Congratulations to IMCB's latest PhD graduate - James Odame ABOAGYE

Friday, 11 Jan 2019

Thesis Title: Virus-Host Interactions of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Nucleocapsid Protein.

The novel human coronavirus MERS-CoV causes severe respiratory distress with a high mortality rate. The nucleocapsid (N) protein of MERS-CoV (MERS-N) interacts with host factors to regulate cellular functions. To determine the processing and subcellular localization of MERS-N as well as its role in regulating host functions, mouse monoclonal antibodies binding to different domains within MERS-N were generated to detect its expression in infected and overexpressed cells. Employing the monoclonal antibodies, the N protein was detected to be cleaved in transfected and infected cells with additional cleavage/degradation and/or modification(s) observed in infected cells. PCR array was used to screen for the regulation of host antiviral response and apoptotic genes in cells overexpressing MERS-N. Several genes were upregulated transcriptionally including IL6, IL8, TNF, CXCL10, and BCL2. However, CXCL10 which is associated with disease severity was upregulated on the translational level via the C-terminal fragment (196-413 aa) of MERS-N. In addition, MERS-N was characterized as a nucleocytoplasmic protein with functional nuclear export and nuclear localization signals.

The localization of MERS-N within the cytoplasm was observed to contribute to the upregulation of CXCL10. This study shed light on the possible role of the N protein in the replication and pathogenesis of MERS-CoV.

Supervisor

A/Prof. Tan Yee Joo

Publication list

Overexpression of the nucleocapsid protein of Middle East Respiratory Syndrome coronavirus up-regulates CXCL10

Aboagye, J. O., Yew, C. W., Ng, O. W., Monteil, V. M., Mirazimi, A., & Tan, Y. J.

Bioscience Reports, 2018

Figure

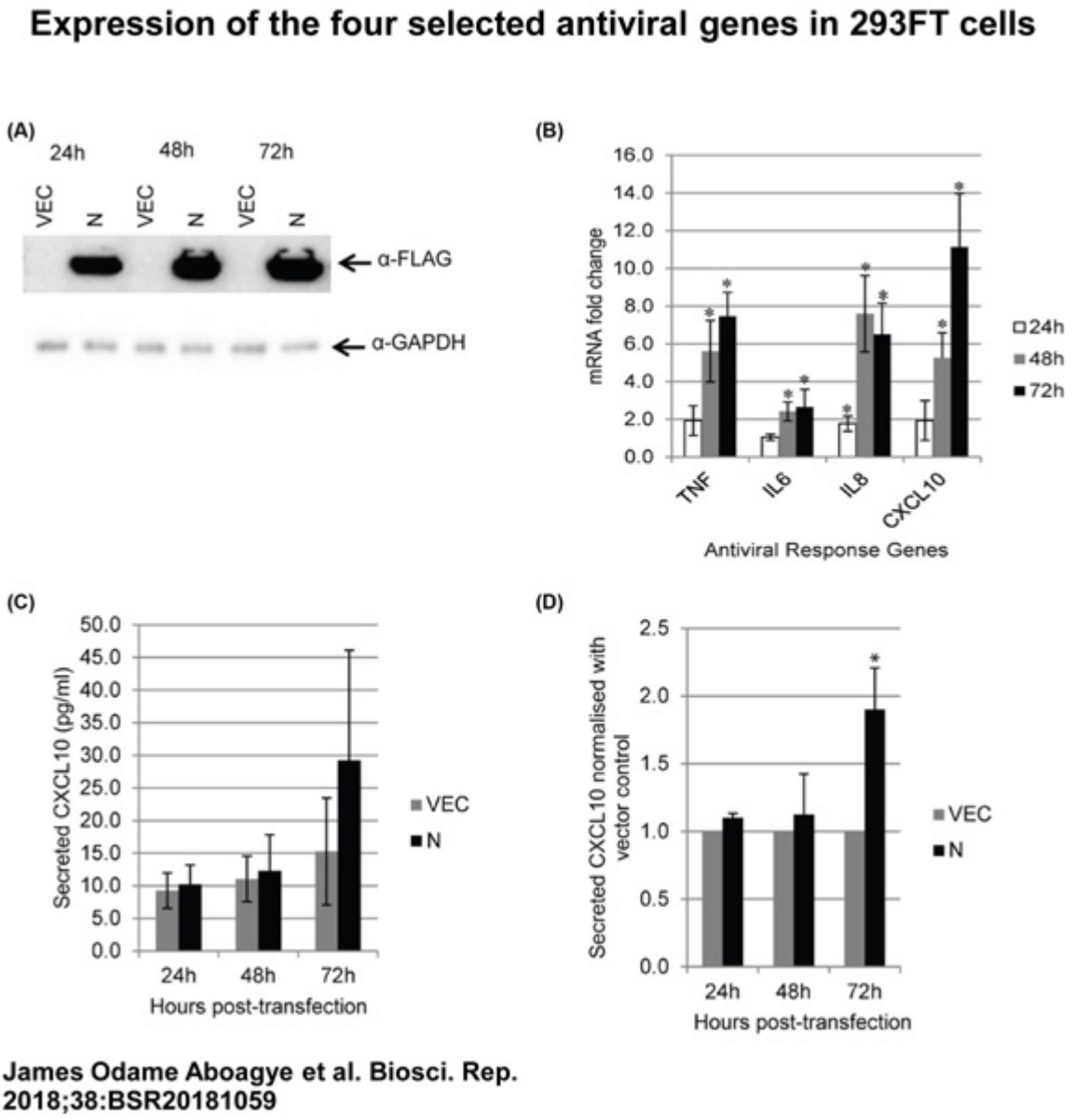

Figure Legend:

Expression of the four selected antiviral genes in 293FT cells (A) 293FT cells were transiently transfected with empty vector and FLAG-tagged MERS-N. Western blot analysis was performed using anti-FLAG antibody. (B) RNA transcripts from transiently transfected 293FT cells were assayed by RT-qPCR with TaqMan probes to analyze the mRNA fold changes of TNF, IL6, IL8, and CXCL10. mRNA expression of genes was first normalized against GAPDH and subsequently, MERS-N up-regulation was normalized against levels in vector-transfected cells. (C) Cell supernatants were harvested and CXCL10 secretion was evaluated using ELISA. (D) The secretion of CXCL10 by MERS-N was normalized against the vector. The results of these experiments were expressed as mean ± S.D. (error bars) of five independent

experiments. Asterisk (*) indicates statistical significance of $P<0.05$ when compared with vector-transfected cells at the respective time-points.