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(please insert ref. above)

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

Understanding the evolution and interspecies transmission of betacoronaviruses by structural and biophysical approaches

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	YUEN, Kwok-Yung (Prof)	GAO, George Fu (Prof)
Post	Chair Professor & Co-director	Professor & Director
Unit / Department / Institution	State Key Laboratory of Emerging Infectious Diseases, Department of Microbiology, The University of Hong Kong	CAS Key Laboratory of Pathogenic Microbiology and Immunology (CASPMI), Institute of Microbiology, Chinese Academy of Sciences
Contact Information	kyyuen@hku.hk	gaof@im.ac.cn
Co-investigator(s) *(with title and institution)*	1. WOO, Patrick Chiu-Yat (Dr, Professor) 2. LAU, Susanna Kar-Pui (Dr, Associate Professor) 3. CHAN, Jasper Fuk-Woo (Dr, Assistant Professor)	1. YAN, Jinghua (Dr, Professor) 2. QI, Jianxun (Dr, Associate Professor) 3. LU, Guangwen (Dr, Assistant Professor)

3. Project Duration

	Original	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1 Jan 2015	
Project Completion date	31 Dec 2018	
Duration *(in month)*	48 months	
Deadline for Submission of Completion Report	31 Dec 2019	

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To understand the evolution and interspecies transmission of pCoVs by identifying and characterizing the structural and functional features of their Spike protein variants identified in our bat surveillance program in mainland China and Hong Kong.

2. To identify and characterize the receptor-recognition of representative pCoVs that are of clinical importance to human or animal with our focus on Tylonycteris bat CoV HKU4, Pipistrellus bat CoV HKU5 by cocrystallization and protein-binding assays. Success with these two viruses will allow us to study other betacoronaviruses such as Rousettus bat CoV HKU9 and human coronavirus HKU1 in the future.

5.2 Revised Objectives

As above

6. Research Outcome

Major findings and research outcome

1. With this funding, we published in Virology. 2017 Jul;507:101-109, by solving the crystal structure of the Spike S1 subunit C-terminal domain of HKU5 (HKU5-CTD),

another BatCoV that is phylogenetically related to MERS-CoV but cannot bind to CD26. There are two subdomains in HKU5-CTD, the core and the external. Despite the low residue conservation among CTDs(pair-to-pair amino acid identity ranging from 17.2% to 58.7%) and the core subdomains (pair-to-pair amino acid identity ranging from 16.6% to 66.7%) in the four lineages, the topology of the latter ones are highly conserved, with five anti-parallel β strands constituting the core-center and the same orientation of secondary elements in the core-peripheral.

2. Evolutionally, BatCoV HKU5 S protein is more diverse than BatCoVHKU4 S protein, and various deletions in loop 1 have been sequenced. This indicates that BatCoV HKU5 is able to generate variants to occupy new ecological niches and might acquire the ability to bind to hCD26 by accumulating mutations and ultimately cause human respiratory infections like MERS-CoV and SARS-CoV. Accordingly, it is very important to perform long-lasting surveillance of BatCoV HKU5 evolution, especially the variety of S protein in the event that the virus breaks the inter-species and/or inter-tissue transmission barriers.

3. Host tropism is predominantly determined by the interaction between coronavirus spikes and their corresponding host receptors. We showed that the spike proteins of coronaviruses such as human MERS coronavirus can recognize a broad range of cell surface molecules, which serve to augment coronavirus attachment or entry. These include the CEACAM5 and GRP78. The finding on CEACAM5 was published in J Virol. 2016 Sep 29;90(20):9114-27. The finding on GRP78 is now published in J Biol Chem.

4. Immunostaining of human lung tissues identified abundant co-expression of DPP4 and CEACAM5 or GRP78 in the epithelial cells along the human airways. Moreover our study identified GRP78 as an attachment factor that might modulate virus entry for two phylogenetically related betacoronaviruses of different lineages, MERS-CoV and bCoV-HKU9. GRP78 may serve to concentrate virus particles on the cell surface, which may then increase the possibility of receptor-mediated virus entry for MERS-CoV and bat CoV-HKU9. Importantly, MERS-CoV infection resulted in an upregulation of GRP78 on the cell surface, which may in turn increase the attachment of MERS-CoV and further enhance the possibility of virus entry in the infected cells.

5. It is tempting to speculate that the capacity of MERS-CoV spike to utilize multiple host surface proteins including CEACAM5, CD9, and GRP78 may give MERS-CoV a physiological advantage in establishing efficient infections, which may contribute to the high pathogenicity of the virus. Our finding of the lineage C MERS-CoV and lineage D bCoV-HKU9 sharing the same host attachment factor GRP78 highlights the importance of monitoring the evolution of bCoV-HKU9, which may jump the interspecies barrier into human leading to another major epidemic in the future.

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

1. Though the grant is used up already, we are still attempting to do interaction dynamics and perhaps co-crystallization of Spike S1 with CEACAM5 and GRP78 in

the presence or absence of DPP4. The recent discovery of host cell sialic acid as another attachment factor of MERS coronavirus and the role of host cell tetraspanin as packaging molecule for host protease to facilitate activation of viral Spike protein. The large variety of attachment factor being discovered for MERS coronavirus may facilitate the evolution of this and other coronavirus in jumping interspecies barrier, and perhaps increased virulence in terms of wider tissue tropism in the individual host.

2. Though we can presently only find the attachment factor for lineage D betacoronavirus bat HKU9, we will continue to hunt for its receptor as recombinants or mutants of lineage D bat betacoronavirus may jump into human leading to another MERS or SARS outbreak. As this interspecies jumping have already happen for lineage A betacoronavirus bovine OC43-like coronavirus, lineage B bat/civet SARS coronavirus and lineage C camel MERS coronavirus, it would be reasonable to prepare for a lineage D coronavirus to cause another interspecies jumping.

3. Besides the implications of CEACAM5 and GRP78 as important host factors in the pathogenesis of MERS coronavirus infection, it would also be important to consider using these as targets for therapeutic intervention since blocking experiments show that blocking antibody or antigen can dramatically decrease viral infection and viral load in the culture supernatants.

7. The Layman's Summary

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

The majority of the emerging virus infections come from animals as illustrated by the betacoronaviruses including lineage A OC-43, lineage-B SARS and lineage-C MERS coronavirus (CoV). Using crystallography, we have demonstrated the stepwise evolution of MERS related HKU5 CoV spike in adapting to human DPP4 receptor which account for interspecies jumping. But the wide tissue tropism of MERS coronavirus suggests that other host entry factors could be important to facilitate the interspecies jumping and virulence. Using a biochemical baiting technology (VOBPA), we demonstrated that CEACAM5 and then GRP78, which are highly expressed in many human tissues including the lung, are other host surface proteins that potently facilitate MERS-CoV attachment and cell entry. Moreover we also found that GRP78 facilitates the attachment of the lineage D bat coronavirus HKU9 onto human cells. Our study has demonstrated the importance of continued surveillance of these animal coronaviruses which can evolve to jump into human, their ability to utilize multiple cell surface proteins as cell entry factors and wide tissue tropism (potential targets for therapeutic intervention), and the possibility of the lineage D bat-CoV being able to jump into human in the future which may lead to another MERS or SARS like outbreaks.

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)*						
2016				Che-Man Chan, Hin Chu, Yixin Wang, Bosco Ho-Yin Wong, Xiaoyu Zhao, Jie Zhou, Dong Yang, Sze Pui Leung, Jasper Fuk-Woo Chan, Man-Lung Yeung, Jinghua Yan, Guangwen Lu, George Fu Gao, Kwok-Yung Yuen*	Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5 Is an Important Surface Attachment Factor That Facilitates Entry of Middle East Respiratory Syndrome Coronavirus. J Virol. 2016 Sep 29;90(20):9114-27. doi: 10.1128/JVI.01133-16.	2016	Yes	Yes	Yes
2017				Han X, Qi J, Song H, Wang Q, Zhang Y, Wu Y, Lu G, Yuen KY, Shi Y, Gao GF.	Structure of the S1 subunit C-terminal domain from bat-derived coronavirus HKU5 spike protein.	2017	Yes	Yes	Yes

2018		Yes		Hin Chu, Che-Man Chan, Xi Zhang, Yixin Wang, Shuofeng Yuan, Jie Zhou, Rex Kwok-Him Au-Yeung, Kong-Hung Sze, Dong Yang, Huiping Shuai, Yuxin Hou, Cun Li, Xiaoyu Zhao, Vincent Kwok-Man Poon, Sze-Pui Leung, Man-Lung Yeung, Jinghua Yan, Guangwen Lu, Dong-Yan Jin, George F. Gao, Jasper Fuk-Woo Chan*, Kwok-Yung Yuen*	Middle East respiratory syndrome coronavirus and bat coronavirus HKU9 both utilize GRP78 for attachment onto host cells; Journal of Biological Chemistry		Yes	Yes	Yes

Publications in collaboration with Mainland collaborators (but funded by mainland NSFC):

1. Obameso JO, Li H, Jia H, Han M, Zhu S, Huang C, Zhao Y, Zhao M, Bai Y, Yuan F, Zhao H, Peng X, Xu W, Tan W, Zhao Y, **Yuen KY,** Liu WJ, Lu L, Gao GF.The persistent prevalence and evolution of cross-family recombinant coronavirus GCCDC1 among a bat population: a two-year follow-up. Sci China Life Sci. 2017 Dec;60(12):1357-1363. doi: 10.1007/s11427-017-9263-6. Epub 2017 Dec 1.

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/2015/ Macau	From SARS to MERS	World Medicine Summit	2016	No	Yes in the oral presentation	July/2015/ Macau
October/ 2016/Wuhan	Relevance of MERS coronavirus induced apoptosis to pathogenesis	Nature Conference on viral infection and immune response	2016	No	Yes in the oral presentation	October/ 2016/Wuhan
November/ 2016/ Futian	Middle East Respiratory Syndrome (MERS) pathogenesis	The Inaugural Conference of Shenzhen International Institute for Biomedical Research	2016	No	Yes in the oral presentation	November/ 2016/ Futian

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
Shuai Huiping	PhD	1 August 2013	24 November 2017

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

NA