The war against infectious respiratory disease goes on: report on the 10th anniversary symposium of anti-SARS

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Ten years ago, severe acute respiratory syndrome (SARS) originated in Guangdong Province of China and swept rapidly to other countries. A war fighting against SARS was subsequently launched worldwide. In commemoration of the anti-SARS war one decade later, the 10th Anniversary Symposium of Anti-SARS was held in Guangzhou on April 12th by China State Key Laboratory of Respiratory Disease. This event was a gathering of renowned specialists and researchers (Figures 1,2) in the field of respiratory medicine, including those from subsidiary institutions of Chinese Academy of Science (Guangzhou Institute of Biomedicine and Health, Wuhan Institute of Virology, Beijing Institute of Microbiology, and Shanghai Pasteur Institute), China National Center for Disease Control (CDC), Chinese Academy of Medical Sciences, Chinese Military Academy of Medical Sciences, Fudan University, Sun Yat-sen University, the University of Hong Kong, and The Chinese University of Hong Kong, to share their latest research findings. Among the keynote speakers to this symposium were Academicians Nanshan Zhong, Guoping Zhao, Joseph Sung, Kwok-Yung Yuen, and Yiling Wu. With an aim to facilitate communications in follow-up studies of SARS and future efforts to combat emerging respiratory diseases, the symposium looked back at major achievements on related topics. The rise of novel avian influenza virus H7N9 in April of 2013 clearly underlines the impending necessity of this symposium.

As the chair of the symposium and a pioneer in the anti-SARS campaign, Dr. Nanshan Zhong gave an opening speech, in which he first led all the attendants to stand in silent condolence for the medical workers and victims who died in this global catastrophe (Figure 3). Professor Chen Wang (Chairman of Respiratory Disease Society, Chinese Medical Association) and Professor Yu Wang (Director of China CDC) presented the great advances so far achieved in prevention and control of emerging respiratory infectious diseases over the past ten years, including the improvement in response system and capacity, the role of Chinese traditional medicine in the treatment of SARS, and the more open system for sharing information and resources.

The symposium consisted of three sections. The first section was to revisit the studies on SARS and other emerging respiratory infectious diseases. The second section focused on the follow-up studies of SARS and the latest advancements of studies on respiratory infectious diseases, and was followed by a press conference to answer the questions from media. Below are briefings for part of keynote speeches in each section.

Section 1: Revisiting the studies on SARS and other emerging respiratory infectious diseases

From SARS to H7N9

Keynote speaker: Nanshan Zhong

Academician, Chinese Academy of Engineering, Former President of Chinese Medical Association (2006-2011), Director of China State Key Laboratory of Respiratory Disease in Guangzhou.

Since the beginning of 2003, there have been unceasing outbreaks of emerging respiratory infectious diseases, ranging from SARS, to H5N1, H1N1, and to H7N9 influenza which occurred early this year. However, China along with the international community has made considerable headway in unraveling the causative pathogens and transmission route to these emerging respiratory infectious diseases. In studies for epidemics with certain viruses, like SARS, H5N1 and H1N1, we first discovered their properties of family clustering and human-to-human transmission before looking into their pathogens, while in the case of H7N9, we first discovered H7N9 as a pathogen and then its victims. Moreover, from SARS to H7N9, the time cost for tracking the transmission route has

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Figure 1. Gathering of renowned specialists and researchers.

Figure 2. The attendees are dedicated to the presentation.

Figure 3. Prof. Nanshan Zhong is giving an opening speech.

been substantially shortened, which significantly improved the prevention and control of emerging respiratory infectious diseases.

Why the fatality rate of emerging respiratory infectious diseases is so high (especially H5N1 and H7N9)? There were experiments *in vivo* proving that transmission of virus causes the immune dysfunction in target cells, and that the release of a large number of cytokine damages lung tissues and a variety of organs. Therefore, its pathogenesis is actually a process that virus sparks the immune inflammation. The increase of cytokine activity (especially the elevation of IP10) in the early stage of infection may be the key that triggers the immune-mediated pulmonary injury during the SARS infection. Immunosuppressive therapy may help relieve the cascade reaction of cytokine release. High viral replication and its inflammatory response are central to the pathogenesis of human-avian influenza. Hence in clinical practices, we should focus on early diagnosis and effective anti-viral therapy to prevent such a fierce response of cytokine.

JAK3 inhibitors [such as JAK3 inhibitor VI and CP-690550 (Tofacitinib: Pfizer)] may reduce the SARS-CoV- or H5N1-induced cytokine cascade, because JAK3 signal channel can control the cascade release of cytokines caused by SARS-CoV or H5N1, thus reducing lung injury. Besides, viral invasion may invoke the autophagic death of alveolar cells *in vivo*. The effect of SARS virus on ACE2 receptor leads to the disorder of renin-angiotensin, consequently resulting in organ damage. According to these conclusions mentioned above, JAK3 inhibitors, ACE inhibitors, and autophagic inhibitors (like chloroquine) are probable to reduce the virus-induced pulmonary injury. The clinical marketed drugs such as Losartan, Valsartan, Irbesartan, Olmesartan, Candesartan and angiotensin AT1R inhibitor are advisable in the treatment of H7N9 victims.

As for effective drugs and vaccines, we have developed homemade Oseltamivir. Peramivir, as a neuraminidase (NA) intravenous agent, was used in China for the first time. The diagnosis and treatment of emerging respiratory diseases with Chinese traditional medicine have transformed from empirical medicine to evidence-based medicine. The effects of Lianghua Qingwen Capsules and Jinhua Qinggan Capsules were first verified by randomized double-blind trials. And the time for exploring vaccines is largely shortened.

In clinical treatment, we're still confronted with a lot of problems. Ten years ago, we concluded that as long as the adrenocortical hormone was applied to the right patients with the right dose at the right time, the survival rate could be elevated. Yet this conclusion failed to be proved in saving critical patients with H5N1 and H1N1. The application of NIV in critical patients at early stage can decrease the intubation rate and increase the survival rate. Though some experiments suggest that NIV would enhance the possibility of air cross infection, this still lacks clinical proofs. The convalescent plasma transfusion is proved effective to save critical patients (e.g., the patients with H5N1 and H1N1), yet the plasma source is limited.

To sum up, the public and scientists have attached much

Figure 4. Prof. Joseph Sung shares his thoughts in clinical treatment of SARS.

more alertness and importance to emerging respiratory diseases, and the transparency of information has been tremendously improved. Encouraging advances have been achieved in the timely detection of pathogen and infective route, rapid diagnosis and the development of reagents. However, the research and development of effective antiviral drugs (e.g., new anti-neuraminidase, SiRNA inhalant), the drugs inhibiting lung injury induced by cytokine storm, as well as the drugs and technology in clinical treatment (e.g., universal antibody), still fall far behind the current demand of prevention and control, whereas all these are in urgent need for the treatment of critical victims. Hence, we still need to make more efforts.

Clinical treatment of SARS

Kenote speaker: Joseph Sung, MD, PhD.
Department of Medicine and Therapeutics, The Chinese University of Hong Kong.

SARS is a tri-phasic illness, which experiences the viral replicative phase, immune hyperactive phase, and pulmonary-destruction phase. There is still no therapy for SARS-Cov infection, but some medicines have certain effect on the treatment of SARS according to the clinical experience in the outbreak of SARS and related studies. Some follow-up studies in human beings and animals confirm interferons's function on SARS, especially in early stage and prophylaxis. Clinical experience and studies also show that protease inhibitors, monoclonal antibody, convalescent plasma, siRNA and TLR agonists work in the treatment of SARS, but they still require further randomized studies. Though corticosteroid has clinical and radiological responses and effect on the suppression of cytokines, it still needs to be cautiously used because of its side effects such as reduced viral clearance, increased bacterial superinfections, metabolic complications of hyperglycaemia, hypertension, hypokalemia, *etc.* Researches on therapies against severe respiratory viral infections are urgently needed.

It is dangerous to expose to exhaled air from patients generally within 1m during common respiratory therapy (e.g., oxygen therapy, NIV, jet nebulizer). 5 case-control and 5 retrospective cohort studies from 2003-2010 related to SARS identified & rated using the GRADE system suggest that an increasing risk of transmission of SARS to healthcare workers are associated with 4 procedures: tracheal intubation, manual ventilation before intubation, tracheotomy and NIV. Thus, preventive measures against transmission should be taken, such as, avoiding high pressure during NIV via single circuit especially connection to whisper swivel device, significant sideway dispersion to 13 and 23 cm occurs during coughing despite coverage by N95 or surgical mask respectively, isolation rooms with negative pressure or adequately ventilated single rooms important for high risk patients. More information about prudent isolation of the patient coupled with protective measures for care providers and other patients are introduced on WHO guidelines (Figure 4).

From SARS to novel animal & human CoV

Keynote speaker: Kwok-Yung Yuen
Chair of Infectious Diseases, Department of Microbiology, University of Hong Kong, Academician, Chinese Academy of Engineering.

The SARS epidemic in 2003 was traced from human to civets at the wild life market of South China. However subsequent studies showed little evidence of infection of wild civets and farm civets. Further animal surveillance showed that the natural reservoir of ancestral virus, the bat SARS coronavirus (CoV), is in the Chinese horseshoe bats. This important finding has sparked a rush for virus hunting in bats. We have then discovered more than 12 novel CoVs in many types of animals and especially in bats and birds which have a unique immune system allowing them to shed virus for prolonged period with no symptoms. More importantly, we discover in 2007 the close relatives of the novel SARS-like virus called Human Coronavirus EMC (hCoV-EMC), now circulating in Middle East, in the Tylonycteris-bat-coronavirus-HKU4(Ty-BatCoV-HKU4) and Pipistrellus-bat-coronavirus-HKU5(Pi-BatCoV-HKU5), which are carried by the lesser bamboo bat (Tylonycteris pachypus) and Japanese Pipistrelle bat (Pipistrellus abramus) in Hong Kong. We discuss the importance of these findings in relationship to the presently looming epidemic at the Middle East in this lecture (Figure 5).

The evidence-based medicine study on the treatment of influenza with Traditional Chinese Medicine (TCM)—the study on the prevention and control of influenza with Lianhua Qingwen Capsules

Keynote speaker: Yiling Wu

Academician, Chinese Academy of Engineering, Famous TCM Expert.

Viral respiratory infectious diseases have become a significant threat to the health of human being. In TCM, there are rich records about the prevention and control of epidemic diseases, which accumulate abundant experience for the prevention and control of viral respiratory infectious diseases. The system "The Diagnosis and Treatment of Collateral Disease" is first innovated on the base of the past experience. It explores the pathogenesis and development of epidemic febrile diseases with collateral spatial concept of "three-dimensional network system", the research framework of collateral disease. Accordingly, the therapeutic strategy of "active intervention" is put forward. Combined with the ancient Chinese therapeutic experience, the new medicine against cold and influenza, Lianhua Qingwen Capsule granted with Chinese national patent, is developed.

Basic research shows that the new drugs have the advantage of integral regulation. It has the functions of broad-spectrum antivirus and apparent inhibition of influenza virus, parainfluenza virus, H1N1, H3N2, SARS, H5N1, H9N2, and EV71, *etc.* It effectively inhibits various bacteria to facilitate the treatment of polyinfection. Moreover, it is antipyretic, anti-inflammatory, antitussive, expectorant, and immunoregulatory. The time that Lianhua Qingwen Capsules takes to turn viral nucleic acid and to relive all the influenza syndromes is no different to that of Oseltamivir ($P>0.05$). Lianhua Qingwen Capsule even excels Oseltamivir in abating the severity of respiratory diseases, relieving the syndromes such as cough, fever, headache, feebleness, and muscular soreness, *etc.* ($P<0.05$, 0.01). No adverse drug effect is found. These results come from 256 multicenter, randomized, double-blind, Oseltamivir-controlled clinical studies led by Beijing You An Hospital Affiliated to Capital Medical University during the outbreak of H1N1 in 2009. Thus, we can see the unique advantages of TCM in treating H1N1, as well as its significance in coping with the virus-induced public health events.

Section 2: Follow-up studies of SARS and the latest advancements of studies on respiratory infectious diseases

Rapid preparation and development of therapeutic antibodies for emergency responding to H7N9 and other unexpected emerging infectious diseases

Keynote speaker: Ling Chen

Deputy Director of State Key Laboratory of Respiratory Disease, Professor of Guangzhou Medical College and Guangzhou Institute of Respiratory Disease, The First Dean of Guangzhou Institute of Biomedicine and Health (2003-2008).

Great achievement has been made in the study of emerging respiratory infectious diseases by State Key Laboratory of Respiratory Disease over the ten years. In 2003, State Key Laboratory of Respiratory Disease first confirmed the pathogen of SARS in Guangdong, later discovered its immune inflammatory mechanisms, established early diagnosing method and put forward three major principles for treatment. Its suggestion of strict control over wildlife markets effectively prevented the reoccurrence of SARS. In 2006, the year of avian influenza, State Key Laboratory of Respiratory Disease presided over the establishment of Guangzhou Cooperation Center for the Prevention and Control of Avian Influenza and swiftly developed the drugs, Oseltamivir and Zanamivir. The respiratory pathogen detecting technique based on the technique of fluorescent quantitative PCR has been invented, particularly, the portable isothermal pathogen detecting system.

How to cope with the emerging respiratory infectious diseases? The State Key Laboratory of Respiratory Disease has explored how to rapidly design and construct antigen (with reverse genetics, Ad-GFP) without pathogen. If there exist convalescents, we can quickly screen humanized monoclonal neutralizing antibody by B-cell clone, high-throughput sequencing, or phage display, *etc.* If there are no or not enough convalescents, we can first immunize macaque, then quickly screen and manufacture the monoclonal neutralizing antibody from macaque by B-cell clone, high-throughput sequencing, and phage display, *etc.* Multiple clinical studies prove that "Human-macaque chimeric mAbs galiximab (IDEC-114, anti-CD80) and lumiliximab (IDEC-152, anti-CD23), in which the VH and VL from cynomolgous macaques were fused with human constant regions. In the clinical studies of phase I and II, no human anti-galiximab or anti-lumiliximab antibodies were detected in humans." (Leonard JP, Friedberg JW, Younes A, Fisher D, Gordon L, *et al.* 2007; Rosenwasser LJ, Busse WW, Lizambri RG, Olejnik TA, Totoritis MC, *et al.* 2003) Hence, after production and preparation, humanized monoclonal antibody could be available to large numbers of people, which is significant in a massive outbreak of emerging respiratory infectious diseases. Macaque antiserum can be used for emergency after sterilization by radiation or chemical means.

As for the study on the H7N9, State Key Laboratory of Respiratory Disease has made the influenza vaccine of H7N7 (A/Netherlands/219/2003) PR8, and Replication-deficient adenovirus vector Ad5-H7N7. The HA sequence of H7N7 (A/Netherlands/219/2003) and that of H7N9 are 96% identical.

The anti-HA7 serum has already been produced from immunized mice. Its antibody can neutralize the H7N7 virus. Because of its 96% similarity, it must be able to identify H7N9. The anti-HA7 serum is readily available to the researches in need (Figure 6).

Figure 5. Prof. Kwok-Yung Yuen.

Figure 6. Prof. Nanshan Zhong and Dr Ling Chen.

The molecular mechanism by which highly pathogenic H5N1 and SARS CoV lead to acute lung injury

Keynote speaker: Chenyu Jiang
Director, Department of Biochemistry and Molecular Biology, Beijing Union Medical College, Researcher, Basic Medical Research Institute of Chinese Academy of Medical Science.

The emerging infectious diseases (e.g., SARS-CoV, H5N1, H1N1) infect people's respiratory system and then lead to Acute Lung Injury and Acute Respiratory Distress Syndrome. Based on the clinical phenomena, our research group conducts researches at patient, animal and molecular levels, explaining its pathogenesis, so as to develop possible drugs for clinical prevention and treatment and promote the study of translational medicine.

Our discoveries are as following:

I. The molecular mechanism: the SARS virus and HPAI H5N1 virus cause the imbalanced regulation of renin angiotensin system and so lead to the pulmonary injury.
II. HPAI H5N1 virus can cause an autophagic death of pulmonary epithelial cells and then results in ALI, yet Chloroquine has remarkable effect on the treatment of it.
III. Monoclonal antibodies IL-17 and IP-10 can be applied to the treatment of H1N1 virus in 2009.

In conclusion, the studies on SARS-CoV, HPAI H5N1 and H1N1 attest that the recombination of ACE2, Chloroquine, Monoclonal antibodies IL-17 and IP-10 can be used to the treatment of ALI caused by corresponding pathogens accordingly.

The study on new mechanisms of CoV replication and new drug target

Keynote speaker: Deyin Guo
Dean, School of Basic Medicine, Wuhan University, Deputy Director, State Key Laboratory of Virology.

Viewing that wild animals (such as bats) still carry SARS or nCoV-like viruses and society and ecology keep change, threat of CoV to human society will exist for a long time. Therefore, it is still of great practical significance and medical values to strengthen the basic research on cross-species transmission and infection pathogenesis of CoV and develop effective prevention and control measures. CoV is the largest RNA virus, and its genome RNA may be also the largest RNA in nature. Therefore CoV evolves some special mechanism to ensure the reliable copy of its huge RNA Genome and maintain moderate variation rate at the same time so as to easily adapt to the new replication environment, for instance, the cross-species transmission. The purpose of this study is to reveal the molecular mechanism of the CoV replication, to uncover new drug targets for the CoV, and establish high-throughput screening system for anti-CoV drugs. Our discoveries are as followings:

I. SARS-CoV is found to encode a special protein (nsp14), which has the activities of both RNA3'-5'exonuclease enzyme and N7-MTase. These two kinds of activities respectively process and modify the 3'- and 5'-end of viral RNA.
II. We Found that the non-structural protein of the SARS-CoV, Nsp10, activate nsp16 to exercise the function of 2'-O-MTase, and explained nsp10/nsp16 complex crystal structure (Chen *et al.*, 2011, PLoS Pathogens).
III. SARS-CoV's synthesis of two non-classical subgenomic RNAs by discontinuous transcription reveals that discontinuous transcription occurs in the synthesis stage of negative-strand.
IV. Secure SARS-CoV replicon carrying reporter gene has been constructed.
V. We have established High-throughput drug screening system based on the yeast targeting SARS-CoV nsp14

Figure 7. Dr. Deyin Guo, Prof Nanshan Zhong, Dr Ling Chen and Prof. Shuwen Liu (from left to right).

Figure 8. Prof. Fu Gao.

(RNA exonuclease and the methyl modified enzyme), and based on nsp10/nsp16 complex structure, the polypeptide inhibitor is designed to inhibit the activity of the 2'-O-MTase. (KE *et al.*, 2012).

In conclusion, these results contribute to a better understanding of CoV' replication and pathogenic molecular mechanism, and lay a theoretical foundation for the research and development of new anti-CoV drugs (Figure 7).

Influenza virus entry and release: from 2009 pH1N1 to bat-derived virus genome and H7N9

Keynote speaker: Fu Gao

Deputy Director, China's CDC, Director, Key Laboratory of Pathogenic microorganisms and Immunology, Chinese Academy of Science.

Influenza A virus entry and fusion are mediated by interaction between its envelop protein hemagglutinin (HA) and the host cell surface sialic acids (SA). Its release is mediated by the viral surface envelop protein NA. The HA and NA of the 2009-pandemic H1N1 influenza virus are characterized by its similar structure to 1918 HA and 150-cavity deficient NA. The recent isolated bat influenza A virus genome encodes distinct HA (H17) and NA (NA-like N10). Our functional and structural analysis of the H17 and N10 reveals that they are not canonical HA or NA and the virus is most likely using a unique entry and release mechanism. Some new receptors must exist for the bat-derived influenza-like virus (Figure 8).

A highly potent and safe SARS submit vaccine based on the receptor-binding domain in SARS-CoV spike protein

Keynote speaker: Shibo Jiang

Director, Institute of pathogenic microorganisms, Fudan University.

The spike (S) protein of SARS CoV, a type I transmembrane envelope glycoprotein, is responsible for virus binding and fusion. Although the full-length S protein can induce neutralizing antibody responses, it also contains some predominant immune components inducing antibodies that do not neutralize, but rather may enhance virus infection. Some of these epitopes may even induce harmful immune or inflammatory responses. Our previous studies have shown that the receptor-binding domain (RBD) in the S protein contains the critical neutralizing domain (CND). We designed a recombinant subunit vaccine containing the RBD and used it to immunize mice and rabbits. We found that this RBD-based vaccine can elicit highly potent neutralizing antibody responses with neutralizing antibody titers as high as 1:15,000, about 20- to 100-fold higher than those of the mouse antisera induced by DNA vaccines and vaccine virus vectors encoding the full-length of SARS-CoV S protein. The vaccinated mice were fully protected from the challenge with different strains of SARS-CoV. Using this vaccine, we have induced a series of neutralizing mAbs that can recognize different conformational epitopes in RBD and these mAbs can cross-neutralizing SRAS-CoV strains isolated from the early and late epidemic stage of SARS and from civet cats. Last year, the NIH of the United States has decided to support the preclinical development of our RBD-based SARS vaccine, but stop to support the further development of other SARS vaccines. Most recently, we have used the similar approach to develop RBD-based vaccine against the novel hCoV-EMC that has caused an outbreak of SARS-like disease in Middle East and Europe.

Modulation of potential respiratory pathogens by pH1 N1 viral infection revealed by next generation sequencing

Keynote speaker: TSUI Kwok Wing, Stephen

Professor, School of Biomedical Science; Head, Division of

Genomics and Bioinformatics; Associate Director, CUHK-BGI Innovation Institute of Tran-somics; Director, Hong Kong Bioinformatics Centre; Director, Centre for Microbial Genomics and Proteomics; The Chinese University of Hong Kong.

While much effort has been made to characterize influenza A pdm09 virus (pH1N1), the flu that was responsible for the fourth influenza pandemic, study on the composition of bacteria that lead to secondary infection is lagging behind to some extent. Therefore, we have recruited pneumonia patients with and without pH1N1 infection in Guangzhou and characterized their oropharyngeal microbiota by the unbiased high-throughput sequencing method. While no significant difference were observed between common bacterial pneumonia-causative agents (Acinetobacter and Streptococcus species), previously unreported Pseudomonas species equipped with chemotaxis and flagellar assembly genes significantly increased (>20-fold) in the pH1N1-infected group. Bacillus and Ralstonia species that also increased significantly (5-10-fold) were also found to possess similar signaling and motility genes. In contrast, no such genes were found in oral commensal Prevotella, Veillonella and Neisseria species, which decreased significantly, or in either Acinetohacter or 10 out of 21 Streptococcus species, including Streptococcus pneumoniae. Our results support the notion that pH1N1 infection provides a niche for previously unnoticed potential respiratory pathogens that were able to access the lower respiratory tract with weakened immunity. Besides the metagenomics study on influenza, similar work on HIV/AIDS and tuberculosis will be briefly introduced (Figure 9).

The prophylactic and therapeutic potentials of siRNAI Inhibitors for fighting emerging respiratory viral infections

Keynote speaker: Yang Lu
Chief scientists of Sirnaomics, U.S.

One of the most challenging concerns for anti-influenza drugs such as NA inhibitors is development of drug-resistant strains (some circulating strains were found to be uniformly resistant to Oseltamivir or Adamantanes). We have developed a proprietary algorithm for designing siRNAs targeting conserved regions of influenza A viruses in an effort to defeat their drug-resistance. We selected potent siRNA duplexes targeting H5N1 (Avian) and H1N1 (swine) viruses through *in vitro* screening in MDCK cells. After evaluating several polymer-based *in vivo* delivery systems, we formulated most potent siRNA inhibitors with polymer nanoparticles and administered these as aqueous solutions into mouse airway via intranasal and intrachacheal instillations. Potent antiviral activities were observed in the mouse lung that protected treated mice when challenged with a 10× lethal dose of H5N1. A "potency enhancing motif" (PEM) was found that was able to enhance prophylactic and therapeutic potencies when it was embedded in the siRNA sequence. We compared antiviral activities among siRNA inhibitors and chemodrugs currently used in the clinic using the mouse model and found that our siRNA drug demonstrated greater efficacy/potency and therapeutic benefit. Using bioinformatics tools we have identified combinations of SiRNAs against different viral segments of the genome that, when combined, cover 99.8% of all flu strains that have infected humans. The newly identified siRNAs showing potent anti-H1 N1 activity in MDCK cells have 100% homology to viral threats from H51\11, H3N2, H7N2 and H9N2, *etc.* We are currently evaluating these siRNAs with polymer-based nanoparticles for broader spectrum anti-influenza activity in animal models.

The ORF3a-like protein of human CoV functions as a viroporin that regulates the virus production

Keynote speaker: Bing Sun
Chinese Director, Shanghai Pasteur Institute.

A locus located between the spike and envelope gene is conserved in all human CoV, and contains a complete or truncated open reading frame (ORF). Previously, we have shown that this locus accessory protein 3a (ORF-3a like protein) from SARS-CoV forms ion channels and regulates virus release. In the present study, we explored whether, the ORF4a protein of HCoV-229E has a similar functions. Our findings showed that ORF4a proteins expressed in the infected cells and localized at the endoplasmatic reticulum/Golgi intermediate compartment (ERGIC). The ORF4a proteins formed homo-oligomers through disulfide bridges and possessed ion channel activity in both Xenopus oocytes and yeast. The HCoV-229E ORF4a ion channel is permeable to K+. Furthermore, the viral production was decreased when the ORF4a protein expression was suppressed by siRNA in the infected cells. Since ORF-3a like protein is relative conserved in human CoV, it is potential target for drug development to control virus infection.

Section 3: The press conference

Geographically, the rural areas seem to have more contacts with poultry (as the source of infection with H7N9), but why urban area like Jiangsu, Zhejiang and Anhui provinces are identified as the high risk areas of H7N9?

The virus spreads along the flyway of migratory birds, covering the Yangtze River Delta. But this is not the only reason as there might be some genetic mutation, conversion or reassortment, which can occur anywhere. Generally speaking, the avian influenza usually strikes populated areas with developed transportation. In this way,

Figure 9. Prof. Kwok Wing Tsui.

Figure 10. Rrof. Nanshan Zhong, Prof Fu Gao and other experts are taking questions in Press conference.

the avian influenza spreads quickly and induces infection once migratory birds or poultry are infected.

Although it remains elusive why urban areas are of high-risk infection, it can be partly explained that the cities mentioned above enjoy developed transportation and frequent gathering and distribution of poultry. However, poultry in rural areas is free-range in fixed places, thus there is less infection among poultry (Figure 10).

Now it is the right season for birds' northward migration. Since genetic reassortment between migratory birds and poultry occurred in Shanghai, Jiangsu and Zhejiang provinces, will the genetic reassortment or mutation happen again in their continuing northward migration? As a doctor and scientist, could you predict the genetic reassortment?

The reoccurrence of genetic reassortment is possible. Avian influenza virus is detected from the analysis of a genetic sequence. The genetic sequence (e.g., H9N2, H11N9) has something in common with that of poultry, and this proves the possibility of the genetic reassortment between wild birds and poultry. As wild birds have their own flyways, there is the possibility that such genetic reassortment occurs during their migration.

Why we now pay more attention to genetic reassortment? That's because the H7N9 with genetic reassortment is characteristically low pathogenic in poultry (i.e., no or slight symptoms), unlike H5N1. But once infected by human, it becomes highly pathogenic. Thus, it is more complicated than previous avian influenza (e.g., H5N1), involving agricultural and sanitary situations. If we detect low pathogenic H7N9, we cannot kill poultry throughout the whole province or China. In the past, poultry was killed within the range of 3 kilometers in case of H5N1. However, this time we adopt the interim strategy of killing only the poultry within the H7N9-stricken area.

Based on the current sci-tech condition, there is no way to study the genetic reassortment. We can only keep close monitoring over H7N9, unable to predict its possible variations. This is very passive. Nevertheless, there is mutative regularity in common influenza (e.g., H1N1, H3N2, *etc.*), that is, small or large mutations will regularly occur over a certain period. Particularly, small mutation can often be predicted, so it is possible to produce some vaccines preventing against influenza A. However, as for H7N9, we are unable to predict its mutation.

As a scientist, could you please explain why the transmission of bird flu was bird-to-bird in the past, while now it becomes bird-to-human?

It is hard to explain. There are many factors involved, such as human's close contact with poultry and travel to various places thanks to the well-developed transportation. But now no one can shed light on the real reason for bird-to-human transmission. After all, the fowl plague was something thousands of years ago.

So far, there are no evidences for human-to-human transmission. Do scientists have any definite evidence to prove that interpersonal transmission is impossible? Once the interpersonal transmission occurs, what shall we do to interfere with it?

There is no evidence to prove the impossibility of human-to-human transmission. At present, we can only say no interpersonal transmission is found according to the fact, but this cannot prove the impossibility of human-to-human transmission. H7N9 is still changing, so there are all kinds of possibilities. For example, SARS at the beginning was low infectious. Latter, its infectivity increased. Currently, we cannot predict that H7N9 is impossible to spread among human beings. The most conservative yet most effective approach is to isolate infected people once the interpersonal transmission occurs. When the swine influenza broke out in 2009, the strategy "blocking entry from outside and preventing proliferation inside" was adopted and proven to be

effective, controlling the spread of virus to considerable extent. During the prevention, we research and develop vaccines and other medicines, for vaccine is a relatively good approach to prevent infection.

If people who have more contacts with poultry are more easily infected, why a large number of confirmed victims without any contact with poultry can also be infected? What kind of people are the most susceptible to H7N9?

This is an urgent problem for our scientific and technological workers to explore. Generally speaking, direct contact leads to infection; but it remains unclear whether indirect contact with poultry (e.g., contact with poultry's excrement and feathers) will result in infection. In one of the H5N1 cases before, a worker in Fujian Province often sat on benches stained with a lot of bird droppings when he passed through the woods replete with migratory birds. Though he had no direct contact with birds, he was infected by the bird flu too. So, indirect contact is a possible reason for infection. Above all, there must be a source of infection, but it is still not clear whether the infective route is direct or not. The infectious source of H5N1 has been confirmed to be poultry, but it is unsure whether that of H7N9 is chicken. It could be waterfowls, migratory birds and pigeons, *etc.* People having contact with poultry are all potentially within the high-risk group. Because on one hand, a high percentage of confirmed victims have contact history with poultry; on the other hand, the gene of H7N9 is mainly from poultry.

For clinicians, how to deal with the infectious diseases like avian influenza?

H7N9 is a sporadic bird-to-human infectious disease. No interpersonal transmission is found so far. Critical patients are difficult to cure, so doctors should be much more alert to monitor virus once unexplained pneumonia and influenza-like symptoms with high hemogram are detected. It cannot be gradually observed as usual if antibiotics fail to work.

Meanwhile, it should be reported immediately if there are influenza symptoms (like a high fever) and contact history with poultry in epidemic area. Hormone should be used when patients develop ARDS, and to the right person at the right time and in the right dose. Using hormone in the early stage is incorrect, for it will slow down the virus clearance and may lengthen virus' stay in body and impair its immunity. So first of all, doctors should enhance their vigilance; second, they should be able to identify the incipient signs of patients' exacerbation. As such disease like H7N9 at a certain stage will develop quickly on some patients, thus it needs to be detected and treated timely (e.g., patients should be sent to the ICU for respiratory support), to avoid pulmonary collapse and consolidation. These are two most important requirements for doctors to cope with the infectious diseases of this kind.

Do scientists and doctors feel passive and frustrated when faced with genetic assortment of avian influenza again and again? After fighting against viruses from SARS to H5N1, do you have more preparation, experience and confidence than a decade ago? As a scientist, are you confident of winning this war?

Passive but not frustrated. "Passive" means we cannot predict and prevent viral mutation; "Frustrated" means we don't monitor virus carefully and let it spread and develop into infectious disease. But in this regard, we are proactive. Up to now, I still greatly support the measures that Guangdong Province adopted ten years ago. If an unexplained and highly infectious disease is detected, the best way is to announce it to the whole province immediately and pay attention to isolation.

I think we can defeat the virus, but it's impossible to avoid mortality, for infectious diseases always follow their own laws. We have already known the strain of this virus and are conducting genetic analysis. With the standard strain, we can contact WHO and produce vaccines quickly. It took less than 5 months for China to produce H1N1 vaccine in 2009, which was a great success, and nearly 100 million people were injected with this vaccine. Therefore, I am very confident in this war. Now we are ready for vaccine's production. Once we get access to the standard strain, we can immediately make adjuvants.

Epilogue

This symposium gathered almost all the prominent Chinese experts, scholars and researchers in the fields of respiratory disease, biomedicine, virology, microbiology, molecular biology, and genomics, *etc.* to report and communicate their achievements of studies in SARS and other emerging respiratory infectious diseases over the past ten years. Hopefully, it will give some insights for the related researchers and scientists and make contribution to the improvement of human's health.

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