

CHINESE MEDICAL SCIENCES JOURNAL

REVIEW

Effect of Autophagy Over Liver Diseases[△]

Dong-qian Yi¹, Xue-feng Yang^{1*}, Duan-fang Liao², Qing Wu¹,
Nian Fu¹, Yang Hu¹, and Ting Cao¹

¹Department of Digestion Internal Medicine, the Affiliated Nanhua Hospital of
University of South China, Hengyang 421000, Hunan, China

²Department of Pathology, Hunan University of Chinese Medicine,
Changsha 410208, China

Key words: autophagy; hepatic fibrosis; fatty liver; viral hepatitis; liver cancer

Abstract In recent years, increasingly evidences show that autophagy plays an important role in the pathogenesis and development of liver diseases, and the relationship between them has increasingly become a focus of concern. Autophagy refers to the process through which the impaired organelles, misfolded protein, and intruding microorganisms is degraded by lysosomes to maintain stability inside cells. This article states the effect of autophagy on liver diseases (hepatic fibrosis, fatty liver, viral hepatitis, and liver cancer), which aims to provide a new direction for the treatment of liver diseases.

Chin Med Sci J 2016; 31(1):65-68

AS one of the important forms of cell death, autophagy is a process for maintaining stability inside cells through degradation of impaired cell organelles, misfolded protein, and intruding microorganisms by lysosomes.¹ Under nutrient deficiency or stress conditions, autophagy would usually be activated in order to protect cell viability.¹ Autophagy includes three types: macroautophagy, chaperone mediated autophagy, and microautophagy. As one important metabolic process inside cells, autophagy is mainly regulated by two signals pathway among mammals: rapamycin (mTOR)-dependent pathway and rapamycin (mTOR)-independent pathway.²

RESEARCH METHODS AND TESTING INDEXES OF AUTOPHAGY

At present, gene knockout, autophagy inhibitor, and mTOR inhibitor are adopted in autophagy studies. The autophagyrelated genes mainly include Beclin1, Atg5 and Atg7, and autophagy inhibitors mainly include Aralen and 3-methyl adenine (3-MA). Aralen, an inhibitor in the later period of autophagy, is mainly used *in vivo* experiments. Whereas, 3-MA, an inhibitor in the early period of autophagy, is mainly used *in vitro* experiment.³ Besides, rapamycin as the pathway inhibitor of mTOR, has become one indispensable tool in the research of autophagy.⁴ Currently, autophagy is assessed by means of electron microscope, western blot and immunofluorescence. Autophagosome or autophagolysosome observed by electron microscope is considered as the gold standard for analyzing autophagy.⁵

Received for publication August 16, 2015.

*Corresponding author Tel: 86-734-8358010, Fax: 86-734-8358399,
E-mail: yxf9988@126.com

△Supported by the National Natural Science Foundation of China (81373465).

AUTOPHAGY AND HEPATIC FIBROSIS

In recent years, accruing evidences indicate that autophagy plays a vital role in the formation of hepatic fibrosis. While hepatic stellate cells (HSCs) as the main source of hepatic fibrosis, mainly located in the perisinusoidal Disse cavity between endothelial cells and hepatocytes, account for about 5% to 15% of the total number of cells in the liver. It is characterized by perinuclear lipid vacuolation that is stored with retinylesters and triglycerides. When acute or chronic liver injury occurs, HSCs proliferate, then liver fibrosis generates and lipid vacuolations disappear. But the mechanism of lipid vacuolation disappearance is still unclear. Thoen *et al*⁶ found that HSC activation resulted in increased autophagy *in vitro* and *vivo* experiments. While reducing the degree of HSC activation decreased autophagy level as well.⁷

Autophagy and activation and proliferation of HSCs

The activation and proliferation of HSCs is a key factor in the development of hepatic fibrosis, which involves various signal molecules and transduction pathways, such as intracellular inflammasome, farnesoid X receptor (FXR), peroxisome proliferator activated receptor (PPAR), vitamin D receptor (VDR), retinoic acid receptor, Rev-erba receptors, liver X-activated receptor (LXR) receptors and Wnt/ β -catenin, gata binding protein 4 (GATA4) and Hedgehog signaling pathways.⁸ Autophagy is a metabolic process and participates in HSC differentiation by various signaling molecules common encoding, including Hedgehog, LXR receptor, Rev-erba receptor, and PPAR- γ .⁹ Hernandez-Gea *et al*¹⁰ have found that autophagy can provide energy for HSC activation by hydrolyzing retinoid material into fatty acids. This finding also explained why retinoids will be lost when HSCs get activated. Other mesenchymal cells is activated by this typical autophagy reaction as well.¹¹ Kluwe *et al*¹² found that HSCs still had the ability of activation in retinoid deficiency model with lecithin retinol Acyl transferase (LRAT) gene knock-out mouse, so there is no necessary connection between HSC activation and retinoid matters. Other studies have found that retinoid compounds can convert retinol into retinal and retinoic acid, which are related to collagen expression and activation of transforming growth factor β (TGF β), thus activating natural killer (NK) cells to phagocytize HSC.^{13, 14} Thus autophagy can reduce phagocytosis of HSC from NK cells by stimulating retinyl esters hydrolysis and promoting retinoid metabolism, and then lead to the development of liver fibrosis. Antophagy inhibitor might provide a new strategy for the treatment of liver fibrosis.

Autophagy and apoptosis of HSCs

As we know, hepatic fibrosis is a reversibly dynamic lesion, which can be reversed by promoting the apoptosis of activated HSC cells. Autophagy, another form of cell death, has a close relationship with apoptosis. As one kind of anti apoptotic factor, Bcl-2 family can not only inhibit apoptosis, but also interact with Beclin and affect autophagy.^{15, 16} Zhan *et al*¹⁷ found that transient receptor potential vanilloid 4 (TRPV4) can inhibit apoptosis of stellate cells through AKT signaling pathway of autophagy. Kawasaki *et al*¹⁸ also found that the accumulation of collagen in HSCs disturbed by heat shock protein 47 (HSP47) could induce cells to death by endoplasmic reticulum stress when autophagy is inhibited. Fu *et al*¹⁹ also found TGF- β 1 (a potent fibrogenic cytokine involved in liver fibrosis) could reduce apoptosis *via* autophagy induction. The above results showed that autophagy could inhibit apoptosis of HSCs. However, how autophagy to inhibit apoptosis is not clear. So, further researchs are still required to understand the mechanism of autophagy inhibiting apoptosis of HSCs.

Autophagy and aging of HSCs

Though the molecular mechanism remains unclear, multiple reports have shown that autophagy is associated with aging. Krizhanovsky *et al*²⁰ found p53 knock-out could aggravate degree of liver fibrosis in rats. Other studies have indicated that cells aging can activate the mammalian target of rapamycin (mTOR) pathway by adjusting up insulin/insulin like growth factor-1(IGF-1) signaling pathway, thus inhibiting autophagy.^{21, 22} In addition, Zhao *et al*²³ found that the ability of autophagy in long-lived naked mole rats was significantly higher than that in short-lived rats. Furthermore, they found that the ratio of apoptotic cells to normal HSCs would increase when treated with 3-MA or rapamycin. From this result, we can conclude that autophagy may inhibit the aging of HSCs.

AUTOPHAGY AND OTHER LIVER DISEASE

Autophagy is not only involved in the formation of liver fibrosis, but also plays an important role in the occurrence and development of other liver diseases such as fatty liver, viral hepatitis, and liver cancer, and so on.

Autophagy and fatty liver

In hepatic cells, autophagy is an approach of fat metabolism by wrapping up partial or even whole lipid droplets to form autophagy lysosome, and then decompose it into free fatty acids.²⁴ For non-alcoholic fatty liver, the mechanism of disease incidence is not clear, but the classic "two

hits" doctrine has been widely recognized. This theory indicates that "the first strike" happens as a result of the liver lipid deposition caused by insulin resistance, while "the second strike", based on the first strike, is a kind of inflammatory reaction due to the accumulation of reactive oxygen species (ROS) in the liver. Stankov *et al*²⁵ found that ROS and lipid accumulation can be removed by autophagy, which contributes to the treatment of non-alcoholic liver disease. In the study of alcoholic fatty liver, it is found that the metabolism of ethanol may decrease the activity of AMPK in the liver, while AMPK inhibits autophagy through mTOR pathway. The ethanol is also can change the transportation of vesicles in the liver cells and reduce the transportation of raw materials to inhibit autophagy. On the one hand, whatever alcohol or non-alcohol fatty liver cells, the inhibition of autophagy can increase the lipid content inside cells.^{26, 27} The degree of steatosis can be improved by expression of autophagy related gene (ATG7).²⁸ Therefore, how to stimulate autophagy mildly under the physiological state may be a new way to treat fatty liver.

Autophagy and viral hepatitis

Autophagy plays an important role in viral replication. While the effect of hepatitis B virus (HBV) on autophagy remains ambiguous. It has also been demonstrated that HBV can induce autophagy and promote its DNA replication.²⁹ This procession is mainly mediated by HBV X protein (HBX), which activates autophagy *via* up-regulating the expression of Beclin1.³⁰ Autophagy is also activated when it is combined with related enzyme at the starting stage of autophagy and class III phosphatidylinositol 3-kinase (C3-PI3K).³¹ Tian *et al*³² knocked out Atg5 in the liver of rats in order to inhibit autophagy, and the serum levels of HBV DNA, HBeAg, HBsAg, and RNA of S gene all decrease. Moreover, HCV also regulated the autophagic flux to enhance its replication.³³ Therefore, HBV and HCV are able to induce autophagy to enhance theirs replication. Inhibiting autophagy can significantly inhibit the replication of hepatitis virus, which may be a potential target for anti-virus treatment.

Autophagy and liver cancer

Hepatocellular carcinoma (HCC) is currently the fifth malignant cancer, and the third cause for cancer death. An interesting phenomenon was found in the study of liver cancer: cell autophagy seems to exert opposite effects on liver cancer. On the one hand, many studies have shown that autophagy, as one self-protective mechanism of cells, can inhibit the occurrence of liver cancer. For example, Takamura *et al*³⁴ successfully created a primary liver cancer model by knocking out autophagy related gene (Atg5) in

rats. On the other hand, a large number of studies have proven that autophagy can increase the survival rate of HCC cells in the given circumstances. For example, faced with the environment of hypoxia and energy deficiency, liver cancer cells can obtain energy from the non-functional organelles and proteins of autophagy. Therefore, the function of autophagy on liver cancer is complex, and how to deal with the suppressant and promotional function of autophagy to liver cancer will provide a new way of treatment for liver cancer.

CONCLUSIONS

In summary, autophagy plays different roles in different liver diseases, participating in the formation of hepatic fibrosis and aggravating fiberization by promoting activation and proliferation of HSCs and inhibiting apoptosis and aging of HSCs. Removing ROS and lipid accumulation in the liver can be helpful for the treatment of fatty liver. In viral hepatitis, autophagy can be activated by the virus, which can promote the replication of DNA of the virus. In liver cancer, autophagy can inhibit the development of cancer while also keeping the hepatoma cells alive. But at present, research on the relationship between autophagy and liver diseases is still at an early stage, and many issues need to be solved. In particular the active mechanism of autophagy in liver diseases remains to be explained, and a thorough study on it may provide new directions for the treatment of liver diseases.

REFERENCES

1. Damme M, Suntio T, Saftig P, et al. Autophagy in neuronal cells: general principles and physiological and pathological functions. *Acta Neuropathol* 2015; 129:337-62.
2. Jewell JL, Russell RC, Guan KL. Amino acid signalling upstream of mTOR. *Nat Rev Mol Cell Biol* 2013; 14:133-9.
3. Ni HM, Bockus A, Wozniak AL, et al. Dissecting the dynamic turnover of GFP-LC3 in the autolysosome. *Autophagy* 2011; 7:188-204.
4. Cai Z, Yan LJ. Rapamycin, autophagy, and Alzheimer's disease. *J Biochem Pharmacol Res* 2013; 1:84-90.
5. Chen Y, Azad MB, Gibson SB. Methods for detecting autophagy and determining autophagy induced cell death. *Can J Physiol Pharmacol* 2010; 88:285-95.
6. Thoen LF, Guimarães EL, Dollé L, et al. A role for autophagy during hepatic stellate cell activation. *J Hepatol* 2011; 55:1353-60.
7. Hernández-Gea V, Ghiassi-Nejad Z, Rozenfeld R, et al. Autophagy releases lipid that promotes fibrogenesis by

- activated hepatic stellate cells in mice and in human tissues. *Gastroenterology* 2012; 142:938-46.
8. Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. *Gut* 2015; 64:830-41.
 9. Chen Y, Choi SS, Michelotti GA, et al. Hedgehog controls hepatic stellate cell fate by regulating metabolism. *Gastroenterology* 2012; 143:1319-29.
 10. Hernandez-Gea V, Ghiassi-Nejad Z, Rozenfeld R, et al. Autophagy releases lipid that promotes fibrogenesis by activated hepatic stellate cells in mice and in human tissues. *Gastroenterology* 2012; 142:938-46.
 11. Hernandez-Gea V, Hilscher M, Rozenfeld R, et al. Endoplasmic reticulum stress induces fibrogenic activity in hepatic stellate cells through autophagy. *J Hepatol* 2013; 59:98-104.
 12. Kluwe J, Wongsiriroj N, Troeger JS, et al. Absence of hepatic stellate cell retinoid lipid droplets does not enhance hepatic fibrosis but decreases hepatic carcinogenesis. *Gut* 2011; 60:1260-8.
 13. Yi HS, Lee YS, Byun JS, et al. Alcohol dehydrogenase III exacerbates liver fibrosis by enhancing stellate cell activation and suppressing natural killer cells in mice. *Hepatology* 2014; 60:1044-53.
 14. Radaeva S, Wang L, Radaev S, et al. Retinoic acid signaling sensitizes hepatic stellate cells to NK cell killing *via* upregulation of NK cell activating ligand RAE1. *Am J Physiol Gastrointest Liver Physiol* 2007; 293:G809-16.
 15. He C, Levine B. The Beclin 1 interactome. *Curr Opin Cell Biol* 2010; 22:140-9.
 16. Robert G, Gastaldi C, Puissant A, et al. The anti-apoptotic Bcl-B protein inhibits BECN1-dependent autophagic cell death. *Autophagy* 2012; 8:637-49.
 17. Zhan L, Yang Y, Ma TT, et al. Transient receptor potential vanilloid 4 inhibits rat HSC-T6 apoptosis through induction of autophagy. *Mol Cell Biochem* 2015; 402:9-22.
 18. Kawasaki K, Ushioda R, Ito S, et al. Deletion of the collagen-specific molecular chaperone Hsp47 causes endoplasmic reticulum stress-mediated apoptosis of hepatic stellate cells. *J Biol Chem* 2015; 290:3639-46.
 19. Fu MY, He YJ, Lv X, et al. Transforming growth factor- β 1 reduces apoptosis *via* autophagy activation in hepatic stellate cells. *Mol Med Rep* 2014; 10:1282-8.
 20. Krizhanovsky V, Yon M, Dickins RA, et al. Senescence of activated stellate cells limits liver fibrosis. *Cell* 2008; 134:657-67.
 21. Chan SH, Kikkawa U, Matsuzaki H, et al. Insulin receptor substrate-1 prevents autophagy-dependent cell death caused by oxidative stress in mouse NIH/3T3 cells. *J Biomed Sci* 2012; 19:64.
 22. Jung HS, Lee MS. Role of autophagy in diabetes and mitochondria. *Ann N Y Acad Sci* 2010; 1201:79-83.
 23. Zhao S, Lin L, Kan G, et al. High autophagy in the naked mole rat may play a significant role in maintaining good health. *Cell Physiol Biochem* 2014; 33:321-32.
 24. Martinez-Lopez N, Singh R. Autophagy and lipid droplets in the liver. *Ann Rev Nutr* 2015; 35:215-37.
 25. Stankov MV, Panayotova-Dimitrova D, Leverkus M, et al. Autophagy inhibition due to thymidine analogues as novel mechanism leading to hepatocyte dysfunction and lipid accumulation. *AIDS* 2012; 26:1995-2006.
 26. Singh R, Kaushik S, Wang Y, et al. Autophagy regulates lipid metabolism. *Nature* 2009; 458:1131-5.
 27. Wu D, Wang X, Zhou R, et al. CYP2E1 enhances ethanol-induced lipid accumulation but impairs autophagy in HepG2 E47 cells. *Biochem Biophys Res Commun* 2010; 402:116-22.
 28. Yang L, Li P, Fu S, et al. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab* 2010; 11:467-78.
 29. Yang H, Fu Q, Liu C, et al. Hepatitis B virus promotes autophagic degradation but not replication in autophagosome. *Biosci Trends* 2015; 9:111-6.
 30. Sir D, Tian Y, Chen WL, et al. The early autophagic pathway is activated by hepatitis B virus and required for viral DNA replication. *Proc Natl Acad Sci USA* 2010; 107:4383-8.
 31. Tang H, Da L, Mao Y, et al. Hepatitis B virus X protein sensitizes cells to starvation-induced autophagy *via* upregulation of beclin 1 expression. *Hepatology* 2009; 49:60-71.
 32. Tian Y, Sir D, Kuo CF, et al. Autophagy required for hepatitis B virus replication in transgenic mice. *J Virol* 2011; 85:13453-6.
 33. Wang L, Tian Y, Ou JH. HCV induces the expression of Rubicon and UVRAG to temporally regulate the maturation of autophagosomes and viral replication. *PLoS Pathog* 2015; 11:e1004764.
 34. Takamura A, Komatsu M, Hara T, et al. Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* 2011; 25:795-800.