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REVIEW ARTICLE

A REVIEW ON FUTURE PERSPECTIVES IN TARGETING THE RNA CAPPING IN DENGUE VIRUS BY NS5 METHYLTRANSFERASE

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ABSTRACT

Dengue is a clinically important arboviral disease and its global estimated burden is worrisome for the national and international health agencies. The Dengue virus (DENV) NS5 is the largest and most conserved non-structural protein among flaviviruses; for its crucial role in RNA synthesis and capping, it has been explored as a therapeutic target. In this review article, we have discussed the role of RNA capping and how it is critical for the viral RNA stability and evasion from the host immune sensors. With the structural information and mutagenesis studies describing DENV NS5 methyltransferase mechanistic role in sequential methylation, selective inhibitors for methyltransferase (MTase) domain have been identified. Despite, extensive studies, we could find only limited number of inhibitors targeting the capping process in vitro; but no clinically relevant inhibitors have been reported till date, particularly owed to the non-specificity and toxicity issues. In the future perspective section, we propose novel enzyme engineering strategies to develop a therapeutic protein that can be administered in severe Dengue cases to bring down the viral load. The proposed engineered MTase can have characteristics not only limited to enhanced affinity to viral RNA than native MTase and accomplishing defective or non-canonical methylation, that can render the viral RNA unstable or prone to host immune sensors. The engineered MTase can be delivered via state of art delivery systems such as nanoparticles, chimeric proteins, protein cages etc.

KEYWORDS

Dengue, Methyltransferase domain, DENV NS5 MTase, RNA capping, DENV NS5 inhibitors

1. INTRODUCTION

Dengue is a viral disease of humans which is transmitted through the bites of infected *Aedes* mosquitoes, predominantly in warm and tropical climates. Recently, the global dengue burden has grown manifold with estimated infections reaching up to 400 million; the major brunt (~ 70%) is borne by Asian countries (Bhatt et al., 2013; Shepard et al., 2016). For its global impact on the national health ecosystems, dengue is in the list of major diseases of the World Health Organization (WHO). Although, majority of the infections are mild and asymptomatic but severe dengue infections causing flu-like illness without appropriate medical interventions can be fatal. India is also a major contributor of dengue cases in South-East Asian region; the distribution of dengue in India is widespread, owed to seasonal outbreaks reported from majority of states depending on climatic conditions (National Center for Vector Borne Disease Control (NCVBDC), 2023).

Dengue virus (DENV) belongs to Flaviviridae family, causing distinct epidemiological patterns associated with its four distinct serotypes (DENV 1-4). Co-circulating DENV serotypes within a region are dangerous which can cause severe manifestations in subsequent dengue infections. In cases of secondary dengue infection with new serotypes antibody-dependent enhancement (ADE) may ensue, where the previous antibodies against DENV facilitates viral entry. Clinically, dengue symptoms include severe headache, pain behind eyes, muscle and joint pain, nausea, vomiting etc. which can escalate to severe dengue with complications like plasma leakage causing dengue shock syndrome, respiratory distress, bleeding, liver enlargement and other organ involvement. Usually, Dengue infection is classified as Dengue fever (DF), Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS). DF is considered as mild form of primary infection lasting for about 2-7 days. In some cases, DF may escalate to DHF that manifests with hemostatic malfunction, increased vascular permeability and increased vascular leakage causing DSS (Roy and Bhattacharjee, 2021). According to WHO, DHF shall be assigned to cases with constant fever (2-7 days), hemorrhage with thrombocytopenia etc., and should be symptomatically managed with intensive medical care.

Dengue infection can be detected by viral isolation, reverse transcriptase real-time PCR and serological methods; however, the latter are more commonly used, which include detection of NS1 protein via rapid diagnostic tests (RDTs) and ELISA for IgM antibodies for current infections. However, there is a need for newer diagnostic tools for Dengue, because of the cross-reactivity of NS1 and IgM-based diagnostic tests with other flaviviruses such as Zika virus (Wellekens et al., 2022). There are ongoing studies to develop novel diagnostic tools based on aptamers that are economically cost effective, robust and have the potential to be implemented in RDT format or on microfluidic chips (Vashisht et al., 2020).

Currently, there is no specific antiviral treatment available for dengue virus infection; supportive care and symptomatic management is the only resort in severe dengue infections. On vaccine front, there are multiple vaccine candidates in clinical trials- live attenuated chimera; inactivated virus; subunit vaccine; DNA vaccine etc. The licensed dengue vaccine (Dengvaxia®- CYD-TDV) has a yellow fever YFV-17D vaccine virus backbone with prM/E genes of the DENVs (1-4). Dengvaxia® has been

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found safe only in participants of 9-45 years of age, who have been previously exposed to DENV infection (Huang et al., 2021). Recently, a new vaccine for dengue- tetravalent live attenuated (QDENGA®- TAK-003) has been approved for use in the European Union for individuals four years of age and older (Takeda, 2022). The QDENGA® proved to be efficacious for symptomatic dengue over 3 years, however the efficacy declined over time, which can be managed with booster doses.

This review article highlights the importance of RNA capping in DENV and further discusses the current state-of-art in targeting the RNA capping. We have also discussed future perspectives in targeting RNA capping with interdisciplinary approaches, that are not only limited to enzyme engineering of the NS5 methyltransferase domain and exploring the natural biodiversity in therapeutic compounds from marine sources.

2. Dengue Virus (DENV)

DENV is an enveloped, single stranded, positive-sense RNA virus with a genome size of approximately 10,700 nucleotides, encoding 3411 amino acids long polyprotein; 3 structural proteins- Capsid-C, precursor membrane-prM and envelope-E and seven non-structural proteins- (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Guzman et al., 2016). A schematic representing the organization of the structural and non-structural proteins is shown in Figure 1.

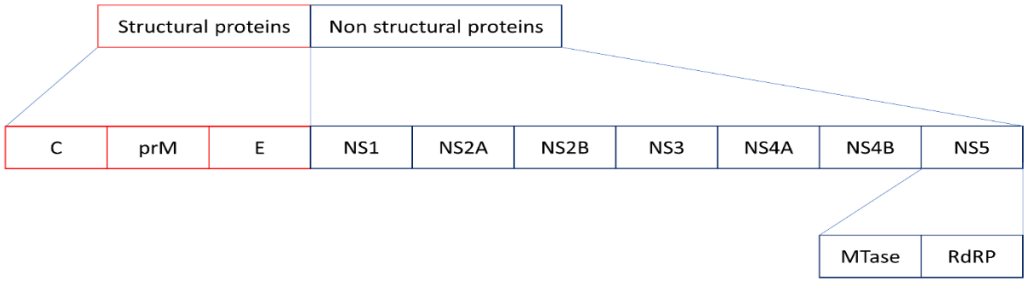

Figure 1: A schematic representation showing the composition of the structural and non-structural proteins of the Dengue virus. C- Capsid; prM- precursor membrane; E- envelope are structural proteins. NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 are non-structural proteins. MTase- methyl transferase domain and RdRp- RNA-dependent RNA polymerase domain.

DENV genomic RNA is complexed with capsid proteins (C), which are surrounded by membrane (M) and envelope (E) proteins. The DENV enters the host cell *via* fusion with host plasma membrane to form an endosome, where the nucleocapsid is released after the pH-dependent fusion of viral and endosomal membranes. The capped viral RNA translates as a polyprotein and further processed by viral and host proteases to form individual proteins. The non-structural (NS) proteins play crucial role in viral replication and packaging along with host endoplasmic reticulum (ER). NS1 & NS2A are involved in viral replication, while the soluble NS1 is released on cell surface and serves as major detection tool for acute dengue infection (Libraty et al., 2002). NS2B acts as a cofactor for NS3 and accomplish the nucleoside triphosphatase and helicase activities during RNA synthesis. NS4A is involved in formation of replication vesicles, while NS4B acts as immune evader by suppressing IFNβ and IFNγ signaling. NS5 is majorly involved in RNA synthesis and evasion from IFN signaling of the host.

2.1 The DENV NS5

NS5 is the largest protein (102 kDa) of DENV and is highly conserved among all the serotypes. Due to its high level of conservation, unique RdRp activity and its role in down regulation of host IFN signaling, NS5 has long been considered as a potential drug target. Architecture of the NS5 protein have been found identical in the flaviviruses- N-terminal methyltransferase domain and C-terminal RNA-dependent RNA polymerase (RdRp), connected by a 5-6 linker residues (El Sahili and Lescar, 2017). The DENV NS5 MTase domain (1-265 residues) is responsible for capping the nascent RNA at positions- N-7 of the guanine cap and the ribose 2′-OH of the first adenine (type I cap structure- $^{m7}GpppA_{m2'-0-}$), which is similar to most eukaryotic and viral RNAs and crucial for mRNA stability and efficient translation (Dong et al., 2010a).

The crystal structure of the DENV MTase domain (PDB ID- 3P97) has been solved, which comprised the peculiar α/β fold similar to other flaviviral MTase domain structures- Japanese Encephalitis Virus (JEV; PDB ID- 4K6M), Zika Virus (ZIKV; PDB ID- 5KQR) and West Nile Virus (WNV; PDB ID- 3LKZ) (Lim et al., 2011; Lim et al., 2011; Lu and Gong, 2013; Coloma et al., 2016; Dong et al., 2010b). Defective capping has been shown to reduce viral multiplication and generation of attenuated viruses capable of eliciting higher IFN signaling and antibody responses (Schmid et al., 2015). The uncapped triphosphate RNA or dsRNA are detected by the pathogen recognition receptors (PRRs) like retinoic acid inducible gene I (RIG-I) or melanoma differentiation-associated protein 5 (MDA5) (Dalrymple et al., 2015). Therefore, the viral RNA capping is critical in the evasion of the virus from host immune sensors (Chang et al., 2016). The RdRp domain (276-900 residues) is responsible for replication of plus strand of the DENV genomic RNA from negative ssRNA strand.

2.2 Capping of nascent RNA by DENV NS5 MTase domain

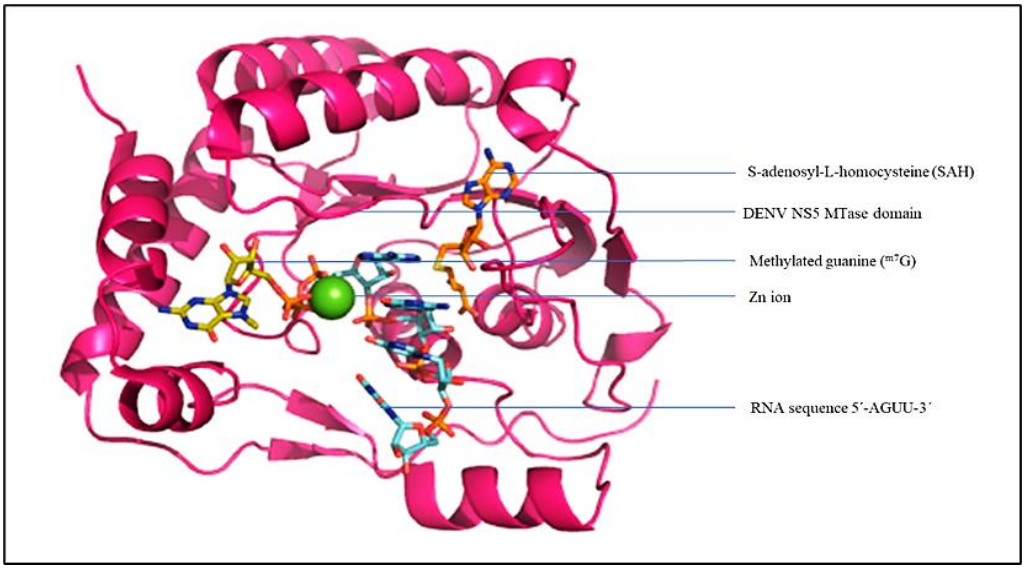

Figure 2: Ternary complex (PDB: 4V0Q) of DENV NS5 MTase domain, S-adenosyl-L-homocysteine (SAH) and cap-0 viral RNA substrate 5′-AGUU-3′. [Adapted from Sahili and Lescar, Viruses, 2017] (El Sahili and Lescar, 2017).

A crystal structure as a ternary complex including full length DENV NS5 protein bound to cap-0 viral RNA substrate and S-adenosyl-L-homocysteine (SAH) has been deduced; a snippet of NS5 MTase domain is represented in Figure 2 (Zhao et al., 2015). After the synthesis of plus strand of the genomic RNA, the capping by the MTase domain follows sequential enzymatic reactions- 1) NS3 hydrolyzes the γ-phosphate from 5´ UTR (RNA phosphatase activity); 2) NS5 MTase transfers GMP moiety to the 5´-diphosphate RNA (guanylyl transferase activity) and 3) sequential methylations at ^{m7}G and $A_{m2'-O-}$ ($G_{0ppp}AG$-RNA→$^{m7}G_{0ppp}AG$-RNA [cap-0]→$^{m7}G_{0ppp}A_{m2'-O-}G$-RNA [cap-1] (Ray et al., 2006). Figure 3 depicts the schematic of the capping of the DENV nascent RNA.

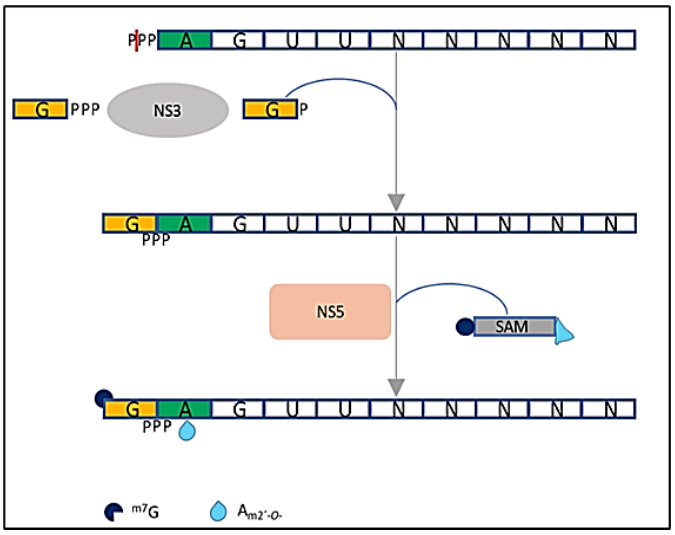

Figure 3: Schematic of the capping of DENV nascent RNA by NS5 MTase in association with NS3. The capping involves dual methyltransferase activities (^{m7}G- cap-0 and $A_{m2'-O-}$–cap-I) accomplished by NS5 with SAM as methyl donor for both.

The sequential methylations followed the order (^{m7}G and $A_{m2'-O-}$) where the ^{m7}G has been shown to be more important than $A_{m2'-O-}$; however, mutations abrogating both methylations were lethal for DENV replication (Dong et al., 2010a). Mutagenesis studies on residues involved in AdoMet binding or catalysis of the DENV NS5 MTase domain have demonstrated that both methylations (^{m7}G and $A_{m2'-O-}$) are accomplished using a single AdoMet binding pocket and changes in the conformation of the MTase domain greatly influenced MTase activities (Kroschewski et al., 2008). While cap-0 (^{m7}G) is essential for translating the viral polyprotein, the importance of cap-1 ($A_{m2'-O-}$) in the viral RNA substrate is highlighted by the fact that missing cap-1 (2´-*O* methylation activity) due to a mutation (E216A in DENV NS5) attenuated the virus in wild-type host cells, but not in *Ifit1*$^{-/-}$ cells (Dong et al., 2014; Daffis et al., 2010). In fact, the cap-1 on the nascent viral RNA protects it from host immune sensors (pathogen recognition receptors)- retinoic acid inducible gene-I (RIG-I), melanoma differentiation-associated protein 5 (MDA5) and IFN induced protein with tetratricopeptide repeats 1 (IFIT1) (Schuberth-Wagner et al., 2015; Daffis et al., 2010; Züst et al., 2011).

2.3 Targeting the RNA capping in DENV

In the current review article, we will discuss only the state-of-art specifically targeting the DENV RNA capping. It is well established that capping (methylation of the nascent RNA) is crucial for efficient translation of the viral polyprotein (Ray et al., 2006). Mutations that altered the cap-0 activity of the MTase were found to be lethal for West Nile Virus (WNV), while cap-I failure allowed the WNV to evade the IFN signaling by the host cells (Zhou et al., 2007; Daffis et al., 2010). Capping of the nascent RNA in DENV involves two separate but concomitant processes- guanylyltransferase and methyltransferase activities accomplished by DENV NS5 MTase; failing successful capping can render the nascent RNA vulnerable to host immune sensors and subsequent degradation of the uncapped RNA by host nucleases. Being a single methyl donor for both methylation activities, SAM appears to be a target of choice for inhibition of viral RNA capping.

A number of strategies have been employed in drug discovery against DENV NS5 that are not only limited to structure based drug design; selective inhibitors for DENV MTase; fragment-based drug design; chemical modifications of antiviral molecules; virtual screening; receptor-based and dynamic pharmacophore modeling etc. (Wangikar et al., 2016). Sinefungin is an analog of SAM and has been demonstrated as an important antibiotic, antiviral, antiparasitic compound, but its lack of specificity and nephrotoxicity rendered it unusable for clinical applications (Podvinec et al., 2010). It is important to note that the core domains of the MTase are largely conserved among the flavivirus and host MTases, therefore, it is challenging to develop selective MTase inhibitors. Amongst the identified NS5 inhibitors, there are only three MTase inhibitors of non-nucleoside inhibitor class that have been reported previously.

Interestingly, structure information of the DENV MTase located a unique pocket adjacent to the SAM binding site, at which SAM analog (compound 10) could bind and selectively inhibited viral MTase (IC_{50} 0.08-0.24 μM), yet demonstrated poor inhibition potency (Lim et al., 2011). A compound Entry 30 was identified with IC_{50} 91 μM, but again demonstrated poor inhibition potency (Benmansour et al., 2017). Another non-nucleoside inhibitor of DENV MTase is NSC 306711 which demonstrated IC_{50} 1μM and is currently under optimization for clinical studies (Brecher et al., 2015). Other efforts on experimental screening to identify inhibitors of DENV MTase did not yield fruitful results; and therefore, researchers resorted to virtual screening for identification of DENV MTase inhibitors. An important limitation of screening of potent MTase inhibitors is requirement of sophisticated technique of scintillation counter, which are not available in most of the biochemistry research laboratories of developing countries.

One study performed virtual screening of 2.1 million compounds and identified 1 inhibitor with IC_{50} 60 μM with recombinant DENV NS5 MTase (Luzhkov et al., 2007). A virtual screening of ~ 5 million compounds identified 4 inhibitors that exhibited IC_{50} < 10 μM in in-vitro methylation assays (Podvinec et al., 2010). Using biophysical techniques- saturation transfer difference (STD)-NMR, new inhibitors of DENV NS5 were identified and further validated by computational studies. The study found 12 ligands which were found to interact with DENV NS5; of these 9 of them were US-FDA approved drugs, while 3 were nature-derived products (Ullah et al., 2023). In a most recent approach to identify DENV MTase inhibitors, a virtual screen of ~ 0.6 million compounds were employed, which identified 10 compounds with tolerable cytotoxicity in Huh-7 cells and IC_{50} < 50 μM (García-Ariza et al., 2022). These limited studies and challenges in identification of specific DENV MTase targeting highlights the case for putting in alternative efforts to target the RNA capping.

3. Future Perspectives

The current state-of-art in targeting DENV RNA capping clearly highlights the lack of any successful MTase inhibitor in clinical studies till date. Taking into account the global dengue risk and lack of any specific anti-dengue treatment, it becomes imperative that new strategies should be employed to discover new DENV RNA capping inhibitors such as therapeutic use of engineered DENV MTase. It is noticeable that synthetic compound libraries are potent source of new drug molecules, but the complexity of naturally sourced extracts from plants or from marine sources cannot be neglected. Marine-derived natural extracts from the rich biodiversity of the oceans of the world have been screened to report new antimalarials- another vector-borne parasitic disease (Prashar et al., 2022). Phytochemicals such as curcumin, quercetin and myricetin have been shown to act as non-competitive inhibitors of the DENV NS2b/NS3 (Saqallah et al., 2022).

In a novel vaccine and antiviral therapy development, it has been successfully demonstrated that DENV MTase activity can be modulated rationally by disruption of the catalytic tetrad (K-D-K-E), which abrogated the $A_{m2'-O}$ methylation (Zust et al., 2013). The resulting viruses lacking $A_{m2'-O}$ methylation were found to be highly sensitive to IFN mediated immune responses. Moreover, immunization of monkeys with 1000 plaque forming units of mutant DENV imparted full protection from DENV infection; primarily by the host's innate immune system. Similar, approaches to abrogate the methylation by disrupting the catalytic tetrad of the MTase domain have been demonstrated in WNV and Tembusu Virus (TMUV) (Ray et al., 2006; Wu et al., 2022). The successful alteration of the methylation potential of MTase domain and its critical role in viral life cycle prompted us to envisage rational design of engineered NS5 MTase as a therapeutic protein in severe Dengue cases.

In fact, our research group is involved in engineering the methyltransferase potential of DENV MTase domain using bacterial produced DENV NS5 MTase (unpublished). We have envisaged engineering the DENV MTase to have altered substrate (vRNA) specificity, a similar approach had been previously demonstrated in *Blastocystis* succinyl-CoA synthetase (SCS) (Vashisht et al., 2017). High viremia has been strongly linked to the disease severity of Dengue, thereby confirming that lowering viral load can be a good therapeutic intervention (Morsy et al., 2020). The viral load control can be achieved either by inhibiting viral replication or by stimulating the immune system. Above mentioned studies fairly supported the idea to target vRNA capping (methylation), which can be achieved either by MTase inhibitors or by administration of engineered MTase with defective capping potential.

Following features of the engineered Dengue NS5 MTase are worth investigating- 1) enhanced vRNA affinity of the engineered MTase compared to the viral-derived NS5 MTase, so that vRNA binds preferentially to engineered MTase to alter viral replication and exposing vRNA to host immune system, 2) defective methylation- engineered MTase capable of methylating selective residues of the vRNA contrary to the canonical cap guanine and first adenine, 3) engineered MTase with slower methylation or altered sequential methylation steps leading to defective capping. We anticipate that administration of engineered NS5 MTase would have following advantages over traditional vaccine strategies- 1) the smaller size of engineered NS5 MTase would elicit lesser non-specific immune response, which can be administered with state-of-art delivery systems including nanoparticles, chimeric proteins, protein cages etc. to improve the bioavailability 2) production of engineered NS5 MTase could be cost effective and less resource intensive compared to a vaccine and 3) availability of engineered NS5 MTase as a therapeutic intervention only required in severe dengue cases would reduce the risk of unnecessary administration of vaccine to a larger number of people.

4. Discussion

DENV NS5 is the largest non-structural protein which accomplish the methylation and RNA-dependent RNA polymerase activities on the nascent RNA. The DENV NS5 interacts with multiple host proteins to modulate the innate immune responses preferentially of the JAK-STAT pathway (Bhatnagar et al., 2021). The crucial role of methylation or RNA capping by the DENV NS5 MTase has been well established in the viral life cycle leading to survival of the DENV by evasion from host immune sensors. Thorough investigations of the viral RNA capping mechanism and structural information of the DENV NS5 MTase have led to the identification of the crucial active site residues, residues that are responsible for maintaining the overall conformation of the MTase and SAM binding pocket. Based on this information, non-nucleoside inhibitors of DENV NS MTase have been identified, but poor inhibition potency and toxicity have presented a major bottleneck for their clinical development. With the literature survey, we found that there are not enough studies focusing on targeting the viral RNA capping.

Except for identification of three non-nucleoside inhibitors of the MTase activity and some other MTase inhibitors from virtual screens that have been demonstrated to target DENV RNA capping, there are no inhibitors that reached clinical trials. In light of the limited advances in the MTase inhibitor development, we propose to divert the attention of the antiviral therapeutic development towards enzyme engineering strategies, particularly focused on modulation of the substrate specificity or defective/non-canonical methylation. Modulation of substrate specificity have been shown to be a crucial approach in enzyme engineering. The tyrosyl-t-RNA synthetase had been engineered to increase the affinity for its substrate ATP by 100-fold with two point mutations (Wilkinson et al., 1984). In *Blastocystis* succinyl-CoA synthetase (SCS), the canonical substrate ATP was replaced with a similar ligand- GTP, by altering the electrostatic properties of the gatekeeper residues and two crucial binding site residues (Vashisht et al., 2017). Mutations in the catalytic tetrad (K-D-K-E) of the MTase have been shown to be crucial for abrogation of RNA capping and in turn generated attenuated viruses or impaired virulence in Tembusu virus (Wu et al., 2021). In addition, mutagenesis studies targeting the residues involved in nascent viral RNA with DENV MTase should be investigated to ensue defective methylation on non-canonical residues.

We are presenting these enzyme engineering strategies to target the DENV MTase and wanted to bring the attention of protein engineers towards the potential DENV NS5 MTase domain engineering, which can be developed as a targeted therapeutic intervention in severe dengue cases. It is critical to note that there are no therapeutic interventions or vaccines available for dengue, till date, except for the clinical management of dengue cases. Learning the lessons from the current COVID-19 pandemic, it becomes more and more imperative to discover therapeutic interventions for viral diseases. Escalating dengue infections across the globe are concerning and lack of approved drugs and specific anti-dengue treatments place dengue as a major arboviral disease. In the present review article, we have discussed the importance of RNA capping in DENV life cycle, where targeting the RNA capping using MTase inhibitors is a promising strategy, but off-target effects and clinical toxicity are major hurdles for drug development. Therefore, we argue that alternative strategies such as engineered NS5 MTase domain can be explored as a potential therapeutic to counter dengue infections in severe cases.

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References

Benmansour, F., Trist, I., Coutard, B., Decroly, E., Querat, G., Brancale, A., Barral, K., 2017. Discovery of novel dengue virus NS5 methyltransferase non-nucleoside inhibitors by fragment-based drug design. Eur J Med Chem., 125, Pp. 865-880. doi.org/10.1016/j.ejmech.2016.10.007.

Bhatnagar, P., Sreekanth, G.P., Murali-Krishna, K., Chandele, A., Sitaraman, R., 2021. Dengue Virus Non-Structural Protein 5 as a Versatile, Multi-Functional Effector in Host–Pathogen Interactions. Frontiers in Cellular and Infection Microbiology, 11. doi.org/10.3389/fcimb.2021.574067.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, G.R., Simmons, C.P., Scott, T.W., Farrar, J.J., Hay, S.I., 2013. The global distribution and burden of dengue. Nature, 496 (7446), Pp. 504-507. doi.org/10.1038/nature12060.

Brecher, M., Chen, H., Liu, B., Banavali, N.K., Jones, S.A., Zhang, J., Li, Z., Kramer, L.D., Li, H., 2015. Novel Broad Spectrum Inhibitors Targeting the Flavivirus Methyltransferase. PLoS One, 10 (6), Pp. e0130062. doi.org/10.1371/journal.pone.0130062.

Chang, D.C., Hoang, L.T., Mohamed Naim, A.N., Dong, H., Schreiber, M.J., Hibberd, M.L., Tan, M.J.A., Shi, P.Y., 2016. Evasion of early innate immune response by 2'-O-methylation of dengue genomic RNA. Virology, 499, Pp. 259-266. doi.org/10.1016/j.virol.2016.09.022.

Coloma, J., Jain, R., Rajashankar, K.R., García-Sastre, A., Aggarwal, A.K., 2016. Structures of NS5 Methyltransferase from Zika Virus. Cell Rep., 16 (12), Pp. 3097-3102. doi.org/10.1016/j.celrep.2016.08.091.

Daffis, S., Szretter, K.J., Schriewer, J., Li, J., Youn, S., Errett, J., Lin, T.Y., Schneller, S., Zust, R., Dong, H., Thiel, V., Sen, G.C., Fensterl, V., Klimstra, W.B., Pierson, T.C., Buller, R.M., Gale, M., Jr., Shi, P.Y., Diamond, M.S., 2010. 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. Nature, 468 (7322), Pp. 452-456. doi.org/10.1038/nature09489.

Dalrymple, N.A., Cimica, V., Mackow, E.R., 2015. Dengue Virus NS Proteins Inhibit RIG-I/MAVS Signaling by Blocking TBK1/IRF3 Phosphorylation: Dengue Virus Serotype 1 NS4A Is a Unique Interferon-Regulating Virulence Determinant. mBio, 6 (3), Pp. e00553-00515. doi.org/10.1128/mBio.00553-15.

Dong, H., Chang, D.C., Xie, X., Toh, Y.X., Chung, K.Y., Zou, G., Lescar, J., Lim, S.P., Shi, P.Y., 2010a. Biochemical and genetic characterization of dengue virus methyltransferase. Virology, 405 (2), Pp. 568-578. doi.org/10.1016/j.virol.2010.06.039.

Dong, H., Fink, K., Züst, R., Lim, S.P., Qin, C.F., Shi, P.Y., 2014. Flavivirus RNA methylation. J Gen Virol., 95 (Pt 4), Pp. 763-778. doi.org/10.1099/vir.0.062208-0.

Dong, H., Liu, L., Zou, G., Zhao, Y., Li, Z., Lim, S.P., Shi, P.Y., Li, H., 2010b.

Structural and functional analyses of a conserved hydrophobic pocket of flavivirus methyltransferase. J Biol Chem., 285 (42), Pp. 32586-32595. doi.org/10.1074/jbc.M110.129197.

El Sahili, A., Lescar, J., 2017. Dengue Virus Non-Structural Protein 5. Viruses, 9 (91).

García-Ariza, L.L., Rocha-Roa, C., Padilla-Sanabria, L., Castaño-Osorio, J.C., 2022. Virtual Screening of Drug-Like Compounds as Potential Inhibitors of the Dengue Virus NS5 Protein. Frontiers in Chemistry, 10. doi.org/10.3389/fchem.2022.637266.

Guzman, M.G., Gubler, D.J., Izquierdo, A., Martinez, E., Halstead, S.B., 2016. Dengue infection. Nature Reviews Disease Primers, 2 (1), Pp. 16055. doi.org/10.1038/nrdp.2016.55.

Huang, C.H., Tsai, Y.T., Wang, S.F., Wang, W.H., Chen, Y.H., 2021. Dengue vaccine: an update. Expert Rev Anti Infect Ther., 19 (12), Pp. 1495-1502. doi.org/10.1080/14787210.2021.1949983.

Kroschewski, H., Lim, S.P., Butcher, R.E., Yap, T.L., Lescar, J., Wright, P.J., Vasudevan, S.G., Davidson, A.D., 2008. Mutagenesis of the dengue virus type 2 NS5 methyltransferase domain. J. Biol Chem., 283 (28), Pp. 19410-19421. doi.org/10.1074/jbc.M800613200.

Libraty, D.H., Young, P.R., Pickering, D., Endy, T.P., Kalayanarooj, S., Green, S., Vaughn, D.W., Nisalak, A., Ennis, F.A., Rothman, A.L., 2002. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. J. Infect Dis., 186 (8), Pp. 1165-1168. doi.org/10.1086/343813.

Lim, S.P., Sonntag, L.S., Noble, C., Nilar, S.H., Ng, R.H., Zou, G., Monaghan, P., Chung, K.Y., Dong, H., Liu, B., Bodenreider, C., Lee, G., Ding, M., Chan, W.L., Wang, G., Jian, Y.L., Chao, A.T., Lescar, J., Yin, Z., Vedananda, T.R., Keller, T.H., Shi, P.Y., 2011. Small molecule inhibitors that selectively block dengue virus methyltransferase. J Biol Chem 286 (8), Pp. 6233-6240. doi.org/10.1074/jbc.M110.179184.

Lu, G., Gong, P., 2013. Crystal Structure of the full-length Japanese encephalitis virus NS5 reveals a conserved methyltransferase-polymerase interface. PLoS Pathog, 9 (8), Pp. e1003549. doi.org/10.1371/journal.ppat.1003549.

Luzhkov, V.B., Selisko, B., Nordqvist, A., Peyrane, F., Decroly, E., Alvarez, K., Karlen, A., Canard, B., Qvist, J., 2007. Virtual screening and bioassay study of novel inhibitors for dengue virus mRNA cap (nucleoside-2'O)-methyltransferase. Bioorg Med Chem., 15 (24), Pp. 7795-7802. doi.org/10.1016/j.bmc.2007.08.049.

Morsy, S., Hashan, M.R., Hieu, T.H., Mohammed, A.T., Elawady, S.S., Ghosh, P., Elgendy, M.A., Le, H.H., Hamad, W.M.A., Iqtadar, S., Dumre, S.P., Hirayama, K., Huy, N.T., 2020. The association between dengue viremia kinetics and dengue severity: A systemic review and meta-analysis. Rev Med Virol., 30 (6), Pp. 1-10. doi.org/10.1002/rmv.2121.

NCVBDC, 2023. Distribution of Dengue/DHF in India. https://ncvbdc.mohfw.gov.in/index4.php?lang=1&level=0&linkid=432&lid=3714. (Accessed 25/02/2023.

Podvinec, M., Lim, S.P., Schmidt, T., Scarsi, M., Wen, D., Sonntag, L.S., Sanschagrin, P., Shenkin, P.S., Schwede, T., 2010. Novel inhibitors of dengue virus methyltransferase: discovery by in vitro-driven virtual screening on a desktop computer grid. J. Med Chem., 53 (4), Pp. 1483-1495. doi.org/10.1021/jm900776m.

Prashar, C., Thakur, N., Chakraborti, S., Areeb Hussain, S.S., Vashisht, K., Pandey, K.C., 2022. The landscape of nature-derived antimalarials-potential of marine natural products in countering the evolving Plasmodium. Frontiers in Drug Discovery, 2. doi.org/10.3389/fddsv.2022.1065231.

Ray, D., Shah, A., Tilgner, M., Guo, Y., Zhao, Y., Dong, H., Deas, T.S., Zhou, Y., Li, H., Shi, P.Y., 2006. West Nile virus 5'-cap structure is formed by sequential guanine N-7 and ribose 2'-O methylations by nonstructural protein 5. J. Virol., 80 (17), Pp. 8362-8370. doi.org/10.1128/JVI.00814-06.

Roy, S.K., Bhattacharjee, S., 2021. Dengue virus: epidemiology, biology, and disease aetiology. Canadian Journal of Microbiology, 67 (10), Pp. 687-702. doi.org/10.1139/cjm-2020-0572 %M 34171205.

Saqallah, F.G., Abbas, M.A., Wahab, H.A., 2022. Recent advances in natural products as potential inhibitors of dengue virus with a special emphasis on NS2b/NS3 protease. Phytochemistry, 202, Pp. 113362. doi.org/https://doi.org/10.1016/j.phytochem.2022.113362.

Schmid, B., Rinas, M., Ruggieri, A., Acosta, E.G., Bartenschlager, M., Reuter, A., Fischl, W., Harder, N., Bergeest, J.P., Flossdorf, M., Rohr, K., Höfer, T., Bartenschlager, R., 2015. Live Cell Analysis and Mathematical Modeling Identify Determinants of Attenuation of Dengue Virus 2'-O-Methylation Mutant. PLoS Pathog., 11 (12), Pp. e1005345. doi.org/10.1371/journal.ppat.1005345.

Schuberth-Wagner, C., Ludwig, J., Bruder, A.K., Herzner, A.M., Zillinger, T., Goldeck, M., Schmidt, T., Schmid-Burgk, J.L., Kerber, R., Wolter, S., Stümpel, J.P., Roth, A., Bartok, E., Drosten, C., Coch, C., Hornung, V., Barchet, W., Kümmerer, B.M., Hartmann, G., Schlee, M., 2015. A Conserved Histidine in the RNA Sensor RIG-I Controls Immune Tolerance to N1-2'O-Methylated Self RNA. Immunity, 43 (1), Pp. 41-51. doi.org/10.1016/j.immuni.2015.06.015.

Shepard, D.S., Undurraga, E.A., Halasa, Y.A., Stanaway, J.D., 2016. The global economic burden of dengue: a systematic analysis. The Lancet Infectious Diseases, 16 (8), Pp. 935-941. doi.org/10.1016/S1473-3099(16)00146-8.

Takeda, 2022. Takeda's QDENGA®▼ (Dengue Tetravalent Vaccine [Live, Attenuated]) Approved for Use in European Union. https://www.takeda.com/newsroom/newsreleases/2022/takedas-qdenga-dengue-tetravalent-vaccine-live-attenuated-approved-for-use-in-european-union/.

Ullah, A., Atia tul, W., Gong, P., Khan, A.M., Choudhary, M.I., 2023. Identification of new inhibitors of NS5 from dengue virus using saturation transfer difference (STD-NMR) and molecular docking studies. RSC Advances, 13 (1), Pp. 355-369. doi.org/10.1039/D2RA04836A.

Vashisht, K., Prashar, C., Tyagi, S., Rawat, G., Kumari, P., Pandey, K.C., 2020. Are aptamers really promising for diagnostic or therapeutic applications in dengue? Dengue, 41, Pp. 31.

Vashisht, K., Verma, S., Gupta, S., Lynn, A.M., Dixit, R., Mishra, N., Valecha, N., Hamblin, K.A., Maytum, R., Pandey, K.C., van der Giezen, M., 2017. Engineering Nucleotide Specificity of Succinyl-CoA Synthetase in Blastocystis: The Emerging Role of Gatekeeper Residues. Biochemistry, 56 (3), Pp. 534-542. doi.org/10.1021/acs.biochem.6b00098.

Wangikar, P., Martis, E., Ambre, P., Nandan, S., Coutinho, E., 2016. Update on methyltransferase inhibitors of the dengue virus and further scope in the field. J. Emerg., Infect Dis, 1 (108), Pp. 2.

Wellekens, K., Betrains, A., De Munter, P., Peetermans, W., 2022. Dengue: current state one year before WHO 2010–2020 goals. Acta Clinica Belgica, 77 (2), Pp. 436-444. doi.org/10.1080/17843286.2020.1837576.

Wilkinson, A.J., Fersht, A.R., Blow, D.M., Carter, P., Winter, G., 1984. A large increase in enzyme-substrate affinity by protein engineering. Nature, 307 (5947), Pp. 187-188. doi.org/10.1038/307187a0.

Wu, X., Pan, Y., Huang, J., Huang, S., Wang, M., Chen, S., Liu, M., Zhu, D., Zhao, X., Wu, Y., Yang, Q., Zhang, S., Ou, X., Zhang, L., Liu, Y., Yu, Y., Gao, Q., Mao, S., Sun, D., Tian, B., Yin, Z., Jing, B., Cheng, A., Jia, R., 2022. The substitution at residue 218 of the NS5 protein methyltransferase domain of Tembusu virus impairs viral replication and translation and may triggers RIG-I-like receptor signaling. Poult Sci., 101 (9), Pp. 102017. doi.org/10.1016/j.psj.2022.102017.

Wu, X., Zhang, Y., Wang, M., Chen, S., Liu, M., Zhu, D., Zhao, X., Wu, Y., Yang, Q., Zhang, S., Huang, J., Ou, X., Zhang, L., Liu, Y., Yu, Y., Gao, Q., Mao, S., Sun, D., Tian, B., Yin, Z., Jing, B., Cheng, A., Jia, R., 2021. Methyltransferase-Deficient Avian Flaviviruses Are Attenuated Due to Suppression of Viral RNA Translation and Induction of a Higher Innate Immunity. Frontiers in Immunology 12. doi.org/10.3389/fimmu.2021.751688.

Zhao, Y., Soh, T.S., Lim, S.P., Chung, K.Y., Swaminathan, K., Vasudevan, S.G.,

Shi, P.Y., Lescar, J., Luo, D.A.O., 2015. Molecular basis for specific viral RNA recognition and 2'-O-ribose methylation by the dengue virus nonstructural protein 5 (NS5). Proc Natl Acad Sci U S A 1(112(48)).

Zhou, Y., Ray, D., Zhao, Y., Dong, H., Ren, S., Li, Z., Guo, Y., Bernard, K.A., Shi, P.-Y., Li, H., 2007. Structure and Function of Flavivirus NS5 Methyltransferase. Journal of Virology, 81 (8), Pp. 3891-3903. doi.org/doi:10.1128/JVI.02704-06.

Züst, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., Siddell, S.G., Ludewig, B., Thiel, V., 2011. Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nat Immunol, 12 (2), Pp. 137-143. doi.org/10.1038/ni.1979.

Zust, R., Dong, H., Li, X.F., Chang, D.C., Zhang, B., Balakrishnan, T., Toh, Y.X., Jiang, T., Li, S.H., Deng, Y.Q., Ellis, B.R., Ellis, E.M., Poidinger, M., Zolezzi, F., Qin, C.F., Shi, P.Y., Fink, K., 2013. Rational design of a live attenuated dengue vaccine: 2'-o-methyltransferase mutants are highly attenuated and immunogenic in mice and macaques. PLoS Pathog, 9 (8), Pp. e1003521. doi.org/10.1371/journal.ppat.1003521.