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博士(医学)　　孫　鎖鋒

論文題目

Involvement of PUF60 in transcriptional and post-transcriptional regulation of hepatitis B virus pregenomic RNA expression

(B 型肝炎ウイルスプレゲノム RNA 発現における PUF60 による転写および転写後調節)

[Introduction]

Hepatitis B virus (HBV) is a hepatotropic, enveloped virus of *Hepadnaviridae* family with a partial double-stranded relaxed circular DNA genome. Although evidence on regulations of HBV lifecycle is accumulating, molecular mechanisms underlying transcriptional- and post-transcriptional regulations of HBV gene expression are still not fully understood. The aim of this study is to identify novel host factors that function as regulators of the viral lifecycle and to elucidate the molecular mechanisms underlying their regulation.

[Materials and Methods]

The viral RNAs in cells where pUC-HB-Ce carrying the 1.24-fold length of HBV genome derived from genotype C and the FLAG-tagged PUF60 (poly-U-binding factor 60kDa)-expressing plasmid (pcDNA-F-PUF60) were co-transfected were analyzed by northern blotting and quantitative PCRs for pregenomic RNA (pgRNA) and the particle-associated viral DNA. Plasmids expressing PUF60 deletion mutants were generated via several PCRs using pcDNA-F-PUF60 as the template. The promoter activity was determined by the reporter luciferase assay. RNA degradation was measured in the presence of actinomycin D. Binding between transcription factors and the HBV DNA sequence *in vitro* was analyzed by a gel mobility shift assay.

[Results]

In the course of our proteomic screening and subsequent siRNA assay, PUF60, a member of the U2 small nuclear ribonucleoprotein auxiliary factor protein family, was identified as an HBV RNA-binding factor that is possibly involved in HBV gene expression. Reporter assays to test an effect of PUF60 on various promoter activities showed that PUF60 is involved in up-regulation of HBV pregenome promoter activity. Further investigation to assess the effect of PUF60 on the HBV post-transcriptional processes demonstrated that PUF60 plays a role in pgRNA degradeation and suppression of pgRNA splicing. When pUC-HB-Ce was introduced into human hepatoma cells with pcDNA-F-PUF60, the pgRNA level was higher at days 1–2 post-transfection but declined thereafter in PUF60-expressing cells compared to viral replication control cells. Deletion analyses showed that the second and first RNA

recognition motifs (RRMs) within PUF60 are responsible for pregenome promoter activation and pgRNA degradation, respectively. Ectopic expression of a PUF60 mutant with deletion of the first RRM led to higher HBV production. PUF60 is involved in up-regulation of HBV enhancer II/basal core promoter activity in pgRNA transcription via interaction with transcription factor 7-like 2 (TCF7L2), which directly binds to the enhancer sequence.

[Discussion]

Findings obtained in this study suggest that PUF60 is potentially a versatile regulator of both transcriptional- and post-transcriptional steps of expression of HBV pregenome. This is the first to demonstrate involvement of a host factor in not only positively but negatively regulating the gene expression and replication of certain virus. RNA decay mechanisms in mammalian cells are now recognized as functional roles in antiviral host defense. Thus, it is likely that PUF60-dependent acceleration of pgRNA degradation is a component of anti-HBV mechanisms, and up-regulation of pgRNA transcription induced by PUF60 might be an evolutionally acquired strategy to reduce antiviral effects via the RNA decay pathway.

[Conclusion]

PUF60 was identified as a new type of regulator of HBV lifecycle, capable not only of transcriptionally up-regulation of pgRNA expression but of post-transcriptional involvement including acceleration of the pgRNA decay and suppression of pgRNA splicing. These findings gain insights on the functional linkage between transcriptional and post-transcriptional regulations on the viral replication and on the mechanism(s) to control antiviral host defense and viral persistence.