracellular clusterin inhibits mitochondrial apoptosis by

- suppressing p53-activating stress signals and stabilizing the cytosolic Ku70-Bax protein complex. *Clin Cancer Res* 15:48–59
- Wang H, Sun RQ, Zeng XY, Zhou X, Li S, Jo E, Molero JC, Ye JM (2015) Restoration of autophagy alleviates hepatic ER stress and impaired insulin signalling transduction in high fructose-fed male mice. *Endocrinology* 156:169–181
- Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci* 9:244–252
- Wang WR, Li TT, Jing T, Li YX, Yang XF, He YH, Zhang W, Lin R, Zhang JY (2017) SIRT1 regulates the inflammatory response of vascular adventitial fibroblasts through autophagy and related signaling pathway. *Cellular physiology and biochemistry : international journal of experimental cellular physiology*. *Biochem Pharmacol* 41:569–582
- Wilson MR, Easterbrook-Smith SB (2000) Clusterin is a secreted mammalian chaperone. *Trends Biochem Sci* 25:95–98
- Xu L, Liu JH, Zhang J, Zhang N, Wang ZH (2015) Blockade of autophagy aggravates endoplasmic reticulum stress and improves Paclitaxel cytotoxicity in human cervical cancer cells. *Cancer Res Treat* 47:313–321
- Zhang H, Kim JK, Edwards CA, Xu Z, Taichman R, Wang CY (2005) Clusterin inhibits apoptosis by interacting with activated Bax. *Nat Cell Biol* 7:909–915
- Zhang Y, Chen ML, Zhou Y, Yi L, Gao YX, Ran L, Chen SH, Zhang T, Zhou X, Zou D, Wu B, Wu Y, Chang H, Zhu JD, Zhang QY, Mi MT (2015) Resveratrol improves hepatic steatosis by inducing autophagy through the cAMP signaling pathway. *Mol Nutr Food Res* 59:1443–1457
- Zhu X, Liu Q, Wang M, Liang M, Yang X, Xu X, Zou H, Qiu J (2011) Activation of Sirt1 by resveratrol inhibits TNF-alpha induced inflammation in fibroblasts. *PLoS One* 6:e27081

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