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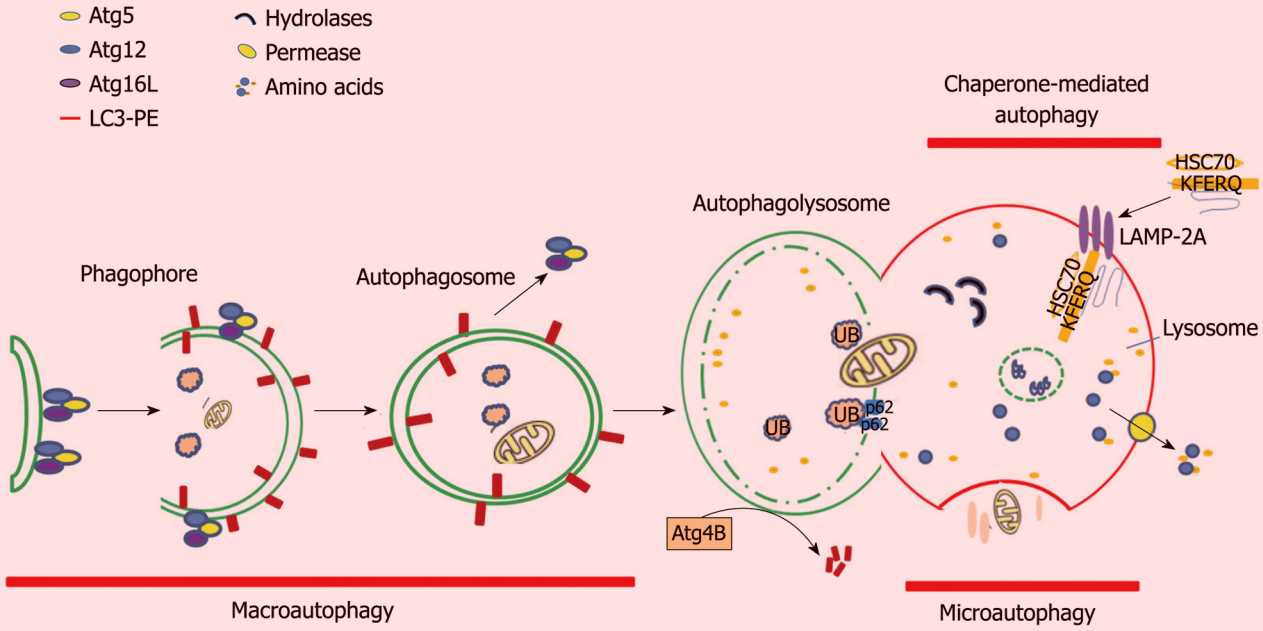
Three forms of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy



WJBC

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EDITORIAL

What is the purpose of launching *World Journal of Biological Chemistry*?

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Abstract

The first issue of *World Journal of Biological Chemistry* (*WJBC*), whose preparatory work was initiated on July 01, 2009, will be published on January 26, 2010. The *WJBC* Editorial Board has now been established and consists of 341 distinguished experts from 37 countries. Our purpose of launching *WJBC* is to publish peer-reviewed, high-quality articles via an open-access online publishing model, thereby acting as a platform for communication between peers and the wider public, and maximizing the benefits to editorial board members, authors and readers.

Key words: Maximization of personal benefits; Editorial board members; Authors; Readers; Employees; *World Journal of Biological Chemistry*

Peer reviewer: Christine Blattner, PhD, Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe, PO Box 3640, 76021 Karlsruhe, Germany

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INTRODUCTION

I am very pleased to announce that the first issue of *World Journal of Biological Chemistry* (*World J Biol Chem*, *WJBC*, online ISSN 1949-8454, DOI: 10.4331) will be published on January 26, 2010. The *WJBC* Editorial Board has now been established and consists of 341 distinguished experts from 37 countries.

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. To realize these desired attributes of a journal and create a well-recognized journal, the following four types of personal benefits should be maximized.

MAXIMIZATION OF PERSONAL BENEFITS

The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others.

Maximization of the benefits of editorial board members

The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it

should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution.

Maximization of the benefits of authors

Since *WJBC* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJBC* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading.

Maximization of the benefits of readers

Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion[1].

Maximization of the benefits of employees

It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal[2,3]. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

CONTENTS OF PEER REVIEW

In order to guarantee the quality of articles published in the journal, *WJBC* usually invites three experts to comment on the submitted papers. The contents of peer review include: (1) whether the contents of the manuscript are of great importance and novelty; (2) whether the experiment is complete and described clearly; (3) whether the discussion and conclusion are justified; (4) whether the citations of references are necessary and reasonable; and (5) whether the presentation and use of tables and figures are correct and complete.

SCOPE

The major task of *WJBC* is to rapidly report the most recent developments in the research by the close collaboration of biologists and chemists in area of biochemistry and molecular biology, including: general biochemistry, pathobiochemistry, molecular and cellular biology, molecular medicine, experimental methodologies and the diagnosis, therapy, and monitoring of human disease.

COLUMNS

The columns in *WJBC* will include: (1) Editorial: to introduce and comment on major advances in rapidly developing areas and their importance; (2) Frontier: to review recent developments, comment on current research status in important fields, and propose directions for future research; (3) Topic Highlight: this column consists of three formats, including: (a) 10 invited review articles on a hot topic; (b) a commentary on common issues associated with this hot topic; and (c) a commentary on the 10 individual articles; (4) Observation: to update the development of old and new questions, highlight unsolved problems, and provide strategies for their resolution; (5) Guidelines for Basic Research: to provide Guidelines for basic research; (6) Guidelines for Clinical Practice: to provide guidelines for clinical diagnosis and treatment; (7) Review: to review systemically the most representative progress and unsolved problems, comment on current research status, and make suggestions for future work; (8) Original Article: to report original and innovative findings; (9) Brief Articles: to report briefly on novel and innovative findings; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: to discuss and reply to contributions published in *WJBC*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: to introduce and comment on quality monographs; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities on basic research and clinical practice.

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EDITORIAL

Role of autophagy in liver physiology and pathophysiology

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Abstract

Autophagy is a highly conserved intracellular degradation pathway by which bulk cytoplasm and superfluous or damaged organelles are enveloped by double membrane structures termed autophagosomes. The autophagosomes then fuse with lysosomes for degradation of their contents, and the resulting amino acids can then recycle back to the cytosol. Autophagy is normally activated in response to nutrient deprivation and other stressors and occurs in all eukaryotes. In addition to maintaining energy and nutrient balance in the liver, it is now clear that autophagy plays a role in liver protein aggregates related diseases, hepatocyte cell death, steatohepatitis, hepatitis virus infection and hepatocellular carcinoma. In this review, I discuss the recent findings of autophagy with a focus on its role in liver pathophysiology.

Key words: Autophagy; Mitophagy; Lipophagy; Alcohol liver

Peer reviewers: Wen-Chun Hung, PhD, Professor, Chair, Institute of Biomedical Sciences, National Sun Yat-Sen University, No. 70, Lien-Hai Road, Kaohsiung 804, Taiwan, China; Dean G Tang, MD, PhD, Professor, Department of Carcinogenesis, The University of Texas M.D Anderson Cancer Center, Science Park-Research Division, 1808 Park Rd. 1C, Smithville, TX 78957, United States

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INTRODUCTION

The term "autophagy" comes from Greek, auto means self and phagos means to eat. It was first described nearly 40 years ago by De Duve *et al*[1] and was based on morphological observations of the sequestration of cytoplasm into closed, membrane-delimited vacuoles. There are three modes of autophagy that differ in how the cytoplasmic materials are delivered to lysosomes. However, they share a common last step by which the materials are degraded in the lysosome with eventual recycling of the degraded materials *via* lysosomal permease to efflux of the amino acids (Figure 1). Microautophagy results in the direct uptake of cytoplasm at the lysosomal surface by invagination, protrusion or septation of the sequestering organelle membrane. In contrast, macroautophagy sequesters a portion of cytoplasm, inclusions (e.g. glycogen) or whole organelles (e.g. mitochondria, endoplasmic reticulum, peroxisomes) into structures with a double membrane called autophagosomes. The contents of the autophagosomes are degraded after fusion with lysosomes called autolysosomes. Chaperone-mediated autophagy (CMA) differs from the other two autophagy processes in that vesicular traffic is not involved. Instead, particular cytosolic molecules biochemically related to KFERQ are recognized by a molecular chaperone complex [including heat-shock protein of 70 kDa (hsp70) and its cochaperones] present in the cytosol and on the lysosomal membrane where it binds to a CMA receptor; i.e. the lysosome-associated membrane protein type-2A (LAMP-2A)[2,3]. Among the three different modes of autophagy, macroautophagy is thought to play a major role

in intracellular degradation. Therefore, this review will focus on macroautophagy (hereafter referred to as autophagy). The molecular machinery and regulatory signals for autophagy have been reviewed extensively recently and thus will not be discussed in detail in this review[4-7]. This review will focus on recent progress regarding the role of autophagy in liver pathophysiology.

SIGNIFICANCE IN BIOLOGY AND MEDICINE

In yeast, induction of autophagy plays an important role in the response to stress, such as nutrient limitation. The primary role of autophagy is to degrade enveloped cytosol and organelles resulting in the recycling of amino acids. Moreover, autophagy also plays a role in development. For instance, it is needed for yeast sporulation, for the *Caenorhabditis* elegans entry into the dauer phase of the life cycle, and for Drosophila *melanogaster* pupa formation. Autophagy is also important for clearance of apoptotic cells during embryonic development[8]. During caloric restriction, autophagy is involved in the extension of the life span[9,10]. Autophagy is also used as a defense mechanism against the invasion of various bacteria and viruses[11]. However, some bacteria or viruses may also subvert autophagy to replicate within the autophagosomes[12]. For example, poliovirus and rhinovirus may use the cellular autophagosome to promote viral replication, probably because the double-membraned structures of the autophagosome provide membranous supports for viral RNA replication complexes[13,14]. Finally, autophagy has been shown to be involved in various human diseases such as cancer[15,16], innate and adaptive immunity by antigen presentation[17,18] and neurodegenerative diseases[19].

AUTOPHAGY IN LIVER PATHOPHYSIOLOGY

Removal of intracellular protein aggregates

Autophagy has now been recognized to be able to help clear up protein aggregates. The two degradation systems, the ubiquitin-proteasome system and autophagy, are both activated by protein aggregates, but they can differentially degrade different forms of the substrates[20]. Autophagy seems to be able to degrade all forms of misfolded proteins whereas proteasomal degradation is likely limited to soluble proteins[21]. Increased accumulation of ubiquitin positive protein aggregates has been observed in Atg7 liver specific knockout mice, suggesting that autophagy is constitutively acting on the turnover of cytoplasmic proteins, a process that has been classified as "basal autophagy"[22,23].

Although autophagy is generally thought to be a non-selective lysosomal degradation pathway, there are many examples showing that autophagy can be selective. In addition to providing nutrition during starvation, selective autophagic degradation of intracellular misfolded proteins plays an important homeostatic function. Insufficient removal of these misfolded proteins may cause protein aggregate-related pathogenesis (discussed below). Accumulating evidence now supports ubiquitination is a candidate signal for autophagic degradation of misfolded and aggregated proteins. Recent studies suggest that this degradative process is mediated through the mammalian protein p62/SQSTM1. p62 directly binds to poly- or mono-ubiquitin through its C-terminal ubiquitin binding domain (UBA) and also binds directly with autophagy proteins light chain-3 (LC3) and GABARAP, and thus acts as a cargo adapter for ubiquitinated proteins and links them to autophagy degradation[24-26]. Using Atg8 as the bait, neighbor of BRCA1 gene 1 (NBR1) is identified as an additional LC3- and Ub-binding protein, which is structurally and functionally like p62 (Figure 2). Inhibition of autophagy leads to the accumulation of protein aggregates that are both p62 and NBR1 positive[27]. Therefore, it is suggested that p62 together with NBR1 promotes autophagic degradation of ubiquitinated proteins. p62 has been found to be localized in Mallory bodies in alcoholic liver disease and p62 may be required for their formations[28].

Disruption of basal autophagy in the liver, by generating the liver specific Atg7 in knockout mice, leads to the accumulation of inclusion bodies, abnormal membrane structures, accumulation of peroxisomes and deformed mitochondria, resulting in hepatomegaly and liver injury. Interestingly, deletion of p62 markedly attenuates liver injury induced by the autophagy deficiency due to the deficiency of Atg7[23,29]. The protective effects of the loss of p62 were thought to be due to the suppression of inclusion bodies formation in the liver; however, recent studies suggest that p62 may have multiple functions. Accumulated p62, due to autophagy defects, can promote oxidative stress, alter nuclear factor-κB (NF-κB) regulation and gene expression, and promote tumorigenesis[30]. In addition, p62 can also promote caspase-8 activation by inducing caspase-8 aggregation[31]. Therefore, homeostasis of p62 *via* autophagy is vital for many cellular functions.

α-1-antitrypsin (AT) deficiency

Study of AT deficiency, which causes liver inflammation and carcinogenesis, was one of the first lines of evidence suggesting a role of autophagy in diseases associated with aggregate-prone proteins[32]. AT, the archetype of the Serpin supergene family, is the principal blood-borne inhibitor of destructive neutrophil proteases including elastase, cathepsin G, and proteinase[33,34]. The classical form of α1AT deficiency affects 1 in 1800 live births in Northern European and North American populations[34]. The normal AT protein is secreted from hepatic cells into the bloodstream, where it inhibits the neutrophil proteases. However, a mutation in the AT gene results in misfolding of the mutant protein, which cannot transport from the endoplasmic reticulum (ER) and becomes stuck in the ER as an aggregated form[20]. In the liver cells of AT deficiency patients, an increased number of autophagosome is readily observed[35]. Autophagy mainly

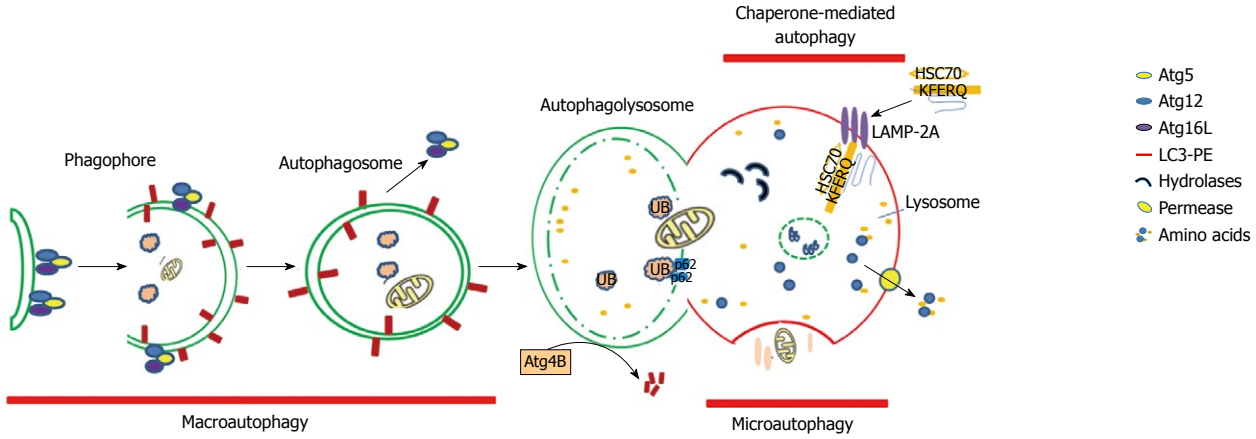

Figure 1 Three forms of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy starts with the *de novo* formation of a cup-shaped isolation membrane or phagophore. The elongation of the isolation membrane is driven by *Atg* genes while engulfing cytosolic components. The formation of double membrane autophagosomes eventually fuse with lysosomes to form autophagolysosomes where engulfed contents are degraded by lysosomal proteases and hydrolases. Amino acids and other small bio-molecules, such as glucose, are transported back into the cytosol for re-use through the lysosomal membrane permease. Microautophagy involves the engulfment of cytosolic proteins, organelles, and even a piece of nuclear material instantly at the lysosomal membrane by invagination, protrusion, and separation. Chaperone-mediated autophagy is a process of direct transport of a group of proteins that contain a KFERQ motif, which associates with hsc70 and its co-chaperones. This complex then binds with LAMP-2A on the lysosomal membrane. All forms of autophagy subsequently lead to the degradation of intra-autophagosomal components by lysosomal hydrolases. PE: Phosphatidylethanolamine.

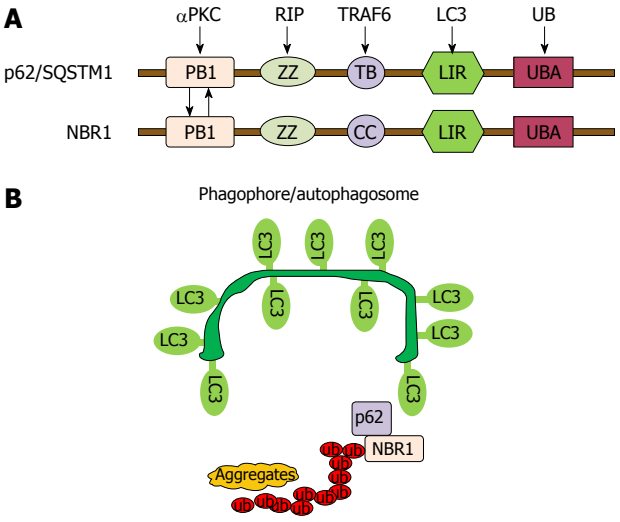

Figure 2 Autophagy regulates protein homeostasis through interaction with p62 and NBR1. A: A schematic diagram showing the domain organization of p62 and NBR1 proteins. PB1: Phox and Bem1p domain; ZZ: Zinc finger domain; TB: TRAF6-binding domain; CC: Coiled-coil domain; LIR: LC3-interacting region; UBA: Ub-associated domain; B: p62 and NBR1 are autophagy receptors that interact with both ubiquitin-positive protein aggregates through their UBA domains and target them to autophagosomes through their LIR regions with LC3 on the autophagosomal membranes, thereby promoting autophagy of ubiquitinated targets.

serves to degrade the mutant AT aggregates in the ER, whereas the soluble mutant proteins are subjected to ER-associated degradation (ERAD) by proteasomes[20,36].

Hypofibrinogenemia

Hypofibrinogenemia is another liver ER storage disease. A mutant form of fibrinogen, named Aguadilla γD, forms protein aggregates in the hepatic ER, causing similar pathological alterations to AT deficiency[37]. Although most of the mutant forms can be degraded *via* the ERAD pathway, autophagy helps to degrade excess aberrant polypeptide formed aggregates within the ER[37]. These studies suggest a protective role of autophagy in relieving the cytotoxicity associated with abnormal protein aggregates in the ER. When the unfolded protein response and ERAD is saturated or impaired, the accumulated abnormal proteins in the ER cause ER stress. The ER stress signaling pathways, such as Ire1/, PERK/eIF2α and JNK, may be involved in the ER-accumulated aggregates induced-autophagy[38,39]. Autophagy may help to remove part of the abnormal ER, presumably together with the accumulated protein aggregates to maintain organelle homeostasis. In this context, autophagy serves as an "ERAD-like" mechanism and contributes to ER quality control[21].

Alcoholic Mallory body

Autophagy may also play a role in alcohol-induced liver pathogenesis. Earlier studies have shown that alcohol fed to rats produced hepatomegaly, associated with enlargement of the hepatocytes and protein accumulation[40,41]. The mechanisms for the alcohol-induced protein accumulation in hepatocytes are not completely known. It is suggested that alcohol exposure can alter the proteolytic activity of hepatic lysosomes[42], alter the trafficking of lysosomal enzymes[43], and probably alter microtubule structures and vesicle protein trafficking in hepatocytes[44]. Moreover, there is also evidence that ethanol administration can inhibit proteasome activity, likely due to the ethanol metabolism and generation of reactive oxygen species (ROS)[45]. Suppression of proteasome activity and induction of ROS have been shown to be able to induce autophagy in other cell types and systems. We recently found that binge drinking of alcohol indeed could induce autophagy in the mouse liver (Ding *et al* manuscript in press).

Besides the general protein accumulation, alcohol exposure also leads to the formation of inclusion bodies known as Mallory bodies in hepatocytes, which are frequently observed in alcoholic hepatitis and cirrhosis[46]. Mallory bodies are filaments of intermediate diameters with intermediate filament components[47]. These structures contain cytokeratin 8 and cytokeratin 18 and ubiquitin positive protein aggregates and share many similar characteristics with other inclusion bodies found in neuronal degenerative diseases, such as Lewy bodies in Alzheimer's disease and Huntington inclusions bodies in Huntington's disease[47]. Although Mallory bodies have no longer been considered just as a marker of alcoholic disease, the biological significance of Mallory bodies in alcohol-induced liver injury is still unclear. Moreover, the mechanisms for the induction of Mallory bodies are also not completely known. Inhibition of proteasome activity by proteasome inhibitor can induce Mallory body-like structures in cultured cells and in mouse liver. Interestingly, induction of autophagy by rapamycin, a mTOR inhibitor, significantly suppresses Mallory body formation both *in vitro* and *in vivo*, suggesting autophagy plays an important role in alcoholic Mallory body formation and induction of autophagy may help to attenuate Mallory body formation[48,49].

Degradation of organelles via autophagy

Unlike proteasomal degradation, autophagy can degrade not only cytosolic proteins but also organelles such as mitochondria, peroxisome and ER.

Degradation of peroxisome by autophagy (pexophagy)

Autophagy selectively removes peroxisomes (pexophagy), which was first discovered in yeast when the culture medium was switched and peroxisomal function was no longer required for growth[50]. It was further found that both macroautophagy and microautophagy could be used to degrade peroxisomes in yeast. For example, in *P. pastoris*, glucose-induced peroxisome degradation mainly occurs through microautophagy, whereas ethanol-induced degradation utilizes macroautophagy[51].

In mammalian cells, autophagic degradation of peroxisomes has been observed in hepatocytes treated with clofibrate or dioctyl phthalate[52], two drugs that activate the peroxisome proliferators-activated receptors to induce accumulation of peroxisome in mammalian cells[52,53]. The requirement of autophagy to degrade peroxisomes in hepatocytes is further proved in a recent study using the liver specific Atg7-deficient mouse challenged with phthalate esters, an agent that can induce marked increase of peroxisome numbers and size in the liver[54].

Pexophagy has been well known to be a selective process, and its mechanisms have been best studied in yeast. In *P. pastoris,* PAtg30 functions as an adapter molecule to interact with peroxisomal membrane proteins PpPex14 and PpPex3 and autophagy proteins PpAtg11 and PpAtg17, and in turn, links peroxisomes to autophagy degradation[55]. In mammalian cells, it seems that peroxisomes can be removed by autophagy similar to the ubiquitinated protein aggregates. A recent study reveals that fusion of a single ubiquitin moiety to a peroxisome integral membrane protein, PMP34, is sufficient to trigger selective autophagy degradation of peroxisomes. Interestingly, this kind of selective pexophagy is also mediated by p62, similar to the role of p62 in the autophagic degradation of protein aggregates as discussed above[56].

Mitophagy

Enveloped mitochondria in autophagosomes have been observed by De Duve *et al*[1] as early as 1966 in drug injected rat liver cells. This process is now termed "mitophagy"[1,57]. Increasing evidence now supports mitophagy as a selective process. In yeast, Atg32, a mitochondria-anchored protein, has recently been found to be essential for selective mitophagy, although a mammalian homologue of Atg32 has not been found[58,59]. Except for Atg32, Uth1p and Aup1 have also been found to be involved in mitochondrial autophagy although it seems that they only play roles in certain models[60,61]. The mechanisms of mitophagy are more complicated in mammalian cells and the following mechanisms discussed below have been implicated in mammalian cell mitophagy.

Mitochondrial permeability transition (MPT)

MPT, an event that has long been proposed to regulate apoptosis and necrosis in mammalian cells, may also play a role in regulating mitophagy. When cultured hepatocytes were deprived of nutrition, mitochondria became depolarized and moved into acidic vesicles[62]. Cyclosporin A, a MPT inhibitor, significantly inhibited mitochondria depolarization and mitophagy during nutrient deprivation in hepatocytes[62]. Besides the nutrient deprivation, mitophagy in hepatocytes was also induced when selected mitochondria inside living hepatocytes were subjected to laser-induced photodamage[63]. Mitophagy after nutrition deprivation was further confirmed by using cultured hepatocytes from the GFP-LC3 transgenic mouse, in which some mitochondria were enveloped by the green GFP-LC3 signals[63]. As mitophagy could selectively remove those damaged mitochondria, it has been proposed that mitophagy could be protective against cell death, as these mitochondria produce toxic free radicals and release mitochondria apoptotic factors[57]. Indeed, in drug induced pathogenesis of Reye syndrome, salicylate induced mitochondria damage by inducing MPT in hepatocyte[64]. Interestingly, autophagic degradation of damaged mitochondria was found in liver biopsies of Reye syndrome patients[65], and also in an influenza B virus model of Reye's syndrome in mice[66].

Mitochondrial fragmentation

Mitochondria are dynamic organelles undergoing fusion and fission constantly. It is tempting to speculate that fragmented mitochondria are more readily taken up by autophagosomes due to their size. In a nitric oxide (NO)-induced neuron damage model, it was found that Fiss1, a protein that regulates mitochondria fission, was involved in mitophagy[67]. We and others found that inhibition of

mitochondria fragmentation such as by overexpression of a mutant form of mitochondrial fission molecular, Drp1K38A, can also suppress mitophagy[67]. Moreover, using the Mfn1 deficient mouse embryonic fibroblasts, in which mitochondria are already fragmented due to the lack of mitochondrial fusion protein Mfn1 in these cells, we found a much higher rate of mitophagy in Mfn1-deficient cells than that of wild type cells (Ding *et al*, unpublished observations). Interestingly, in the nutrition deprivation-induced mitophagy in hepatocytes, it is found that only a portion of individual mitochondria becomes sequestered, in some cases sequestered from both the ends and middle parts of mitochondria[63]. These data tend to support that the mitochondrial fission process may also be coordinated with autophagosome formation[63].

Nix and BNIP3

How the damaged mitochondria are recognized by the autophagy machinery in mammalian cells is not clear. BNIP3 (Bcl-2/E1B-19kDa interacting protein 3) was first identified in a yeast two-hybrid screen for proteins that interact with adenovirus E1B 19 kDa[68]. BNIP3 is a pro-apoptotic mitochondrial protein that contains a Bcl-2 homology 3 (BH3) domain and a carboxyl terminal transmembrane (TM) domain[69,70]. BNIP3 is inserted into the outer mitochondrial membrane through its C-terminus transmembrane domain while its N-terminus is exposed in the cytoplasm. Unlike other BH3-only pro-apoptotic proteins, the TM domain of BNIP3, but not its BH3 domain, is required for mitochondria targeting and pro-apoptotic function[70,71]. Nix/BNIP3L is a homolog of BNIP3 and they share 53%-56% amino acid sequence identity[72]. In addition to apoptosis, BNIP3 has been implicated in necrosis and autophagic cell death[73-75]. However, BNIP3 is not ubiquitously expressed under normal conditions. It is only expressed in skeletal muscle and brain at a low level under physiological conditions. It is markedly expressed in regions of solid tumors or normal tissue in response to hypoxia and appears to be regulated by hypoxia-inducible factor (HIF), which binds to a site on the BNIP3 promoter[76,77].

BNIP3 has been found to be important for ceramide or arsenic trioxide induced autophagy in malignant glioma cells[75,78]. Using cultured mouse embryonic fibroblast (MEF) cells, it is demonstrated that mitophagy is induced by hypoxia. This mitophagy requires the HIF-1-inducible expression of BNIP3. Mitophagy serves as an adaptive metabolic response to prevent increased levels of ROS *via* removal of damaged mitochondria, and in turn to mitigate cell death[79]. The critical role of BNIP3 in mitophagy has further been supported by an elegant genetic model. During the maturation, reticulocytes completely eliminate their mitochondria partly through autophagy, a process that provides a physiological model to study mitophagy. In Nix-deficient mice, mitochondrial clearance in reticulocytes is significantly inhibited or retarded, suggesting that Nix is required for the selective elimination of mitochondria[80]. Later on, it was discovered by another group that the role of Nix for mitophagy is likely due to the loss of mitochondria membrane potential (MMP) induced by Nix, because treatment with a mitochondria uncoupler or a BH3 mimetic, induces the loss of MMP and restores the sequestration of mitochondria into autophagosomes in Nix-deficient erythroid cells[81]. Their results thus suggest that Nix-dependent loss of MMP is important for targeting damaged mitochondria to autophagosomes. This notion may also help to explain why mitochondrial permeability transition is involved in hepatocytes mitophagy, because in most cases, the onset of mitochondria permeability transition can lead to the loss of MMP.

Parkin and ubiquitin

As discussed above, ubiquitin plays an important role for the autophagic removal of not only protein aggregates but also organelles such as peroxisomes. This is mainly achieved through several adapter molecules, such as p62 and NBR1, which can directly interact with poly- and mono-ubiquitin and LC3. Therefore, it is very tempting to hypothesize that ubiquitin may also play a role in mitophagy. Indeed, it is recently found that Parkin, an ubiquitin E3 ligase, could be recruited selectively to impaired mitochondria and to promote their degradation *via* autophagy[82]. Interestingly, although *Parkin* was first identified as a gene implicated in autosomal recessive Parkinsonism, *Parkin* knockout mice have enhanced hepatocyte proliferation and hepatocellular carcinoma (HCC)[83]. It is not known whether the lack of Parkin in the hepatocytes would affect the hepatocyte mitochondrial turnover resulting in an increased number of damaged mitochondria, increased levels of oxidative stress and genome instability, which contribute to tumorigenesis. Although direct experimental evidence is not yet available to show the role of ubiquitination of mitochondria in mitophagy, it has been noted that sperm-derived mitochondria are completely eliminated after fertilization to ensure that only maternal mitochondrial DNAs are inherited. Interestingly, sperm mitochondria have been found to be tagged with ubiquitin, although whether autophagy was involved in this process has not been determined.

ER-phagy

The ER was first identified as being selectively sequestrated by autophagic vacuoles as early as 1973, when hepatocytes were previously treated with phenobarbital followed by cessation of the treatment[84]. In this case, based on morphological study, the elimination is mainly of smooth ER. This was later confirmed by a study using a biochemical approach, in which two typical ER membrane proteins, phenobarbital (PB)-inducible cytochrome P-450 and NADPH-cytochrome P-450 reductase, were selectively degraded by autophagy in rat liver when rats were treated with phenobarbital followed by removal[85]. It will be interesting if this model can also be applied in the liver specific Atg-7 knock out mouse.

Currently, how ER is selectively removed by autophagy is not known. The ER is a major intracellular site for

proper protein folding and posttranslational modifications. Disrupting the oxidized environment of ER by dithiothreitol (DTT), calcium homeostasis by thapsigargin, or inhibition of glycosylation by tunicamycin, can all lead to the accumulation of misfolded proteins in the ER and causes the so called unfolded protein response (UPR)[21]. We and others have demonstrated that ER stress can induce autophagy, likely through the UPR components such as Ire1, perk and eif2α or the ER calcium leakage[38,39,86,87].

Lipohagy

Lipid droplets (LDs) are intracellular storage depots for neutral lipid that are found in all kinds of cells, ranging from bacteria to human. The LD has been considered as an organelle with a polar lipid monolayer membrane that envelops the hydrophobic core of triglycerides (TGs), diacyglycerol (DG), cholesterol ester (CE), and other esters in various proportions[88]. The phospholipid composition of the LD is very similar to the ER membrane, which includes phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI)[89]. There are also a variety of proteins associated with the LD membrane. For example, more than 10 Rab proteins, including Rab5, -7, -11 and -33, have been detected in isolated LDs. However, among them, only Rab18 has been confirmed by microscopic co-localization studies[90]. In addition to the Rab proteins, PAT proteins are perhaps the most characterized LD associated proteins. PAT proteins, named after perilipin, ADRP, and the tail-interacting protein of 47 kDa (TIP47), mainly regulate cytosolic lipase mediated lipolysis, which has been thought to be a major pathway for the regulation of lipid homeostasis[88]. However, recent work by the Czaja and Cuervo groups clearly demonstrates that autophagy also plays an important role in lipid homoeostasis in hepatocytes by autophagic lipolysis[91]. Suppression of the autophagic pathway, either by a genetic or pharmacological approach, leads to the accumulation of LDs in hepatocytes and other cells. The autophagic marker LC3-II is highly enriched in the LD fractions, and LDs are found to be enveloped by GFP-LC3 positive vesicles. More importantly, it seems that autophagy plays an important role in the clearance of the accumulated LDs in hepatocytes, in particular, in response to the methionine- and choline-deficient (MCD) diet or oleate addition-induced lipid load[91]. However, in starvation-induced hepatic lipid accumulation, it is found that knockout of Atg7 actually leads to less lipid accumulation in the liver, suggesting that different stress-induced lipid accumulation or a different source of lipids maybe differentially regulated by autophagy or some of the Atg proteins may have non-autophagic functions such as to regulate the LD formation[92]. Nevertheless, these findings open a new possible therapeutic approach for treating liver steatosis induced by a high fat diet or obesity *via* induction of autophagy.

Xenophagy for hepatitis virus

There is an increasing body of evidence now supporting autophagy and/or the autophagy genes as having both anti-viral and pro-viral capacities against various viruses. Autophagy can directly recognize and enwrap virions and/or viral components and target them for degradation in lysosomes, a process termed as "xenophagy"[11,93,94]. Autophagy may also regulate the innate and adaptive immune system to protect against viral infections. In order to counteract autophagy to survive, it is not surprising that some viruses can use some mechanisms to either inhibit autophagy or escape from autophagy recognition. In support of this concept, it has been shown that herpes viruses and lentiviruses can use some viral proteins to inhibit autophagy. For example, ICP34.5, a neurovirulence protein from Herpes simplex virus type 1 (HSV-1), binds protein phosphatase 1α to counter PKR-mediated phosphorylation of eIF2α and in turn suppresses autophagy. In addition, ICP34.5 may also suppress autophagy by binding to the autophagy-promoting protein Beclin 1[93]. Some other intracellular pathogens can escape from autophagic degradation by either suppressing the fusion of autophagosomes with lysosomes or escaping autophagy recognition[95,96].

In the liver, both hepatitis B and C viruses have been shown to be involved in the regulation of autophagy. Beclin-1, an essential autophagy protein, is found to be upregulated in hepatitis B virus-infected cancerous liver tissues. Enforced expression of HBV X protein induces Beclin-1 upregulation in cultured hepatoma cells and, more importantly, enhanced starvation-induced autophagy[97]. In contrast to hepatitis B, hepatitis C virus replication is more complicated. Transfection of HCV viral RNA into Huh7.5 cells leads to the accumulation of autophagosomes and this induction seems to depend on HCV virus-induced ER stress and an unfolded protein response (UPR)[98]. However, this autophagic response is not complete because the long lived protein degradation is not changed, suggesting accumulated autophagosomes are either due to a defect of fusion with lysosomes or alterations of the lysosomes due to the infection of HCV. Interestingly, siRNA knockdown of some essential autophagy genes, such as *Atg7*, *LC3*, *Beclin-1*, *Atg5* and *Atg12* all suppress HCV replication[98,99]. Moreover, chloroquine, an autophagy inhibitor by increasing lysosomal pH, also significantly suppresses HCV replication in hepatocytes[100]. However, it is found that HCV proteins failed to co-localize with autophagy proteins in infected cells, suggesting the HCV replication complex does not assemble on autophagic vesicles[101]. It remains unknown exactly how autophagy proteins affect HCV replication, and it is possible that the autophagy pathway may provide an initial membranous support for translation of incoming RNA before the accumulation of viral proteins or some autophagy proteins may have non-autophagic effects for viral replication.

AUTOPHAGY IN LIVER TUMORIGENESIS AND TUMOR METASTASIS

As one protein degradation and recycling pathway, autophagy has been generally believed to be a pro-survival

pathway. In nutrient starvation conditions, the pro-survival function of autophagy has been very well characterized[102]. Under the conditions of nutrient starvation, autophagy can recycle the macromolecules and thus help to overcome the moment of stress[102]. This hypothesis is clearly supported by the fact that deletion of autophagy genes leads to increased cell death under nutrient deprivation. Autophagy's role in organism survival has been observed in yeast, plants, worms, flies and mice. Atg5, Atg3 or Atg7 knockout mice die during the neonatal period when the placental blood is no longer supplied. Atg5 knockout mice exhibit reduced amino acid concentrations in plasma and tissues and show signs of energy depletion. This situation can be considered a form of starvation, during which autophagy is critical for survival[23,103]. Autophagy also acts in a protective role during other cell stress, and in this setting, autophagy is used as a strategy to remove either toxic protein aggregates or damaged mitochondria and mitochondrial-generated ROS that could activate apoptosis[30,38,39]. However, autophagy can also contribute to cell death if the process is over activated and deregulated, resulting in excessive catabolism and/or hijacking of the apoptosis machinery[104,105]. When hepatocytes are under starvation conditions, it was reported that ferritin could be degraded in the autophagosomes. The subsequent generated pool of free iron sensitized hepatocytes to be killed by oxidative stress, likely through the iron-mediated Fenton-reaction and, in turn, enhanced oxidative stress[106].

Although constitutive autophagy is important for cellular homeostasis and cell survival, paradoxically, loss of autophagy has been found to promote tumorigenesis. An essential autophagy gene, *Beclin 1*, was frequently found monoallelically deleted in many human cancers, such as breast, prostate and ovarian cancers[107]. Mice with allelic loss of *Beclin 1* are prone to HCC, lung adenocarcinoma, mammary hyperplasia, and lymphoma. Loss of heterozygosity of UVRAG, a Beclin 1 interacting protein, is frequently observed in colon cancers[108,109]. Moreover, loss of other autophagy regulatory genes, such as *bif-1* and *atg4C,* also increased tumorigenesis in mice[109,110]. To further support the concept that autophagy may suppress tumorigenesis, many other known tumor suppressor genes, such as *Lkt*, *Ampk*, *Pten,* are positive regulators of autophagy[111-114]. In contrast, many oncogenes products, including phosphatidylinositol 3-kinase, Akt and anti-apoptotic Bcl-2 family proteins, suppress autophagy[115].

Mice that have autophagy defects develop liver injury, steatohepatitis and HCC[16,29,91,115]. Autophagy defects can lead to an increased level of oxidative stress, accumulation of damaged mitochondria and intracellular p62, an adaptor protein that functions to direct polyubiquitinated proteins to autophagosomes for degradation. Sustained p62 expression resulting from autophagy defects is sufficient to alter NF-κB regulation and gene expression and to promote tumorigenesis. In contrast, suppression of ROS production and p62 expression inhibit tumorigenesis[30]. Increased levels of p62 have been documented in alcoholic liver disease as a major component of Mallory body and alcoholic liver injury and have been implicated to promote HCC[28,116]. However, whether p62 contributes to alcoholic related HCC is not known. Steatohepatitis has been implicated to promote HCC but also could result from autophagy suppression. Moreover, hepatitis C virus can also inhibit autophagy and thus may provide an additional mechanism to promote HCC[98]. Taken together, autophagy plays multiple essential roles in liver pathophysiology by removing misfolded proteins, regulating hepatocellular organelle turn over, maintaining hepatic lipid homeostasis, and influencing hepatitis virus infection (Figure 3). Therefore stimulation of autophagy in liver may thus have therapeutic effects to mitigate steatohepatitis, mitochondria damage, accumulation of 62 and virus infection and may provide a novel means to suppress HCC.

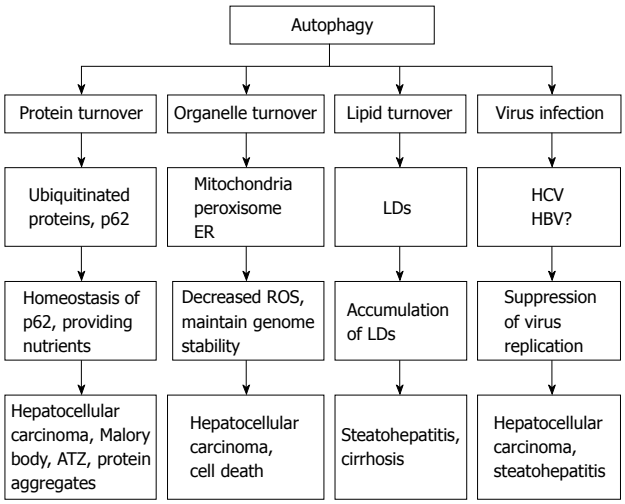

Figure 3 Role of autophagy in liver pathophysiology. At least 4 different roles that autophagy may play in liver physiology and liver diseases: remove misfolded proteins, regulate hepatocellular organelle turn over, maintain hepatic lipid homeostasis, and influence hepatitis virus infection. As a result, defects in autophagy may lead to accumulation of alcoholic Mallory bodies, α-antitrypsin deficiency-induced liver injury, increased hepatocyte cell death, steatohepatitis and hepatocellular carcinoma. ER: Endoplasmic reticulum; ROS: Reactive oxygen species; LDs: Lipid droplets.

CONCLUSION

Research progress on autophagy has been growing substantially in the past few years and understanding of the molecular mechanisms of its regulation and its impact on human diseases has increased. As a vital cellular process, autophagy plays an important role in maintaining cellular homeostasis by removing toxic protein aggregates, damaged or superfluous organelles and protects cells by mitigating ER and oxidative stress and by providing energy and macromolecules to maintain essential cellular process. As outlined in this review, autophagy plays significant roles in at least four areas of liver pathophysiology: removal of misfolded proteins and balance of nutrients and energy, regulating organelle turn over, maintaining lipid homeostasis, and affecting hepatitis virus infection and replication (Figure 3). Defects or suppression of autophagy can lead to hepatocyte cell death, steatohepatitis and hepatocellular carcinoma.

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REVIEW

Role of post-translational modifications of HTLV-1 Tax in NF-κB activation

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Abstract

Human T-cell leukemia virus type 1 (HTLV-1), the first human retrovirus discovered, is the etiological agent of adult-T-cell leukemia/lymphoma. The HTLV-1 encoded Tax protein is a potent oncoprotein that deregulates gene expression by constitutively activating nuclear factor-κB (NF-κB). Tax activation of NF-κB is critical for the immortalization and survival of HTLV-1-infected T cells. In this review, we summarize the present knowledge on mechanisms underlying Tax-mediated NF-κB activation, with an emphasis on post-translational modifications of Tax.

Key words: Adult-T-cell leukemia/lymphoma; Human T-cell leukemia virus type 1; Nuclear factor-κB; Human T-cell leukemia virus type 1-associated myelopathy/tropical spastic paraparesis; IKK

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INTRODUCTION

The human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of adult-T-cell leukemia/lymphoma (ATLL) and a neuroinflammatory disease termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[1-3]. Ten million to 20 million people worldwide are infected with HTLV-1, predominantly in endemic areas in Southern Japan, the Caribbean, Western Africa and South America. ATLL develops in approximately 5% to 10% of HTLV-1-infected individuals, after a long period of latency, suggesting a multistep process of T-lymphocyte transformation[4]. In ATLL patients, the malignant cells typically consist of oligoclonal or monoclonal outgrowths of CD4+ and CD25+ T lymphocytes carrying a complete or defective provirus of HTLV-1[5]. ATLL consists of four clinical subtypes including acute, lymphoma, chronic and smoldering. The current therapies for acute ATLL, which is the most aggressive form, are largely ineffective since the average time of survival after diagnosis with acute ATLL is only 6 mo[6]. HTLV-1 can be transmitted through sexual contact, blood transfusions, and from mother to child *via* breast-feeding[7-9]. The route of transmission appears to be one of the factors that determines the type of disease that occurs, with blood transmissions increasing the risk for HAM/TSP and mucosal transmissions (breast-feeding) increasing the risk for ATLL[4]. HTLV-1 predominantly infects CD4+ T cells *in vivo*, although recent studies indicate that other cell types such as CD8+ T-cells and dendritic cells (DCs) may also serve as reservoirs for HTLV-1[10].

HTLV-1 infects cells by transmission of virions between cells (infectious transmission) or by transmission of a provirus to the two daughter cells of a dividing infected cell (mitotic transmission). At least two receptors for HTLV-1 have been identified, including glucose transporter type 1 and neuropilin-1 (NRP1)[11,12]. Heparin sulfate proteoglycans also play an important role in facilitating the entry of HTLV-1[13,14]. There is also evidence for cell-type specific receptors since a recent study has reported that HTLV-1 enters DCs by binding to the receptor DC-SIGN[15]. Infected cells that express viral antigens are rapidly targeted by cytotoxic T cells, therefore the viral load is maintained predominantly by cells harboring silent provirus spread by mitotic transmission[12]. HTLV-1 transmission by free virions is very inefficient, at least in T cells, however, recent studies indicate that cell-free HTLV-1 virions are highly infectious for DCs[16]. When an HTLV-1 infected cell contacts an uninfected cell, a microtubule-organizing center (MTOC) is polarized at the cell-cell junction, and then viral proteins, such as Gag and viral genome RNA, accumulate at this junction allowing viral products, such as Gag and viral genome RNA, to accumulate at the junction with subsequent transfer of the viral complex into the target cell[17]. In HTLV-1-infected cells, expression of intercellular adhesion molecule 1 (ICAM-1) is upregulated, which increases the polarization of the MTOC at the point of contact in HTLV-1-infected cells, suggesting that increased expression of ICAM-1 facilitates cell-to-cell transmission of HTLV-1[18]. The frequencies of HTLV-1 provirus integration into transcription units (from the first exon to the last exon) are 26.8% (15/56) in carriers and 33.9% (20/59) in ATL, equivalent to the frequency calculated based on random integration (33.2%)[19]. However, there is evidence that HTLV-1 provirus is prone to integration near the transcriptional start sites in leukemic cells[19].

The HTLV-1 genome is 9032 nucleotides in length and encodes the structural proteins necessary to form the viral core particle (Gag, Env, and Pol) and the enzymatic retroviral proteins (reverse transcriptase, integrase and protease) and is flanked on both ends by long terminal repeats (LTRs) that contain cis-elements that regulate viral gene expression[20]. In addition, the HTLV-1 genome contains a cluster of at least five open reading frames (ORFs) within the pX region that are generated by alternative splicing[1]. The *tax* gene is the most extensively studied and encodes a protein of 40 kDa. The other pX genes encode $p12^{I}$, $p27^{I}$, $p13^{II}$, and $p30^{II}$ and all function as HTLV-1 accessory proteins[21]. The HTLV-1 accessory proteins encoded in the pX region have diverse functions, many of which involve modulation of host signaling pathways[22]. For example, p12 triggers early interleukin-2 (IL-2) expression by increasing the level of intracellular calcium and selectively activating nuclear factor of activated T cells (NFAT)[23]. p13 protein accumulates in mitochondria and may function as a negative regulator of cell growth[24]. The p30 protein modulates cell cycle and apoptosis regulatory genes[25]. Very little is known regarding p27 function. Recently, a novel ORF has been identified in the complementary strand of the pX region and encodes the HTLV-1 basic leucine zipper gene (*HBZ*)[26]. There are two transcripts of *HBZ* representing spliced and unspliced forms. The spliced form of *HBZ* is expressed in ATLL and has been proposed to regulate cell proliferation[27,28]. *HBZ* also functions as a repressor of HTLV-1 transcription by forming heterodimers with CREB, CREB-2, CREM, and ATF-1 and forming inactive complexes impaired in binding to Tax-responsive elements[26,29]. During the late stages of ATLL, HBZ, which is probably the only viral product expressed at this time[30,31], may support proliferation and growth of ATLL cells.

THE HTLV-1 ONCOPROTEIN TAX

Tax is a 40 kDa phosphoprotein that contains both nuclear localization (NLS) and nuclear export sequences that enable it to shuttle between the nucleus and cytoplasm[32-35]. Tax is a trans-activating protein that regulates both viral and cellular gene expression[36,37]. With regard to viral gene expression, Tax recruits the transcription factor CREB, and the co-activators CBP/p300 and PCAF, to the HTLV-1 LTR viral promoters[38-40]. The expression of Tax is required for HTLV-1 viral gene expression. In addition to regulating viral gene expression, Tax also regulates cellular proliferation, apoptosis, genetic instability, telomerase activation, and inactivation of tumor suppressors[41-43]. Tax modulates the activation of host transcription factors to deregulate gene expression, which favors cell growth and survival[44]. Nuclear factor-κB (NF-κB) is a key target of Tax since Tax mutants, unable to activate NF-κB, are defective for cell immortalization[45]. Furthermore, NF-κB is required for the survival of HTLV-1 transformed cells[46].

Tax plays an essential role in HTLV-1-mediated leukemogenesis, in part, by driving cellular proliferation and enhancing cell survival[47]. Consistent with these functions, Tax was shown to be necessary and sufficient for the immortalization of CD4+ T-cells, a hallmark of ATLL[45,48]. Transgenic mice, expressing Tax under the control of the HTLV-1 LTR promoter, develop neurofibromas and mesenchymal tumors[49]. When Tax expression is regulated by the granzyme B promoter, mice developed large granular lymphocytic leukemias comprising CD8+ T cells and natural killer cells[50]. Two recent studies with novel Tax transgenic mice have yielded phenotypes that more closely resemble ATLL[51,52]. Transgenic mice expressing Tax under the *Lck* proximal promoter were shown to develop thymus-derived immature T-cell leukemia with clinical, pathological, and immunologic features characteristic of acute ATLL[51]. In an independent study, Tax expressed in lymphocytes in a conditional manner resulted in progressive alopecia, hyperkeratosis and skin lesions commonly observed in

the preleukemic phase of ATLL[52]. Importantly, mice expressing the Tax M22 point mutant, defective for NF-κB activation, did not develop this phenotype[52]. Collectively, these studies provide *in vivo* evidence that Tax is both necessary and sufficient for tumor formation.

The expression of Tax promotes the dysregulation of hundreds of cellular genes including proto-oncogenes, cytokines, growth factor receptors, cyclin-dependent kinases, inhibitors of cyclin-dependent kinases, and genes involved in DNA repair and cell adhesion[47,53]. Tax also upregulates the expression of the T-cell growth factor IL-2 as well as its high affinity receptor IL-2R (also known as CD25)[54]. In the early phases of infection, HTLV-1-infected cells are dependent upon the presence of IL-2, possibly contributing to the early clonal expansion of infected T cells through an IL-2/IL-2R autocrine/paracrine loop. Disease progression, however, occurs in the absence of IL-2 secretion or expression. HTLV-1-infected cells are not dependent on IL-2, which is concomitant with constitutively activated Janus kinases and signal transducers and activators of transcription, leading to the induction of Shc/Ras/Raf/mitogen-activated protein kinase and PI3K/AKT pathways[55]. Tax mainly exerts its pleiotropic functions through direct interaction with numerous cellular proteins, many of which regulate signal transduction pathways[56-59]. In this review, we will focus on recent studies illustrating the importance of Tax post-translational modifications as well as Tax targeting of NF-κB negative regulatory proteins.

REGULATION OF NF-κB BY TAX

In mammalian cells, NF-κB is composed of five structurally related proteins, RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2), organized in different homo- and hetero-dimer NF-κB complexes. NF-κB1 and NF-κB2 are translated as precursor proteins, p105 and p100, for which proteasome-mediated processing generates the mature NF-κB subunits, p50 and p52, respectively. All NF-κB proteins share a common Rel-homology domain mediating their dimerization, DNA binding and NLS. NF-κB is normally sequestered as an inactive form through physical interaction with inhibitory κB (IκB) regulatory proteins in the cytoplasm. There are two distinct NF-κB signaling pathways: the canonical and noncanonical or alternative pathways. Generally, the canonical pathway regulates inflammation and cell survival, whereas the noncanonical pathway regulates lymphoid organogenesis and B-cell survival (Figure 1)[60]. The canonical NF-κB pathway is induced in response to diverse stimuli, including the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and IL-1, engagement of the T-cell receptor or exposure to viral and bacterial products. Following induction by various stimuli, the IκBs are phosphorylated by the IKK complex, which is composed of the catalytic subunits IKKα and IKKβ and a non-catalytic scaffolding subunit IKKγ/NEMO, leading to their ubiquitination and degradation, thus freeing NF-κB dimers to translocate to the nucleus[61]. The noncanonical pathway regulates the processing of p100 to p52 and is induced by TNF superfamily members, including CD40 Ligand, CD70, B-cell activating factor and RANK Ligand. In response to these specific TNF superfamily ligands, the MAP3K, NF-κB inducing kinase (NIK) phosphorylates IKKα, which in turn, phosphorylates p100 triggering proteasome-dependent processing to p52[62,63]. NIK stability, and hence p100 processing, is regulated by an E3 ubiquitin ligase complex consisting of TRAF2, TRAF3, cIAP1 and cIAP2[64,65].

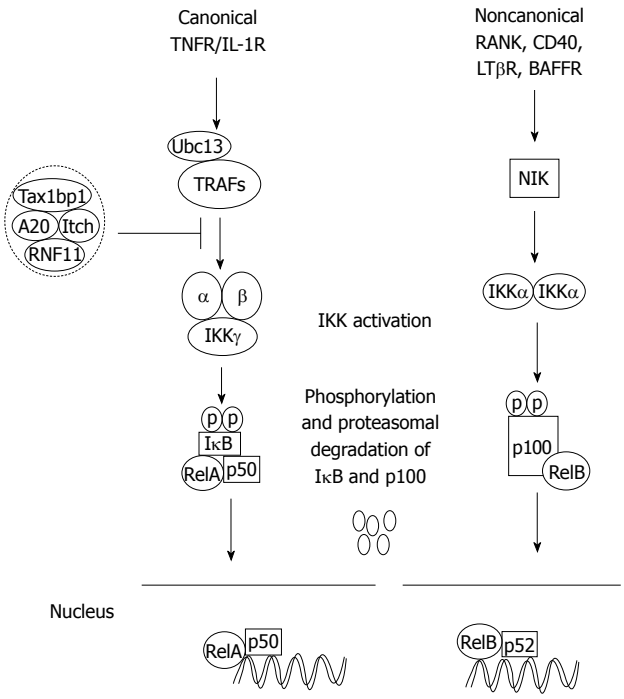

Figure 1 Canonical and noncanonical nuclear factor-κB (NF-κB) activation pathways. The binding of a specific ligand to a receptor (i.e. tumor necrosis factor-α (TNF-α) binding to TNFR1) leads to the recruitment and activation of an IKK complex comprising IKKα, IKKβ catalytic subunits and the regulatory subunit IKKγ/NEMO. The IKK complex then phosphorylates IκBα leading to degradation by the proteasome and concomitant translocation of NF-κB to the nucleus where it activates target genes. The NF-κB negative regulators, A20, TAX1BP1, Itch and RNF11, form a complex and inhibit activation of NF-κB upstream of IKK in the canonical pathway. In the noncanonical pathway, NIK is activated downstream of select TNFR superfamily members, and phosphorylates IKKα that in turn phosphorylates p100 resulting in its ubiquitination, limited degradation by the proteasome and nuclear mobilization of RelB/p52 dimers. Ubc13: Ubiquitin-conjugating enzyme 13.

Most human cancers exhibit constitutively activated NF-κB[66], in stark contrast to the transient NF-κB activation observed upon stimulation of cells with proinflammatory cytokines TNF-α or IL-1. NF-κB is constitutively activated in both HTLV-1-transformed T-cell lines and freshly isolated ATL cells[67]. Tax stimulates both canonical and non-canonical pathways, and constitutively activates NF-κB in HTLV-1 infected cells, by interacting with several NF-κB members, including RelA, p50, and p52[66,68], and also members of the IκB family such as IκBα and the precursor proteins p105 and p100. Tax interaction with NF-κB transcription factors does not

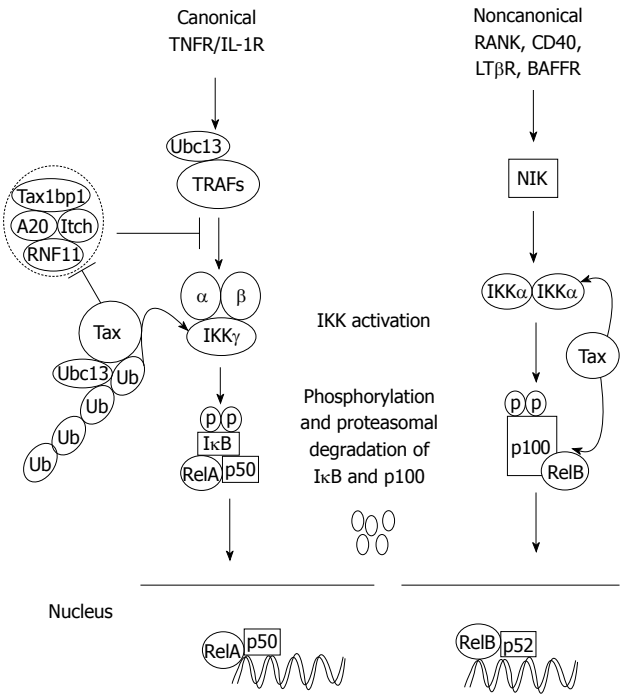

Figure 2 Mechanisms of Tax activation of the canonical and noncanonical NF-κB pathways. In the canonical pathway, Tax interacts with TAX1BP1 to disrupt the formation and function of the A20 ubiquitin-editing complex. Ubiquitinated Tax interacts with IKKγ and activates the canonical NF-κB pathway. Tax triggers activation of the noncanonical pathway downstream of NIK by recruiting IKKα to p100 thus stimulating phosphorylation, ubiquitination, and processing to p52.

fully explain Tax-mediated NF-κB activation since the completion of this process also requires cytoplasmic events. A key event in Tax-mediated NF-κB activation is binding with IKKγ/NEMO[69-72]. Tax interacts with IKKγ/NEMO in transfected cells as well as HTLV-1 transformed cell lines[73]. Notably, Tax activation of NF-κB is defective in T-cells genetically deficient for IKKγ[74]. Thus, it is likely that Tax binds to IKKγ/NEMO as a mechanism to be assembled into IKK complexes[75]. Tax interactions with IKKγ/NEMO are also essential for activation of the noncanonical pathway as well; however, Tax does not require NIK to trigger p100 processing[75]. Tax likely triggers the activation of the IKK catalytic subunits by recruiting upstream kinases, such as TGF-β activating kinase 1, to IKK[72]. Tax therefore promotes IκB degradation at multiple levels, thereby allowing nuclear translocation of NF-κB independently of external stimuli (Figure 2). In HTLV-1 transformed cell lines, Tax has been shown to promote the relocalization of IKK subunits to a perinuclear compartment co-localizing to the Golgi apparatus[73,76]. Consistent with these finding, another study has indicated that Tax hijacks IKK to lipid raft microdomains in the Golgi where it is activated[77]. Therefore, the Golgi appears to be a cellular compartment where Tax triggers the activation of IKKs.

In order to promote a persistent NF-κB response, it can be predicted that Tax would impair the function of NF-κB inhibitory proteins. Indeed, recent studies from our laboratory demonstrated that Tax targets the NF-κB negative regulatory protein A20 for inactivation. A20 (also known as TNFAIP3) is a zinc finger protein that is essential for the termination of NF-κB signaling. A20-deficient mice die prematurely because of uncontrolled multi-organ inflammation and cachexia[78]. A20 functions as an ubiquitin-editing enzyme that targets ubiquitinated signaling proteins, such as RIP1 and TRAF6, for inactivation[79-81]. A20 contains a deubiquitination domain of the ovarian tumor family and seven C-terminal zinc finger domains[82]. A20 first removes lysine K63 (K63)-linked polyubiquitin chains from RIP1 and then polyubiquitinates RIP1 with lysine 48 (K48)-linked chains leading to its degradation[82]. A20 is an essential component of a ubiquitin-editing complex together with the regulatory proteins TAX1BP1, Itch and RNF11[79]. TAX1BP1 was originally cloned as an interacting protein of Tax in a yeast two-hybrid screen[83,84]. Mice lacking TAX1BP1 have been generated by two groups, and TAX1BP1 has been shown to be an essential negative regulator of NF-κB by serving as an adaptor molecule for A20[80,85]. Shembade and coworkers have shown that ectopic expression of Tax leads to the disruption of the A20 ubiquitin-editing complex[79-81]. The mechanism by which Tax disrupts the A20 ubiquitin-editing enzyme complex is unclear, although Tax may potentially impair protein-protein interactions by steric hindrance or by modifying post-translational modifications.

POST-TRANSLATIONAL MODIFICATION OF TAX

Post-translational modifications of Tax are important in the constitutive activation of NF-κB pathways, inhibition of DNA repair, activation of the p53 tumor suppressor and cell cycle control. Tax undergoes numerous post-translational modifications including phosphorylation, ubiquitination and sumoylation[86-89]. Phosphorylation of Tax at multiple sites on serine residues is important for Tax localization to nuclear bodies and for Tax-mediated activation of gene expression *via* both the ATF/CREB and NF-κB pathways[90,91]. However, the kinases for Tax phosphorylation and activation of NF-κB have not yet been identified.

Polyubiquitination of Tax leads to its cytoplasmic retention and is critical for the activation of IKK and NF-κB[86-88,92]. Shembade and others have shown Tax polyubiquitination is predominantly composed of K63-linked polyubiquitin chains[86,93,94]. Tax polyubiquitination can occur on multiple lysine residues, although lysine 263, 280, and/or 284 are the most critical sites[87]. Physical interaction of Tax with ubiquitin-conjugating enzyme 13, an E2 enzyme for K63 linked polyubiquitination, is essential for Tax ubiquitination and interaction with NEMO[86]. However, the Tax E3 ligase is currently unknown, although it is likely to be an E3 ligase capable of K63-linked polyubiquitination. Recently, the regulatory molecules TAX1BP1 and NRP/Optineurin were shown

to be required for Tax polyubiquitination and activation of NF-κB[76]. However, how these molecules promote Tax polyubiquitination is not completely understood.

In addition to phosphorylation and polyubiquitination, Tax also undergoes sumoylation[87]. Tax sumoylation leads to its nuclear retention and the formation of nuclear bodies that include NF-κB, p300 and CBP as well as components of the transcription and splicing machineries[87]. The sites of Tax sumoylation overlap with polyubiquitination[87] thus the localization of Tax may determine whether it becomes polyubiquitinated or sumoylated. Nevertheless, it is clear from published studies that ubiquitination and sumoylation act in concert for Tax-mediated activation of gene expression *via* the NF-κB pathway.

Yet another post-translational modification of Tax is acetylation, which modulates transcription factor functions such as DNA binding affinity, stability and ability to interact with coactivators and corepressors[95,96]. Tax acetylation occurs at a lysine residue at amino acid position 346 in the carboxy-terminal domain of Tax by the transcriptional coactivator p300[95]. When Tax is acetylated, it favors activation of gene expression *via* the NF-κB pathway, suggesting that Tax oncogenic potential depends on Tax acetylation[95]. This modification may also compete with ubiquitination or sumoylation for overlapping targeted lysine residues.

CONCLUSION

HTLV-1 Tax interacts with several host proteins to activate IKK and NF-κB for proliferation and transformation of HTLV-1 infected cells. Tax activates both the canonical and noncanonical NF-κB pathways. It is clear that Tax ubiquitination is critical for interaction with IKKγ/NEMO for NF-κB activation; however it is unknown whether Tax ubiquitination is important for activation of the noncanonical NF-κB pathway. Future studies will be necessary to identify host factors such as adaptor molecules and E3 ligases that Tax requires to activate NF-κB.

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Lorne, Australia
31st Lorne Genome Conference on the Organization and Expression of the Genome

February 26-27
Manchester, United Kingdom
The 5th Annual Biomarkers Congress

February 27-March 5
Innsbruck, Austria
3rd FEBS Special Meeting on ABC Proteins-ABC2010

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London, United Kingdom
3rd annual Advances in Synthetic Biology conference and exhibition

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The 4th Annual China Chemical Focus 2010

April 24-28
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2010 2nd ASM Conference on Mobile DNA

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

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16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

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Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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