THE m6A METHYLATION SYSTEM LIMITS HEPATITIS B VIRUS REPLICATION

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N6-methyladenosine (m6A) is a common RNA modification, which plays a critical role in RNA fate and regulating such aspects as splicing, stability, nuclear export, and translation efficiency. The introduction, removal, and recognition of m6A modifications in RNA are regulated by a number of factors, known as writer, eraser, and reader proteins. It is known that the m6A modification can play an important role in the life cycle of viruses, including hepatitis B virus. The m6A methylation system has a significant impact on the hepatitis B viral cycle (HBV), particularly, on stability of mRNA transcripts, encapsidation efficiency, and reverse transcription of HBV pgRNA. In this study, we assessed the effect of knockout and activation of expression of several factors of the m6A methylation system on the HBV viral cycle, including pregenomic RNA (pgRNA) and circular covalently closed DNA (cccDNA). The study was carried out using the StCas9 nuclease system for knockout and the dCas9-p300 system for activation of gene expression. The levels of pgRNA and cccDNA were estimated by real-time PCR. The data obtained show the restriction of the viral cycle at the basal level by the factors METTL3, METTL14, METTL16, FTO, JMJD6, and hnRNPA2B1, as well as suppression of the viral cycle with overexpression of all of the above factors, except for hnRNPA2B1.

Keywords: hepatitis B; m6A factors; cccDNA; pgRNA

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INTRODUCTION

Hepatitis B virus (HBV) is a DNA virus that belongs to the *Hepadnaviridae* family [1]. The HBV virion consists of a lipid supercapsid with short (S-HBs), medium (M-HBs), and long (L-HBs) antigens on its surface. An icosahedral nucleocapsid consisting of the HBc protein (the "core" protein is located inside the supercapsid. The nucleocapsid contains a genome covalently linked to the viral DNA polymerase (Pol). The HBV genome is represented by partially double-stranded relaxed circular DNA (rcDNA) approximately 3.2 thousand base pairs long [2, 3]. HBV infection of hepatocytes initially involves low-affinity interaction with heparan sulfate proteoglycans followed by subsequent high affinity interaction with sodium taurocholate cotransport polypeptide (NTCP), which is expressed in hepatocytes. This leads to internalization of the virus via receptor-mediated endocytosis. In the cell cytoplasm, the virus is released from the supercapsid and then imported into the nucleus through the nuclear pore complex [4]. In the nucleus, the nucleocapsid disassembles to release rcDNA, which is converted into circular covalently closed DNA (cccDNA) through a multi-step process involving cellular enzymes [5]. cccDNA is a template for the synthesis of all viral mRNAs, as well as pregenomic RNA (pgRNA), which is the basis for reverse transcription to form cccDNA [6]. cccDNA is the main factor responsible for the chronic course of HBzV infection due to high stability and maintenance of the cccDNA pool due to the processes of hepatocyte reinfection and re-import of rcDNA into the nucleus [7–9].

N6-methyladenosine (m6A) is the most common epitranscriptomic modification, which was found in all types of RNA. The m6A modification is mainly localized in conserved DRACH motifs (D = G/A/U, R = G/A, H = A/U/C) [10]. Transcriptome analysis has shown that ~37% of m6A tags are located within coding sequences, about 20% have been found in the 3′-untranslated region; 28% of the tags are detected within -200 to +200 nucleotides after the stop codon, while 12% of m6A tags are located in the transcription start region. In addition, the highest enrichment of m6A tags was found in the stop codon region and the transcription start region [11, 12]. The introduction, removal, and reading of m6A tags is a dynamic process. Methylases, including METTL3, METTL14, METTL16, known as writer proteins, introduce m6A tags. Demethylases, also called eraser proteins, remove m6A tags. In this context demethylases ALKBH5 and FTO play the role of erasers. Reader proteins are responsible for the recognition of m6A tags [10]. Reader proteins

are localized in the nucleus and cytoplasm. Nuclear readers include YTHDC1, hnRNPA2B1, and hnRNPG proteins. Cytoplasmic readers include YTHDF1, YTHDF2, YTHDF3, and YTHDC2 [11]. Reader proteins play a decisive role in the fate of m6A-modified RNA, influencing splicing, nuclear export, translation and stability [13].

It is known that HBV interacts with the m6A system [14]. For example, METTL3/METTL14-dependent m6A modification of the 3′ ε-loop reduces the stability of viral transcripts, while a similar modification of the 5′ ε-loop of pgRNA is necessary for the reverse transcription of pgRNA into rcDNA [15]. The m6A modification of the 5′ ε-loop allows pgRNA to interact with the HBc core protein and ensures efficient packaging of pgRNA into the nucleocapsid, which is necessary for the assembly of viral particles [16]. It has also been shown that m6A methylation in the coding sequence of HBx, the main transcriptional activator of the virus, is necessary for efficient HBV replication [17]. In addition, HBx directly binds to the METTL3/METTL14 writer protein complex, stimulates their nuclear import, and promotes binding to cccDNA, providing co-transcriptional modification of viral transcripts [18].

In addition to influencing the viral cycle, there is evidence that of m6A influences the innate immune response to HBV. For example, METTL3/METTL14-dependent modification of viral RNA leads to a decrease in RIG-I-dependent recognition of HBV RNA. This occurs due to binding of the YTHDF3 and YTHDF2 factors to HBV RNA and blocking its recognition by the RIG-I sensor of foreign RNAs [19]. HBx protein can also induce m6A methylation of *PTEN* gene mRNA, a factor required for nuclear import of the interferon transcription factor IRF-3 [17, 20]. This reduces the stability and mRNA levels of the *PTEN* gene, which is involved in the nuclear import of IRF-3, leading to a decrease in interferon synthesis. Therefore, the m6A modification of HBV RNA may contribute to immune evasion. At the same time, the m6A modification may cause degradation of HBV RNA by the antiviral RNase ISG20 [21].

Thus, the role of the m6A methylation system and, particularly, individual m6A methylation components in the HBV life cycle and HBV interactions with human cells remains unclear.

In this study, we have assessed the effect of expression blockade by CRISPR/Cas9-mediated gene knockout and overexpression of individual factors of the m6A methylation system on HBV replication in human cells, namely, the writer proteins METTL3, METTL14, METTL16, the eraser protein FTO, and the reader proteins JMJD6 and hnRNPA2B1. These factors were selected because we wanted to investigate in this study at least one factor from each group (writers, readers, erasers). For factor knockout, a highly specific CRISPR/Cas9 system from the *Streptococcus thermophilus* (StCas9) was used [22, 23]. Using knockout instead of knockdown it was possible to evaluate the effect at late time points, namely, on days 10 and 14.

The results of this study have shown that knockout of the *METTL3*, *METTL14*, *METTL16*, *FTO*, *JMJD6*, or *hnRNPA2B1* genes leads to an increase in the level of pgRNA and HBV cccDNA. Overexpression of any of the above factors, except hnRNPA2B1, leads to a decrease in the levels of HBV cccDNA in the cell. Therefore, these factors may participate in the suppression of HBV replication.

MATERIALS AND METHODS

Cell Culture

Experiments were carried out using the HepG2 cell line [24] (Fig. 1). The cells were cultured in a complete DMEM medium supplemented with 10% fetal bovine serum (Capricorn Scientific, Germany), 4.5 g/l glucose (PanEco, Russia), 2 μM L-glutamine (Capricorn Scientific), and 1% penicillin-streptomycin (PanEco).

Preparation of the Recombinant HBV Genome

To study the effect of knockdowns on the hepatitis B viral cycle, the cell line was co-nucleofected with recombinant cccDNA obtained using the minicircle technology [25], described earlier [26]. Briefly, the sequence between the attP and attB recombination sites in the pMC.CMV-MCS-EF1α-GFP-SV40polyA vector was replaced with the sequence encoding HBV cccDNA by means of the Gibson Assembly Cloning kit (New England Biolabs, USA). The resulting plasmid was transformed into ZYCY10P3S2T cells capable of expressing PhiC31 integrase, required for minicircle plasmid recombination in the presence of arabinose. Cell colonies were then inoculated into Terrific Broth medium (Dia-M, Russia) and incubated for 16 h in an IKA KS4000 ic shaker-incubator (IKA-Werke GmbH & Co. KG, Germany) at 37°C and 250 rpm. After 16 h, Induction Media (LB broth containing 0.2% arabinose) was added to the overnight culture. After that, the bacterial culture was incubated for 3 h at 30°C and 250 rpm and 1 h at 37°C and 250 rpm to obtain recombinant cccDNA (rcccDNA). Plasmid isolation was performed using the Maxi kit for plasmid DNA isolation from bacterial cells (Biolabmix, Russia).

Nucleofection

For nucleofection, the HepG2 cell monolayer was dissociated with trypsin (PanEco) and resuspended in the OptiMem™ medium (Thermo Fisher Scientific, Gibco™, USA). Then, the plasmid and PCR product were added to the resulting suspension (10 μg of plasmid and 200 ng of PCR product per 1 million cells). The resulting suspension

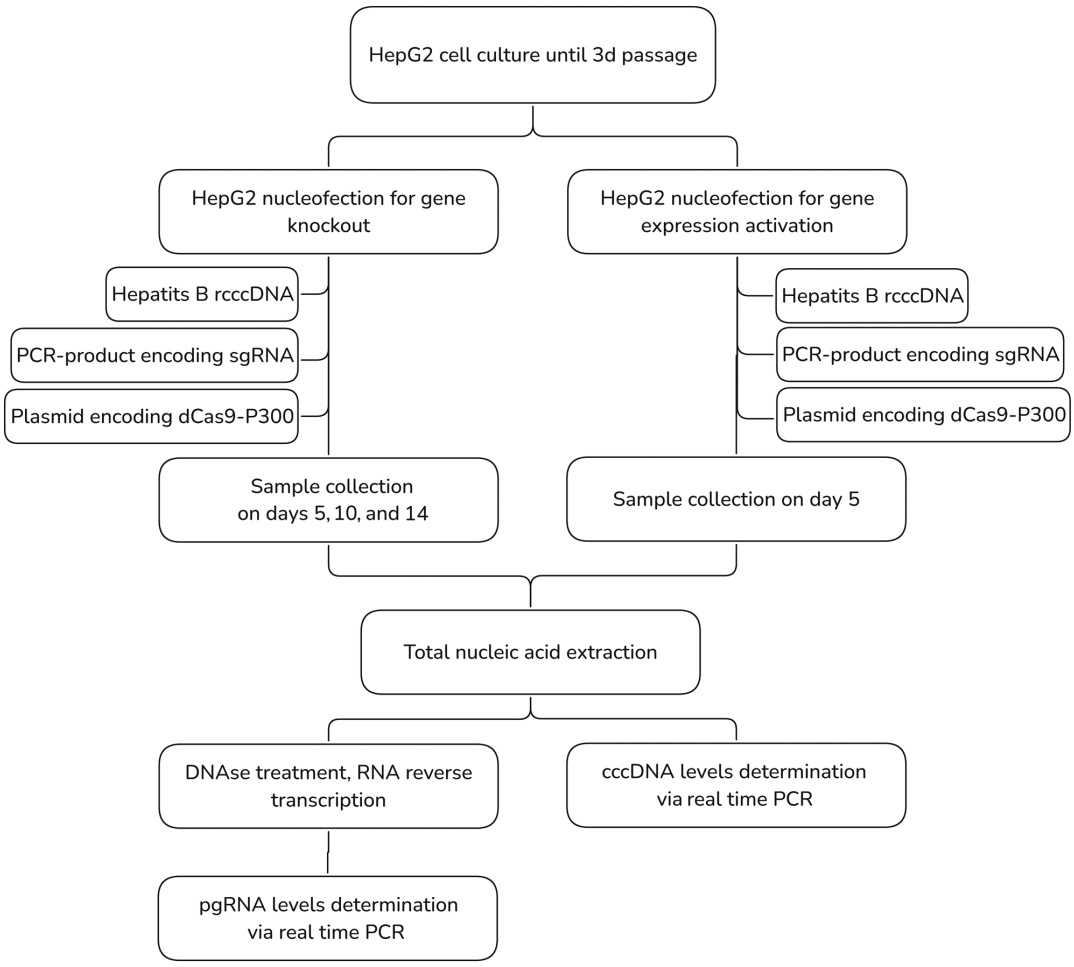

Figure 1. The experimental setup.

was transferred to an Electroporation Cuvette 0.2 cm gap (BioRad, USA) and electroporation was performed using a GenePulser Xcell electroporator (BioRad) with a pre-installed factory electroporation protocol for COS7. After that, the cells were seeded on 6-well plates and cultured as described earlier.

Gene Knockout

The StCas9 nuclease system was used for gene knockdown. For gene knockout using this system, the guide RNA is selected in such a way that the cleavage occurs in the region of an exon that is present in all transcripts of the target gene. After cleavage, the resulting DNA ends are repaired by non-homologous end joining; this leads to the occurrence of frameshift mutations, appearance of stop codons, and subsequent nonsense-mediated RNA decay [27].

Using the CHOPCHOP software [28], guide RNAs for StCas9 were selected for the exons of the target genes (Fig. 2) in such a way that the target sequence was 20 nucleotides in length, while there were no non-target regions of the genome with two or fewer nucleotide mismatches (Table 1). PCR products encoding the U6 promoter, target sequence, and guide RNA scaffold were obtained by overlapping PCR. The U6 promoter and guide RNA scaffold were obtained from the MST1 sgRNA plasmid (Addgene, USA), and the target sequence was formed by a 20-nucleotide overlapping primer sequence.

HepG2 cells (the 54th passage according to the ATCC accompanying documentation, and the 3rd passage in the laboratory) were co-nucleofected with rcccDNA, a PCR product encoding a guide RNA, and a plasmid encoding the StCas9 protein (Addgene).

Activation of Gene Expression

A chimeric protein consisting of dCas9 with p300 histone acetyltransferase fused to it was used to activate gene expression. The dCas9 protein lacks its nucleolytic activity due to point mutations in the nucleolytic domains but retains the ability to target the DNA strand. Such delivery of p300 to the distal enhancer region allows activation of transcription of the target gene [29]. Guide RNAs for gene expression activation were selected for promoter regions by means of the CHOPCHOP software [28] for SpCas9 so that there were no off-target interactions with two or fewer nucleotide mismatches and the target sequence was 20 nucleotides in length (Table 2).

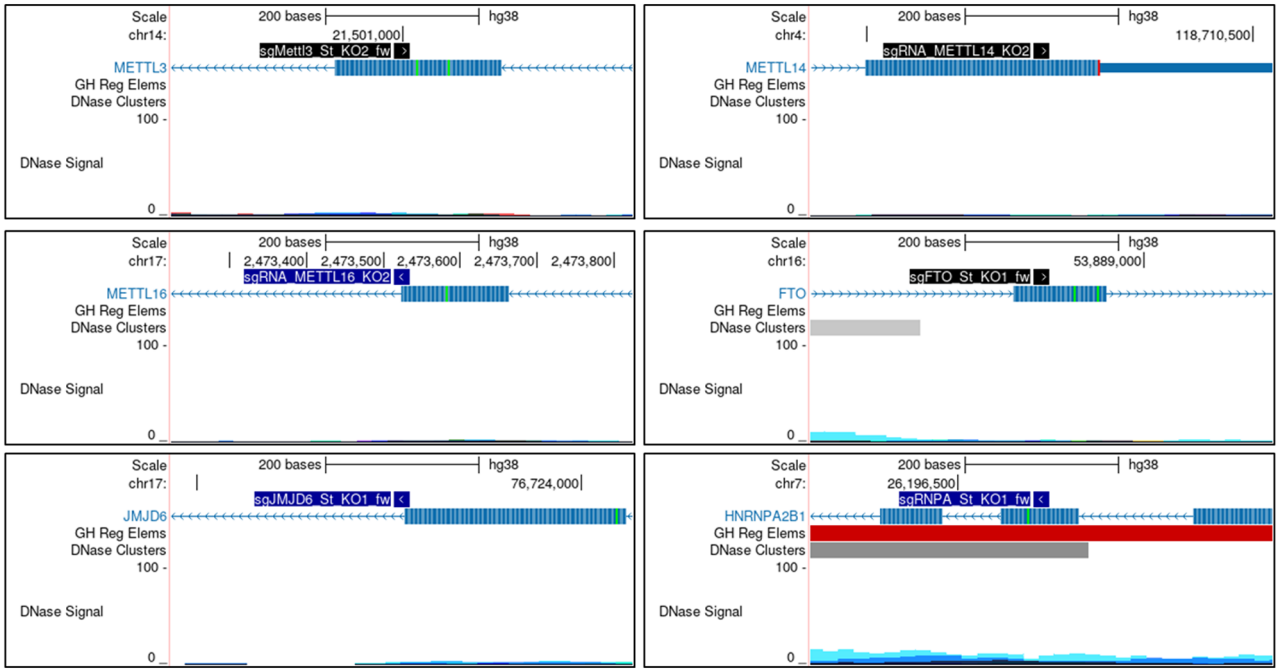

Figure 2. Design of guide RNAs to gene exons for CRISPR knockout of m6A genes. Guide RNAs were designed using CHOPCHOP, CCTop, and the UCSC browser. The design of guide RNAs took into account the promoter location, the presence of RNA isoforms, the presence of DNase I sensitivity sites, and possible off-target targets of the guide RNAs.

Table 1. Target sequences selected for knockout of genes encoding factors of the m6A system

Name of guide RNA	Sequence
METTL3 KO1	CACCTCAGGTACCTGTCTGC
METTL3 KO2	GGTCTTTGCTGCCAGGGGCC
METTL14 KO1	GAACTTCTGCTGGCCGTGGA
METTL14 KO2	GAAATAGATCTAACTTCCGA
METTL16 KO1	GATATGTGTTTCAACTATGC
METTL16 KO2	TCTCATAAAAGGTTAGCGCC
JMJD6 KO 1	TACCAGGTATAGATGAACTG
JMJD6 KO2	GGCCCCATCACAAACCACCT
hnRNPA2B1 KO1	TAAGAAAGGCTTTGTCTAGA
hnRNPA2B1 KO2	TCTGAATTTTTCATTCCAGT
FTO KO1	TTGGTTTCAAGGCAATCGAT
FTO KO2	GATCATCTACAGGAAAAACA

Table 2. Target sequences of expression activation of genes encoding factors of the m6A system

Name of guide RNA	Sequence
METTL3	GTTGCTTATAGACGCGCATG
METTL14	CGCGCCGGAAGTCTCTACTG
METTL16	AGAAACCCTCGCGACACCTG
FTO	CTATAGCGCCGACAGCGTGG
hnRNPA2B1	CTGGGCGAGCACGATTTCCG
JMJD6	TACGCGACGGCCTGACGTAG

Each PCR product encoded the U6 promoter, target sequence and guide RNA scaffold. The U6 promoter and scaffold were obtained from the pLX-sgRNA plasmid (Addgene). The target sequence was formed by a 20-nucleotide overlapping primer sequence.

HepG2 cells (passage 54 of stock according to ATCC documentation, passage 3 in the laboratory) were nucleofected with recombinant cccDNA, a PCR product encoding a guide RNA under the control of the U6 promoter, and a plasmid encoding the dCas9-P300 system (Addgene).

Nucleic Acid Extraction Isolation

Total RNA and DNA were isolated from cells using a RIBO-prep kit (Central Research Institute of Epidemiology, Russia) according to the manufacturer's protocol. The resulting sample was analyzed for HBV cccDNA levels as described below. A portion of the total DNA and RNA was subjected to DNAse I treatment (New England Biolabs) to remove DNA. The obtained RNA was re-extracted using the RIBO-prep kit to remove DNase, and then reverse transcribed using the REVERTA L kit (Central Research Institute of Epidemiology). The resultant sample containing cDNA was analyzed for HBV pgRNA levels as indicated.

Real-Time PCR

The cellular pgRNA levels were normalized to the GAPDH mRNA level. The cellular cccDNA levels were normalized to the β-globin gene. Forward GGTCCCCTAGAAGAAGAACTCCCT and reverse CATTGAGATTCCCGAGATTGAGAT primers were used to determine the pgRNA level, and GAPDH mRNA levels were determined using CCAGGTGGTCTCCTCTGACTT and GTTGCTGTAGCCAAATTCGTTGT after reverse DNase treatment and reverse transcription. cccDNA levels were determined using the primers CCGTGTGCACTTCGCTTCA and GCACAGCTTGGAGGCTTGA. The β-globin gene level was determined using the V31-FEP-CE — AmpliSens® HPV HCR-Screen (CRIE) kit (Central Research Institute for Epidemiology).

Statistical Analysis

Statistical analysis of results was performed using the GraphPad Prism program. Statistical significance relative to the control was evaluated using the Student's *t*-test. The differences were considered statistically significant at $p<0.05$.

RESULTS

Design of CRISPR/Cas9 Systems for Cleavage of Target DNA Regions

The activity of StCas9 with selected guide RNAs was assessed in the nucleolytic cleavage reaction *in vitro*. For this purpose, DNA amplification of the target gene fragment from the HepG2 cell genome was performed using PCR, and DNA was incubated with a complex of the recombinant StCas9 protein and the selected guide RNA. Target DNA cleavage was recorded by the formation of DNA bands with a lower molecular weight, formed as a result of target DNA cleavage in the central region. High nucleolytic activity was demonstrated with up to 100% cleavage of the target DNA (Fig. 3). A number of guide RNAs did not demonstrate efficient cleavage. As a result, one guide RNA was selected for each gene for cleavage and gene knockout.

Knockout of Genes Encoding Proteins of the m6A Methylation System Affects the Level of HBV pgRNA

After validation of the guide RNAs, HepG2 cells were nucleofected with the CRISPR/Cas9 system targeting specific genes and HBV rcccDNA. The CRISPR/Cas9 system with a non-targeted guide RNA was used as control. The transfection efficiency was ~85% [30, 31]. The effect of factor knockout on HBV replication was dynamically assessed on days 5, 10, and 14 after nucleofection by changes in the levels of pgRNA (Fig. 4) and rcccDNA (Fig. 5) of HBV.

Results have shown that on day 5, knockout of any of the genes encoding writer proteins (*METTL3*, *METTL14*, *METTL16*) caused a significant ($p<0.005$) increase in pgRNA levels (Fig. 4A). Knockout of *METTL3* resulted in a more than twofold increase in pgRNA levels (Mean (M): 2.37; $p = 0.000006$), knockout of *METTL16* caused almost 100-fold increase (M: 97.63; $p = 0.001563$), and knockout of *METTL14* caused up to 2.5 thousand-fold (Knockout 1 (KO1) — M: 2.65; $p = 0.000547$; KO2 — M: 2505.98; $p = 0.000339$). Therefore, writer proteins can reduce the stability of HBV RNA, and knockdown of m6A writers can promote HBV replication. These results are consistent with results of a previously published study that showed stabilization of HBV pgRNA upon knockdown of both *METTL3*/*METTL14* genes [15]. Subsequently, pgRNA levels gradually decreased and by day 14 they were at or even below the control values. This may be due to a decrease in the oncogenic properties of HepG2 model cells, a decrease in viability as a result of knockout of writer genes, or due to mechanisms of interaction between the m6A methylation system and HBV pgRNA that remain currently unexplored.

Knockout of the *FTO* eraser gene also led to a significant increase in pgRNA levels (*FTO* KO2 M: 21.01; $p = 0.0002$) (Fig. 4B). It was previously found that *METTL3*/*METTL14* knockdown led to a decrease in pgRNA reverse transcription, while FTO knockdown, on the contrary, led to its enhancement. Similarly, double knockdown of *METTL3*/*METTL14* increased the expression of HBc and HBs proteins, while knockdown of *ALKBH5* and *FTO* genes decreased HBc and HBs levels [15].

Knockout of the reader genes *JMJD6* and *hnRNPA2B1* resulted in increased pgRNA levels as early as day 5 (Fig. 4C). In *hnRNPA2B1* knockout samples, pgRNA levels were increased by more than 490-fold (M: 496.9; $p = 0.000008$), while *JMJD6* knockout resulted in more than 10-fold increase

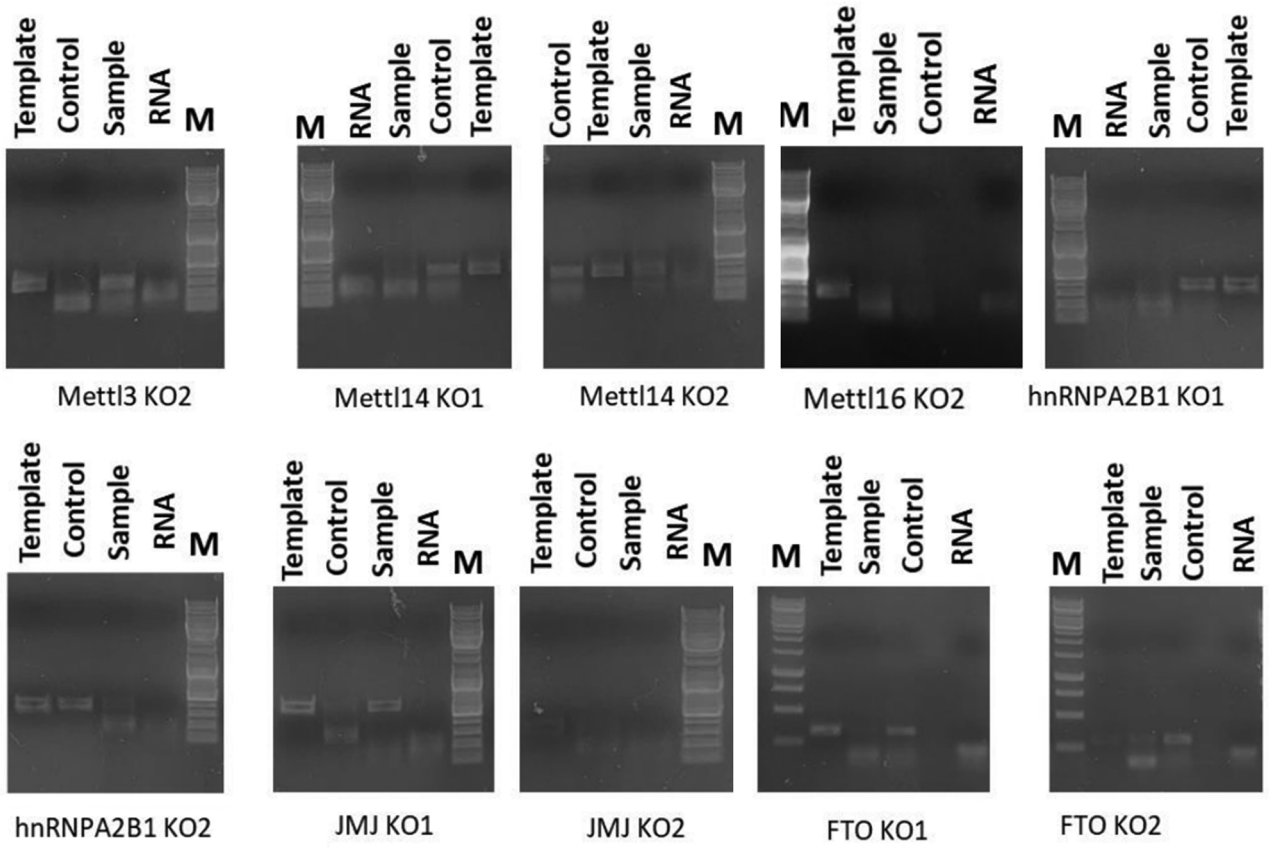

Figure 3. Results of *in vitro* cleavage of target DNA by StCas9 with the corresponding guide RNA. The results of *in vitro* cleavage with guide RNAs to the *hnRNPA2B1*, *METTL3*, *METTL14*, *JMJD6*, *FTO*, and *METTL16* genes are shown. KO1 and KO2 are guide RNAs to targets 1 and 2, respectively. M is a molecular weight marker; RNA – control RNA without template DNA and StCas9 protein; Sample – sample containing all reaction components; Control – control sample containing all reaction components but lacking StCas9 protein; Target DNA – sample of target DNA.

in pgRNA levels (M: 10.81; $p = 0.007818$). However, over time, *hnRNPA2B1* knockout resulted in decreased pgRNA levels: by day 14, their levels were about one-third of the control value (M: 0.032318; $p<0.000001$). In contrast, *JMJD6* gene knockout resulted in a sharp increase in pgRNA levels by day 14 (M: 8364; $p = 0.000599$).

These data indicate that each of the studied factors limits HBV transcription at the basal expression level. The factors METTL14, METTL16, METTL3, hnRNPA2B1, and JMJD6 exhibited the greatest activity in limiting HBV transcription.

Knockout of m6A Methylation System Proteins Affects the Level of HBV cccDNA

In addition to transcription, one of the key stages of the HBV life cycle is the formation and maintenance of a cccDNA pool in the nuclei of infected cells. Analysis of the effect of knockout of m6A system genes on cccDNA levels demonstrated results that were almost identical to the data on the assessment of HBV transcription. It was shown that knockout of the *METTL14*, *METTL16*, and *METTL3* genes dramatically increased cccDNA levels in HepG2 cells (Fig. 5A-C). At the same time, knockout of the *FTO* gene significantly decreased cccDNA levels. Knockouts of the *hnRNPA2B1* and *JMJD6* reader protein genes also decreased cccDNA levels, but only on day 5. No significant differences from control levels were found on days 10 and 14 (Fig. 5B).

The Effect of Knockout and Activation of Key Factors of the m6A System on HBV Replication

For direct comparison of knockout and overexpression of m6A system genes, HepG2 cells were transfected with HBV rcccDNA by using the CRISPR/Cas9 nuclease system (for gene knockout) or the CRISPRa with the dCas9-p300 system (for overexpression) and the HBV rcccDNA levels were analyzed on day 5 after the start of the experiment. It was not possible to compare later time points due to the transient nature of transcription activation using the dCas9-p300 system. For the group of the writer factor genes *METTL3*, *METTL14*, *METTL16* (Fig. 6A), the *FTO* eraser gene (Fig. 6B), and the *JMJD6* reader gene (Fig. 6C), the proviral effect of gene knockout and the antiviral effect of gene overexpression were demonstrated. Therefore, these factors limit

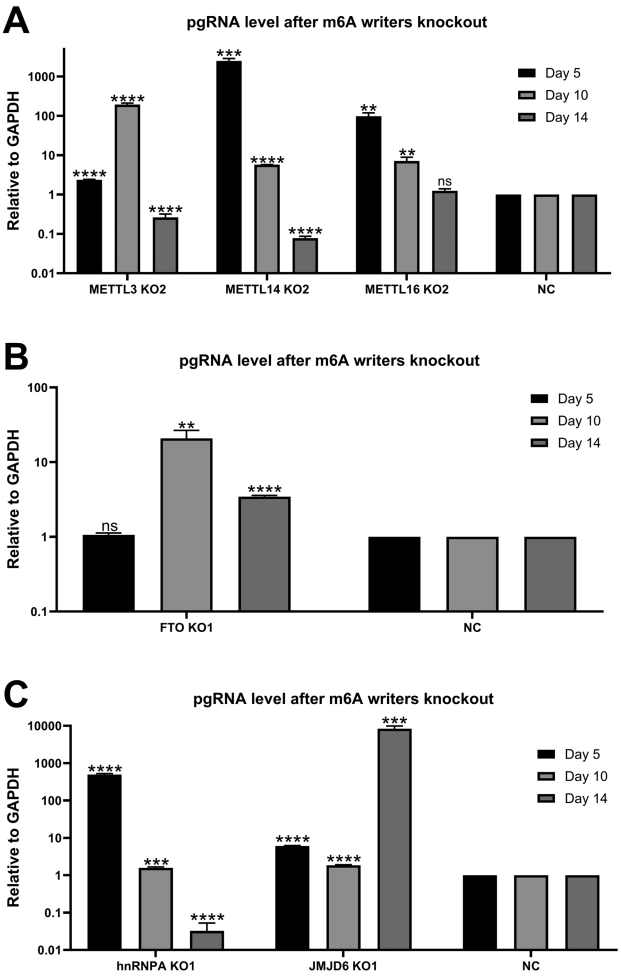

Figure 4. Levels of HBV pgRNA normalized to GAPDH mRNA in cells with knockout of m6A system factors. HepG2 cells were co-nucleofected with HBV rcDNA, plasmid encoding StCas9, and a PCR product encoding guide RNA and U6 promoter in triplicates. pgRNA levels in samples were analyzed on days 5, 10, and 14 by qPCR. Guide RNA (NC), not directed to targets in the cell and virus genomes, was used as a control. Error bars correspond to standard deviations. Statistical significance was calculated using Student's *t*-test. ***p*<0.01, ****p*<0.001, *****p*<0.0001, ns – insignificant. pgRNA – HBV pregenomic RNA. KO – knockout. NC – guide RNA for which there is no complementary sequence in the HBV genome and the human genome.

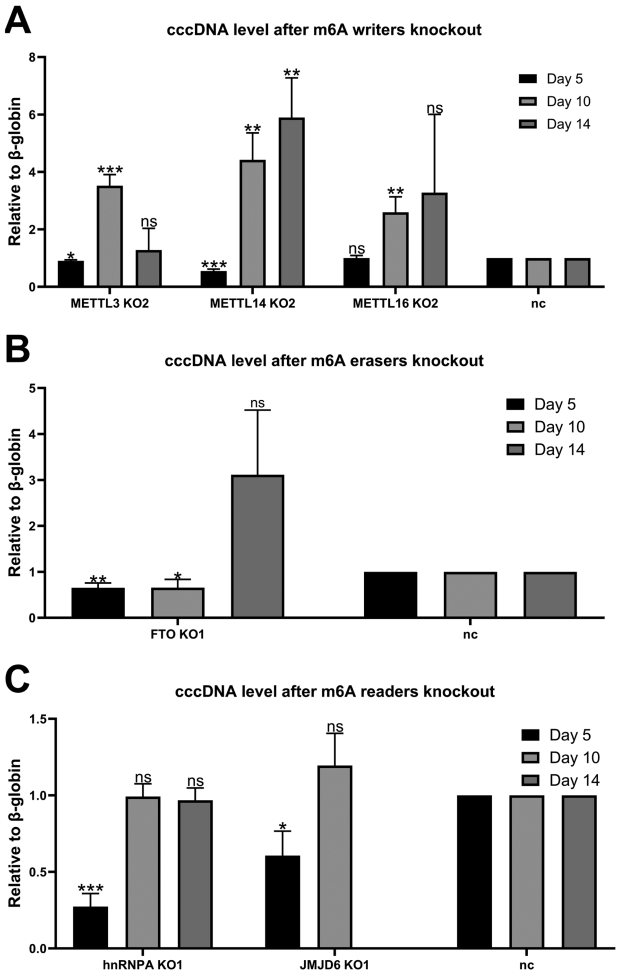

Figure 5. Dynamics of changes in HBV cccDNA levels during knockout of m6A factors. HepG2 cells were co-nucleofected with recombinant cccDNA, a plasmid encoding stCas9, and a PCR product encoding guide RNA and the U6 promoter. Analysis of cccDNA levels was performed on days 5, 10, and 14 after nucleofection. Error bars correspond to standard deviations. Statistical significance was calculated using the Student's *t*-test. **p*<0.05, ***p*<0.01, ****p*<0.001, ns – nonsignificant. cccDNA – short covalently closed HBV DNA. KO – knockout. NC – guide RNA that lacks a complementary sequence in the HBV genome and the human genome.

HBV replication levels at the basal expression level, and can exert an antiviral effect when overexpressed. At the same time, *hnRNPA2B1* overexpression (Fig. 6C) did not affect HBV cccDNA levels.

DISCUSSION AND CONCLUSIONS

The effects of knockdowns of m6A methylation factors on HBV pgRNA levels have been assessed in a number of studies. For example, Imam et al. found that double knockdown of *METTL3*/*METTL14* led to a significant increase in pgRNA stability (from 6.5 h to 15.9 h) [15]. At 48 h after *METTL3*/*METTL14* knockdown, an increase in total HBV RNA levels was noted [16, 18]. In this study, an increase in pgRNA levels occurred both with single knockout of the *METTL3* or *METTL14* genes and with knockout of *METTL16*. A similar proviral effect of m6A writer factor knockout was also observed during evaluation of HBV cccDNA levels.

Since ALKBH5 and FTO demethylases remove m6A methylation, their knockdown often produces effects opposite to the effect of methyltransferase knockdown. For example, knockdown of *FTO* and *ALKBH5* resulted in decreased expression of HBc and HBs, while knockdown

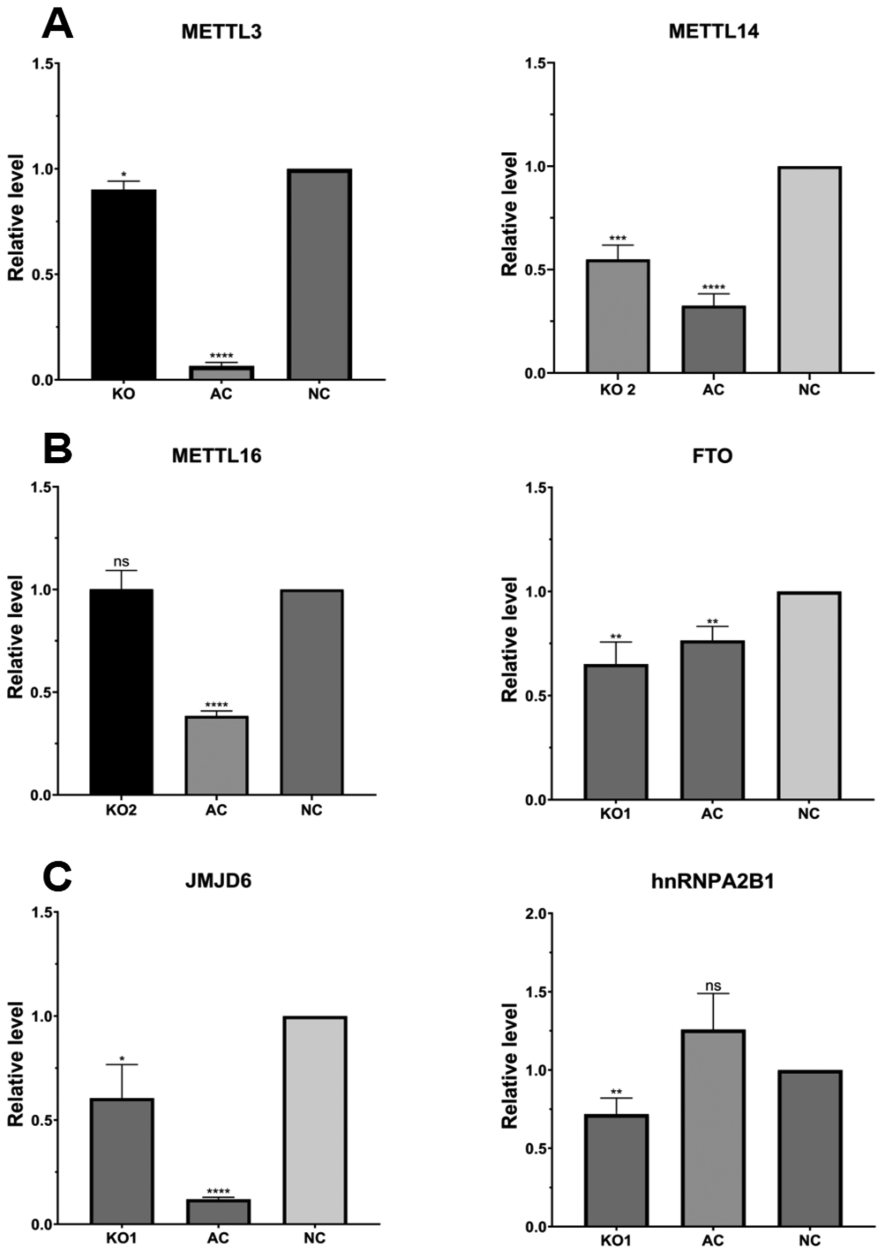

Figure 6. The effects of knockouts (KO) and CRISPR-activated transcription (ACT) of individual genes on HBV cccDNA levels. KD1 and KD2 designate use of different guide RNAs to different regions of the target gene. The presence of one KD means that only one of two guide RNAs was active based on the results of the *in vitro* target DNA cleavage assay. Statistical significance was evaluated using the Student's *t*-test. ***p*<0.01, ****p*<0.001, *****p*<0.0001, ns – nonsignificant. cccDNA – short covalently closed HBV DNA. KO – knockout. NC – guide RNA for which there is no complementary sequence in the HBV genome and the human genome.

of *METTL3* and *METTL14* resulted in their increased expression [15]. However, *FTO* knockdown increased cellular pgRNA levels by more than 20-fold versus control. In addition, in this study we have demonstrated for the first to examine the effects of knockdown of *JMJD6* and *hnRNPA2B1* readers. We have found that *hnRNPA2B1* knockout had no effect, while *JMJD6* knockout dramatically (>8000-fold) increased HBV transcription and replication parameters.

Experiments on factor overexpression confirmed the role of METTL3, METTL14, and METTL16 in limiting HBV replication. Their switch off by means of the CRISPR/Cas9 nuclease systems had a proviral effect, while overexpression had an antiviral effect. Thus, it can be concluded that these factors prevent HBV replication in human cells at the basal expression level. Separately, it should be noted that knockout of genes encoding FTO, JMJD6, or hnRNPA2B1 factors led to an increase in HBV pgRNA transcription, but caused a decrease in the cccDNA level at least on day 5 of the experiment. Further studies should determine the mechanism of action of these factors, their interaction with each other, with viral RNA and protein factors and human cells.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or the use of animals as objects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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СИСТЕМА м6А МЕТИЛИРОВАНИЯ ОГРАНИЧИВАЕТ РЕПЛИКАЦИЮ ВИРУСА ГЕПАТИТА В

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N6-метиладенозин (м6А) — распространённая модификация РНК, которая играет определяющую роль в судьбе РНК, регулируя такие аспекты, как сплайсинг, стабильность, ядерный экспорт и эффективность трансляции. Внесение, удаление и распознавание м6А модификации в РНК регулируется рядом факторов, а именно белками-писателями, стирателями и читателями. Известно, что м6А-модификация может играть важную роль в жизненном цикле вирусов, в том числе вируса гепатита В. Доказано, что система м6А метилирования оказывает влияние на вирусный цикл гепатита В (ВГВ), в частности на стабильность мРНК-транскриптов, эффективность энкапсидации и обратной транскрипции пгРНК ВГВ. В данном исследовании оценивали влияние нокаута и активации экспрессии ряда факторов системы м6А метилирования на вирусный цикл ВГВ, а именно — прегеномную РНК (пгРНК) и кольцевую ковалентно-замкнутую ДНК (ккзДНК). Исследование проводили с использованием системы нуклеаз StCas9 для нокаута и системы dCas9-p300 для активации экспрессии генов. Уровни пгРНК и ккзДНК оценивали методом ПЦР в реальном времени. Полученные данные показывают ограничение вирусного цикла на базальном уровне факторами METTL3, METTL14, METTL16, FTO, JMJD6 и hnRNPA2B1, а также подавление вирусного цикла при гиперэкспрессии всех вышеперечисленных факторов, за исключением hnRNPA2B1.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: гепатит В; факторы системы м6А; ккзДНК; пгРНК

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