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Microbiology Congress 2018: PCR array profiling of antiviral genes in human embryonic kidney cells expressing Human corona virus OC43 structural and accessory proteins - Meshal Beidas - Kuwait University

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Background & Objective: Human corona virus OC43 (HCoV-OC43) causes common cold and is associated with severe respiratory symptoms in infants, elderly and immune compromised patients. HCoV-OC43 is a member of Betacoronavirus genus that also includes the Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS) coronaviruses. Both SARS-CoV and MERS-CoV were shown to express proteins with the potential to evade early innate immune responses. However, the ability of HCoV-OC43 to antagonize the intracellular antiviral defense has not yet been investigated. The objective of this study was to investigate the role of HCoV-OC43 structural (membrane and nucleocapsid) and accessory (ns5a and ns2a) proteins in the modulation of antiviral gene expression profile in human embryonic kidney 293 (HEK-293) cells using PCR array analysis.

Human coronavirus OC43 (HCoV-OC43) is an envelope, positive-sense RNA virus classified as a *Betacoronavirus*, the same genus as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) coronaviruses. HCoV-OC43 infection has been associated mainly with upper respiratory tract symptoms and exacerbation of asthma and pneumonia in some groups and institutional settings. The virus has also been associated with severe neurological disorders like acute disseminated encephalomyelitis in children.

While most studies have focused their attention on the immunopathology of SARS-CoV and MERS-CoV, there has not been the same interest for HCoV-OC43. Indeed, there are few studies concerned with the molecular mechanisms governing HCoV-OC43 infection and its effect on the intracellular host defenses. HCoV-OC43 is over 30 kb in length, and similarly to the other coronaviruses, it is composed of the structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N). Uniquely, there are two accessory proteins interspaced between these structural proteins called ns2a and ns5a . These proteins are not essential for replication; however, they might play a role in the pathogenesis of coronavirus infection.

The interferon (IFN) induction and signaling pathway is the major branch in the innate immune response against viruses. The induction of type I IFN is initiated by molecular sensing of viral RNA by pattern recognition receptors such as TLR, RIG-I, and MDA5. The adaptor protein MAVS mediates the signals from RIG-I and MDA5 to activate the kinases TBK1 and IKKε, which in turn phosphorylate IRF3 and IRF7 transcription factors. IRF3 and IRF7 dimerize to undergo nuclear translocation and initiate the transcription of type I IFN. The adaptor proteins MYD88 and TRIF mediate signals from TLRs and activate IRAK1, TRAF6, and the aforementioned kinases. The IRFs along with nuclear factor kappa B (NF-κB) can then be phosphorylated to translocate into the nucleus and establish the transcription of type I IFN by binding cognate sites on the IFN-β promoter. NF-κB specifically binds to the NF-κB response element (NF-κB-RE) to regulate the expression of pro-inflammatory and cell survival genes.

Method: HCoV-OC43 membrane (M), nucleocapsid (N), ns5a and ns2a mRNA were amplified and cloned into the pAcGFP1-N expression vector (Clontech), followed by transfection in HEK-293 cells. Expressions of M, N, ns5a and ns2a proteins were confirmed by indirect immunofluorescence test. Three days post-transfection, the cells were challenged by Sendai virus. The human antiviral response PCR array system (Qiagen) was used to profile the antiviral gene expression in HEK-293 cells, using the fold regulation comparison and the manual normalization methods.

Result: Around 50-60 genes were down-regulated by HCoV-OC43 proteins, the most prominent genes being those critical for the activation of transcription factors involved in the antiviral response like interferon regulatory factors (IRFs) and activator protein-1 (AP-1). Among the most important down-regulated genes were those coding for interferons (IFNs) mitogenactivated protein kinases (MAPKs), pro-apoptotic and pyroptotic proteins (Caspases, cathepsins, tumor necrosis factor), proinflammatory cytokines (interleukins), pattern recognition receptors (PRRs; toll-like receptors and NOD-like receptors) and their signaling transduction proteins (TICAM1, MAVS).

Conclusion: This study shows for the first time that similarly to SARS-CoV and MERS-CoV, HCoV-OC43 has the ability to down-regulate the transcription of genes critical for the activation of different antiviral signaling pathways.

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