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Comment

Bats as Animal Reservoirs for the SARS Coronavirus: Hypothesis Proved After 10 Years of Virus Hunting

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Recently, the team led by Dr. Zhengli Shi from Wuhan Institute of Virology, Chinese Academy of Sciences, and Dr. Peter Daszak from Ecohealth Alliance identified SL-CoVs in Chinese horseshoe bats that were 95% identical to human SARS-CoV and were able to use human angiotensin-converting enzyme 2 (ACE2) receptor for docking and entry. Remarkably, they isolated the first known live bat SL-CoV that replicates in human and related cells. Their findings provide clear evidence that some SL-CoVs circulating in bats are capable of infecting and replicating in human (Ge X Y, et al., 2013).

The severe acute respiratory syndrome (SARS) was the first pandemic of the new millennium. It started in November 2002 in Southern China and had spread over 33 countries, causing 8096 infections and 774 dead cases (fatality rate of 9.6%), along with huge economic losses. The etiological agent of SARS was identified as a novel coronavirus (SARS-CoV) (Drosten C, et al., 2003; Ksiazek T G, et al., 2003). However, the origin of SARS-CoV remains elusive. Although it is suggested that bats are the natural reservoirs for SARS-CoV, isolation of a SARS like virus (SL-CoV) from bats have been unsuccessful.

To trace the origin of the sudden emerging SARS-CoV, molecular epidemiological studies have been conducted by different research groups. In 2003, Guan et al. isolated SARS-CoVs from Himalayan palm civets and two other species in a live-animal market in Guangdong, China (Guan Y, et al, 2003). The Chinese SARS molecular epidemiology consortium suggested that the early-phase human SARS-CoV strains may have originated from wild animals (The Chinese SARS Molecular Epidemiology Consortium, 2004). These and other evidences suggested that palm civets were the direct source since the isolates from civets were highly related to human isolates from 2002-3 and 2003-4 SARS pandemic (Guan Y, et al, 2013; Song H D, et al., 2005; Wang M, et al, 2005). Since 2004, SL-CoVs have been identified from bats by several research groups including Dr. Shi's lab (Li W, 2005; Lau S K, et al, 2005). These bat isolates are more genetically diverse and share an overall nucleotide identity of 88% to 92% to the SARS-CoVs from humans or civets, resulting in the hypothesis that bats may be the natural hosts of SARS-CoV.

However, there are still some missing links between previously characterized SL-CoVs from bats and SARS-CoV that precipitated the 2002-3 outbreaks. 1) albeit the overall genome sequence similarity, there are significant differences in spike (S) protein between the previously known SL-CoVs and SARS-CoVs. The sequence identity of S1 fell to 64%, accompanying with insertions and (or) mutations in this region. S1 contains the receptor binding domain (RBD), which plays a key role in receptor recognition and is a major determinant of host range and cross-species infection of SARS-CoV. It was suggested that the previously known bat SL-CoV stains cannot jump from bats to civets or humans owing to the significant differences between their RBDs (Li F, 2013); 2) although SL-CoVs have been identified from different bat species, isolation of a live SL-CoVs from bats never succeed; 3) no native SL-CoV from bats could use ACE2 as receptors and infect human cells, only when its RBD is replaced with the counterpart from a human SARS-CoV strain (Li W, et al, 2003; Becker M M, et al, 2008; Ren W, et al, 2008). Therefore, these SL-CoVs seem unlikely to be the immediate precursors of civet or human SARS-CoVs (Li F, 2013).

After 10 years virus hunting, Ge et al. are now able to fill in important

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missing links associated with SARS origin and epidemic (Fig. 1). Firstly, they identified two novel SL-CoV stains RsSHC014 and RS3367 in Chinese horseshoe bats, which show higher sequence similarity (~95% identity) with human SARS-CoV than observed previously (Lau S K, et al, 2005; Li F, 2013; Ren W, et al, 2006; Tong S, et al, 2009). Particularly, their RBDs are more closely related to (85% aa identity for RsSHC014 and 96% for RS3367) SARS-CoV RBD, with perfect sequence alignment and absence of any deletion or insertion. Secondly, they reported the first isolation of a live bat SL-CoV strain (SL-CoV-WIV1) with Vero E6 cells. This isolate is almost identical to its parental virus Rs3367, with 99% nt identity and 100% aa identity even in the highly variable S1 region. Thirdly, by using this valuable isolate WIV1, Ge et al. demonstrated for the first time that a bat SL-CoV strain can exploit human-, civet- and bat-derived ACE2 as its cellular entry receptors and replicated efficiently in HeLa cells which express these receptors. They further assessed the host ranges of WIV1 with a variety of cell lines and found that cells from different species (human, pig, and bat) are able to support the virus replication at different levels. In addition, WIV1 can be neutralized by different SARS-patient sera collected in 2003. These findings suggested a much closer relationship between bat WIV1 strain and SARS-CoV.

Interestingly, although not being further discussed in their publication, one of the five key residues in RBD, the 479 aa, is an asparagine in the RS3367. The residue 479 is known to be an asparagine only in human SAR-CoVs, but not in the previously identified bat SL-CoVs or civet SAR-CoVs. It is proposed that an asparagine at position 479 has a higher binding affinity with human ACE2 and is likely to determine whether the virus can infect humans (Li F, 2013). The change of the position 479 may have played a role in the adaptation of RS3367 to an ACE2 receptor and the cross-species infections of the virus.

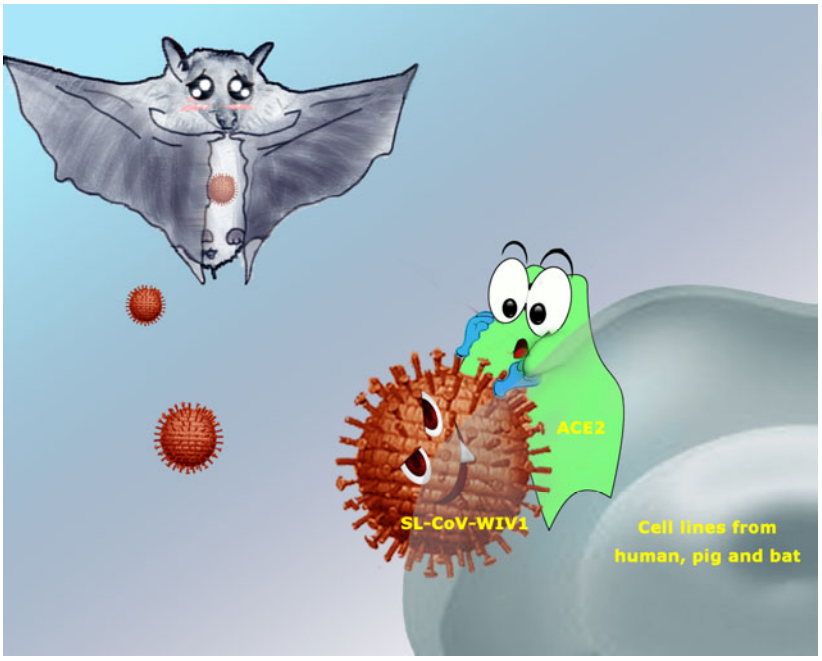

Fig. 1 Bat SL-CoV-WIV1 uses ACE2 to directly infect human cells.
A newly isolated wild-type bat SL-CoV-WIV1 is found to use ACE2 as a cellular entry receptor and replicate in human alveolar basal epithelial cells (A549), pig kidney-15 cells (PK15) and Chinese horseshoe bat kidney cells (RSKT). (Figure provided by Meng Wang, Wuhan Institute of Virology.)

In summary, Ge et al.'s findings solved fundamental questions surrounding the genesis of the SARS-CoV 2003 epidemic. They provided a plausible scenario of direct bat-to-human jump of SARS-CoV without the transmission by interim hosts. Bats are known to be important natural reservoirs of many zoonotic viruses, for example, the recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV) (Chan J F, et al, 2012). The publication by Ge et al. emphasized bats as public health threats and highlighted the importance of epidemiological and laboratory studies of bats-harboring pathogens.

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