L-06
ROLE OF E2/E3 UBIQUITIN LIGASE, UBE2O, IN HEPATITIS B VIRUS REPLICATION

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Chronic hepatitis represents a life-long liver disease caused by infection of Hepatitis B virus (HBV) and affecting more than 250 million people worldwide. Since HBV replication is completely dependent upon host cell protein pathways, the study of virus-host interactions promises to reveal novel cellular targets for development of new therapies. Our strategy is to identify and characterize key cellular proteins and pathways that are essential for different steps of viral replication[1,2]. Using mass spectrometry, we identified a novel HBV Core (HBc) – interacting host protein, UBE2O[3]. UBE2O is an E2/E3 hybrid ubiquitin-protein ligase that displays both E2 and E3 ligase activities and mediates mono-ubiquitination of several chromatin-associated proteins, such as INO80, BAP1 and CXXC1, affecting their subcellular location[4]. Notably, UBE2O is also implicated in endosomal protein trafficking through its ubiquitination of the WASH regulatory complex[5].

Here, we confirmed the interaction between UBE2O and HBc protein using co-immunoprecipitation. Further analysis of various HBc deletion mutants demonstrated that HBc interacted with UBE2O via its C-terminal domain. Co-expression of HBc and UBE2O resulted in HBc protein mono-ubiquitination. Interestingly, single Ser-to-Ala mutations of two major phosphorylation sites involving serines at positions 164 and 172 of a 185-aa HBc variant resulted in increased UBE2O-mediated mono-ubiquitination, suggesting that HBc hypo-phosphorylation is vital for efficient ubiquitination.

The role of UBE2O in viral lifecycle was investigated in HBV-infected HepG2-NTCP cells as well as primary human hepatocytes (PHH) upon downregulation of endogenous UBE2O. Two days prior to HBV infection, the cells were transfected with UBE2O-specific siRNAs and five days post-infection, the levels of intracellular viral DNA/RNA, and extracellular HBe antigen were determined by (RT)qPCR and ELISA, respectively. In addition, the formation and stability of intracellular nucleocapsids and the secretion of viral particles was also examined. When compared to control cells (transfected with non-specific siRNAs), the downregulation of UBE2O led to decreased levels of both HBV DNA/RNA and HBeAg. Interestingly, the formation of intracellular viral nucleocapsids and secretion of virions was significantly impaired in cells with reduced expression of UBE2O. These data suggested that UBE2O may play important role in nucleocapsid maturation and viral secretion.

Recently, a compartment of the late endocytic pathway, the multivesicular body (MVB), has been shown to participate in the final stages of HBV maturation and release. The HBV transiently resides in the MVB and disruption of this compartment was shown to result in decreased virion secretion. Furthermore, the efficient HBV maturation and virion secretion was reported to employ the endosomal sorting complexes required for transport (ESCRT)-II, -III, and the AAA ATPase Vps4[6–8].

To link the cellular pathway involving UBE2O with HBV maturation and release, we analyzed the localization of UBE2O in HBV-infected HepG2-NTCP cells by immunofluorescence microscopy. As shown in Fig. 1A, the endogenous UBE2O protein resides in the cytosol in vesicular compartments that are also positive for the marker of MVBs, CD63. We also analyzed HBV core particles using an antibody that specifically recognized viral capsids. Interestingly, the co-staining of viral capsids and UBE2O (Fig. 1B) resulted in similar co-localization pattern, indicating the possible association of both UBE2O and core particles within MVBs.

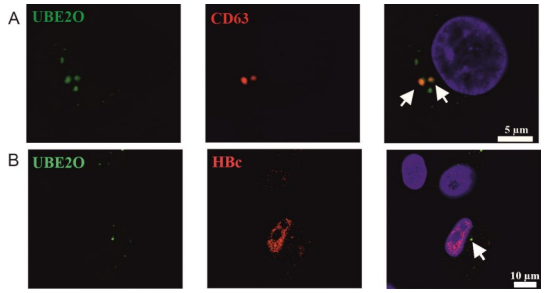

Fig. 1. **Association of UBE2O and viral capsids with multivesicular bodies (MVBs). HepG2-NTCP cells were infected with HBV (MOI of 1000 VGE/cell) and 5 days post-infection the cells were fixed and stained with antibodies to UBE2O and CD63 (A), or UBE2O and capsid/HBc (B). The nuclei were stained with DAPI, and the distribution of UBE2O, CD63 and capsids were visualized with a confocal fluorescence microscope.**

In conclusion, our results implicated UBE2O in host MVB functions and suggested that UBE2O is an important cellular regulator required for efficient maturation and release of enveloped HBV virions. Based on these findings, UBE2O may be a valuable target for HBV control.

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