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PREDICTING THE POSSIBLE BIOLOGICAL MARKERS AS TARGETED THERAPY FOR DENGUE VIRAL INFECTIONS

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Abstract

The incidence rate of dengue infections in Indonesia shows an upward trend every year. Recently, dengue infection treatment is still limited to symptom management and especially efforts to restore the body's immune system to fight the infection. There is still no immediate treatment and prevention mechanism for dengue infection. This study aimed to predict the possible targeted proteins in Dengue infection as biomarkers in further molecular assays in part of future drug development. *In silico* pathway analysis based on KEGG's database was performed to determine the target protein and visualized them into the 3D model through SWISS-Model and PyMOL. Each protein also tested for their interaction using STITCH based on two bioprocesses, namely the cytoplasmic pattern recognition receptor signaling and the innate immune response pathway. The analysis showed that three of the target proteins are RIG-I, MDA5, and MAVS, convincingly interacting in both pathways with very high predictions for specific actions value (0.800), compelling experimental/ biochemical data (0.990 to 0.999), and a low false discovery rate value. RIG-I and MDA5 play an essential role as the viral RNA primary receptors in the early stages of DENV infection. Even by inhibiting these two proteins, it has a severe effect on the cytokine response. The MAVS protein was an essential antiviral protein that serves as a bridge between sensory proteins and responder proteins in innate immune cell mechanisms against DENV infections.

INTRODUCTION

Dengue infection is the most widely spread mosquito-borne disease in the world. Southeast Asia has become an endemic region because of its tropical climate and enormous urban and rural areas [1,2]. Indonesia is the world's second-largest country in terms of cases of dengue infections among the 30 endemic countries in the world [3]. Dengue infection was discovered in Indonesia after an outbreak in Surabaya and Jakarta in 1968 [4]. From 1968 to 2016, the dengue infection incidence rate has shown an increasing trend each year [3]. Dengue infection case is found in all provinces in Indonesia with the highest cases occurred in three provinces, West Java, Central Java, and East Java with more than 5000 cases per year, with a fatality rate of 1%, while significantly higher in East Indonesia around 2% fatality rate [5]. If the patient got improper medical treatment, it could jump to 25-40% [1].

Dengue infection is caused by the dengue virus (DENV), which has four serotypes: type 1, 2, 3, and 4. In Southeast Asia, the most dominant DENV serotypes are DENV 2 and 3. In contrast, for subtropical regions where the temperature is colder, the predominant DENV serotypes are DENV 1 and 4 [6]. Dengue infection in humans occurs through the bite of a DENV's infected mosquito. When this mosquito sucks human blood, the DENV in the mosquito's saliva also going through the human skin tissue. The DENV infected dendritic cells, Langerhans cells, and another neighboring cell in the tissue. Furthermore, DENV was transmitted to the lymphatic nodes that will infect macrophages, monocytes, and dendritic cells [7].

It will replicate inside the infected cell by hijacking its host machinery. The DENV's genetic material is double-stranded RNA (dsRNA), which encodes the capsid, membrane, envelope, and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Dengue infection is divided into three phases: dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. The last two phases are the most dangerous phase, leading to several complications provoking a particular organ's failure. Some cases are asymptomatic, but typical symptoms in patients with dengue infection are sudden high fever, liver enlargement, hypotension, and internal bleeding [8].

The current dengue treatment method is only available to cure the symptoms caused by the disease. Existing clinical dengue treatments use acetaminophen and paracetamol IV as the pain killer, ranitidine as an anti-nausea. In some cases, the use of antibiotics for secondary infections caused by the disease. The high fatality risk of dengue occurs in infants and children [9]. Several actions have also been proposed to lower or even eliminate dengue risk, such as disturbing its vector life cycle [3], direct-acting and host-directed antiviral drug, and DENV vaccine. However, there is no sufficient DENV vaccine to restrain infection until now. The effective and safe antiviral drugs that act directly are also yet to be discovered [3]. Host-directed antiviral is considered a promising strategy since viruses interfere with the biological pathways of host cells to facilitate replication. Therefore, we predicted the possible biological markers as potent targets for future anti-DENV development programs, specifically targeting either host or viral protein that will lower the viral load or prevent the virus from inhibiting the host's innate immune responses.

MATERIALS AND METHODS

Materials

The primary data obtained from the UniProt protein (https://www.uniprot.org/), while the protein's genomics data as comparative data retrieved from NCBI GenBank (https://www.ncbi.nlm.nih.gov/).

DENV Infection Pathway Analysis

The infection pathway analysis in the host cells based on KEGG's database (https://www.genome.jp/kegg/) to identify potential target proteins of the host cell immune mechanism against infection of DENV.

Protein Data Mining

After determining the potential target protein, the structural data were collected, especially the amino acid sequences for each protein from UniProt and NCBI GenBank, simultaneously. The proteins are DDX58/RIG-1 (O95786), MDA5/IFIH1 (Q9BYX4), and C indicates MAVS/IPS-1 (Q7Z434).

Homology Protein Modeling

Each protein's structural data modeled by a homology protein algorithm using SWISS-Model (https://swissmodel.expasy.org/) to determine their 3D structure, then visualized using PyMOL. A cartoon model with a rainbow color scheme was generated for this purpose.

Protein-Protein Interaction and Pathway Analysis

Predicted target proteins were analyzed for their interactions using STITCH (http://stitch.embl.de/). We used molecular interactions, fusion genes, co-occurrence, and co-expression, with the minimum required interaction score of 0.900 (highest confidence) as parameters. Proteins could be considered potential target proteins analyzed with two major biological process pathways, the cytoplasmic pattern recognition receptor signaling pathway (GO: 0002753) and the innate immune response (GO: 0045087).

RESULTS AND DISCUSSION

The KEGG pathway analysis generated only one pathway related to DENV, specifically the mechanism of cell response against dengue infection through the RIG-I-like receptor signaling pathway (map04622). Two proteins that were predicted to have essential roles in the early stage of viral entry are RIG-1 (O95786 isoforms 1) and MDA5 (Q9BYX4 isoform 1). It still unclear whether these sorts of viral genetic material affect receptor proteins involved in the viral recognizing stages, so we assume that both RIG-1 and MDA5 are engaged in the DENV RNA recognizing stage. To confirm whether RIG-1 and MDA5 are the key proteins in viral detection, both of them analyzed using STITCH with the virus detection process pathway (GO: 0009597). The analysis shows a positive result in Figure 1. It is confirmed that the RIG-1 and MDA5 are the proteins that play a role in virus detection on the host cell.

In the RIG-I-like receptor signaling pathway, either RIG-1 and MDA5 have the same downstream: the MAVS protein (Q7Z434 isoform 1). They are considered as potential target proteins (Figure 2B) and three-dimensional models shown in Figure 2A.

Figure 3A shows the DDX58/RIG-1 interacts with several proteins such as TRIM25, UBB, UBC, and the MAVS. If we referred to the previous result, which shows that downstream of DDX58/RIG-1 is MAVS/IPS-1, DDX58/RIG-1 also interacts with MAVS in two different stages. The first one is DDX58/RIG-1 binds to the MAVS with very high predictions for specific actions value (0,800). The second stage is DDX58/RIG-1 activates the MAVS with very high predictions for specific actions value (0,800). Both of these interactions have compelling experimental/biochemical data values (0,999). Figure 3B shows that virtually identical results are also found in the analysis of MDA5/IFIH1. The data in the database indicates that MDA5/IFIH1 interacts with two proteins, UBC and MAVS. MAVS also acts as the downstream of MDA5/IFIH1. We conducted further analysis on the interaction of these two proteins. The interaction between MDA5/IFIH1 and MAVS identical to the interaction between DDX58/RIG-1 and MAVS. MDA5/IFIH1 binds to the MAVS; also, MDA5/IFIH1 activates the MAVS; both have a value of specific actions Predictions for very high (0,800). Both of these interactions have compelling Experimental/Biochemical data value (0,990) — analysis of the MAVS/IPS-1 conducted as a crosscheck to the previous two protein interaction analysis. Based on the study confirmed that the results of the last two protein analyses showed consistent results. This analysis also indicates that there is no direct interaction between the RIG-1 and MDA5.

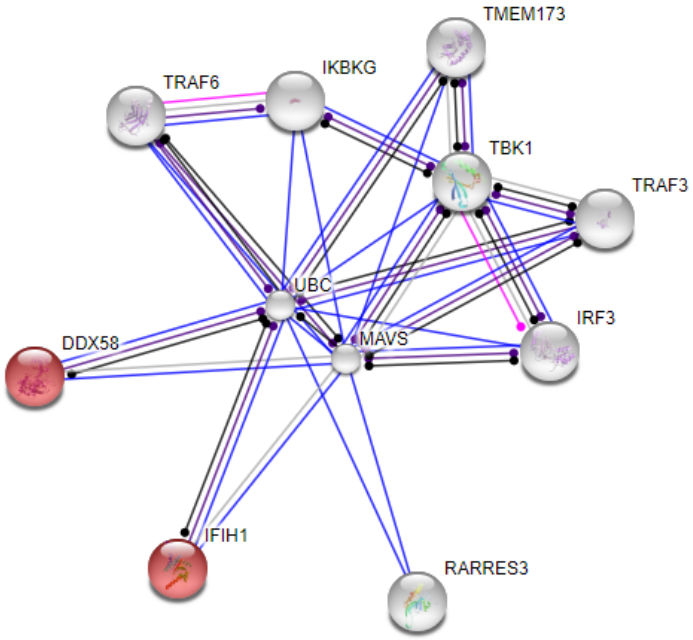

Figure 1. Analysis of protein interactions through STITCH with the biological process of virus detection (GO: 0009597) with a minimum required interaction score of 0.900 (highest confidence) shows node DDX58 (RIG-1) and IFIH1 (MDA5) turns red, which means confirmed that both proteins have an essential role in DENV RNA detection process in the host cells.

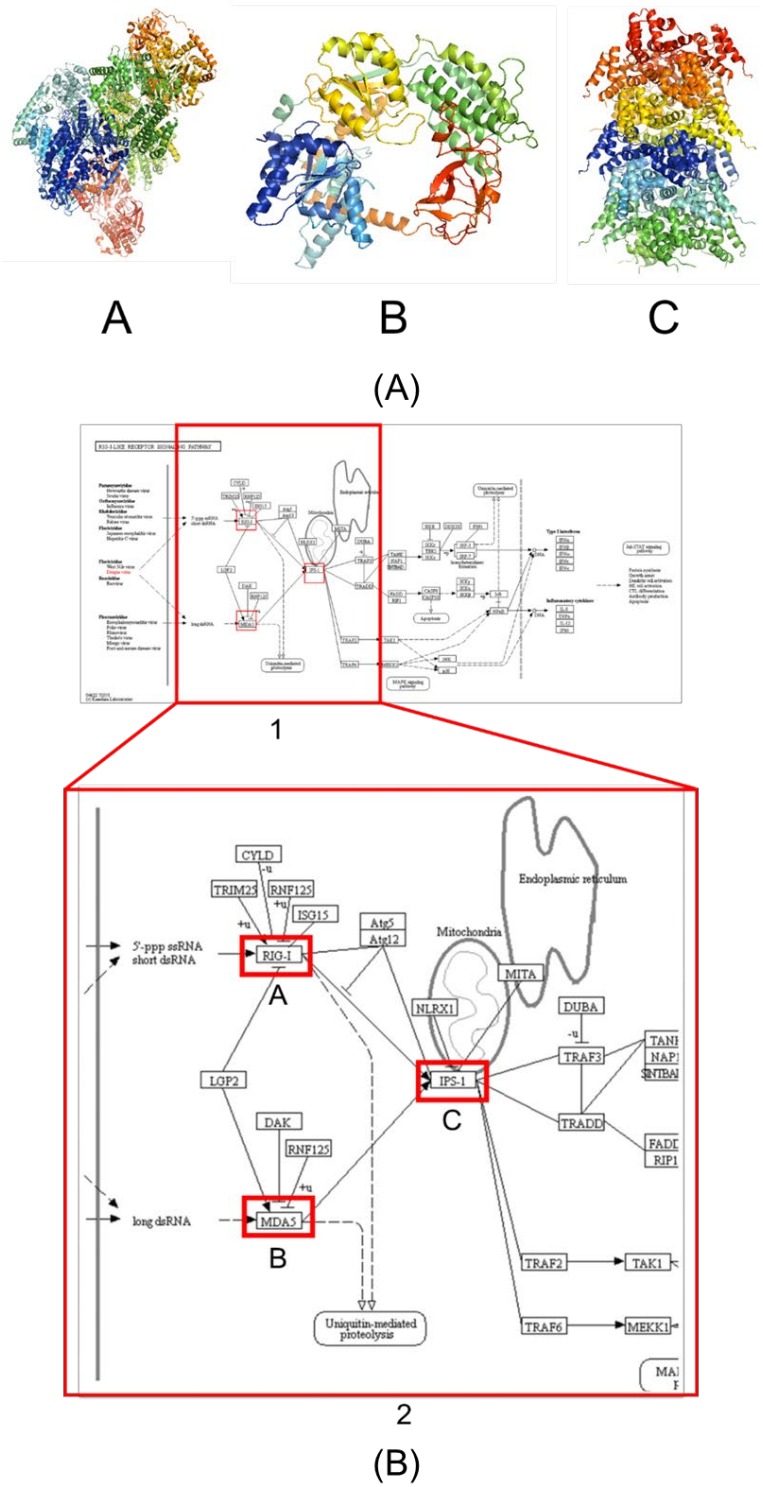

Figure 2. (A) The three-dimensional model of the target proteins. A indicates DDX58/RIG-1 (O95786 isoform 1), B indicates MDA5/IFIH1 (Q9BYX4 isoform 1), and C indicates MAVS/IPS-1 (Q7Z434 isoform 1). The colors represent the protein's amino acid chains. **(B)** The RIG-I-like receptor signaling pathway (map04622) is one of several pathways that describes the cell responses against the viral infection. DENV is recognized by the host cells through this pathway. The first part shows the RIG-I-like receptor signaling pathway as a whole piece; the big red box shows the viral RNA detection stage, which is the earliest stage in the cell response mechanism against DENV infection. The second part shows the viral RNA detection stages. The small red boxes indicated the critical proteins involved in the early detection of the viral RNA mechanism. A indicates RIG-1/DDX58 protein, B indicates MDA5/IFIH-1 protein, and C indicates IPS-1/MAVS protein.

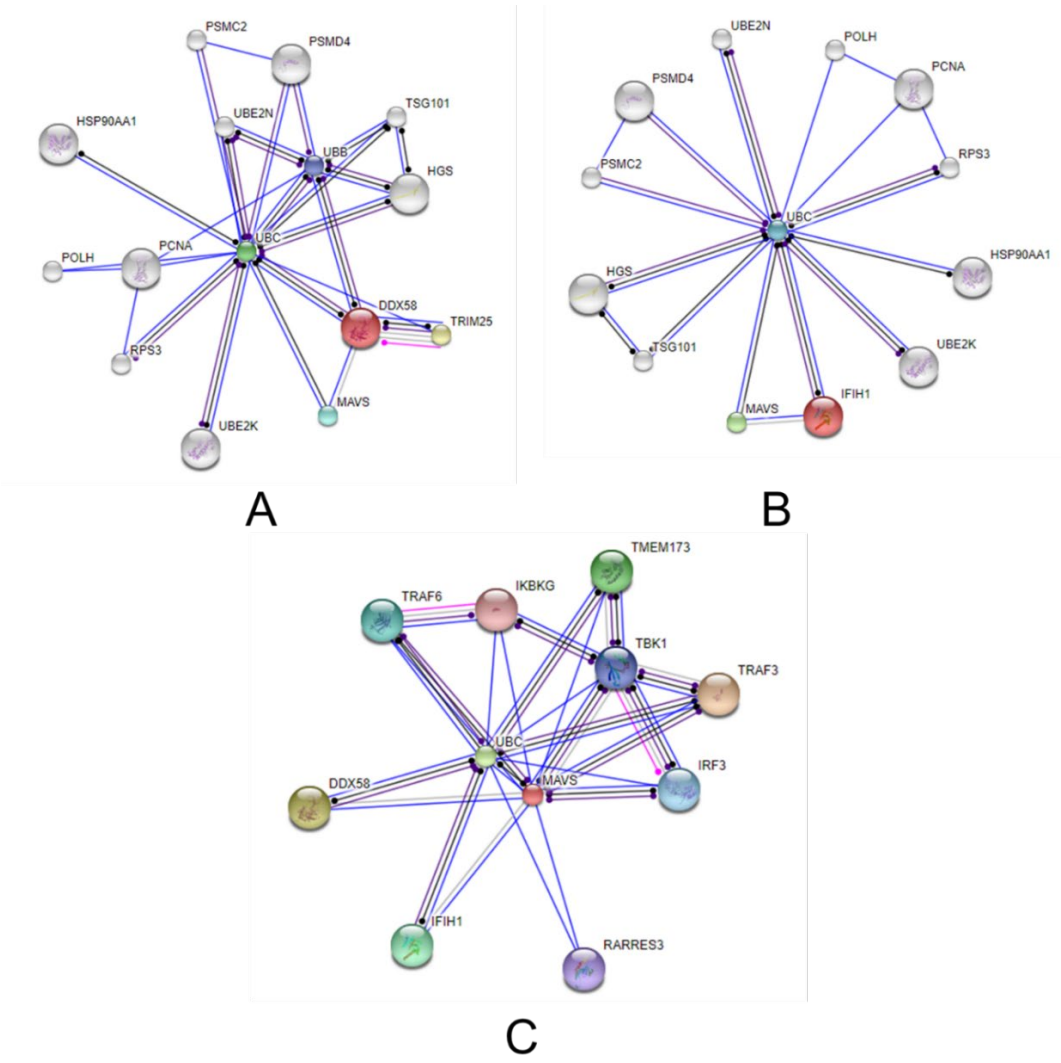

Figure 3. The STITCH protein interaction analysis with molecular interaction network edges, active interaction sources such as experiments, fusion genes, co-occurrence, and co-expression, with the minimum required interaction score of 0.900 (highest confidence). (A) showed the results of DDX58/RIG-1 protein interactions, (B) showed the results of MDA5/IFIH1 protein interactions, and (C) showed the results of MAVS/IPS-1 protein interactions. The line thickness connecting two nodes illustrates the strength of the interaction between proteins. Red nodes indicate the inputted protein; the blue line represents the bond between proteins; posttranslational modification describes the pink line. The black line represents the reaction between the proteins. The purple line illustrates the catalysis process. The arrow-shaped line indicates positive interaction, the rounded-shaped line indicates uncertain interaction, and the right-angled line indicates negative interaction.

Figures 4 and 5 show that these three proteins are involved in two significant pathways; the cytoplasmic pattern recognition receptor signaling pathway (GO: 0002753) and the innate immune response pathway (GO: 0045087). Those pathways were chosen because they were found in all three proteins. Also, they matched the primary function of these proteins, especially in the viral recognition mechanism on the RIG-I-like receptor signaling pathway. The DDX58/RIG-1 analysis with the cytoplasmic pattern recognition receptor signaling pathway (GO: 0002753) indicates the false discovery rate value of 1.21×10^{-7}. The MAVS and DDX58 nodes, all in red, suggested that those proteins interact in the same pathway as before. With the same parameters, MDA5/IFIH1 shows the value of a false discovery rate of 6.84×10^{-6}, while the IFIH1 and MAVS nodes are also shown in red, indicating that the two proteins interact the same pathway. The previous data showed that the DDX58, IFIH1, and MAVS nodes are in red, which means these three proteins interact with each other with a false discovery rate value of 1.63×10^{-14}. The analysis between these three target proteins using the innate immune response parameter (GO: 0045087) shows that the red nodes were identical with the results using the cytoplasmic pattern recognition receptor's parameter (GO: 0002753). In the DDX58/RIG-1 analysis, the false discovery rate was 1.02×10^{-6}, while the MDA5/IFIH1 analysis shows the false

discovery rate of 1.64×10^{-4} MAVS analysis showed the value false discovery rate of 1.47×10^{-10}.

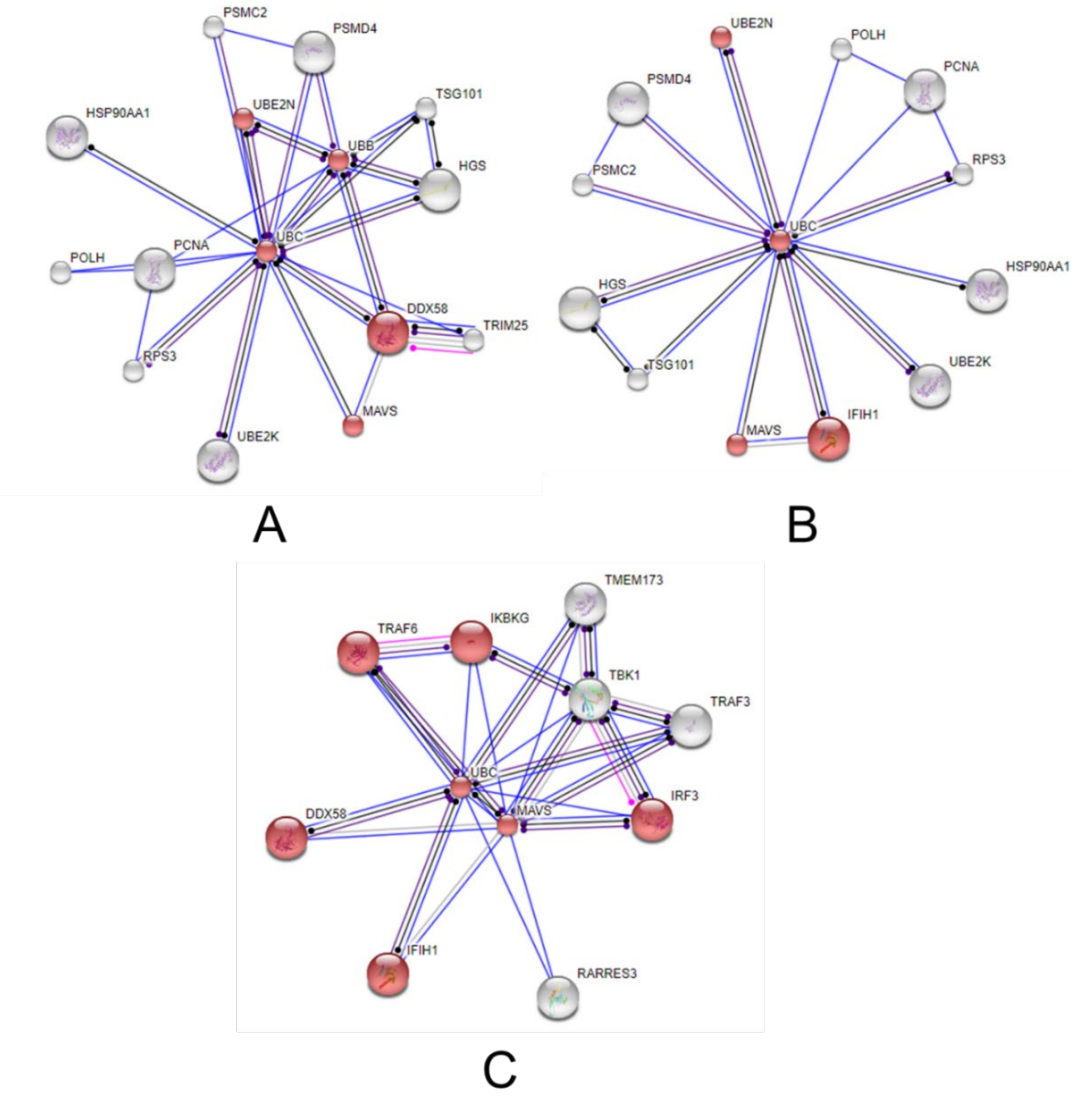

Figure 4. The target protein interactions analysis using Cytoplasmic Pattern Recognition Receptor Signaling Pathway parameter (GO: 0002753). A shows the DDX58/RIG-1's interactions, B shows the MDA5/IFIH1's interactions, and C shows the MAVS/IPS-1's interactions. The red node shows the proteins that interact within the Cytoplasmic Pattern Recognition Receptor Signaling Pathway (GO: 0002753).

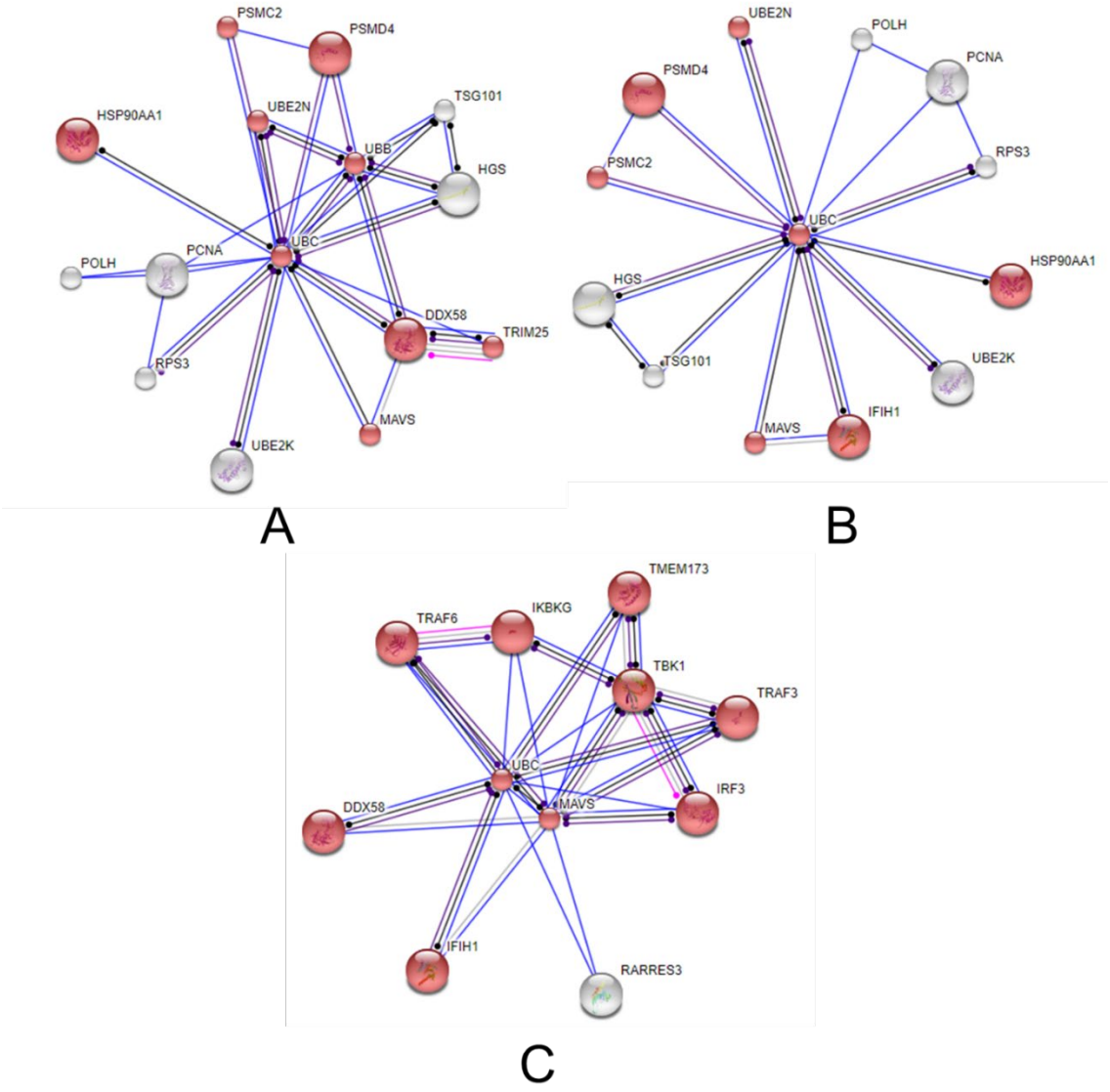

Figure 5. The target protein interactions analysis using Innate Immune Response Pathway parameter (GO: 0045087). A shows the DDX58/RIG-1's interactions, B shows the MDA5/IFIH1's interactions, and C shows the MAVS/IPS-1's interactions. The red node shows the proteins that interact within the Innate Immune Response Pathway (GO: 0045087).

Dengue infection recognition pathway analysis through KEGG shows that the only pathway involved in the viral detection and cell response against DENV infection only RIG-I-like Receptor Signaling Pathway. However, RIG-I-like Receptor Signaling Pathway is not exclusive to DENV. However, it can also identify several families of viruses such as Reoviridae, Picornaviridae, Rhabdoviridae, and Flaviviridae, just like DENV, West Nile virus, Zika virus, and Hepatitis C virus. Viral detection and cell response against viral infection in the pathway involving two receptor proteins can recognize viral RNA as a sign of an infection. The proteins are RIG-I and MDA5, both of which are family RIG-I-like receptor (RLR) and Cytoplasmic Pattern Recognition Receptors [10,11]. Based on the pathway, it is still unclear whether RIG-1 or MDA5 recognized the DENV RNA. The DENV RNA is a positive-sense single-stranded RNA (ssRNA) with a length ranging between 10.7-11 kb [12,13]. RIG-I generally able to recognize both viral single-stranded RNA (ssRNA) and double-stranded RNA (ssRNA) but less than 2000 nucleotide length [14,15], similar to MDA5, which also be able to recognize both viral single-stranded RNA (ssRNA) and double-stranded RNA (ssRNA), but with more than 2000 nucleotides length [14]. It is also capable of being activated by RNA complexes with high molecular weight [11]. The RIG-I generally recognizes the negative-strand RNA, while MDA5 generally recognizes the

positive-strand RNA [16]. However, RIG-I also can be activated by the presence of viral RNA replication processes that produce 5'triphosphorylated RNA [17].

The RIG-I and MDA5 have MAVS as their downstream. Mitochondrial antiviral-signaling protein (MAVS) is a protein located in the outer mitochondrial membrane that acts as a hub in the RIG-I-like Receptor Signaling Pathway (RLR). To activate the MAVS that triggers an innate immune reaction to the virus, the domain Caspase recruitment domains (CARDS) on RIG-I and MDA5 should be open because the interactions between proteins occur in the CARDS domain. When there is no viral RNA in the cell, the RIG-I and MDA5 CARDS are inactive, so they do not trigger a downstream reaction and binds to the MAVS. The CARDS activation on RIG-I and MDA5 occurs when the RNA virus is detected in the cell's cytoplasm.

Viral RNA triggers the conformational change in these proteins, exposing their CARDS and bind to their downstream protein [17]. Like the Receptor Signaling Pathway mechanism, RIG-I cannot separate from the mitochondrial dynamics because the MAVS protein acts as a mitochondrial outer membrane protein. The activation of MAVS by RIG-I and MDA5 triggers TANK-binding kinase 1 (TBK1) and IκB kinase-ε (IKKε) by recruiting the TRAF3-TBK1 complex into mitochondria. This mechanism also triggers downstream reactions that lead to cell apoptosis as well as the synthesis of IFN as the cell defense mechanism of the virus [17]. The entire detection signals received by RIG-I and MDA5 will lead to the MAVS. The DENV's protease is predicted to prevent the mitochondrial fusion process by inhibiting the host cell immune response in dengue infection. The NS2B3 is expected to cleave the mitofusin (MFN) that mediates mitochondrial fusion, both MFN1 and MFN2. The DENV's NS2B3 is predicted to inhibit the MFN2, preventing the cells apoptosis so the viral replication process can still occur in the infected host cell [18]. The RLR signaling activation triggers mitochondrial elongation. The signal will trigger the phosphorylation of IRF-3 and Ikβα, optimum when the mitochondria are undergone fusion. Mitochondria also facilitate interaction between the MAVS with a signaling protein located in the endoplasmic reticulum called STING/MITA that has a role in signaling for the type I interferon synthesis. The DENV NS2B3 can also inhibit STING, so the type I interferon synthesis declined by more than 50% [17,19].

The NS2A and NS2B protease synthesized from DENV RNA can inhibit the activation of RIG-I, MDA5, MAVS, and TBK1, which affect the phosphorylation process of IRF3 and affect the inhibition of IFNβ, which affect the host cell innate immune response [17]. RIG-I and MDA5 silencing mechanism performed by DENV's protease will also cause a significant drop of the IL-1b, IL-6, and TNF expression, which is predicted to cause viral replication in the host is uninterrupted. The DENV proteases that inhibit both RIG-I and the MAVS make both of them unable to recognize the DENV RNA undergoing replication on the cell's cytoplasm. The silencing of the MAVS also resulted in the inhibition of cytokines expression that is important in the cellular immune response against DENV infection [20].

This study concluded that RIG-I, MDA5, and MAVS were valuable target proteins in future anti-DENV and other antivirus development. Those proteins have primary function and interaction in the early stages of innate immune responses against DENV infection. These proteins are common silencing targets of the viral protease, such as the DENV's NS2A and NS2B that significantly inhibit their response in the innate immune response pathway. The inhibition of innate immune response blocking mechanism by the viral protease using an antivirus is predicted to lower the viral load and preventing more severe pathogenesis. This prediction must be confirmed in further comprehensive studies using in vivo or in vitro technique.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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