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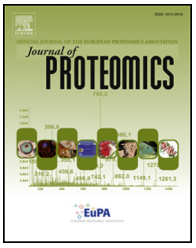

Proteomic profiling of chikungunya virus-infected human muscle cells: Reveal the role of cytoskeleton network in CHIKV replication

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ABSTRACT

Chikungunya virus (CHIKV) is an arthropod-borne, positive-sense, single-stranded RNA virus belonging to genus *Alphavirus* and family *Togaviridae*. The clinical manifestations developed upon CHIKV-infection include fever, myositis, arthralgia and maculopapular rash. Thus, the re-emergence of CHIKV has posed serious health threats worldwide. Due to the fact that myositis is induced upon CHIKV-infection, we sought to understand the dynamic proteomic regulation in SJCRH30, a human rhabdomyosarcoma cell line, to gain insights on CHIKV pathogenesis. Two-dimensional gel electrophoresis (2DE) in combination of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to profile differential cellular proteins expression in CHIKV-infected SJCRH30 cells. 2DE analysis on CHIKV-infected cells has revealed 44 protein spots. These spots are found to be involved in various biological pathways such as biomolecules synthesis and metabolism, cell signaling and cellular reorganization. siRNA-mediated gene silencing on selected genes has elucidated the biological significance of these gene-translated host proteins involved in CHIKV-infection. More importantly, the interaction of vimentin with non-structural protein (nsP3) of CHIKV was shown, suggesting the role played by vimentin during CHIKV replication by forming an anchorage network with the CHIKV replication complexes (RCs).

Biological significance

Chikungunya virus (CHIKV) is a re-emerging virus that has caused various disease outbreaks in Africa and Asia. The clinical symptoms of CHIKV-infection include fever, skin rash, recurrent joint paint, and myositis. Neuronal implications and death may be resulted from the severe viral infection. Up to date, there are no effective treatments and vaccines against CHIKV-infection. More importantly, little is known about the differential regulation of host proteins upon CHIKV infection, hence deciphering the viral-host cell interactions during viral infection provide critical information on our understanding on the mechanisms of virus infection and its dependency of host proteins for replication. In light

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of the muscle-related clinical manifestations of myositis resulting from CHIKV-infection, human rhabdomyosarcoma cells, SJCRH30 were utilized in this protein profiling study, in order to decipher the pathogenesis of CHIKV. This study has identified an arrays of host proteins that are differentially regulated upon CHIKV infection including that of the cytoskeletal protein, vimentin that plays significant role in aiding the replication of CHIKV within the host cells through 2DE assay. Immunofluorescence assay further shows that the novel interaction between cytoskeleton structure and CHIKV replication complex by forming an intercalating network around the replication complexes and facilitating various stages of the virus life cycle. This novel finding has inevitably led to a deeper understanding of CHIKV pathogenesis in revealing the importance of host proteins during CHIKV replication, as well as contributing to the development of specific antiviral strategies against this medically important viral pathogen

1. Introduction

Chikungunya virus (CHIKV) is an arthropod-borne, positive-sense, single-stranded RNA virus, which is readily transmitted by mosquito vectors, *Aedes aegypti* and *Aedes albopictus* [1–3], in various tropical countries such as Africa, Thailand, Cambodia, Vietnam, Indonesia, Malaysia, the Philippines and Singapore [4]. It was first isolated from a patient from Mankonde Plateau in Tanzania, Africa in 1952 [1,2,5]. The term "Chikungunya" is referring the stooped posture resulted from the recurrent and debilitating joint pain induced upon CHIKV-infection [6].

CHIKV belongs to the genus *Alphavirus* and family *Togaviridae* [1–3]. The CHIKV RNA genome, which contains a 5′ capping and 3′ polyadenylated tail, encodes for four non-structural (nsP1, nsP2, nsP3 and nsP4) and five structural proteins (capsid, E1, E2, 6 K and E3) that are driven by two open reading frames (ORFs) respectively [1,2]. Each non-structural protein plays different roles in CHIKV replication. nsP1 is involved in the synthesis of negative strand of viral RNA genome, and RNA capping and methylation. nsP2, on the other hand, functions as RNA helicase and RNA triphosphatase. It is also involved in proteinase activity as well as in shutting-off of host transcriptional activity. nsP3 mainly functions as the cofactor of viral RNA dependent RNA polymerase (RdRp), which is encoded by nsP4 [1,2]. These non-structural proteins will then form the replication complex (RC) that facilitates the downstream synthesis of viral genome. The structural proteins, E1 and E2 glycoproteins, are responsible for the virus fusion with the cell membrane in low pH environment and virus attachment on the host cells, respectively [1–3,6].

The clinical manifestations induced by CHIKV-infection include high fever, skin rash, rigor, myalgia, headache, photophobia, polyarthritis and myositis [1,7]. In addition, the more recent outbreak of CHIKV was reported with more severe neurological and haemorrhagic diseases, and deaths [1]. Due to the absence of vaccine and specific treatment for CHIKV, early diagnosis for CHIKV-infection becomes essential [1,2]. This is not only to minimize the further spread of CHIKV unknowingly among the patients but also to reduce the possibility of misdiagnosis with other symptoms-alike viral infection, such as dengue virus (DENV) infection. Currently, the available treatments for CHIKV-infection mainly focus on the alleviation of clinical symptoms.

Noting the rise of CHIKV-infection cases, studies have been done to investigate the replication processes of CHIKV in human host [8–10]. Besides from the intrinsic enzymatic activities of the structural and non-structural proteins of CHIKV, the interactions between CHIKV proteins and human host proteins have also been studied to understand the mechanisms on how the virus hijacks the host machineries in order to block antiviral response, as well as to achieve successful replication followed by virions assembly. In the study performed by Bourai and co-workers, they have shown that CHIKV proteins were found to be closely associated with human host RNA processing and translation proteins, pro-apoptotic proteins and autophagy proteins during the early phase of CHIKV-infection [11].

Previous proteomic studies on human host of CHIKV-infection were mainly performed on neuronal cells and hepatocytes-like cells [8–10,12]. Studies have revealed that some host proteins were found to be associated with CHIKV replication, either to facilitate the replication process or to inhibit the reproduction of CHIKV virions. Apart from developing fever, rash, headache and polyarthralgia, CHIKV-infection was also found to result in myositis in patients [1,7]. The outbreak of CHIKV in the Réunion has documented 97.7% of myositis incidence upon CHIKV infection [13]. Hence, the investigation of CHIKV-infection in human muscle cell lines may have revealed novel evidences of the mechanisms played by host cellular proteins involving in viral replication as well as the disease progression in human host cells.

In light of the muscle-related clinical manifestations resulting from CHIKV-infection [6], human rhabdomyosarcoma cells, SJCRH30 were utilized in this protein profiling study, in order to decipher the pathogenesis of CHIKV. Two-dimensional gel electrophoresis (2DE) in combination of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to profile differential cellular protein expression in CHIKV-infected SJCRH30 cells. The proteomic analysis of CHIKV-infected SJCRH30 cells has revealed various host proteins, which involve in biological processes such as biomolecules synthesis and metabolism, cell signaling and cellular reorganization, to be differentially regulated. This study, for the first time, has showed the close interaction between vimentin with CHIKV, suggesting the supporting role played by vimentin during CHIKV replication. These discoveries may provide a new avenue for developing a novel antiviral strategy against this essential host-viral protein interaction.

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