Autophagy: A new therapeutic target for liver fibrosis

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Abstract

Hepatic fibrosis is a wound-healing response to liver injury and the result of imbalance of extracellular matrix (ECM) accumulation and degradation. The relentless production and progressive accumulation of ECM can lead to end-stage liver disease. Although significant progress has been achieved in elucidating the mechanisms of fibrogenesis, effective anti-fibrotic strategies are still lacking. Autophagy is an intracellular process of self-digestion of defective organelles to provide material recycling or energy for cell survival. Autophagy has been implicated in the pathophysiology of many human disorders including hepatic fibrosis. However, the exact relationships between autophagy and hepatic fibrosis are not totally clear and need further investigations. A new therapeutic target for liver fibrosis could be developed with a better understanding of autophagy.

Key words: Autophagy; Liver fibrosis; Hepatic stellate cells; Antifibrotic target

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Core tip: Autophagy plays dual roles in hepatic fibrosis. On the one hand, it attenuates fibrosis by reducing hepatic injury via inhibiting inflammatory reaction and maintaining cellular homeostasis. On the other hand, it fuels activation of hepatic stellate cells (HSCs) by lipophagy and induces type I collagen synthesis. More studies using Atg selective knockdown mice or primary HSCs derived from Atg-deleted mice are needed. Selective inhibition of autophagy in HSCs is an attractive antifibrotic strategy.

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FIBROSIS

Liver fibrosis is a wound-healing response to a range of chronic liver diseases of different etiology, and drives
the progression of chronic hepatic diseases towards advanced liver cirrhosis and even hepatic carcinoma. Effective therapies are lacking besides diet control and physical exercise. Persisting parenchymal cell injury results in recruitment of immune cells, and activation and accumulation of fibrogenic cells. As the main source of liver fibrogenic cells, hepatic stellate cells (HSCs) lose cytoplasmic lipid droplets composed of retinyl esters to transdifferentiate from quiescent cells to activated myofibroblasts upon liver injury[1]. Myofibroblasts synthesize and secrete extracellular matrix (ECM) in an attempt to limit liver injury[2]. In addition, they also produce a wide range of matrix metalloproteinases (MMPs) that degrade ECM, and specific tissue inhibitors of metalloproteinase to inhibit activation of MMPs[3]. In brief, hepatocyte injury, immune cell recruitment, and fibrogenic cell activation contribute to the imbalance of ECM accumulation and degradation, which ultimately lead to fibrosis.

**AUTOPHAGY**

Autophagy is a catabolic intracellular pathway, targeting defective or excessive organelles to the lysosomes for degradation into amino acids, free fatty acids or other small molecules used for material recycling or energy harvest. Autophagy, usually stimulated by energy restriction, stress or inflammation, is regarded as a survival mechanism that plays a critical role in maintaining cellular homeostasis, which is involved in many human disorders including fibrotic disease. Three different kinds of autophagy are defined based on how the substrates are delivered to the lysosomes for degradation: macroautophagy, microautophagy, and chaperone-mediated autophagy, with macroautophagy being the major type. Although it is regarded as a cell-protective mechanism, excessive autophagy can cause cell death, known as type II programmed cell death[4]. However, it is unclear whether autophagy directly executes cell death or is a secondary effect of apoptosis. Autophagy can be considered a double-edged sword[5], and more investigations are needed to explore the complicated roles of autophagy.

**AUTOPHAGY IN FIBROSIS: "HERO" OR "VILLAIN"?**

**Autophagy reduces fibrosis by hepatocyte injury attenuation**

An increasing body of evidence supports the notion that autophagy participates in the pathophysiology of many human disorders including hepatic fibrosis. However, whether it is a hero or villain in hepatic fibrosis is still controversial.

Recent studies have demonstrated that autophagy impairment results in liver disease exacerbation due to reduction of degradation of defective organelles and unfolded proteins, which causes oxidative and endoplasmic reticulum stress[6-9] (Figure 1). Autophagy is increased in mice treated acutely with alcohol, in parallel with a marked reduction of serum inflammatory markers and tissue triglyceride level[10]. Autophagy may degrade activated caspase-8, a death receptor[11], thus exhibiting an antifibrotic effect by limiting liver injury. Furthermore, in α-1 antitrypsin (AT) deficiency, a disease in which the α-1 AT mutant Z protein results in protein aggregation and chronic liver injury, an autophagy-enhancing drug was demonstrated to reduce the hepatic load and reversed fibrosis[12]. Collectively, all the studies consistently supported that autophagy acted as a hero in hepatic fibrosis.

**Controversial issues of autophagy and HSC activation**

It had been unclear whether autophagy participates in HSC activation until the study of Zhu et al[13] in 1999, which demonstrated that rapamycin, a known immunosuppressive agent, inhibited HSC proliferation and limited fibrogenesis in mouse models treated with carbon tetrachloride (CCl4). They further demonstrated that rapamycin decreased HSC proliferation. As an immunosuppressant, rapamycin inhibited growth factor signaling in nonimmune as well as immune cells[14], which may largely explain its antifibrotic effect. The authors pointed out that mammalian target of rapamycin (mTOR) negatively regulated autophagy. The binding of rapamycin and mTOR appeared to block interleukin-2-dependent proliferation of T cells and even other cells[15]. Similar results were gained in another two studies[16,17]. However, it is unfortunate that no one has detected any change in autophagy during improvement of fibrosis, because rapamycin or its analogs stimulate autophagy by inhibiting mTOR. The antifibrotic effect of rapamycin depends on its antiproliferative effect on fibrotic cells or the indirect effect of autophagy stimulation remains unclear.

Fortunately, 10 years later, another study discovered that autophagic flux was increased during HSC activation and was inhibited by baflomycin A1, an autophagy inhibitor. HSC activation was blocked by 3-methyladenine (MA) or chloroquine, suggesting that inhibition of HSC activation could be achieved by interruption of different phases of autophagy[18]. This evidence strongly indicates that autophagy is involved in HSC activation (Figure 1). Another discovery that merits further consideration is that platelet-derived growth factor BB, which activates HSCs, stimulates the location of microtubule associated protein light chain 3 (LC3), an important biomarker protein of autophagy and lipid droplets, implying a potent relationship between HSC activation and lipid metabolism.

Hernández-Gea et al[18] have shown that autophagy releases lipid that promotes fibrogenesis by activating HSCs in mice and in human tissues. Inhibition of autophagy by pharmacological antagonism or Atg5 and Atg7 knockout in mice also resulted in attenuation of fibrogenesis, as well as increased lipid content in stellate...
Autophagy and collagen degradation

Gene and pharmacological inhibition of autophagy in mice resulted in increased levels of type I collagen in mouse kidneys and primary mesangial cells, suggesting that autophagy promotes intracellular degradation of type I collagen, which is a major component of ECM. Autophagy attenuates endoplasmic reticulum stress by eliminating misfolded procollagen. Furthermore, beta-(2)-adrenergic stimulation triggers autophagy in cardiac fibroblasts and promotes intracellular collagen degradation and inhibits cardiac fibrosis. However, this effect has been demonstrated in other organs, and whether it exhibits the same effect in liver remains unclear.

The above studies marked a milestone in the exploration of the role of autophagy in hepatic fibrosis. Autophagy is mostly a cell survival mechanism that attenuates hepatic inflammatory injury and ultimately inhibits liver fibrosis. Given more insight into the role of autophagy in HSC activation, we have realized a new perspective that autophagy is responsible for activation of HSCs and other hepatic fibrogenic cells, by intracellular lipid degradation, leading to fibrosis. TGF-beta induces autophagy, therefore, its role in liver fibrosis needs further investigation. We have to take into account that although autophagy may be a critical pathway in ameliorating hepatic injury in the short term, its long-term effect in fibrogenic cells may worsen chronic liver diseases, which could be regarded as a side effect in antifibrotic therapy.

We suggest that if autophagy could be selectively inhibited in HSCs and other fibrotic cells, autophagy special blocker would be an attractive candidate of antifibrotic strategies. Nonetheless, inhibition of cell-specific autophagy is exciting, yet more challenging in a tissue containing various types of cells. Further research is needed, targeting different receptors on the cell surface that may activate different effect of autophagy. It would also be useful to determine whether HSC activation is completely blocked by autophagy inhibition or just partly reversed to a quiescent phase, and the appropriate extent and time of autophagy should be seriously considered. Several genes participate in induction of autophagy. This raises the question of whether there is a link between autophagy and HSC phenotypic transformation.

Controversial issues of autophagy and mTOR

The data from Thoen et al. seem to contradict the HSC-activating yet autophagy-inhibiting effect of mTOR, because mTOR contributes to cell proliferation, including HSC. Likewise, it has been demonstrated that rapamycin, an mTOR target inhibitor and autophagy stimulator, reduces liver fibrosis, which is contradicted by later studies showing that autophagy induces HSC activation. Liu et al. have indicated that autophagy inhibitor 3-MA significantly
inhibits proliferation and activation of HSCs by arresting the cells in G2 phase. Whether autophagy inhibits or promotes HSC proliferation is controversial. Whether the inhibitory effect on proliferation of fibrogenic cells depends on mTOR inhibition itself or an indirect action on autophagy remains unclear. In a recent study, TGF-β rapidly activated its canonical Smad signaling pathway, and recruited a noncanonical pathway involving mTOR kinase to induce matrix protein collagen I expression, thus inducing fibrosis. Therefore, it is essential to investigate the relationship among mTOR, autophagy, and HSC proliferation. Few studies have focused on lipid metabolism and HSC activation, leaving the mechanism of intracellular lipid degradation poorly understood. More research, especially with selective knockdown of Atg in mice, or HSCs derived from Atg-deleted mice, will shed light on this.

Finally, we hypothesize that since fibrosis is the result of imbalances of ECM accumulation and degradation, could it be a new direction to focus on the translocation of ECM turning into cell from extracellular matrix. Then intracellular matrix could be enclosed by autophagosome and subsequently fuses with lysosome to be degraded. Since autophagy has been demonstrated to promote the degradation of type I collagen in kidney, some level of autophagy may help in treatment of hepatic fibrosis.

CONCLUSION

Autophagy is a novel target playing dual roles in human diseases including liver fibrosis. Autophagy may help cells to live through stress conditions and attenuate inflammation, leading to fibrosis reduction. Autophagy is involved in collagen degradation, which may contribute to fibrosis attenuation. However, autophagy fuels HSCs to be activated and promote fibrosis. The effect of autophagy on liver fibrosis is complex and still controversial. With a better understanding of the effects of autophagy on hepatic fibrosis, autophagy may have potential as a target of antifibrotic therapy.

REFERENCES

1. Schluger LK, Sheiner PA, Thung SN, Lau JY, Min A, Wolf DC, Fiel I, Zhang D, Gerber MA, Miller CM, Bodenheimer HC. Severe recurrent cholestatic hepatitis C following orthotopic liver transplantation. Hepatology 1996; 23: 971-976 [PMID: 8621177 DOI: 10.1002/hep.510230505]
2. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008; 134: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]
3. Iredale JP, Thompson A, Henderson NC. Extracellular matrix degradation in liver fibrosis. Biochemistry and regulation. Biochim Biophys Acta 2013; 1832: 876-883 [PMID: 23149387 DOI: 10.1016/j.bbadis.2012.11.002]
4. Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. Nat Rev Mol Cell Biol 2008; 9: 1004-1010 [PMID: 18971948 DOI: 10.1038/nrm2529]
5. Shintani T, Klioussi DJ. Autophagy in health and disease: a double-edged sword. Science 2004; 306: 990-995 [PMID: 15528435 DOI: 10.1126/science.1099993]
6. Yin XM, Ding WX, Gao W. Autophagy in the liver. Hepatology 2008; 47: 1773-1785 [PMID: 18393362 DOI: 10.1002/hep.22146]
7. Wu D, Cederbaum AI. Inhibition of autophagy promotes CYP2E1-dependent toxicity in HepG2 cells via elevated oxidative stress, mitochondrial dysfunction and activation of p38 and JNK MAPK. Redox Biol 2013; 1: 552-565 [PMID: 24273738 DOI: 10.1016/j.redox.2013.10.008]
8. Wang Y, Singh R, Xiang Y, Craja MJ. Macrophagocyte and chaperone-mediated autophagy are required for hepatocyte resistance to oxidant stress. Hepatology 2010; 52: 266-277 [PMID: 20579144 DOI: 10.1002/hep.23645]
9. Zhang Y, Qi H, Taylor R, Xu W, Liu LF, Jin S. The role of autophagy in mitochondria maintenance: characterization of mitochondrial functions in autophagy-deficient S. cerevisiae strains. Autophagy 2007; 3: 337-346 [PMID: 17404498]
10. Ding WX, Li M, Chen X, Ni HM, Lin CW, Gao W, Lu B, Stolz DB, Clemens DL, Yin XM. Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. Gastroenterology 2010; 139: 1740-1752 [PMID: 20639474 DOI: 10.1053/j.gastro.2010.07.041]
11. Hou W, Han J, Lu C, Goldstein LA, Rabinoewich H. Autophagic degradation of active caspase-8: a crosstalk mechanism between autophagy and apoptosis. Autophagy 2010; 6: 891-900 [PMID: 20724831 DOI: 10.4161/auto.6.7.13038]
12. Hidvegi T, Ewing M, Hale P, Dippold C, Beckett C, Kemp C, Maurice N, Mukherjee A, Goldbacb, Watkins S, Michalopoulos G, Perlmutter DH. An autophagy-enhancing drug promotes degradation of mutant alpha-antitrypsin Z and reduces hepatic fibrosis. Science 2010; 329: 229-232 [PMID: 20522742 DOI: 10.1126/science.1190554]
13. Zhu J, Wu J, Frizzel E, Liu SL, Bashey R, Rubin R, Norton P, Zern MA. Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an in vivo model of liver fibrosis. Gastroenterology 1999; 117: 1198-1204 [PMID: 10535884]
14. Gummert JF, Ikenen T, Morris RE. Newer immunosuppressive drugs: a review. J Am Soc Nephrol 1999; 10: 1366-1380 [PMID: 10361877]
15. Neef M, Ledermann M, Saegesser H, Schneider V, Reichen J. Low-dose oral ramapycin treatment reduces fibrogenesis, improves liver function, and prolongs survival in rats with established liver cirrhosis. J Hepatology 2006; 45: 785-796 [PMID: 17050028 DOI: 10.1016/j.jhep.2006.07.030]
16. Patsenker E, Schneider V, Ledermann M, Saegesser H, Dorn C, Hellerbrand C, Stickel F. Potent antiobiotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis. J Hepatology 2011; 55: 388-398 [PMID: 21168455 DOI: 10.1016/j.jhep.2010.10.044]
17. Thoen LF, Guimarães EL, Dóllé L, Mannerts L, Najimi M, Sokal E, van Grunsven LA. A role for autophagy during hepatic stellate cell activation. J Hepatology 2011; 55: 1353-1360 [PMID: 21803012 DOI: 10.1002/hep.24107]
18. Hernández-Gea V, Ghissi-Nejad Z, Rozenfeld R, Gordon R, Fiel MI, Yue Z, Craja MJ, Friedman SL. Autophagy releases lipid that promotes fibrogenesis by activated hepatic stellate cells in mice and in human tissues. Gastroenterology 2012; 142: 938-946 [PMID: 22240484 DOI: 10.1016/j.gastro.2011.12.044]
19. Hernández-Gea V, Friedman SL. Autophagy fuels tissue fibrogenesis. Autophagy 2012; 8: 849-850 [PMID: 22617442 DOI: 10.4161/auto.19947]
20. Kiyono K, Suzuki HI, Matsuyama H, Morishita Y, Komuro A, Kanro MG, Sugimoto K, Miyazono K. Autophagy is activated by TGF-beta and potentiates TGF-beta-mediated growth inhibition in human hepatocellular carcinoma cells. Cancer Res 2009; 69: 8844-8852 [PMID: 19903843 DOI: 10.1158/0008-5472.CAN-08-4401]
21. Choi ME, Ding Y, Kim SI. TGF-β signaling via TAK1 pathway: role in kidney fibrosis. Semin Nephrol 2012; 32: 244-252 [PMID: 22835455 DOI: 10.1053/j.semnephrol.2012.04.003]
22. Ding Y, Kim JK, Kim SI, Na HJ, Jun SY, Lee SJ, Choi ME. TGF-β: [beta]1 protects against mesangial cell apoptosis via induction of autophagy. J Biol Chem 2010; 285: 37909-37919 [PMID: 20876581 DOI: 10.1074/jbc.M110.93724]
23. Kim SI, Na HJ, Ding Y, Wang Z, Lee SJ, Choi ME. Autophagy...
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promotes intracellular degradation of type I collagen induced by transforming growth factor (TGF)-β1. J Biol Chem 2012; 287: 11677-11688 [PMID: 22351764 DOI: 10.1074/jbc.M111.308460]

24 Ishida Y, Yamamoto A, Kitamura A, Lamandé SR, Yoshimori T, Bateman JF, Kubota H, Nagata K. Autophagic elimination of misfolded procollagen aggregates in the endoplasmic reticulum as a means of cell protection. Mol Biol Cell 2009; 20: 2744-2754 [PMID: 19357194 DOI: 10.1091/mbc.E08-11-1092]

25 Aránguiz-Urroz P, Canales J, Copaja M, Troncoso R, Vicencio JM, Carrillo C, Lara H, Lavandero S, Diaz-Araya G. Beta(2)-adrenergic receptor regulates cardiac fibroblast autophagy and collagen degradation. Biochim Biophys Acta 2011; 1812: 23-31 [PMID: 20637865 DOI: 10.1016/j.bbadis.2010.07.003]

26 Thoen LF, Guimarães EL, Grunsven LA. Autophagy: a new player in hepatic stellate cell activation. Autophagy 2012; 8: 126-128 [PMID: 22082960 DOI: 10.4161/auto.8.1.18105]

27 Hsu CC, Schwabe RF. Autophagy and hepatic stellate cell activation - partners in crime? J Hepatol 2011; 55: 1176-1177 [PMID: 21856271 DOI: 10.1016/j.jhep.2011.08.001]

28 Gäbele E, Reif S, Tsukada S, Bataller R, Yata Y, Morris T, Schnur LW, Brenner DA, Rippe RA. The role of p70S6K in hepatic stellate cell collagen gene expression and cell proliferation. J Biol Chem 2005; 280: 13374-13382 [PMID: 15677443]

29 Liu M, He Y, Zhang J. [Effect of autophagy inhibitor 3-methyladenine on proliferation and activation of hepatic stellate cells]. Xibao Yu Fenzimian Yixue Zazhi 2013; 29: 809-812 [PMID: 23948405]

30 Das F, Bera A, Ghosh-Choudhury N, Aboubd HE, Kasinath BS, Choudhury GG. TGF-β-induced dector suppression recruits mTORC1 and not mTORC2 to enhance collagen I (α2) gene expression. PLoS One 2014; 9: e109608 [PMID: 25333702 DOI: 10.1371/journal.pone.0109608]

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