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# HNF1A regulates the crosstalk between innate immune responses and MAFLD by mediating autophagic degradation of TBK1

Yunfei Qin[a,b], Dongbo Qiu[b], and Qi Zhang[a,b]

[a]Biotherapy Center, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, PR China; [b]Institute of Vaccine, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, PR China

**ABSTRACT**

The selective macroautophagy/autophagy pathway is an important pathway of protein degradation, regulating signal transduction pathways via selective degradation of certain signaling complexes. TBK1 functions as a key protein in innate immunity or metabolic-associated fatty liver disease (MAFLD); however, the degradation of TBK1 has not been fully investigated. Recently, we have found that HNF1A functions as a novel cargo receptor to bridge TBK1 and MAP1LC3/LC3, hence promoting the degradation of TBK1 and regulating antiviral innate immunity and MAFLD.

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Innate immunity activation is involved in the regulation of many cellular activities, including antiviral processes and MAFLD. However, the regulation of innate immune signaling in MAFLD is poorly understood. To evaluate the possible roles of transcription factors or genes related to liver metabolism in innate immune responses, we performed a screen using candidate genes and identified HNF1A as a negative regulator of innate immunity activation [1]. HNF1A associates with the TBK1 rather than other proteins to reduce IRF3 activation (Figure 1). Notably, virus infection can enhance the association between HNF1A and TBK1. By detecting the protein abundance, we found that HNF1A specifically degrades TBK1. In addition, we found that HNF1A mediates the autophagic degradation of TBK1. Although HNF1A has been implicated in the regulation of TBK1 stability, HNF1A only degrades TBK1 having kinase activity.

We predicted and determined that HNF1A can bind with LC3 using its LIR motifs, and the interaction is also enhanced after viral infection. As expected, HNF1A loses the ability to degrade TBK1 without the LIR domains. HNF1A has LIR motifs and can bridge TBK1 and LC3 (Figure 1), so we considered that HNF1A may act as a novel cargo-recognition receptor that mediates the selective autophagic degradation of TBK1.

It is well known that cargo-recognition receptors often recognize substrates through ubiquitin chains; we analyzed the 7 different types of poly-ubiquitination of TBK1 and found that HNF1A markedly enhances the K33-linked ubiquitination of TBK1, but has no appreciable effect on the ubiquitination of TBK1 having other linkages (Figure 1). At the same time, we also found that K33 ubiquitination of TBK1 is significantly upregulated after virus infection, whereas K33 ubiquitination is inhibited in HNF1A-deficient cells. In order to discover the detailed mechanism underlying TBK1 ubiquitination, we generated a series of mutant constructs by substituting lysine (K) residues with arginine (R) with a focus on key lysine residues in the carboxyl terminus of TBK1 through identifying the ubiquitination of different domains. We found that HNF1A loses the ability to degrade the TBK1$^{K670R}$ mutant, and this mutant does not display an enhanced K33-linked ubiquitination by HNF1A when compared with wild-type TBK1. Furthermore, we also found that K33 ubiquitination of TBK1 without kinase activity is not regulated by HNF1A compared to the wild-type TBK1. These lines of evidence suggest that HNF1A regulates K33 ubiquitination of kinase active TBK1 at site K670.

The phosphorylation and activation of TBK1 are critical for antiviral innate immunity and MAFLD. We further determined whether HNF1A-mediated TBK1 degradation is involved in the regulation of antiviral innate immunity and MAFLD. To confirm the inhibitory effect of HNF1A on antiviral immune responses, we used RNA or DNA virus to infect human cells and found that HNF1A promotes RNA or DNA virus infection and inhibits the RIGI/RIG-I- or STING1-mediated type I IFN signaling pathway (Figure 1). We next evaluated the potential role of HNF1A in MAFLD and found that HNF1A is markedly suppressed in NAFLD and NASH liver biopsy specimens. Interestingly, we also found that the active form of TBK1 (*p*-TBK1) is significantly upregulated in these samples. Palmitate can active TBK1 through CGAS-STING1 in MAFLD, so we established a cell model by treating liver cells with palmitate. As shown in the specimens, HNF1A expression is significantly inhibited by palmitate, and p-TBK1 is significantly increased concomitant with the decrease of HNF1A expression (Figure 1). Notably, we also observed that the association of endogenous HNF1A with p-TBK1 and LC3 is enhanced after palmitate stimulation. We previously reported that STING1-TBK1 suppresses the degradation of lipid droplets. Therefore, we next studied whether

**CONTACT** Yunfei Qin qinyf6@mail.sysu.edu.cn; Qi Zhang zhangq27@mail.sysu.edu.cn The Third Affiliated Hospital, Sun Yat-sen University, NO. 600 Tianhe Road, Guangzhou, Guangdong 510630, PR China

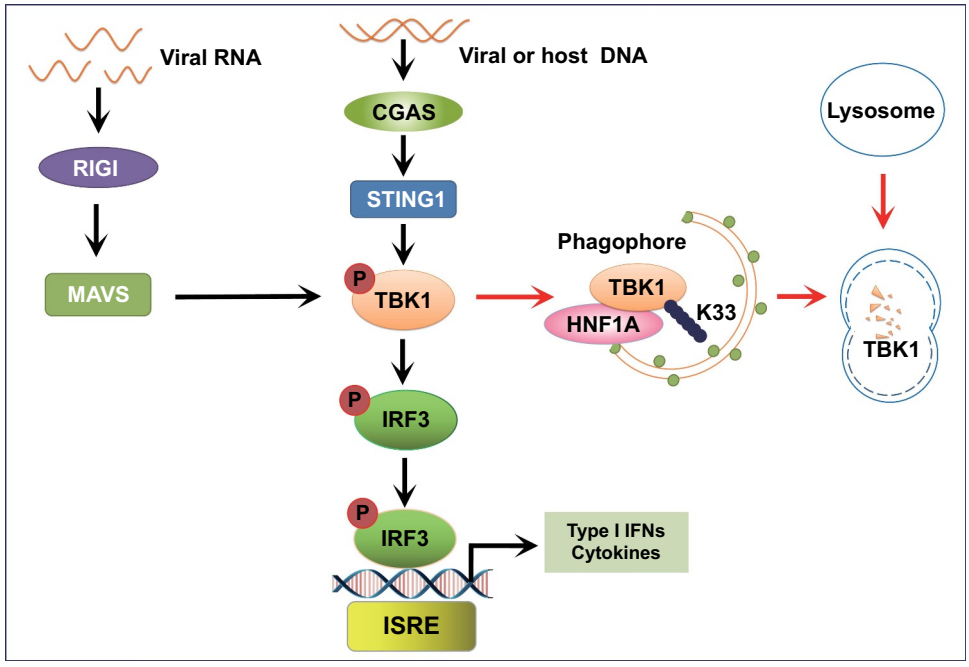


**Figure 1.** A working model of HNF1A-mediated TBK1 degradation in the regulation of antiviral innate immunity and MAFLD. After viral infection or palmitate treatment, HNF1A promotes the K33-linked ubiquitination of TBK1 and facilitates its binding to LC3; subsequent degradation of TBK1 suppresses *p*-IRF3 and type I IFNs. In MAFLD and metabolic dysfunction-associated steatohepatitis/mash, the downregulated expression of HNF1A leads to sustained activation of *p*-TBK1; excessive levels of interferon and some inflammatory factors exacerbate MAFLD and MASH. ISRE, interferon-sensitive response element.

HNF1A affects lipid droplets and triglycerides, and found that this is indeed the case.

In summary, our study provides evidence that HNF1A is not only involved in the regulation of innate immunity, but also associated with MAFLD. Some reports have also suggested that HNF1A has a pivotal role in MAFLD; however, the function in innate immunity remains largely unknown. In our study, we think that TBK1 is another important target of HNF1A function in innate immunity and MAFLD. HNF1A has been repeatedly reported to exercise roles as a transcription factor, whereas little is known about its non-transcription factor function. In this work, we think that HNF1A can serve as a novel cargo recognition receptor to induce the selective autophagic degradation of TBK1. Even though HNF1A serves as a negative regulator in TBK1 activation, generation of tissue-specific *hnf1a* KO mice to reveal the in vivo role of HNF1A in antiviral responses and MAFLD is needed. In addition, HNF1A is not a direct ubiquitination enzyme, so why it can promote the K33 ubiquitination of TBK1 is still worth investigating. Some E3 ubiquitin ligase enzymes are reported to be involved in the regulation of TBK1 ubiquitination, and we also predicted and determined that RNF14 is involved in the regulation of K33 ubiquitination of TBK1 by binding to HNF1A. It has been reported that many molecules can regulate TBK1 degradation, and how these molecules cooperate still needs to be further investigated. Growing evidence suggests that innate immune pathways are critical in MAFLD. Therefore, further study should be carried out in this field in order to develop strategies for the treatment and prevention of MAFLD.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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