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論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	DU KAILI
論文題名 Title	Identification of the Interaction between Minichromosome Maintenance Proteins and the Core Protein of Hepatitis B Virus (HBVコアタンパク質とミニ染色体維持複合体因子との相互作用の同定)

論文内容の要旨

〔目　的(Purpose)〕

Approximately 350 million people worldwide are suffering from chronic hepatitis B virus (HBV) infection. Currently, thorough measures to prevent HBV infection are still insufficient. It is critical to these efforts that researchers elucidate the details of the HBV infection cycle inside host cells. HBV core protein composes the HBV capsid. In addition to the nucleocapsid formation, multiple roles in cccDNA function and the interaction with cellular proteins have been reported. Furthermore, core protein allosteric modulators (CpAMs) are promising as key components of hepatitis B curative therapies, and identifying factors that interact with core proteins is an attractive approach for therapeutic drug development. We attempted to identify core protein interacting factors using highly purified core proteins. Mass spectrometry of the core interacting factors identified several promising and attractive factors, including MCM2, a component of the minichromosome maintenance proteins (MCMs) complex, which are components of the pre-replicative complex (pre-RC). Here we demonstrate the interaction of the HBV core protein with MCM2 and the inhibitory effects of MCM2 on HBV life cycle. And finally, we found that the HBV core protein interacted with the MCM complex. This is the first report showing that MCMs interact with the HBV core protein. These results have implications for our understanding of the unknown functions of core proteins in viral replication.

〔方法ならびに成績(Methods/Results)〕

To demonstrate the interaction of the HBV core protein with MCMs and their effects on HBV life cycle in detail, fir, we established HBV core protein-expressing HepG2 stable cell lines by transducing HepG2 cells with lentiviral vectors expressing His_6-tagged core protein. Next, we examined core particles formation with cesium chloride (CsCl) density gradient ultracentrifugation. The result showed that formation of core particles was not affected by His_6 tag. Then, we applied the stable cell line to pull down endogenous MCM2 with Ni-NTA agarose, besides MCM2, other components of the helicase MCM2-7, MCM5, were also found to interact with the core protein. The six eukaryotic MCMs usually form a hetero hexamer complex, MCM2-7. And thus, in order to investigate whether the whole MCM2-7 complex was involved in the interaction with the core protein, each Halo™-tagged MCM (HaloMCM2, 3, 4, 5, 6 and 7) was individually expressed in the HEK293T cells. The cell lysate of HEK293T cells expressing HaloMCM2 was mixed with lysate of HepG2/HisCore and pulled down with Halo™-link resin. Halo™-link resin bound HaloMCM2 pulled down MCM3, 4, 5, 6, and 7 as well as the core protein. Likewise, each Halo-tagged MCM pulled down HisCore together with the other MCMs (data not shown). These results indicated that HaloMCMs were functional to make a complex with other MCMs and the HBV core could interact with one of them. To further confirm the interaction of HBV core protein with MCM proteins, the localization of HisCore and HaloMCMs was examined by IFA. HisCore protein was colocalized with each of the HaloMCMs, mainly in the cytoplasm. To examine the effect of MCM2 on HBV infection cycle, we established MCM2-knocked down NTCP/HepG2 (C4) cell lines (MCM2KD/C4). The knockdown efficiency was confirmed by Western blot analysis. At 9 days post infection (dpi), HBeAg levels into the supernatants of MCM2KD/C4 cells, levels of cccDNA, core-associated HBV DNA and extracellular particle-associated HBV DNA were all significantly increased compared with the control cells (shNC/C4).

〔総　括(Conclusion)〕

Our results suggested that a) Minichromosome Maintenance Complex (MCM) interacted with the HBV core protein; b) MCMs should be involved in HBV entry/infection establishment and/or cccDNA accumulation in the internal cycle; c) MCMs should serve as restriction factors for HBV replication.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

マウス肝細胞においては何らかの因子がHBVエントリーを阻害するということが想定され、この因子がHBV ヌクレオキャプシドを組成するコア蛋白に関わっている可能性が高いことを示しているが、

本研究で、His-コア蛋白質と相互作用する宿主因子としてMCMの構成因子であるMCM2を初めて同定した。

MCM2のノックダウンした感染系で、HBVの遺伝子発現効率や複製効率が上がることから、MCM2は侵入過程やcccDNA形成過程で抑制的にはたらいていることが明らかとなった。

この現象の詳細なメカニズムは不明だがヒトNTCPを発現するマウス肝細胞がHBVの感染を許容しない一つの理由になっている可能性もあり、今後の研究の発展が期待される。

以上、学位に値する研究と考える。