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The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Molecular dissection of NSs virulence factor in severe fever-with-thrombocytopenia syndrome virus (SFTSV), a novel bunyavirus identified in China

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Dong-Yan Jin	Prof. Mifang Liang
Post	Professor	Professor
Unit / Department / Institution	The University of Hong Kong	Institute of Viral Disease Control and Prevention, China CDC
Contact Information	3/F Lab Block, 21 Sassoon Road, Pokfulam, Hong Kong; dyjin@hku.hk	155 Changbai Road, Changping, Beijing 102206, China; mifangl@vip.sina.com
Co-investigator(s) *(with title and institution)*	Dr. Kin-Hang Kok The University of Hong Kong	Prof. Dexin Li China CDC

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	January 1, 2013		
Project Completion date	December 31, 2016		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	September 30, 2017		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1) Activation of innate antiviral response in SFTSV-infected cells: innate IFN response; inflammasome activation; programmed cell death

2) Preparation and characterization of NSs-deficient SFTSV: plasmid construction by reverse genetics; creation of minireplicon system

3) Characterization of NSs-mediated inhibition of innate IFN response: verification of IFN-antagonizing effect of NSs; activity profile of NSs

4) Molecular mechanism of NSs-mediated inhibition of innate antiviral response: mechanistic study in transfected cells; mechanistic study in infected cells

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____
_____

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

1) We found that infection with SFTSV suppresses type I and type III IFN production through multiple mechanisms including the inhibition of MAVS activity (Chaudhary et al., 2015). The induction of IFN-α1, IFN-β, IFN-λ1 and IFN-λ2 by Sendai virus and several other stimuli was blunted by SFTSV. The primary function of this suppression of the production of type I and type III IFNs with potent antiviral activity is to facilitate SFTSV replication. This suppressive activity is shared by various types of DNA and RNA viruses.

2) We demonstrated that SFTSV suppresses type I and type III IFN signaling but augments that of type II IFN (Chaudhary et al., 2015, 2017a, 2017b). Furthermore, we extended our analysis to other pathogenic viruses including Zika virus and showed a common trend in the ability to differentially modulate IFN signaling (Chaudhary et al., 2017a). In other words, suppressing IFN-β signaling and potentiating the action of IFN-γ are a feature shared by several human viral pathogens, although the strategies used by different viruses such as Zika virus and SFTSV are distinct. The outcome of this differential modulation includes enhancement of viral replication and spreading as well as induction of pro-inflammatory cytokines that cause pathological inflammation and severe disease. This indicates that the innate immune response triggered by SFTSV and other viruses is a double-edge sword or a stone that hits two birds. The activation of IFN-γ signaling by SFTSV is surprising and unexpected. This challenges existing model in the field and reveals another level of complexity in viral modulation of innate immunity. Thus, it is a conceptual advance that will instruct the design and development of new antiviral and immunomodulatory agents. For example, the utility of JAK2 inhibitors such as AG490 in viral infection and viral induction of inflammation merits further analysis.

3) We further showed that SFTSV NSs protein is both required and sufficient for the ability to circumvent type I and type III IFN signaling and to boost type II IFN signaling. Likewise, Zika virus NS5 protein exerts opposite effects on type I/III IFN signaling and type II IFN signaling (Chaudhary et al., 2017a, 2017b). We therefore proposed that they belong to the same group of viral IFN modulators that suppress type I/III IFN signaling but activate type II IFN

signaling. We found that the induction of antiviral ISGs such as OAS1 and MxA was indeed abrogated or dampened by SFTSV NSs whereas the production of IRF1 and CXCL10 that mediate pro-inflammatory response was potentiated (Chaudhary et al., 2015, 2017a, 2017b). Mechanistically, SFTSV NSs interacts with both STAT1 and STAT2. This impedes the interaction between STAT1 and STAT2 but does not affect the formation of STAT1-STAT1 homodimer. As a result, ISGF3 (STAT1-STAT2-IRF9) assembly at ISRE was perturbed whereas GAF (STAT1-STAT1) recruitment to GAS was enhanced. The use of a key virulence factor such as NSs to facilitate viral replication and infection on one hand and to cause pathological inflammation and severe disease on the other is a new viral strategy for combating host defense.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

Getting our two papers on selective activation of IFN-γ signaling by Zika virus NS5 and SFTSV NSs proteins published in *Journal of Virology* or another mainstream international journal such as *Cellular and Molecular Immunology* is at the top of our priority list. The first paper was already under review and the second paper will be submitted soon. We will revise our papers as per reviewers' comments and suggestions. We expect that some new experiments might be requested. We expect that these two papers will ultimately be accepted for publication in a mainstream international journal in our specialty within 2017. Next, since our two sides have greatly strengthened our collaboration on the study of emerging infectious diseases during the execution of this project, we plan to apply for another RGC-NSFC JRS grant to further our mechanistic study on the modulation of innate immune response by Zika virus NS5 protein. This is an extension of the completed study supported by this JRS grant and it will bring our collaborative work to the next level of excellence in the study of the interaction between emerging viral pathogens and host innate immunity. In the case of Zika virus NS5 protein, the mechanism for selective activation of IFN-γ signaling is different from that mediated by SFTSV NSs (Chaudhary et al., 2017a). Particularly, Zika virus NS5 preferentially induces K48-linked polyubiquitination and proteasomal degradation of STAT2, leading to enhanced formation of STAT1-STAT1 homodimeric complex and its subsequent occupancy of GAS in IFN-γ-stimulated genes. Built on the success of the current project, we will shed additional light on how NS5 promotes STAT2 degradation. Since Prof. Liang and Prof. Li are in charge of the prevention and control of Zika virus infection in China, the two sides have complementary resources and expertise. We are looking forward to another fruitful collaboration between the two sides.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Severe fever-with-thrombocytopenia syndrome virus (SFTSV) is an emerging viral pathogen discovered in 2012 in China and subsequently found in other Asian countries and other parts of the world. Severe cases of SFTSV infection are not uncommon and could be fatal. NSs protein of SFTSV is a major virulence factor but it is not understood how it causes severe diseases in infected people. We show in this project that SFTSV NSs protein plays dual roles in viral subversion of host defense. On one hand, NSs protein inhibits the production and function of type I and type III interferons that have antiviral activity. This enhances the replication of SFTSV in infected cells. On the other hand, NSs protein promotes the function of type II interferon, which plays a role in the induction of inflammation. Thus, SFTSV relies on its NSs protein not only to boost viral replication but also to cause severe diseases. Existing pharmaceutical agents that are known to inhibit the action of NSs to affect the function of host interferons might prove useful in combating SFTSV infection and pathogenesis. The general mechanism revealed in our study might also operate in the infection of other viral pathogens such as Zika virus.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) ***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
2014				Siu KL, Yeung ML, **Kok KH**, Yuen KS, Kew C, Lui PY, Chan CP, Tse H, Woo PCY, Yuen KY, **Jin* DY.**	Middle East respiratory syndrome coronavirus 4a protein is a double-stranded RNA-binding protein that suppresses PACT-induced activation of RIG-I and MDA5 in innate antiviral response. *J. Virol.*, 88:4866-76	2015	No	Yes	Yes
2013				**Kok KH, Jin* DY.**	Balance of power in host-virus arms races. *Cell Host Microbe*, 14:5-6	2015	No	Yes	Yes
2015				Chaudhary V, Zhang S, Yuen KS, Li C, Lui PY, Fung SY, Wang PS, Chan CP, **Li D, Kok KH, Liang* M, Jin* DY.**	Suppression of type I/III interferon signalling by NSs protein of SFTSV through inhibition of STAT1 phosphorylation and activation. *J. Gen. Virol.*, 96:3204-11	2017	Yes	Yes	Yes
		2017a		Chaudhary, V., Yuen, K.-S., Chan, J.F.-W., Chan, C.-P., Wang, P.-H., Cai, J.-P., Zhang, S., **Liang, M., Kok, K.-H.,** Chan, C.-P., Yuen, K.-Y., **Jin, D.-Y.**	Selective activation of interferon-γ signaling by Zika virus NS5 protein. *J. Virol.* (under review)	2017	Yes	Yes	Yes

Note: Another research paper (Chaudhary et al., 2017b) in which we report on the activation of IFN-γ signaling by SFTSV NSs protein is in preparation and will be submitted to *Journal of Virology* soon.

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
July 2013, Xi'an, China	Differential roles of RNA-binding proteins TRBP and PACT in RNA silencing and sensing	The 14th Society of Chinese Bioscientists in America International Symposium	2015	No	Yes	Yes
October – November 2014, Lorne, Australia	Viral suppression of type I interferon production through PACT targeting.	The Yin & Yang of the Interferon System. International Cytokine and Interferon Society Satellite Symposium 2014	2015	No	Yes	Yes
June 2014, Quebec City, Canada	Interplays between RNA-binding proteins determine viral infection outcome.	RNA 2014: The 19th Annual Meeting of the RNA Society	2015	No	Yes	Yes
June 2014, Fort Collins, Colorado, USA	The double-stranded RNA-binding protein PACT activates cytoplasmic viral sensor MDA5 by promoting its oligomerization.	The American Society for Virology 33rd Annual Meeting 2014	2015	No	Yes	Yes
June 2015, Taipei	Suppression of innate interferon production and signaling by NSs protein of severe fever-with-thrombocytopenia syndrome virus.	The 15th International Symposium of the Society of Chinese Bioscientists in America (SCBA)	2017	Yes	Yes	Yes
August 2015, Hong Kong	NSs nonstructural protein of severe fever-with-thrombocytopenia syndrome virus is an innate immunosuppressive protein that subverts interferon production and signaling.	Hong Kong Immunology Forum 2015: Annual General Meeting of the Hong Kong Society for Immunology	2017	Yes	Yes	Yes
November 2016, Beijing, China	Selective activation of interferon-γ signaling by Zika virus NS5 protein.	2016 World Life Science Conference	2017	Yes	Yes	Yes

November 2016, Beijing, China	Differential modulation of type I and type II interferon signaling by severe fever-with-thrombocytopenia syndrome virus NSs protein.	2016 World Life Science Conference	2017	Yes	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Vidyanath Chaudhary	PhD	September 2013	February 2017

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

Dr. Chan, Chi Ping (Postdoc) won a Best Presentation Award given by the Society of Chinese Bioscientists in America for his presentation on viral suppression of innate immune signaling in July 2013. Mr. Vidyanath Chaudhary (PhD student) won a Best Poster Presentation Award at the Society of Chinese Bioscientists in America (SCBA) International Symposium in June 2015. He also won a Best Poster Award at the Hong Kong Immunology Forum 2015: Annual General Meeting of the Hong Kong Society for Immunology in August 2015.

Prof. Dexin Li, Prof. Mifang Li and colleagues received a First-Class Award for Scientific Achievements presented by Chinese Society for Preventive Medicine (中華預防醫學會科學技術獎一等獎) for their discovery of and study on SFTSV in December 2013. Dr. Kin-Hang Kok was promoted to a tenure-track Assistant Professor in the Department of Microbiology, The University of Hong Kong in the middle of 2014. Dr. Shuo Zhang, a key member of the group, was promoted to Associate Professor in China CDC at the end of 2014. Prof. Dong-Yan Jin was awarded the Croucher Senior Research Fellowship (the Croucher Award) 2014-2015. He was also awarded an Outstanding Research Student Supervisor Award of the University of Hong Kong in 2014. In 2016, he was endowed as Clara and Lawrence Fok Professor in Precision Medicine.