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Sphingolipid signaling modulates trans-endothelial cell permeability in dengue virus infected HMEC-1 cells

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ABSTRACT

Dengue has emerged as a major mosquito-borne disease in the tropics and subtropics. In severe dengue, enhanced microvascular endothelial permeability leads to plasma leakage. Direct dengue virus (DENV) infection in human microvascular endothelial cells (HMEC-1) can enhance trans-endothelial leakage. Using a microarray-based analysis, we identified modulation of key endothelial cell signaling pathways in DENV-infected HMEC-1 cells. One among them was the sphingolipid pathway that regulates vascular barrier function. Sphingosine-1-phosphate receptor 2 (S1PR2) and S1PR5 showed significant up-regulation in the microarray data. In DENV-infected cells, the kinetics of S1PR2 transcript expression and enhanced *in vitro* trans-endothelial permeability showed a correlation. We also observed an internalization and cytoplasmic translocation of VE-Cadherin, a component of adherens junctions (AJ), upon infection indicating AJ disassembly. Further, inhibition of S1PR2 signaling by a specific pharmacological inhibitor prevented translocation of VE-Cadherin, thus helping AJ maintenance, and abrogated DENV-induced trans-endothelial leakage. Our results show that sphingolipid signaling, especially that involving S1PR2, plays a critical role in vascular leakage in dengue.

1. Introduction

Dengue virus (DENV) circulates in nature as four distinct serotypes, DENV-1 to DENV-4. *Aedes* species of mosquitoes transmit them among humans [1]. Recent estimates predict about 390 million DENV infections globally. Of the 96 million apparent infections among them, 70% occur in Asian countries [2]. Dengue disease can manifest as uncomplicated dengue fever or more severe dengue haemorrhagic fever. The latter is accompanied with abnormal haemostasis or plasma leakage in peritoneal and pleural cavities with manifestation of shock syndrome [1]. In dengue, the critical phase of the disease correlates with the onset of defervescence and usually lasts for 24–48 h. In these patients, plasma leakage in organs such as lungs leads to pleural effusion and respiratory shock [3]. In most cases, it occurs 3–6 days after the onset of fever, and these severe cases are characterized by marked endothelial dysfunction and increased microvascular permeability [4].

Previous studies have implicated the involvement of many serum factors in causing enhanced vascular permeability in dengue [5,6]. These factors are produced mainly in response to virus infection of immune cells such as monocytes, macrophages and mast cells. Elevated plasma levels of pro-inflammatory and immunosuppressive cytokines are observed in dengue patients [7]. Circulating levels of vascular permeability mediators such as vascular endothelial growth factor (VEGF) [8], platelet-activating factor (PAF) [9], Angiopoietin-2 (Ang-2) [10], chymase and tryptase [11,12] were found to be higher in patients with severe disease. In these patients, the levels of endothelial barrier protecting agents such as sphingosine-1-phosphate (S1P) [13,14] and Angiopoietin-1 (Ang-1) [15] were found to be low. In severe dengue, the plasma level of soluble VEGF receptor-1 (VEGFR-1) increases while soluble VEGFR-2 decreases [16]. Studies have also shown that dengue virus non-structural protein 1 (NS1), a secretory protein circulating in the blood in acute dengue patients, can act on the vascular endothelium and cause hyperpermeability [17–19].

Vascular endothelial cell lining forms the primary fluid barrier of the vascular system. It helps in regulating the fluid and cellular efflux from capillaries [20,21]. In dengue infection, there is a pronounced endothelial activation and immune response transforming the quiescent monolayer into a pro-inflammatory, pro-coagulant and pro-adhesive state that may contribute to vascular permeability [22]. These activated endothelial cells express increased levels of cell adhesion molecules like VCAM-1 and ICAM-1 [5]. Reflecting this increased expression, it has been shown that the serum level of soluble VCAM-1 is elevated in severe dengue patients as compared to those having dengue fever [23].

The role of direct DENV infection of endothelial cells in enhancing

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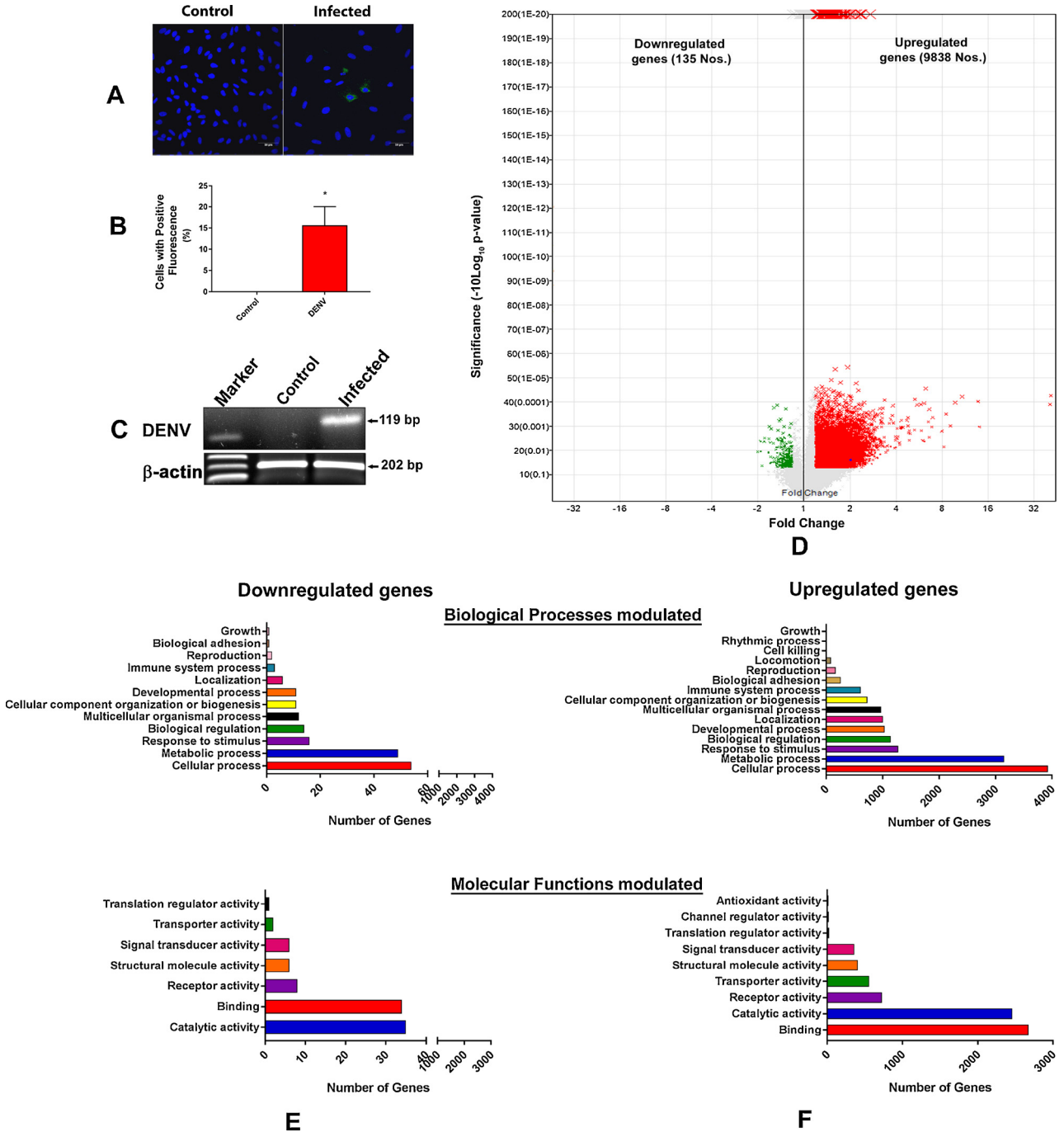

Fig. 1. Microarray analysis of DENV-infected HMEC-1 cells (A) Immunofluorescence detection of DENV-infected HMEC-1 cells using anti-DENV envelope protein antibody. (B) Quantitation of virus infection in HMEC-1 cells. Number of fluorescent cells in four independent fields in three experiments were counted and expressed as % with respect to the total number of cells stained with DAPI. (C) Detection of DENV RNA in the samples used for microarray analysis by RT-PCR (D). Volcano plot representing the number of mRNAs modulated at a statistically significant level in microarray. Red represents mRNA with increased expression and green represents mRNA with decreased expression. Gene Ontology classification of differentially expressed genes identified in microarray using PANTHER database, representing downregulated genes (E) and upregulated genes (F). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

vascular permeability is less clear. Endothelial cell infection, albeit to a very less extent, has been documented in human tissues in autopsy studies [24,25] and in mice studies [26]. Several studies [27–35] have shown that DENV can infect cultured endothelial cells, and infection does not cause cytopathic effect in these cells [36,37]. Studies by others and our own earlier study have shown that DENV infection of endothelial cells can lead to enhanced permeability across the confluent monolayer in endothelial cells in culture [38–40].

At present, there is only limited understanding on the molecular mechanisms behind this enhanced trans-endothelial cell permeability. In the present study, in order to gain further knowledge on the intracellular pathways regulating the barrier dysfunction, we used a microarray-based approach to decipher the gene expression changes in human microvascular endothelial cells (HMEC-1) upon DENV infection. We also explored the functional implications of the modulation of key molecules in Sphingolipid signaling, one of the key endothelial cell