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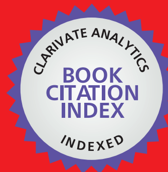

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Chapter 8

Modulation of Autophagy by Free Fatty Acids

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/61484

Abstract

Fatty acids are important molecules with multiple biological properties. Emerging evidence suggests that fatty acids can modulate autophagy. Saturated fatty acids contribute to pancreatic β-cell dysfunction in type 2 diabetes. Palmitic acid, one of the long-chain saturated fatty acids (LCFA), induces autophagy of β-cells which protects them from dysfunctions and apoptotic cell death. Short-chain fatty acids (SCFA) possess antitumor activity in colon cancer cells by promoting autophagy. SCFAs can induce autophagy by suppressing the activity of mTOR signaling. As the most common monosaturated fatty acid (MUFA) in daily nutrition, oleic acid could induce autophagy, which is responsible for the regulation of lipids metabolism in hepatocytes. The ω-3 and ω-6 polyunsaturated fatty acids (PUFA) are essential in normal physiology and metabolism and play a contributory role in the incidence and progress of a series of disease including cancer. Autophagy triggered by ω-3 PUFAs contributes to the cytotoxicity in cancer cells by enhancing apoptosis, while autophagy mediated by ω-6 PUFAs led to the increase in *Caenorhabditis elegans* lifespan. The recent findings illustrate the potential involvement of autophagy regulation by fatty acids in a number of biological and pathological processes.

Keywords: Autophagy, fatty acids, lipids, apoptosis, cancer

1. Introduction

Fatty acids are aliphatic carboxylic acids consisting of a hydrocarbon chain and a terminal carboxyl group. Fatty acids are classified as several groups with respect to their structure and biological functions. Saturated fatty acids, which have no double bond between individual carbon atoms of the hydrocarbon chain, are divided into short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), and very long-chain fatty

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acids (VLCFA) according to their chain length. The introduction of double bonds in the hydrocarbon chain results in the formation of the unsaturated fatty acids. Monounsaturated fatty acids (MUFA) have only one carbon–carbon double bond which can occur in different positions. Polyunsaturated fatty acids (PUFA) contain two or more double bonds along their carbon backbones. PUFAs are classified into subgroups including ω-3 and ω-6 PUFAs according to the position of the first double bond starting from methyl end.

Fatty acids provided by diets are ingested as triglycerides and then broken down into free fatty acids and monoglycerides for absorption in small intestine. In the intestinal mucosa cells, free fatty acids are re-esterified to triglycerides which are transported via lymphatic vessels to circulation as part of chylomicrons. Eventually, they bind to the membranes of hepatocytes, adipocytes, or muscle fibers where they are either stored or oxidized for supplying energy. Apart from serving as important energy substrates, fatty acids have multiple biological functions. The ω-3 and ω-6 PUFAs are essential in normal physiology and metabolism through their roles in membrane structural lipids, signal transduction pathways, eicosanoids metabolism, and gene expression. Saturated fatty acids have been related to adverse health effects whereas unsaturated fatty acids, especially monounsaturated and ω-3 polyunsaturated, are thought to be protective. In addition, trans fatty acids have been shown to play a negative role on health whereas conjugated fatty acids might be beneficial [1].

Autophagy is a catabolic process in which cytoplasmic components are sequestered in double-membrane vesicles and degraded by fusion with lysosomal compartments. The process of autophagy begins with the formation and elongation of phagophores. The cytoplasmic cargo is then sequestered and the double-membrane autophagosome fuses with a lysosome to generate the autolysosome. Finally, degradation is achieved through the hydrolytic enzymes within the autolysosome [2]. Autophagy maintains cellular homeostasis by eliminating superfluous or damaged proteins and organelles as well as provides substrates for energy generation and biosynthesis. Deregulation of autophagy is linked with susceptibility to various disorders including degenerative diseases, metabolic syndrome, aging, infectious diseases, and tumorigenesis.

Emerging evidence indicates the ability of fatty acids to induce autophagy. Mice fed with a high-fat diet lead to the formation of double-membrane autophagosomes in livers and increase the level of LC3-II which serve as a marker of autophagosome formation and the activity of autophagic flux [3]. Autophagy has been demonstrated to be involved in the biological function of fatty acids. Our recent studies reported that SCFAs promote autophagy in colon cancer cells which serves as an adaptive strategy for retarding apoptotic cell death [4]. In this chapter, the regulation of autophagy by fatty acids will be discussed including the mechanism of action involved and the biological significance implicated.

2. Saturated fatty acids

Saturated fatty acids are saturated with hydrogen and can be divided into subgroups according to their chain length. LCFAs increase levels of low-density lipoprotein-cholesterol, which is

http://dx.doi.org/10.5772/61484

connected with pathogenesis in multiple organs. SCFAs influence a wide array of cellular functions, especially affecting colonic health. Autophagy triggered by LCFAs in pancreatic β-cell and cardiomyocytes plays a critical role in the molecular pathogenesis of lipotoxicity. SCFAs induce autophagy in colon cancer and protect against mitochondria-mediated apoptotic cell death.

2.1. Long-chain saturated fatty acids

2.1.1. Palmitic acid

Palmitic acid (16:0) is the most abundant free saturated fatty acid in bloodstream. As its name indicates, it is a major component of the palm oil but also be found in meats and diary products. The stimulatory effect of palmitic acid on autophagy has been extensively examined in rat pancreatic beta cell INS-1 and has also been observed in human pancreatic islet and many other human cell lines including in neuroblastoma, myoblasts, hepatocytes, and endothelial cells [5-7]. After exposure to palmitic acid, INS-1 cells displayed a marked increase in the number of typical autophagosomes characterized by double-membranous vacuoles engulfing cytoplasmic structures. When monodansylcadaverine (MDC) was used to label autophagic vacuoles, the proportion of cells with MDC-stained dots dramatically increased after palmitic acid treatment. In GFP-LC3-expressed INS-1 cells, the number of GFP-LC3 puncta began to increase from 6 h after palmitic acid treatment and size of the dots gradually increased during palmitic acid incubation [5]. Autophagic flux was also accelerated by palmitic acid as evidenced by the enhanced conversion of LC3-I to LC3-II and the fusion between autophagosome and lysosomes. During autophagy, long-lived proteins were engulfed by autophagosomes and delivered to the lysosomes for degradation. The degradation rate of long-lived protein significantly increased in palmitate-treated cells. When autophagy was suppressed by ATG7 knockdown with siRNA, the proteolytic rate induced by palmitic acid significantly declined [6].

While mammalian target of rapamycin (mTOR) has been well established as the key regulator of the autophagic process, palmitic acid does not change phosphorylated status level of mTOR or its substrate p70S6K, suggesting that autophagy triggered by palmitic acid is mTOR-independent [8]. c-Jun N-terminal kinases (JNK) have been implicated in the induction of autophagy by various stimuli including starvation, cytokine stimulation, and endoplasmic reticulum (ER) stress. One mechanism by which JNK contributes to autophagy involves phosphorylation of the antiapoptotic protein Bcl-2. Phosphorylation of Bcl-2 by JNK causes its dissociation from Beclin-1 resulting in induction of autophagy. In addition, JNK could regulate autophagy by enhancing the expression of autophagic genes including Beclin-1, ATG5, and ATG7 [9]. JNK1 was phosphorylated shortly after exposure to palmitate in INS-1 cells, and inhibition of JNK with chemical inhibitor or molecular methods significantly decreased palmitate-induced conversion of LC3-I to LC3-II, indicating that JNK1 activation is responsible for palmitic-acid-triggered autophagy [6,10].

Another kinase involved in palmitate-induced autophagy is protein kinase C (PKC). There are 10 PKC isoenzymes classified as conventional, novel, and atypical in mammalian system. Several PKC isoenzymes including PCKδ, PKCθ, and PCKα have been implicated in autophagy. When taken up by cells, excess free fatty acids are converted into acyl-coA derivatives which can be incorporated and stored in the cells as neutral lipids like diacylglycerol (DAG) and triacylglycerol (TAG). Treatment with palmitic acid resulted in an increase in the relative levels of DAG, which serves as a natural agonist to recruit PKC proteins to plasma membrane for activation [11]. Conventional PKCα has been demonstrated to be activated and responsible for the autophagy by palmitic acid as evidenced by the following observations:

1. The formation of GFP-LC3 puncta and the conversion of LC3-I to LC3-II induced by palmitic acid could be significantly reduced by the chemical inhibitors of general and conventional PKCs.,

2. There was enhanced phosphorylated PKCα in palmitic-acid-treated cells compared to the control group. and/or

3. siRNA knock-down of PKCα reduced the level of LC3-II and autophagic flux induced by palmitic acid [8].

Lipotoxicity is a metabolic syndrome that excessive accumulation of lipid in non-adipose cells leads to cellular dysfunctions and death. Lipotoxicity is believed to be critically involved in type 2 diabetes mellitus which is characterized with the decline of β-cell and insulin resistance. Chronic exposure to elevated levels of free fatty acids leads to pancreatic β-cells lipid overload, dysregulation of insulin secretion, and apoptotic cell death [12]. The lipotoxicity of palmitic acid was predominant when cells were exposed to high level of saturated fatty acids. However, palmitic-acid-mediated autophagy induction has been suggested to be a protective mechanism against free fatty acids-induced β-cell dysfunction, apoptotic cell death, and insulin resistance [5,13-14]. Therefore, autophagy plays a crucial role in the preservation of pancreatic β-cell function. Further, altered autophagic activity has been implicated in the progression of obesity to type 2 diabetes through impairment of β-cell functions and development of insulin resistance [15]. Blockage of mTOR signaling pathway with rapamycin increases the formation of autophagosomes but attenuates palmitic-acid-induced β-cells death. On the contrary, reduction of autophagosome formation by knocking down the ATG5, inhibition of fusion between autophagosome and lysosome by bafilomycin A1, or inhibition of proteolytic degradation with E64d/pepstatin A could significantly augment palmitic-acid-induced β-cells death [5].

2.1.2. Myristic acid

Myristic acid (14:0) accounts for small amounts of total fatty acids in animal tissue, but is more abundant in milk fat and copra and palmist oils. Like other dietary saturated fatty acids, myristic acid is usually associated with negative consequences for human health. Russo et al. [16] found that there was high expression level of LC3 and BECN1 in whole heart lysate and increased number of LC3 puncta in left ventricles of mice fed with milk fat-based diet (MFBD). Because of the high myristate content of MFBD, the effects of myristate on autophagy in isolated mouse cardiomyocytes were examined. Treatment with

myristate promoted the overexpression of autophagy marker BECN1 which was sufficient to increase cardiac autophagy. Furthermore, myristate led to increased formation of GFP-LC3 puncta and the conversion of LC3-I to LC3-II, suggesting the ability of myristate to active autophagic flux in cardiomyocytes. In addition, treatment with palmitate did not induce expression of autophagy marker, indicating that myristate but not palmitate is responsible for the autophagy induced by MFBD [16].

The molecular mechanism underlying myristate-mediated autophagy in mouse cardiomyocytes is involved in the upregulation of C14-ceramide and ceramide synthase 5 (CerS5). Ceramide, which is composed of sphingosine and fatty acid, is a sphingolipid bioactive molecule that can trigger autophagy by interfering with the mTOR signaling pathway and dissociation of the Beclin-1:Bcl-2 complex in a JNK1-mediated Bcl-2 phosphorylation-dependent manner [17]. Myristate treatment increased C14-ceramide levels 10-folds in isolated mouse cardiomyocytes. Meanwhile, the induction of C14-ceramide was consistently observed in the heart of mice fed with MFBD. Ceramide synthesis occurs through N-acylation of sphingoid base by one of 6 CerS isoforms, and CerS5 is responsible for the C14-ceramide synthesis in the mouse heart. Gain- and loss-of-function experiments demonstrated the requirement of CerS5 in sphingolipid-induced autophagy in cardiomyocytes. Overexpression of CerS5 induced BECN1 expression in isolated cardiomyocytes even in the absence of myristate treatment. siRNA-mediated knockdown of endogenous CerS5 abrogated the induction of BECN1 expression, the formation of GFP-LC3 puncta, and the conversion of LC3-I to LC3-II in isolated mice cardiomyocytes treated with myristate, indicating the critical role of CerS5 in myristate-triggered autophagy.

Autophagy has been identified as an important process upregulated in cardiac hypertrophy and dysfunction in responses to pressure overload. However, whether autophagy plays a beneficial or detrimental role in cardiac hypertrophy remains unclear [18]. Mice fed with MFBD developed left ventricular hypertrophy and functional reduction in ejection fraction. Myristate, but not palmitate, treatment increased the size of isolated cardiomyocytes, suggesting that myristate is responsible for MFBD-induced cardiac hypertrophy. In fact, myristate-induced hypertrophy could be completely prevented by LC3 knockdown or treatment with autophagy inhibitor 3-methyladenine (3-MA), indicating that autophagy induced by myristate plays a prohypertrophic role in cardiac lipid overload [16].

2.2. Short-chain fatty acids

Short-chains fatty (SCFAs) acids are the major byproducts of bacterial fermentation of undigested dietary fiber within the intestinal lumen. The main SCFA production of fiber fermentation in colon is acetate (2:0), propionate (3:0), and butyrate (4:0) in a molar ratio about 3:1:1. More than 95% of the SCFAs produced in intestine are rapidly absorbed and metabolized by the host. SCFAs exert potent effects on a variety of colonic mucosal function such as inhibition of inflammation reinforcing various components of colonic defense barrier and decreasing oxidative stress. Furthermore, SCFAs have antitumor activity in the colon by promoting apoptotic cell death [19].

We have reported that SCFAs, particularly propionate, induced autophagy characterized by increased LC3 puncta formation, enhanced conversion of LC3-I to LC3-II, and upregulated expression of LAMP-2 in colon cancer cells [4]. In addition, autophagy could be induced by butyrate in gingival epithelial cells and Chinese hamster ovary (CHO) cell, which play a potential role of cell survival mechanism [20,21].

Treatment of colon cancer HCT116 cells with propionate led to a strong time-dependent reduction in the phosphorylation state of mTOR at Ser2481 while there was no change in the total mTOR levels. Phosphorylation of eukaryotic initiation factor 4E-binding proteins (4E-BP1), a key downstream effector of mTOR activity, also markedly decreased in a dose- and time-dependent manner after exposure to propionate. Furthermore, reduced level of phosphorylated p70S6K, another key downstream effector of mTOR, was noted by 7 h following propionate treatment. Collectively, these observations demonstrate that downregulation of the mTOR signaling pathway is a mechanism for propionate to induce autophagy [4]. To further characterize the mechanisms underlying the reduced mTOR activation and concomitant autophagy induction by SCFA, we examined the activation status of PI3/Akt pathway which activates mTOR in response to the introduction of nutrient and growth factors, but no change of the phosphorylation state of Akt at S473 or Thr308 or the total Akt were observed. However, we observe that the AMP-activated protein kinase (AMPK), an inhibitor of the mTOR protein, was significantly activated by propionate treatment [4], suggesting that AMPK pathway was involved for SCFA to induce autophagy.

AMPK signaling activation induced by propionate was associated with mitochondrial defect-mediated cellular ATP depletion and oxidative stresses. Mitochondria are organelles that primarily produce ATP via oxidative phosphorylation in the inner membrane. Reactive oxygen species (ROS) is an inevitable by-product of mitochondrial metabolism and can cause mitochondrial damage. Such damage subsequently induces the mitochondrial membrane permeability transition (MPT), mitochondria swelling, and cell death. Mitophagy, a selective form of autophagy by which mitochondria are degraded in autolysosomes, plays an essential role in maintaining mitochondrial functional and genetic integrity [22]. Propionate induced ATP reduction and ROS generation in colon cancer cells due to the induction of MPT and loss of the mitochondrial membrane potential. The proportion of mitochondria with lower fluorescence intensity, which represents the depolarized mitochondria, was increased by propionate treatment in a dose- and time-dependent manner in the colon cancer cells. Furthermore, propionate treatment led to co-localization of mitochondria and GFP-LC3 puncta in colon cancer cells. COXIV, a mitochondrial marker, was reduced and localized as defective mitochondria by autolysosomes. An ubiquitin-binding protein-p62, which interacts with LC3 and regulates autophagosome formation, significantly co-localized with mitochondrial COXIV. Consistently, inhibition of autophagic degradation by CQ dramatically increased the accumulation of defective mitochondria [4]. The results suggest the ability of propionate to induce mitophagy, which selectively targets mitochondria with a depolarized membrane potential.

The induction of autophagy by SCFAs may serve as an adaptive strategy for colon cancer cells to retard apoptotic cell death. Application of an autophagy inhibitor was found to enhance the

rate of apoptosis after treatment with SCFAs. Co-treatment of HCT116 cells with propionate/3-MA significantly reduced the percentage of GFP-LC3 formation. Meanwhile, the number of apoptotic cells increased as indicated by the high annexin-V staining. Western blot analysis also indicated the increased cleavages of the pro-apoptotic cascapase-7 and executioner caspase-3. Chloroquine, another inhibitor of autophagy, enhanced apoptosis in HCT116 cells especially at the later stages of treatment. When autophagy was inhibited with molecular shRNA targeted to AMPKα or ATG5, there was a decreased ability of propionate to induce GFP-LC3 puncta formation in HCT116. Consistent with the pharmacologic inhibitory experiment, AMPKα or ATG5 depletion cells displayed a significantly enhanced apoptosis after propionate treatment [4]. The findings suggest that autophagy confers a protective role in propionate-mediated cell death in colon cancer cells.

3. Unsaturated fatty acids

An unsaturated fatty acid is a fatty acid in which there is at least one double bond within the hydrocarbon chain. The most common MUFAs in daily nutrition is oleic acid (18:1 n-9), followed by palmitoleic acid (16:1 n-7) and vaccenic acid (18:1 n-7). Oleic acid could increase the ratio of HDL to LDL cholesterol and decrease aggregation of thrombocytes. ω-3 and ω-6 PUFAs are termed as essential fatty acids because they are necessary for health but cannot be synthesized de novo in humans. Autophagy has been reported to be induced by unsaturated fatty acids and involved in the regulation of lipid metabolism in hepatocytes and apoptosis in cancer cells.

3.1. Monounsaturated fatty acid

As one of the most abundant fatty acid in the diet and serum, oleic acid (18:9 n-1) is a monosaturated fatty acid with the double bond at its ω-9 position. Autophagy triggered by oleate was observed in mammary epithelial cells, hepatocytes, and osteosarcoma cells [3,23-24]. Furthermore, oleate-triggered autophagy was demonstrated by in vivo experiment. Oleate administered via intraperitoneal injection could cause a rapid autophagic response in the heart, liver, and skeletal muscle of mice characterized by LC3 lipidation, p62 degradation, and phosphorylation of AMPK [23]. Treatment with oleic acid in HepG2 cells significantly increased the number of double-membrane autophagosomes with enveloped cytosolic contents. Meanwhile, oleic acid treatment led to the increase of GFP-LC3 puncta number in a concentration-dependent manner, indicating that oleic acid could induce autophagy in hepatocytes [3]. Oleic acid did not suppress mTOR activity as determined by the level of phosphorylated 4EBP1 and p70S6K at different time points and various concentrations. However, oleic-acid-induced autophagy required ROS formation and the classic PI3 kinase complex. The level of ROS production was increased in oleic-acid-treated cells via activation of the NADPH oxidase enzyme complex [25], and the number of GFP-LC3 puncta formation induced by oleic acid could be markedly suppressed by antioxidant NAC and PI3 kinase inhibitor.

Autophagy triggered by oleic acid contributed to the regulation of lipids metabolism in hepatocytes [3]. Free fatty acids taken up by hepatocytes convert into triglyceride (TG) for storage with cholesterol in lipid droplets (LD). When energy is required, the stored TG is hydrolyzed via activation of lipolytic pathways. Knockdown of autophagic gene ATG5 increased TG levels in hepatocytes cultured with oleate or a second endogenous stimulus for TG formation, methionine- and choline-deficient medium (MCDM). Pharmacological inhibition of autophagy with 3-MA also markedly increased the TG content. Consistent with the increased TG levels, lipid staining revealed increased LD number and size in hepatocytes cultured with oleate or MCDM that were further increased by 3-MA addition or ATG5 knockdown [26]. Autophagy did not change TG synthesis as the comparable increase in TG synthesis occurred in ATG5 knockdown and control cells in response to oleate or MCDM. However, ATG5 knockdown led to much lesser increase in the rate of β-oxidation, inductive of the level of free fatty acids generated by TG hydrolysis as well as significant decrease in TG breakdown in hepatocytes cultured in oleate or MCDM [26]. The regulation of lipid metabolism by autophagy is further supported by the association of autophagic pathway components with LDs. Oleate treatment increased co-localization of LDs with the lysosome-associated membrane protein type 1 (LAMP1). Inhibition of autophagosome formation with 3-MA or autophagosome–lysosome fusion with vinblastine markedly reduced LD/LAMP1 co-localization. Moreover, LD co-localization with autophagosome marker LC3 demonstrates a direct association between LDs and autopaghosomes.

Niso-Santano et al. [23] reported recently that oleate-induced non-canonical autophagy in human osteosarcoma U2OS cells required an intact Golgi apparatus. Oleate treatment promoted the co-localization of LC3 with several proteins of trans-Golgi network including trans-Golgi network protein 2 (TGOLN2) and galactose-1-phosphateuridylytransferase (GALT). Similarly, GFP-LC3 puncta induced by oleate co-localized with lectin mannose-binding 1 (LMAN1), a marker of the endoplasmic reticulum–Golgi intermediate compartment and RAB7A, a protein residing in late endosomes. In addition, transmission electron microscopy assessment confirmed the co-localization of vacuolar structures induced by oleate with the Golgi apparatus to the nuclear periphery. Although the disruption of Golgi apparatus could induce autophagy in human cell lines [27], the structural and functional integrity of Golgi apparatus were not affected by the administration of oleate. Moreover, disruption of Golgi apparatus with brefeldin A reduced the ability of oleate to promote autophagic activity, suggesting that oleate possesses the capacity to stimulate autophagic responses but does not compromise the integrity of the Golgi apparatus.

3.2. ω-3 polyunsaturated fatty acids

Omega-3 PUFAs docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5n-3), which are found primarily in cold-water fish and fish oils, reduce the incidence and progress of a series of human diseases including cancer. The ability of DHA and EPA to induce autophagy has been reported in myocardioblasts and diverse human cancer cells [28-32]. DHA treatment dramatically increased the formation of visualized autophagic vacuoles and LC3 puncta. The number of lysotracker-positive vesicles as well as the co-localization of LC3 with

lysotracker was also markedly increased. Chloroquine promoted the induction of LC3-I by DHA. These results collectively suggest that DHA triggers autophagy via activation of the autophagic flux [30]. Dietary DHA and EPA could be converted to their ethanolamide derivatives, docosahexenoyl ethanolamine (DHEA) and eicosapentaenoyl ethanolamine (EPEA). The level of autophagy gene Beclin-1 in breast cancer cell MCF-7 could be significantly increased after treatment with DHEA or EPEA. Furthermore, ethanolamides reduced the association between Beclin-1 and Bcl-2, indicating that DHEA and EPEA could induce autophagy in human breast cancer cells [33].

The molecular mechanism underlying DHA-triggered autophagy is related to the p53/AMPK/mTOR signal pathway. The tumor suppressor p53 plays a dual role in the control of autophagy. On one hand, nuclear p53 can induce autophagy through transcriptional effects. On the other hand, cytoplasmic p53 may acts as a master repressor of autophagy [34]. Tissues from adult mice with an expression of a GFP-LC3 transgene on a p53–/– background showed a higher level of GFP-LC3 puncta than p53+/+ and heterozygous littermate. Consistently, inhibition of p53 with siRNA or pharmacological inhibitor in human cancer cell induced the accumulation of GFP-LC3 puncta. In human cervical and breast tumor cell lines, DHA dose-dependently downregulated the level of p53 and triggered autophagy through a signaling pathway similar to p53 inhibition mediated autophagy. When p53 degradation was inhibited by MG132, the accumulation of GFP-LC3 puncta induced by DHA was significantly reduced, indicating that loss of p53 is responsible for DHA-induced autophagy in cancer cells.

The AMPK/mTOR signaling pathway is responsible for p53 inhibition mediated autophagy. In p53–/– cells, AMPK and its substrates tuberous sclerosis complex 2 (TSC2) and acetyl CoA carboxylase (ACCα) were hyperphosphorylated whereas p70S6K was hypophosphorylated. siRNA-mediated depletion of AMPKα1 and AMPKα2 or inhibition of mTOR with rapamycin eliminated the difference in autophagy between p53-inhibited and control cells [34]. DHA treatment increased the level of phosphor–AMPK and phosphor–ACCα expression and decreased the activity of mTOR pathway, suggesting that DHA induces autophagy through p53-mediated AMPK/mTOR signaling.

Autophagy induced by EPEA and DHEA in breast cancer cells has been attributed to the activation of proliferator-activated receptor gamma (PPARγ). DHA and EPA as well as their ethanolamide derivatives act as the activator of PPARγ, which has been reported to induce autophagy in breast cancer cells [35]. PPARγ modulates the transcription of phosphatase and tensin homolog on chromosome ten (PTEN), a unique phosphatase with the ability to decrease the levels of phosphorylated AKT and consequently AKT-mediated pathway [36]. Via trans-activation of PPARγ in MCF7 cells, DHEA and EPEA enhanced PTEN protein levels and subsequently decreased the activity of AKT/mTOR signaling pathway which is responsible for the induction of autophagy [33].

Omega-3 PUFAs can induce apoptosis by activating both intrinsic and extrinsic apoptotic pathways. DHA-induced autophagy contributes to the cytotoxic activity by enhancing apoptosis in cancer cells. While it is generally accepted that autophagy function as a mechanism for cells to survive from stresses, there is also a substantial body of literature suggesting

that autophagy can promote cell death under certain circumstances [37,38]. Autophagy might degrade cellular components so that the cell eventually activates the apoptosis machinery. DHA-induced apoptosis could be partially blocked when autophagy was inhibited by 3-MA. Consistently, ATG5 and ATG7 knockdown by siRNA reduced both autophagy and apoptosis response to DHA treatment in human cancer cells, indicating that autophagy enhances DHA-induced apoptosis and inhibition of autophagy prevents DHA-apoptotic cell death [30].

3.3. ω-6 polyunsaturated fatty acids

Omega-6 PUFAs are characterized by the presence of at least two carbon–carbon double bonds with the first bond at the sixth carbon from the methyl terminus. The ability of omega-6 PUFA to induce autophagy was observed in *C. elegans* [39]. As in mammalian cells, autophagy occurs at basal levels during normal growth conditions in *C. elegans*, but is rapidly upregulated in response to certain environmental stresses. Using *C. elegans* that express the GFP::LGG-1 reporter as an integrated transgene, autophagy was found to be activated by starvation in the pharyngeal muscle. Enrichment of ω-3 PUFA EPA and ω-6 PUFAs arachidonic acid (AA, 20:4 n-6) and di-homo-γ-linoleic acid (DGLA, 20:3 n-6) was observed in fasted *C. elegans*. Supplementation with AA and DGLA, but not with EPA, was sufficient to activate autophagy in *C. elegans*, indicating the role of ω-6 PUFAs in starvation-triggered autophagy. The *C. elegans* fatty acid desaturases fat-6 and fat-7 are required for long-chain unsaturated fatty acids synthesis, and fat-1 is required for the conversion of ω-6 to ω-3 PUFAs. RNAi targeted to fat-6, fat-7, or fat-1 does not affect the intensity or distribution of LGG1 puncta in well-fed animals, suggesting that the effects of ω-6 PUFAs on autophagy is achieved only when their levels relative to fatty acids are above a certain threshold, which cannot be recapitulated by inactivating the enzymes responsible for the synthesis of ω-6 PUFAs or their conversion to ω-3 PUFAs [39].

Autophagy can contribute lifespan extension of *C. elegans*. Mutations in the insulin-like growth factor (IGF-1) receptor DAF-2 display an increase in adult *C. elegans* longevity. However, autophagy inactivation by RNAi of BEC-1 (the ortholog of Beclin-1), ATG7, and LGG3 (the ortholog of ATG12) decreased lifespan of DAF-2 mutants, indicating that autophagy genes are required for lifespan extension [40]. Dietary restriction plays an evolutionarily conserved role in lifespan extension in yeasts, flies, mammals, and *C. elegans*. The correlation between increased autophagy and lifespan in feeding-defective *C. elegans*, *eat-2*, *eat-3*, and *pha-3*, provides a clue that autophagy might be involved in dietary restriction-mediated lifespan extension [41,42]. Chronic long-term dietary supplementation with AA and DGLA extended *C. elegans* lifespan under the condition of food abundance. Inactivation of the essential autophagy genes BEC-1, LGG-1 (the ortholog of ATG8/MAPLC3), and ATG16.2 (the ortholog of ATG16p/ATG16l1) suppressed the life extension induced by ω-6 PUFAs. These results collectively revealed that ω-6 PUFAs increased *C. elegans* lifespan through the activation of autophagy in well-fed condition. While the mechanisms by which autophagy mediates lifespan extension are not yet understood, the possibility is that autophagy removes damaged mitochondria, decreased levels of intracellular reactive oxygen species, and subsequently protects against oxidative damage. Many of the long-lived mutants in *C. elegans* were resistant to oxidative stress and many mutations that decrease mitochondrial electron transport are

long-lived whereas, conversely, mutations that increase oxidative damage shorten lifespan in *C. elegans*. Thus, longevity in *C. elegans* may be mediated either by mutations that directly affect cellular generation or breakdown of reactive oxygen species or indirectly decrease reactive oxygen specified via upregulation of autophagic turnover of damaged organelles that generate these harmful species.

ω-6 PUFAs are supposed to induce autophagy in mammalian cells. Dietary supplementation of ω-6 PUFAs improve several human conditions that overlap with the pathologies associated with autophagy malfunctions such as chronic inflammation, neuro-degenerative disease, and cancer. Supplementation with AA and DGLA but not EPA leads to the increase of autophagic marker LC3-II and the formation of LC3 puncta in HeLa cells accompanied with the decreased level of p62 [39]. Furthermore, the number of LC3 puncta in HeLa cells treatment with AA or DGLA could be augmented by the inhibition of lysosomal enzymes and impaired by the inactivation of autophagy gene ATG161L indicated the ability of ω-6 PUFAs to trigger autophagy in HeLa cells via activation autophagic flux.

3.4. Trans fatty acids

Trans fatty acids (TFA) have one or more double bonds in *trans* configuration instead of the usual *cis* configuration. The primary dietary trans TFAs are vaccenic acid (18:1 n-7t) and elaidic acid (18:1 n-9t). Vaccenic acid is a naturally occurring TFA found in the fat of ruminants and diary products, whereas elaidic acid is the major TFA found in hydrogenated vegetable oils [43]. TFAs increase LDL and decrease the beneficial HDL levels resulting in a less desirable LDL/HDL ratio, indicating the deleterious effects of TFAs consumption on human health. Extensive evidence has proved the direct connection of TFAs with coronary heart disease, cancer, Alzheimer's disease, diabetes, and other diseases. TFAs-induced autophagy in primary rat cardiac myofibroblasts has been recently reported [44]. Both vaccenic acid and elaidic acid could induce autophagosome formation, LC3 lipidation, LC3-II formation, increased beclin-1 concentration and ATG5–ATG12 complex formation. Considering that inhibition of lysosome–autophagosome fusion with bafilomycin A1 further increased TFAs-mediated LC3-II formation, the authors believe that TFAs-triggered autophagy is related to de novo autophagosome formation and subsequent turnover [44].

TFAs treatment led to marked apoptotic cell death of primary cardiac myofibroblasts, which is dependent upon the activation of autophagy [44]. Both vaccenic acid and elaidic acid induced cell death in a concentration-dependent manner. Meanwhile, caspase-3 and 9, but not caspase-8 or Bid, were activated by TFAs treatment, indicating that the intrinsic apoptosis was induced by TFAs. The role of autophagy in TFAs-induced apoptosis was confirmed in ATG3 and ATG5 knockout mouse embryonic fibroblasts (MEFs). Absence of ATG3 and ATG5 significantly reduced the cytotoxic effects of TFAs. Moreover, ATG3 or ATG5 knockout decreased the activation of caspase 3 and caspase 7 induced by TFAs exposure, demonstrating that autophagy is necessary for TFAs-induced apoptotic cell death [44]. As TFAs treatment led to the generation of ROS and decreased mitochondrial membrane potential [45], it has been hypothesized that the mechanism underlying the interplay between autophagy and apoptosis upon TFAs treatment may be related to the disruption of the mitochondrial metabolism and

membrane potential which serves as a powerful trigger for the induction of apoptosis and autophagy.

3.5. Conjugated fatty acids

Conjugated fatty acids (CFAs) are a mixture of positional and geometric isomers of polyunsaturated fatty acids with conjugated double bonds. The most abundant fatty acids with a conjugated system of double bonds are isomers of linoleic acid (conjugated linoleic acid, CLA), which are found mostly in the meat and dairy products derived from ruminants. Several CLA isomers exist due to positional and geometrical isomerism of the conjugated double bonds and the major naturally existing one of which is referred to as 9Z11E-18:2 [1]. Most recently, CLA has been reported to induce nonalcoholic steatohepatitis in an animal model. The liver of mouse feeding with CLA-containing diet displayed more LC3-positive cells compared with those fed with control diet, indicating the ability of CLA to increase autophagy in liver cells [46].

In addition to CLA, many other CFAs occur naturally in plant seeds and marine algae. Alpha-eleostearic acid (ESA, 9Z11E13E-18:3) is a linolenic acid isomer with a conjugated triene system. ESA makes up approximately 80% of the fatty acids in tung oil and 60% of bitter gourd seed oil. Autophagy induction by ESA was observed in Hela cells as evidenced by the autophagic vacuoles as well as the conversion of LC3-I to LC3-II [47]. ESA decreases phosphorylation of P70KS and AKT effectively in both time- and dose-dependent manner. On the contrary, ERK1/2 phosphorylation could be increased by ESA treatment, indicating that autophagy induced by ESA is involved in the regulation of ERK1/2 and AKT/mTOR signal pathway [47]. Although ESA possesses strong suppressive effects on tumor growth, the induction of autophagy by ESA functions as a protective mechanism against cell death in HeLa cells. When autophagy was inhibited with 3-MA, the viability of HeLa cell was markedly reduced in response to ESA treatment [47].

4. Conclusion

Fatty acids are important molecules with multiple physiological properties including serving as major metabolic fuel, essential components of all membranes, and gene regulators. As an evolutionarily conserved catabolic process, autophagy is generally thought as a survival mechanism in dealing with stress by clearing damaged proteins, organelles, or pathogens or by providing the cells with energy and anabolic building blocks during starvation. Autophagy could be triggered by fatty acids and contributes to the pathologic and physiological processes mediated by fatty acid such as the metabolism of lipid, lipotoxicity, life extension, and antitumor activity (Figure 1). While the regulation of ROS generation, ceramide synthesis, and multiple signal pathways like AKT/mTOR, JNK, and PKC are involved in fatty-acids-induced autophagy, the molecular mechanisms involved and the implications in different physiological or pathological processes need to be further elucidated.

http://dx.doi.org/10.5772/61484

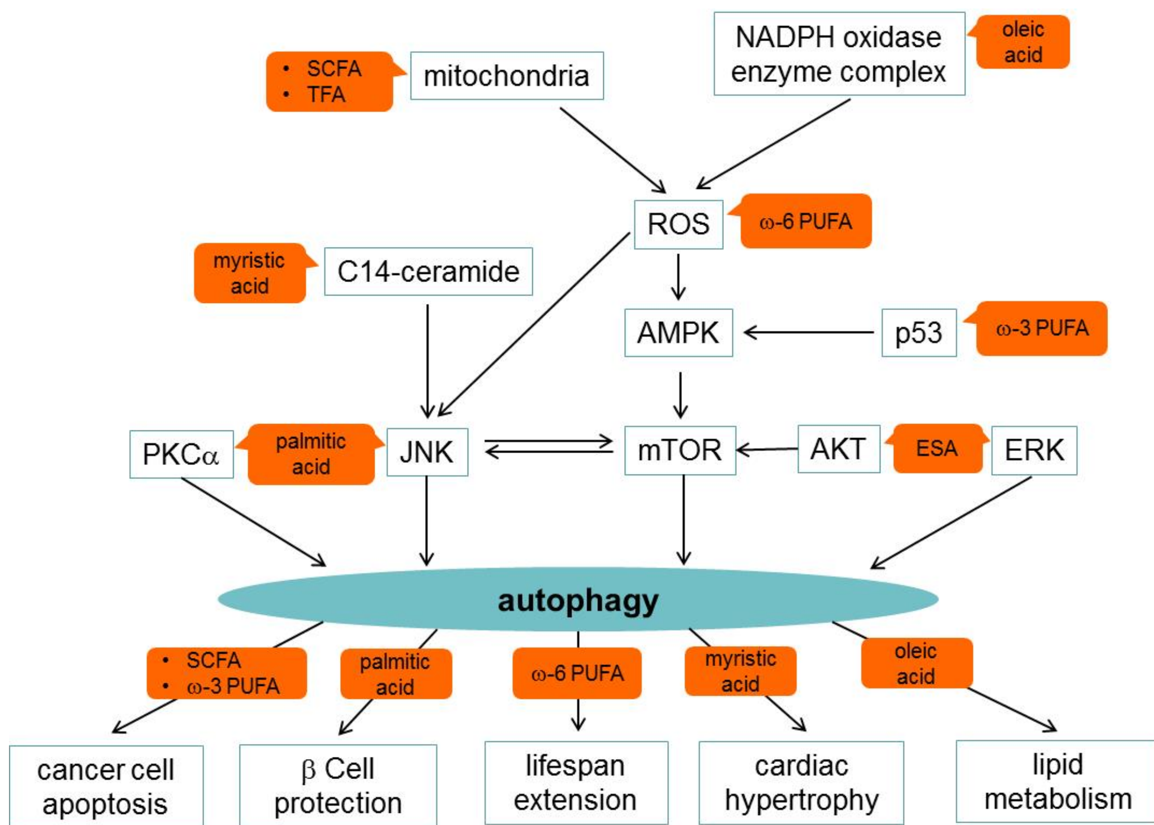

Figure 1. The regulation of autophagy by fatty acids and its biological significances.

Multiple signaling pathways are involved in fatty-acids-mediated autophagy. JNK is responsible for autophagy triggered by long-chain saturated fatty acids, palmitic acid and myristic acid. ROS plays an essential role in autophagy regulation through AMPK/mTOR pathway. SCFA, TFA, oleic acid, and ω-6 PUFA-mediated autopahgy is attributed to the increase of ROS via disruption of mitochondrial metabolism, or activation of NADPH oxidase enzyme complex. ω-3 PUFAs induce autophagy through p53-dependent AMPK/mTOR signaling. Other signaling pathways such as AKT/mTOR and PKCα are also involved in autophagy regulation by fatty acids. Autophagy triggered by fatty acids contributes to diverse pathological and physiological processes including apoptosis of cancer cells, β cell protection and insulin resistance, life extension, lipid metabolism, and cardiac hypertrophy.

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References

[1] Tvrzicka E, Kremmyda LS, Stankova B, and Zak A. Fatty acids as biocompounds: their role in human metabolism, health and disease--a review. Part 1: classification, dietary sources and biological functions. Biomed Pap Med Fac Univ Palacky. Olomouc Czech Repub 2011;155:117–30.

[2] Mizushima N. Autophagy: process and function. Genes Dev 2007;21:2861–73.

[3] Mei S, Ni HM, Manley S, Bockus A, Kassel KM, Luyendyk JP, Copple BL, Ding WX. Differential roles of unsaturated and saturated fatty acids on autophagy and apoptosis in hepatocytes. J Pharmacol Exp Ther 2011;339:487–98.

[4] Tang Y, Chen Y, Jiang H, Nie D. Short-chain fatty acids induced autophagy serves as an adaptive strategy for retarding mitochondria-mediated apoptotic cell death. Cell Death Differ 2011;18:602–18.

[5] Choi SE, Lee SM, Lee YJ, Li LJ, Lee SJ, Lee JH, Kim Y, Jun HS, Lee KW, Kang Y. Protective role of autophagy in palmitate-induced INS-1 beta-cell death. Endocrinology 2009;150:126–34.

[6] Komiya K, Uchida T, Ueno T, Koike M, Abe H, Hirose T, Kawamori R, Uchiyama Y, Kominami E, Fujitani Y, Watada H. Free fatty acids stimulate autophagy in pancreatic β-cells via JNK pathway. Biochem Biophys Res Commun 2010;401:561–7.

[7] Martino L, Masini M, Novelli M, Beffy P, Bugliani M, Marselli L, Masiello P, Marchetti P, De Tata V. Palmitate activates autophagy in INS-1E β-cells and in isolated rat and human pancreatic islets. PLoS One 2012;7:e36188.

[8] Tan SH, Shui G, Zhou J, Li JJ, Bay BH, Wenk MR, Shen HM. Induction of autophagy by palmitic acid via protein kinase C-mediated signaling pathway independent of mTOR (mammalian target of rapamycin). J Biol Chem 2012;287:14364–76.

[9] Lorin S, Pierron G, Ryan KM, Codogno P, Djavaheri-Mergny M. Evidence for the interplay between JNK and p53-DRAM signalling pathways in the regulation of autophagy. Autophagy 2010;6:153–4.

[10] Tu QQ, Zheng RY, Li J, Hu L, Chang YX, Li L, Li MH, Wang RY, Huang DD, Wu MC, Hu HP, Chen L, Wang HY. Palmitic acid induces autophagy in hepatocytes via JNK2 activation. Acta Pharmacol Sin 2014;35:504–12.

[11] Sridharan S, Jain K, Basu A. Regulation of autophagy by kinases. Cancers 2011;3:2630–54.

[12] Giacca A, Xiao C, Oprescu AI, Carpentier AC, Lewis GF. Lipid-induced pancreatic β-cell dysfunction: focus on in vivo studies. Am J Physiol Endocrinol Metab 2011;300:E255–62.

[13] Ebato C, Uchida T, Arakawa M, Komatsu M, Ueno T, Komiya K, Azuma K, Hirose T, Tanaka K, Kominami E, Kawamori R, Fujitani Y, Watad H. Autophagy is important in

islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. Cell Metab 2008;8:325–32.

[14] Mir SU, George NM, Zahoor L, Harms R, Guinn Z, Sarvetnick NE. Inhibition of autophagic turnover in β-cells by fatty acids and glucose leads to apoptotic cell death. J Biol Chem 2014;290:6071-85.

[15] Barlow AD, Thomas DC. Autophagy in diabetes: β-cell dysfunction, insulin resistance, and complications. DNA Cell Biol 2015;34:252–60.

[16] Russo SB, Baicu CF, Van Laer A, Geng T, Kasiganesan H, Zile MR, Cowart LA. Ceramide synthase 5 mediates lipid-induced autophagy and hypertrophy in cardiomyocytes. J Clin Invest 2012;122:3919–30.

[17] Pattingre S, Bauvy C, Levade T, Levine B, Codogno P. Ceramide-induced autophagy: to junk or to protect cells? Autophagy 2009;5:558–60.

[18] Gatica D, Chiong M, Lavandero S, Klionsky DJ. Molecular mechanisms of autophagy in the cardiovascular system. Circ Res 2015;116:456–67.

[19] Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. Review article: the role of butyrate on colonic function. Aliment Pharmacol Ther 2008;27:104–19.

[20] Tsuda H, Ochiai K, Suzuki N, Otsuka K. Butyrate, a bacterial metabolite, induces apoptosis and autophagic cell death in gingival epithelial cells. J Periodontal Res 2010;45:626–34.

[21] Lee JS, Lee GM. Effect of sodium butyrate on autophagy and apoptosis in Chinese hamster ovary cells. Biotechnol Prog 2012;28:349–57.

[22] Kim I, Rodriguez-Enriquez S, Lemasters JJ. Selective degradation of mitochondria by mitophagy. Arch Biochem Biophys 2007;462:245–53.

[23] Niso-Santano M, Malik SA, Pietrocola F, Bravo-San Pedro JM, Mariño G, Cianfanelli V, Ben-Younès A, Troncoso R, Markaki M, Sica V, Izzo V, Chaba K, Bauvy C, Dupont N, Kepp O, Rockenfeller P, Wolinski H, Madeo F, Lavandero S, Codogno P, Harper F, Pierron G, Tavernarakis N, Cecconi F, Maiuri MC, Galluzzi L, Kroemer G. Unsaturated fatty acids induce non-canonical autophagy. EMBO J 2015;34:1025-41.

[24] Pauloin A, Chat S, Péchoux C, Hue-Beauvais C, Droineau S, Galio L, Devinoy E, Chanat E. Oleate and linoleate stimulate degradation of β-casein in prolactin-treated HC11 mouse mammary epithelial cells. Cell Tissue Res 2010;340:91–102.

[25] Hatanaka E, Dermargos A, Hirata AE, Vinolo MA, Carpinelli AR, Newsholme P, Armelin HA, Curi R. Oleic, linoleic and linolenic acids increase ros production by fibroblasts via NADPH oxidase activation. PLoS One 2013;8:e58626.

[26] Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ. Autophagy regulates lipid metabolism. Nature 2009;458:1131–5.

[27] Naydenov NG, Harris G, Morales V, Ivanov AI. Loss of a membrane trafficking protein αSNAP induces non-canonical autophagy in human epithelia. Cell Cycle 2012;15:4613–25.

[28] Fukui M, Kang KS, Okada K, Zhu BT. EPA, an omega-3 fatty acid, induces apoptosis in human pancreatic cancer cells: role of ROS accumulation, caspase-8 activation, and autophagy induction. J Cell Biochem 2013;114:192–203.

[29] Hsu HC, Chen CY, Chiang CH, Chen MF. Eicosapentaenoic acid attenuated oxidative stress-induced cardiomyoblast apoptosis by activating adaptive autophagy. Eur J Nutr 2014;53:541–7.

[30] Jing K, Song KS, Shin S, Kim N, Jeong S, Oh HR, Park JH, Seo KS, Heo JY, Han J, Park JI, Han C, Wu T, Kweon GR, Park SK, Yoon WH, Hwang BD, Lim K. Docosahexaenoic acid induces autophagy through p53/AMPK/mTOR signaling and promotes apoptosis in human cancer cells harboring wild-type p53. Autophagy 2011;7:1348–58.

[31] Shin S, Jing K, Jeong S, Kim N, Song KS, Heo JY, Park JH, Seo KS, Han J, Park JI, Kweon GR, Park SK, Wu T, Hwang BD, Lim K. The omega-3 polyunsaturated fatty acid DHA induces simultaneous apoptosis and autophagy via mitochondrial ROS-mediated Akt-mTOR signaling in prostate cancer cells expressing mutant p53. Biomed Res Int2013;2013:568671.

[32] Yao QH, Zhang XC, Fu T, Gu JZ, Wang L, Wang Y, Lai YB, Wang YQ, Guo Y. ω-3 polyunsaturated fatty acids inhibit the proliferation of the lung adenocarcinoma cell line A549 in vitro. Mol Med Rep 2014;9:401–6.

[33] Rovito D, Giordano C, Vizza D, Plastina P, Barone I, Casaburi I, Lanzino M, De Amicis F, Sisci D, Mauro L, Aquila S, Catalano S, Bonofiglio D, Andò S. Omega-3 PUFA ethanolamides DHEA and EPEA induce autophagy through PPARγ activation in MCF-7 breast cancer cells. J Cell Physiol 2013;228:1314–22.

[34] Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, Criollo A, Morselli E, Zhu C, Harper F, Nannmark U, Samara C, Pinton P, Vicencio JM, Carnuccio R, Moll UM, Madeo F, Paterlini-Brechot P, Rizzuto R, Szabadkai G, Pierron G, Blomgren K, Tavernarakis N, Codogno P, Cecconi F, Kroemer G. Regulation of autophagy by cytoplasmic p53. Nat Cell Biol 2008;10:676–87.

[35] Zhou J, Zhang W, Liang B, Casimiro MC, Whitaker-Menezes D, Wang M, Lisanti MP, Lanza-Jacoby S, Pestell RG, Wang C. PPARgamma activation induces autophagy in breast cancer cells. Int J Biochem Cell Biol 2009;41:2334–42.

[36] Patel L, Pass I, Coxon P, Downes CP, Smith SA, Macphee CH. Tumor suppressor and anti-inflammatory actions of PPARgamma agonists are mediated via upregulation of PTEN. Curr Biol 2001;11:764–8.

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

[38] Gump JM, Thorburn A. Autophagy and apoptosis: what is the connection? Trends Cell Biol 2011;21:387–92.

[39] O'Rourke EJ, Kuballa P, Xavier R, Ruvkun G. ω-6 Polyunsaturated fatty acids extend life span through the activation of autophagy. Genes Dev 2013;27:429–40.

[40] Meléndez A, Tallóczy Z, Seaman M, Eskelinen EL, Hal DH, Levine B. Autophagy genes are essential for dauer development and life-span extension in C. elegans. Science 2003;301:1387–91.

[41] Kaeberlein TL, Smith ED, Tsuchiya M, Welton KL, Thomas JH, Fields S, Kennedy BK, Kaeberlein M. Lifespan extension in Caenorhabditis elegans by complete removal of food. Aging Cell 2006;5:487–94.

[42] Lee GD, Wilson MA, Zhu M, Wolkow CA, de Cabo R, Ingram DK, Zou S. Dietary deprivation extends lifespan in Caenorhabditis elegans. Aging Cell 2006;5:515–24.

[43] Dhaka V, Gulia N, Ahlawat KS, Khatkar BS. Trans fats-sources, health risks and alternative approach – a review. J Food Sci Technol 2011;48:534–41.

[44] Ghavami S, Cunnington RH, Yeganeh B, Davies JJ, Rattan SG, Bathe K, Kavosh M, Los MJ, Freed DH, Klonisch T, Pierce GN, Halayko AJ, Dixon IM. Autophagy regulates trans fatty acid-mediated apoptosis in primary cardiac myofibroblasts. Biochim Biophys Acta 2012;1823:2274–86.

[45] Ahs M, Prasad A, Aminov Z, Carpenter DO. Mechanisms of cell death of thymocytes induced by polyunsaturated, monounsaturated and trans-fatty acids. J Cell Biochem 2011;12:3863–71.

[46] Adkins Y, Schie IW, Fedor D, Reddy A, Nguyen S, Zhou P, Kelley DS, Wu J. A novel mouse model of nonalcoholic steatohepatitis with significant insulin resistance. Lab Invest 2013;93:1313–22.

[47] Eom JM, Seo MJ, Baek JY, Chu H, Han SH, Min TS, Cho CS, Yun CH. Alpha-eleostearic acid induces autophagy-dependent cell death through targeting AKT/mTOR and ERK1/2 signal together with the generation of reactive oxygen species. Biochem Biophys Res Commun 2010;391:903–8.

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