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COMMENTARY

Understanding bat SARS-like coronaviruses for the preparation of future coronavirus outbreaks — Implications for coronavirus vaccine development

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ABSTRACT

The severe acute respiratory syndrome coronavirus (SARS-CoV) first emerged in 2003, causing the SARS epidemic which resulted in a 10% fatality rate. The advancements in metagenomic techniques have allowed the identification of SARS-like coronaviruses (SL-CoVs) sequences that share high homology to the human SARS-CoV epidemic strains from wildlife bats, presenting concrete evidence that bats are the origin and natural reservoir of SARS-CoV. The application of reverse genetics further enabled that characterization of these bat CoVs and the prediction of their potential to cause disease in humans. The knowledge gained from such studies is valuable in the surveillance and preparation of a possible future outbreak caused by a spill-over of these bat SL-CoVs.

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The severe acute respiratory syndrome coronavirus (SARS-CoV) first emerged as an infectious agent in 2003, causing severe and sometimes fatal respiratory disease in humans. The virus had spread rapidly from Southern China to Hong Kong and to the rest of the world, resulting in the SARS epidemic which lasted for 4 months and was eventually put to an end through the implementation of intensive public health measures. The epidemic caused a total of more than 8000 infections with a fatality rate of 10% and a considerable amount of social hardship and economic loss.[1]

Ever since the emergence of SARS, much effort has been made to understand the origin and the emergence of SARS-CoV. Cross-species jumping events have led to the emergence of SARS-CoV in humans. Small animals such as palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procynonoides*) sold in live-animal wet markets in Guangdong Province of Southern China were the immediate sources of the virus transmitted to humans during the 2003 SARS outbreak.[2] Nonetheless, numerous observations suggest that palm civets and other small animals were merely conduits for SARS-CoV transmission to humans rather than the natural wild-life reservoir harboring the virus. Firstly, viral RNA detection and anti-SARS sera were only detected in civets from marketplace but not in farmed or wildlife civets, indicating that palm civets are not widely infected by SARS-CoV.[3] In addition, sequence comparison of various civet SARS-CoV isolates revealed high non-synonymous/synonymous nucleotide substitution ratio, indicating ongoing mutation and evolving process of the virus in civets, further suggesting that palm civets are unlikely the natural reservoir of the virus.[4] High prevalence of anti-SARS-CoV antibodies was detected in serological surveys involving people not infected with SARS-CoV but worked in retail business of palm civets, indicating that cross-transmissions of a precursor SARS-CoV probably occurred before the actual SARS epidemic.[2]

In the past, the inability to culture and isolate some viruses greatly hindered the study of these viruses and the understanding of viral ecology and diversity. The development of metagenomic sequencing technology has enabled the discovery of new viral species in a culture-independent and sequence-independent manner, contributing significantly to the field of viral metagenomics.[5] With the advancements made to the technology, it is now able to carry out high throughput sequencing of large viral genomes with only miniscule amount of viral DNA samples.[6] This greatly aided in the discovery of many coronaviruses, which are the largest RNA viruses known so far, particularly in bats. Since the SARS epidemic, a huge diversity of SARS-like coronaviruses (SL-CoVs), which are coronaviruses of high sequence homology to human SARS-CoV epidemic strains, have been discovered from bats in many countries across the world, including South East Asian, European and African countries (for a review see ref.[7]). However, most of these bat SL-CoVs failed to grow in cell culture, presenting barriers to understand viral replication and pathogenesis. Improvements in the reverse genetic systems for coronavirus have allowed these methods to be widely used in the construction of live SARS-CoV.[8] Through the use of these systems, the enormous amount of information derived from metagenomics sequencing could be utilized to create synthetic coronavirus clones, allowing studies of these viruses to be performed and to identify those which have potential to emerge and cause diseases in humans. Herein, we summarized the findings in some recent publications to highlight the importance of these technological advances in the study of SL-CoVs which have the potential to cause future pandemics.

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In 2005, 2 separate groups reported the identification of genomic sequences of SL-CoVs from horseshoe bats (genus *Rhinolophus*) in China.[9,10] This was an important breakthrough in identifying bats as the natural reservoir of SARS-CoV. These bat SL-CoVs share high genomic sequence homology of 88% to 92% as the human SARS-CoV epidemic strains. However, variations of the spike (S) gene sequence hovered between76 to 78%, with greater sequence differences in the S1 domain (68%) compared to the S2 domain (92 to 96%). The S gene of coronavirus encodes the surface spike (S) protein, which consists of the S1 and the S2 regions responsible for receptor binding and cell-viral membrane fusion respectively during viral entry process.[11] Unlike the human SARS-CoV which utilizes the angiotensin-converting enzyme 2 (ACE2) as the receptor for viral entry, these bat SL-CoVs cannot recognize the ACE2 receptor and hence are unable to infect human cells,[12] suggesting that they are unlikely the direct progenitor of human SARS-CoV. More recently in 2013, Ge *et al* reported the discovery of novel bat SL-CoV sequences RsSHC014 and Rs3367 from Chinese horseshoe bats (*Rhinolophus sinicus*) that share high sequence homology as human SARS-CoV, particularly in the receptor binding domain (RBD) of the S1 region of the S protein which is required for the binding to the ACE2 receptor.[13] These SL-CoV sequences represent the closest related ancestor of the human SARS-CoV. In the same study, a live bat SL-CoV termed WIV1 with high sequence identity (99.9%) to Rs3367 was successfully isolated through culturing in Vero E6 cells for the first time, and it was demonstrated to be able to infect cells of both bat and human origin via the recognition of ACE2 receptor. This not only provided concrete evidence that SARS-CoV originated from bats, but also demonstrated the possibility of a re-emergence or emergence of SARS and other SARS-like viruses in humans with the continual persistence of SL-CoVs in bat reservoirs.

Using the metagenomic findings on bat SL-CoVs, Menachery *et al* described the use of the reverse genetic system to generate full-length WIV1-CoV and SHC014-CoV, as well as chimeric viruses consisting of the WIV1 and SHC014 S protein in the backbone of the SARS-CoV mouse adapted strain MA15.[14,15] By *in vitro* and *in vivo* methods, these SL-CoVs were characterized. Full-length WIV1-CoV and SHC014-CoV, as well as chimeric WIV1-MA15 and SHC014-MA15, replicated efficiently in Vero cells through the binding to human ACE2 receptor. Replication was also observed in primary human epithelial cells at levels similar to the human SARS-COV epidemic strain Urbani. In mouse models, both WIV1-MA15 and SHC014-MA15 caused weight loss but limited disease as opposed to MA15 which resulted in serious disease and mortality. In addition, both full-length WIV1-CoV and SHC014-CoV, although unable to result in any observable weight loss in mice, were able to replicate in lungs at an attenuated level compared to human SARS-CoV Urbani. All together, these findings suggest the inability of WIV1-CoV and SHC014-CoV to cause serious disease, and further adaptations of the viruses would be necessary for efficient infection and replication in human. Nonetheless, augmented replication of WIV1-CoV and SHC014-CoV in the presence of the human ACE2 receptor signifies their potential pathogenicity and emergence in human.

In the same studies of WIV1-CoV and SHC014-CoV, Menachery *et al* also evaluated the effects of some therapeutic monoclonal antibodies (mAbs) and a vaccination approach of SARS for the treatment and protection against WIV1-CoV and SHC014-CoV. MAbs that target the human SARS-CoV S1 protein within the RBD region could cross-neutralize WIV1-MA15 *in vitro* and *in vivo*, while a mAb that target S1 outside the RBD region could only confer partial neutralization.[14] On the other hand, the same mAbs were unable to neutralize SHC014-MA15 as efficiently compared to WIV1-MA15.[15] This is attributed to the different binding affinity of the mAbs to the epitopes on the WIV1 and SHC014 S proteins. In mice vaccinated with inactivated whole SARS-CoV virion, protection against WIV1 and SHC014 viruses was found to be incomplete.[14,15] These findings indicate that the use of mAbs targeting the S1 region of the SARS-CoV S protein and inactivated whole SARS-CoV virions particles to treat and prevent SARS-CoV infection may not be capable of conferring full protection against WIV1-CoV and SHC014-CoV in an event of an outbreak caused by these SL-CoVs.

Knowing that SL-CoVs circulating in bats have certain potential to emerge in humans and that current therapeutic and vaccine strategies may not be effective enough to protect against bat SL-CoVs, it is important to develop novel therapeutics and vaccines that are able to protect against not only the human SARS-CoV, but also bat SL-CoV strains. Passive immunotherapy involving the administration of mAbs is a promising antiviral treatment and prophylactic strategy, as evident from ZMapp and palivizumab which can effectively prevent Ebola and respiratory syncytial virus infections in humans respectively.[16,17] A number of neutralizing mAbs, which act by binding to the S protein of SARS-CoV to inhibit viral entry, have been characterized pre-clinically, serving as potential candidates for passive immunotherapy for SARS (for a complete review see ref. 18). MAbs that bind to the RBD of the S1 domain neutralizes viral infection by preventing RBD interaction with the ACE2 receptor while anti-S2 SARS-CoV-neutralizing mAbs inhibit viral entry by disrupting the viral-cell membrane fusion process. Since the S2 region of the human SARS-CoV and bat SL-CoVs are more conserved compared to the S1 region, mAbs that target the S2 domain are broadly neutralizing and can confer cross-protection against bat SL-CoVs.[18-20] The identification of broad-spectrum inhibitors targeting highly conserved proteins in human SARS-CoV and bat SL-CoVs, such as the 3C-like protease, as well as inhibitors that target important host proteins required for viral entry and pathogenesis, such as host surface and endosomal cysteine proteases, are other feasible ways to develop novel broadly-neutralizing SARS therapeutics (for a full review see ref.[21]). For vaccine development, a comprehensive understanding of CoV-induced immunity is necessary. Identification of conserved epitopes in human SARS-CoV and bat SL-CoVs that give rise to cross-neutralizing antibody and T cell responses can lead to vaccine strategies that cross-protect against all the viruses.

The emergence of another coronavirus, the Middle East respiratory syndrome coronavirus (MERS-CoV), a decade after the SARS epidemic, certainly proves that the introduction of novel coronavirus to human from zoonotic sources is not a one-off event. Although MERS-like coronaviruses were found

in wildlife bats suggesting their bat origin, dromedary camels play a critical role as the main reservoir for the continual zoonotic transmission of the virus to the human population in the Middle East.[22] MERS-CoV, like the SARS-CoV, causes serious lower respiratory tract infections in human as well as extrapulmonary manifestations which can be fatal, although dissimilarities in transmission, clinical presentation and pathogenesis between the 2 could be observed.[23] As of 27th July 2016, the World Health Organization (WHO) has reported a total of 1791 MERS cases with 640 deaths, representing a fatality rate of around 35.7%, a rate much higher than that of SARS. While the SARS epidemic occurred swiftly and was effectively brought to an end after 4 months of intensive public health efforts, MERS-CoV has persisted for more than 3 y and the number of affected individuals continues to escalate. Fortunately, MERS-CoV remains incapable of sustained human-to-human transmission compared to SARS-CoV that is relatively well-adapted to transmission between humans. Intensive research efforts are now being placed on MERS-CoV to understand this novel and more virulent coronavirus, as well as to develop therapeutics and vaccines (for a full review see ref.[24]).

Although SARS-CoV and MERS-CoV are both classified under the order nidovirales and family coronaviridae, they are phylogenetically distinct, with SARS-CoV belonging to lineage B and MERS-CoV belonging to lineage C of the betacoronavirus genus.[25] Nonetheless, several research groups have reported the development of broad-spectrum and pan-coronavirus treatment and vaccine strategies, including those effective against both SARS-CoV and MERS-CoV. For instance, drugs that target the coronavirus protease, papain-like protease (PL-pro), inhibit both SARS-CoV and MERS-CoV *in vitro*.[26] A glycopeptide antibiotic, known as teicoplanin, block viral entry of SARS-CoV and MERS-CoV into host cells by inhibiting cathepsin L, an enzyme required for viral entry via the endosomal pathway.[27] Vaccination of a conserved $CD4^+$ T cell epitope located within the MERS-CoV nucleocapsid protein in transgenic mouse models was able to induce cross-reactive T cells that could provide cross-protection against SARS-CoV challenge.[28] In the light of a dwindling interest in SARS research in the last 5 years, MERS research has contributed to advancing the development of pan-coronavirus therapeutic options that are also effectively against SARS-CoV.

Coronaviruses are capable of mutating at high frequency due to the infidelity of their RNA-dependent RNA polymerases and their high rates of homologous RNA recombination (for a full review see ref. 29). This provides the opportunity for coronaviruses to achieve high diversity and species spill-over, which can lead to possible future outbreaks in human. Ultimately, the knowledge in zoonotic bat SL-CoVs, as well as other coronavirus species, of high potential to emerge in humans will be of paramount importance in the surveillance of future outbreaks caused by coronaviruses and will steer future research direction of treatment and vaccine development.

Abbreviations

SARS-CoV	Severe acute respiratory syndrome coronavirus
SL-CoV	SARS-like coronavirus
MERS-CoV	Middle East respiratory syndrome coronavirus

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