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Proteomic profile of human monocytic cells infected with dengue virus

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ABSTRACT

Objective: To identify the changes in the proteome of U937 cells infected with dengue virus (DENV).

Methods: In this study, differentiated U937 cultures were infected with two DENV-2 strains, one of which was associated with dengue (DENV-2/NG) and the other one with severe dengue (DENV-2/16681), with the aim of determining the cellular proteomic profiles under different infection conditions. Cellular proteins were extracted and separated by two-dimensional electrophoresis, and those proteins with differential expression profiles were identified by mass spectrometry. The obtained results were correlated with cellular viability, the number of infectious viral particles, and the viral DNA/protein quantity.

Results: In comparison with non-infected cultures, in the cells infected with the DENV-2/NG strain, nine proteins were expressed differentially (five were upregulated and four were downregulated); in those cultures infected with the DENV-2/16681 strain, six proteins were differentially expressed (two were downregulated and four were upregulated). The downregulated proteins included fatty acid-binding protein, heterogeneous nuclear ribonucleoprotein 1, protein disulfide isomerase, enolase 1, heat shock 70 kDa protein 9, phosphotyrosyl phosphatase, and annexin IV. The upregulated proteins included heat shock 90 kDa protein AA1, tubulin beta, enolase 1, pyruvate kinase, transaldolase and phospholipase C-alpha.

Conclusions: Because the monocyte/macrophage lineage is critical for disease pathogenicity, additional studies on these proteins could provide a better understanding of the cellular response to DENV infection and could help identify new therapeutic targets against infection.

1. Introduction

Dengue is the most common disease in humans that is transmitted by arthropods. It is caused by the dengue virus (DENV) [1], which is transmitted by the bite of infected mosquitoes, principally those of the species *Aedes aegypti* and *Aedes albopictus* [2]. This virus, whose genome is made up of positive-strand RNA, belongs to the Flaviviridae family and the *Flavivirus* genus [3]. Although, in general, the existence of four serotypes that share antigenic similarities but are genetically different has been reported in the urban viral cycle setting (DENV-1 to DENV-4), the existence of a fifth serotype has recently been reported (DENV-5) in a wild cycle setting [4].

Dengue affects the populations of tropical countries, where more than 2 500 billion people are at risk of being infected, and it is endemic in over 100 of these countries [5]. Its prevalence has increased dramatically in the last few decades, and recent studies estimate that 96 million cases occur on a yearly basis, although

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this number may be underestimated in some regions due to poor clinical surveillance [1]. From a clinical perspective, dengue is a self-limiting febrile illness characterized by headaches, muscle aches, and skin eruptions. The most severe form of the disease is known as severe dengue in which there is increased vascular permeability that results in plasma leakage; other symptoms include thrombocytopenia, hemorrhagic manifestations, and compromised organs, such as the liver, heart, and central nervous system [6].

Although the pathogenesis of severe dengue is not completely clear, some of the risk factors involved in its development have been described (some related to the host and some to the virus). Of the factors related to the virus, it has been reported that the viral genetic variations partly determine virulence and, therefore, epidemic potential [7], which allows certain strains with higher replicative potential to spread more easily in a primary infection. In addition, some serotypes and strains have been associated with the development of severe forms of the disease [8]. In this regard, some DENV strains within serotype 2 have been reported to be associated with the development of mild or severe forms of the disease, as is the case for the New Guinea strain (DENV-2/NG) (originally isolated from a dengue patient) [9], and the DENV-2/16681 strain (isolated from a patient with severe dengue) [10]. Based on their origin and etiology, the DENV-2/NG strain has been associated with the development of dengue [11], and the DENV-2/16681 strain has been associated with severe dengue [12]. Regarding host-associated factors, much has been discussed regarding the pathogenic role of the immune response to a secondary infection. In this regard, the principles of antibody-dependent enhancement and original antigenic sin are the most studied [13,14]. Independent of the factors favoring the development of severe dengue, whether in a primary or secondary infection, there are some cells that play a very important role in the initial capture of the virus, its transport to specific sites within the host, and its replication and propagation.

Monocytes, macrophages, and dendritic cells are considered the primary targets of DENV infection *in vivo* [15,16], although the presence of the virus in different cell types such as hepatocytes, lymphocytes, endothelial, epithelial, neuronal, and muscle cells has also been reported [17]. The monocyte/macrophage lineage, which is normally involved in the processes of the innate and adaptive immune responses, is successful in the elimination of most pathogens, although pathogens sometimes use this strategy to their advantage. In the case of dengue, macrophages are not only the main targets of DENV for its replication but also responsible for spreading the virus to different parts of the hosting organism after its transmission and for enhancing proinflammatory cytokine production, responsible in great measure for the development of severe dengue [15,18]. Within this lineage, the U937 cell line has been widely used to study different aspects of DENV infection.

The U937 cell line, isolated from human histiocytic lymphoma, shows characteristics of immature monocytes, but its differentiation promoted with phorbol esters converts it to a cell line with the morphologic and functional characteristics of macrophages [19]. This cell line can be infected with DENV by two different mechanisms: through cell surface receptors (heat shock 70 kDa protein and heat shock 90 kDa protein), thus allowing the study of conventional forms of viral entry into the host cell during primary or secondary infection, and through Fc receptors that bind viral complexes with antibodies during secondary infections [20,21]. This cell line has been used for studies on viral replication [22], on the evaluation of antiviral agents [23–25], on the immune response [26], *etc*.

Despite the importance and broad use of the U937 cell line, no studies have been performed in which protein expression changes have been determined in this cell line when infected by DENV. For this reason, the present study identified the modifications in the U937 proteome after infection with two strains of DENV-2 clinically associated with dengue: the New Guinea strain DENV-2/NG (associated with dengue) and the DENV-2/16681 strain (associated with severe dengue) under primary infection conditions.

2. Materials and methods

2.1. Cell maintenance and viral stock production

Differentiated-U937 cells (human monocyte derived from lymphoma), and C6/36HT cells (derived from *Aedes albopictus*) were a kind gift from Dr. Jaime E. Castellanos at the El Bosque University (Bogotá, Colombia) and Dr. Guadalupe Guzman at the Pedro Kouri Institute (Havana, Cuba). Both cell lines were grown in Dulbecco's modified Eagle medium (Gibco/Invitrogen, Grand Island, NY, USA) supplemented with 0.25 μg/mL of amphotericin B, 100 μg/mL streptomycin, 100 units/mL penicillin, and either 2% (U937 cells) or 10% (C6/36HT cells) fetal bovine serum (FBS, Gibco). Cell cultures were incubated with 5% CO_2, at either 37 °C (U937 cells) or 34 °C (C6/36HT cells). For all assays, two serotype 2 reference strains were used: strain DENV-2/NG (dengue reference strain) and strain DENV-2/16881 (severe dengue reference strain). Both strains were a kind gift from Dr. Jorge Osorio at the University of Wisconsin (Madison, WI, USA). The viral stocks were expanded in the C6/36HT cell line (five passages for each viral strain) and stored at −70 °C until used.

2.2. MTT viability assay

A colorimetric MTT assay was used to measure the effect of the infection on U937 cell viability. For this assay, 2.5×10^4 cells were seeded in 96-well plates and were infected [multiplicity of infection (MOI): 1] at 24 h. The infected cells were incubated for an additional 48 h. Then, 50 μL of MTT (0.5 mg/mL) were added, and the cultures were incubated for 3 h at 37 °C. Then, 100 μL of dimethyl sulfoxide (Fisher Scientific Inc., Rockford, IL, USA) were added, and the absorbance was read at 450 nm using a Varioskan Flash reader (Thermo Scientific Inc., Rockford, IL, USA). The data were processed by comparing the absorbance of the infected cultures with the absorbance of the uninfected control cultures. The results were expressed as the mean of at least three independent experiments, each with two replicates ($n = 6$).

2.3. Cell infection

A total of 3×10^6 U937 cells were seeded in 25 cm^2 cell culture flasks. After 24 h, the cultures were incubated with each of the reference stocks (DENV-2/NG or DENV-2/16681) for 2 h at 37 °C at an MOI of 1. Afterwards, the virus was removed, and the cultures were cultured for an additional 48 h, after which the supernatant of each culture was collected and stored

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