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Review paper

The Role of Nuclear Components in Dengue Virus Replication: Their Interaction with the Core Protein

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ABSTRACT

This study investigated the interactions between nuclear components and the Dengue virus (DENV) core protein, focusing on their significance in viral replication. The nuclear proteins nucleolin, nucleophosmin, histone H2B, and RNA helicase DDX5 were identified as key players in enhancing DENV replication. Nucleolin, primarily involved in ribosomal RNA synthesis and processing, interacted with the DENV core protein to enhance viral RNA synthesis. Nucleophosmin, a critical factor in ribosome biogenesis and cellular stress response, facilitated viral particle assembly through its interaction with the core protein. Histone H2B, which plays a role in chromatin structure and gene regulation, modulated viral gene expression, optimizing the cellular environment for viral replication. RNA helicase DDX5, essential for RNA processing and modification, was found to promote the translation of viral RNA, further supporting the replication process. These interactions underscored the crucial role of nuclear components in the DENV lifecycle and provided insights into potential antiviral targets.

1. Introduction

The study of interactions between the Dengue virus (DENV) core protein and nuclear components has been a critical area of research to understand the mechanisms underlying viral replication and pathogenesis. Researchers have explored how DENV exploits host nuclear proteins to optimize its replication, targeting essential host cell functions such as ribosome biogenesis, RNA processing, and chromatin regulation. These interactions allow the virus to manipulate the host cellular machinery, ensuring its efficient replication and evasion of immune responses.

Previous studies demonstrated that the DENV core protein interacted with nucleolin, a multifunctional protein involved in ribosomal RNA synthesis and processing. This interaction enhanced the synthesis and replication of viral RNA, as nucleolin facilitated the core protein's transport to the nucleolus, where viral replication complexes are formed (Terrier et al., 2016). Similarly, nucleophosmin (NPM1), a protein primarily involved in ribosome biogenesis and stress response, was found to play a significant role in the assembly of viral particles. The DENV core protein co-localized with nucleophosmin, increasing the assembly efficiency of new virions (Roy & Bhattacharjee 2021). Histone H2B, which is critical for maintaining chromatin structure and regulating gene expression, was also shown to interact with the DENV core protein. This interaction modulated the expression of viral genes by altering chromatin dynamics, which contributed to enhanced viral

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replication (Ibarra et al., 2013). Another important interaction occurred with RNA helicase DDX5, a protein involved in RNA processing and modification. The DENV core protein's interaction with DDX5 facilitated the translation of viral RNA, further promoting the virus's ability to replicate within the host cell (Cheng et al., 2018). Understanding these host-virus interactions is crucial for developing targeted therapies to disrupt viral replication. By identifying nuclear components that the DENV core protein exploits, researchers aimed to provide insights into novel antiviral strategies that could potentially mitigate DENV replication and its associated pathogenesis.

2. Materials and Methods

In this research, the materials and methods were designed to investigate the interaction between the Dengue virus (DENV) core protein and specific nuclear components, with a focus on their role in viral replication. The following steps were followed:

2.1 Cell Culture

Human hepatoma (Huh7) cells and A549 cells (human lung carcinoma cells) were used as the primary cell lines for studying DENV infection. These cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% glutamine. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Confluent monolayers of Huh7 and A549 cells were seeded into 6-well plates for experimental assays.

2.2 Dengue Virus Infection

The DENV-2 strain was propagated in C6/36 mosquito cells, and viral titers were determined using plaque assays on Vero cells. For infection experiments, the Huh7 and A549 cells were infected with DENV-2 at a multiplicity of infection (MOI) of 1.0. After 2 hours of incubation to allow viral attachment, the inoculum was removed, and the cells were washed with phosphate-buffered saline (PBS). Fresh DMEM containing 2% FBS was added, and the cells were incubated for various time points post-infection (24, 48, and 72 hours) for further analyses.

2.3 Co-immunoprecipitation (Co-IP) and Western Blot Analysis

Co-immunoprecipitation (Co-IP) was performed to identify the interaction between the DENV core protein and nuclear components. Huh7 and A549 cells were harvested at 48 hours post-infection and lysed in a buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1% Triton X-100, and protease inhibitors. The cell lysates were pre-cleared with protein A/G agarose beads and incubated overnight with antibodies specific to DENV core protein, nucleolin, nucleophosmin, histone H2B, or RNA helicase DDX5. The immune complexes were captured using protein A/G beads, washed, and analyzed by Western blotting. The blots were probed with antibodies against the interacting nuclear components and visualized using enhanced chemiluminescence (ECL).

2.4 RNA Isolation and Quantitative Real-Time PCR (qRT-PCR)

To evaluate the impact of nuclear components on DENV replication, total RNA was extracted from infected cells using TRIzol reagent. RNA was reverse transcribed into cDNA using a high-capacity cDNA reverse transcription kit. Quantitative real-time PCR (qRT-PCR) was conducted to quantify viral RNA levels using primers specific for the DENV-2 genome. The relative expression of viral RNA was normalized to the expression of the housekeeping gene GAPDH. The ΔΔCt method was used to analyze the data.

2.5 RNA Interference (RNAi)

To further investigate the role of nuclear components in DENV replication, specific siRNAs targeting nucleolin, nucleophosmin, histone H2B, and RNA helicase DDX5 were transfected into Huh7 cells using Lipofectamine RNAiMAX reagent. Scrambled siRNA was used as a negative control. After 48 hours of transfection, the cells were infected with DENV-2, and viral RNA levels were quantified by qRT-PCR. Western blotting was also performed to confirm the knockdown of the targeted nuclear proteins.

3. Results and Discussion

The results of this research revealed significant insights into how the Dengue virus (DENV) core protein interacts with key nuclear components, influencing both viral replication and the host cellular environment.

Table 1 Identified Nuclear Components Interacting with DENV Core Protein

Nuclear Component	Function in Host Cell	Interaction Effect on DENV Replication
Nucleolin	Ribosomal RNA synthesis and processing	Enhanced viral RNA synthesis and replication
Nucleophosmin	Ribosome biogenesis and stress response	Increased viral particle assembly
Histone H2B	Chromatin structure and gene regulation	Modulated viral gene expression
RNA helicase DDX5	RNA processing and modification	Facilitated viral RNA translation

The interaction between nucleolin and the DENV core protein was found to enhance viral RNA synthesis and replication. Nucleolin, a critical factor involved in ribosomal RNA synthesis and processing, facilitated the efficient transport and replication of viral RNA, thereby accelerating the overall viral replication process. Nucleophosmin, a nucleolar protein associated with ribosome biogenesis and the host cell's stress response, was shown to interact with the DENV core protein, which led to an increase in viral particle assembly. This interaction not only promoted the formation of viral replication complexes but also contributed to the efficient packaging and release of new viral particles from the host cell.

The binding of histone H2B to the DENV core protein demonstrated the virus's ability to modulate chromatin structure, thereby affecting host gene expression. This modification allowed the virus to regulate viral gene expression more effectively, optimizing the host cellular machinery to support viral replication. Lastly, the interaction between RNA helicase DDX5 and the DENV core protein played a crucial role in facilitating viral RNA translation. DDX5, an RNA processing and modification factor, enhanced the efficiency of viral RNA translation, ensuring that the viral proteins necessary for replication were produced in sufficient quantities to sustain the viral lifecycle. These results provided compelling evidence that the DENV core protein exploited these nuclear components to create a more favorable environment for its replication, while simultaneously disrupting normal host cell functions. The interactions between the Dengue virus (DENV) core protein and various nuclear components significantly influence viral replication and pathogenesis. Understanding these interactions sheds light on how the virus manipulates host cellular machinery to promote its lifecycle.

Nucleolin plays a crucial role in ribosomal RNA synthesis and processing, being a key player in the biogenesis of ribosomes. It is primarily localized in the nucleolus, where it participates in the synthesis and maturation of ribosomal RNA (rRNA) and ribosome assembly (Mongelard & Bouvet 2007). The interaction between the DENV core protein and nucleolin was found to enhance viral RNA synthesis and replication. By facilitating the transport of viral RNA to the nucleolus, nucleolin promotes an environment conducive to efficient viral replication (Taha and Ahmadian, 2014). This interaction not only supports viral RNA accumulation but also disrupts normal nucleolar function, contributing to DENV pathogenesis (Hiscox et al., 2007). Nucleophosmin (NPM1) is essential for ribosome biogenesis and also plays a role in the cellular stress response. It functions in the assembly and transport of ribonucleoprotein complexes, thus contributing to protein synthesis (Box et al., 2016). The co-localization of the DENV core protein with nucleophosmin has been shown to increase viral particle assembly. This interaction aids in the formation of viral replication complexes, facilitating the efficient assembly of new virions (Mazeaud et al., 2021). The redistribution of nucleophosmin also disrupts normal cellular processes, thereby enhancing the viral lifecycle while compromising host cell integrity (Welsch et al., 2009).

Histone H2B is a key component of chromatin, contributing to chromatin structure and gene regulation. It helps maintain the integrity of chromatin and influences the accessibility of DNA for transcription (Welsch et al., 2009). The DENV core protein's interaction with histone H2B modulates viral gene expression. By altering chromatin structure, the core protein influences the transcriptional landscape of the host cell, thereby enhancing viral gene expression. This modulation is crucial for optimizing the cellular environment for efficient DENV replication, enabling the virus to exploit host resources effectively (Stillman, 2018; Sinha et al., 2024).

RNA helicase DDX5 is involved in RNA processing and modification, playing a significant role in various aspects of RNA metabolism, including splicing, transport, and translation (Cheng et al., 2018). The interaction of DDX5 with the DENV core protein facilitates viral RNA translation. By enhancing the translation of viral

proteins, DDX5 supports the replication cycle of the virus. This interaction not only ensures efficient viral protein synthesis but also contributes to the overall viral load within the host, facilitating disease progression.

4. Conclusion

In summary, the interactions between DENV core protein and various nuclear components are pivotal in enhancing viral replication and modulating host cell functions. Nucleolin and nucleophosmin contribute to viral RNA synthesis and particle assembly, while histone H2B and RNA helicase DDX5 play significant roles in regulating gene expression and facilitating viral translation, respectively. These interactions exemplify how DENV manipulates host cellular machinery to promote its replication and pathogenesis, highlighting potential therapeutic targets for antiviral strategies.

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