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To the Editor

Is SARS-CoV-2 a Product of Reverse Genetics Using Vaccinia Virus-Based Recombination?

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Dear Editor,

Infection with a novel coronavirus SARS-CoV-2 is now widespread, and as of May 19th, 2020, approximately 5,000,000 cases of COVID-19 have been confirmed around the world, with more than 300,000 deaths. There has been a considerable discussion on the origin of the causative virus, SARS-CoV-2 with some evidence that it is not a laboratory construct or a purposefully manipulated virus (1).

Two independent internationally recognized research groups leaded by Prof. Ralph Baric (University of North Carolina at Chapel Hill, U.S.A.) and Dr. Zhengli-Li Shi (Wuhan Institute of Virology, China) have recently generated chimeric SARS-like viruses using reverse genetic systems and have pointed on a potential risk of SARS-CoV re-emergence from viruses currently circulating in bat populations (2).

Recent advances in molecular genetics have led to the possibility of using large DNA viruses, such as vaccinia virus (VacV), as a biological delivery system. VacV, the poxvirus used as the vaccine against smallpox, has gained widespread use as a general vector for expressing foreign proteins in mammalian cells. The ability to take up large inserts of DNA and express high levels of a foreign protein in a wide variety of cell lines has made VacV an attractive biological delivery vehicle (3). VacV vectors have been used to express and characterize glycoproteins of numerous pathogens, and some of those are being evaluated as candidates for developing prophylactic and therapeutic vaccines (4).

The most common method used to produce recombinant viruses involves the insertion of foreign genes into the thymidine kinase (TK) gene of the VacV via homologous recombination. This is accomplished through the construction of a recombination plasmid containing the VacV TK gene into the middle of which the gene of interest is inserted, appended to an efficient VacV promoter element of the desired temporal class. Confluent monolayers of cells are infected with wild-type VacV and transfected with the plasmid DNA to allow homologous recombination to occur.

This inactivates the endo-genous TK gene-producing TK-negative virus that can be biochemically selected using bromodeoxyuridine, and recombinants can be identified by a variety of screening methods (5). The frequency of such homologous recom-bination accounts for ~0.1% of the total virus (6).

Thiel *et al.* (2001) showed a possibility to clone a full-length cDNA copy of the human coronavirus genome into the VacV genome followed by production of infectious RNA transcripts from this template that can be used as a reverse-genetic system for the generation of recombinant coronaviruses (7).

van den Worm *et al.* (2012) reported about the successful development of a reverse genetic system for SARS-CoV (strain HKU-39849) that is based upon the cloning, propagation and mutagenesis of a SARS-CoV cDNA in a VacV vector. They have shown that the process of VacV mediated homologous recombination is a powerful tool to introduce mutations into the coronavirus cDNA (8).

Based on this information, the decision of checking the novel SARS-CoV-2 virus for possible homological recombination traces of the VacV TK gene was made.

The initial alignment of the cDNA sequences of the following coronaviruses:

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1. Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1 (Wuhan CoV) (NCBI Reference Sequence: NC_045 512.2);
2. Bat coronavirus isolate PREDICT PDF-2180 (Bat CoV) (NCBI Reference Sequence: NC_034440.1);
3. Human coronavirus 229E (229 CoV) (NCBI Reference Sequence: NC_002645.1);
4. Human coronavirus OC43 strain ATCC VR-759 (OC43 CoV) (NCBI Reference Sequence: NC_006213.1);

with the cDNA of the VacV TK gene (published by Weir & Moss (1983) (9):

AAGCTTTTGCGATCATAAACTGGATCACAACCAGTATCTCTTAACGATGTTCTTCGCAGATGATGATTCATTTTTTAAGTATTTGGCTAGT
CAAGATGATGAATCTTCAITTATCTGATATATTGCAAATCACTCAATATCTAGACTTTCTGTTATTATTATTGATCCAATCAAAAAATAAAT
TAGAAGCCGTGGGTCATTGTTATGAATCTCTTTCAGAGGAATACAGACAATTGACAA**AATTCACAGACTT**TCAAGATTTTAAAAAACTG
TTTAACAAGGTCCCTATTGTTACAGATGTAGATACATAGATCCTCGTCGCAATATCGCATTTTCTAACGTGATGGATATATTAAAGTCGAA
TAAAGTGAACAATAATTAATTCTTTATTGTCATCATGAACGGCGGACATATTCAGTTGATAATCGGCCCCATGTTTTCAGGTAAAAGTAC
AGAATTAATTAGACGAGTTAGACGTTATCAAATAGCTCAATATAAATGCGTGACTATAAAATATTCTAACGATAATAGATACGGAACGG
GACTATGGACGCATGATAAGAATAATTTTGAAGCATTGGAAGCAACTAAACTATGTGATGTCTTGGAATCAATTACAGATTTCTCCGTGA
TAGGTATCGATGAAGGACAGTTCTTTCCAGACATTGTTGAATTCTGTGAGCGTATGGCAAACGAAGGAAAAATAGTTATAGTAGCCGCAC
TCGATGGGACATTTCAACGTAAACCGTTTAATAATATTTTGAATCTTATTCCATTATCTGAAATGGTGGTAAAACTAACTGCTGTGTGTAT
GAAATGCTTTAAGGAGGCTTCCTTTTCTAAACGATTGGGTGAGGAAACCGAGATAGAAATAATAGGAGGTAATGATATGTATCAATCGG
TGTGTAGAAAGTGTTACATCGACTCATAATATTATATTTTTTATCTAAAAAACTAAAAATAAACATTGATTAAATTTTAATATAATACTTA
AAAATGGATGTTGTGTCGTTAGATAAACCGTTTATGTATTTTGAGGAAATTGATAATGAGTTAAATTACGAACCAGAAAGTGCAAATGAG
GCCGCAAAAAAACTGCCGTATCAAGGACAGTTAAAACTATTACTAGGAGAATTATTTTTTCTTAGTAAGTTACAGCGACACGGTATATTA
GATGGTGCCACCGTAGTGTATATAGGATCTGCTCCCGG

was performed using the Pairwise Sequence Alignment on-line tool (https://www.ebi.ac.uk/services).

As the result, one 13 nucleotides region in the TK gene of the VacV sequence (AATTCACAGACTT) that was 100% specific for just one site in the severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1 (position: 16236-16248) was clearly identified (Fig 1).

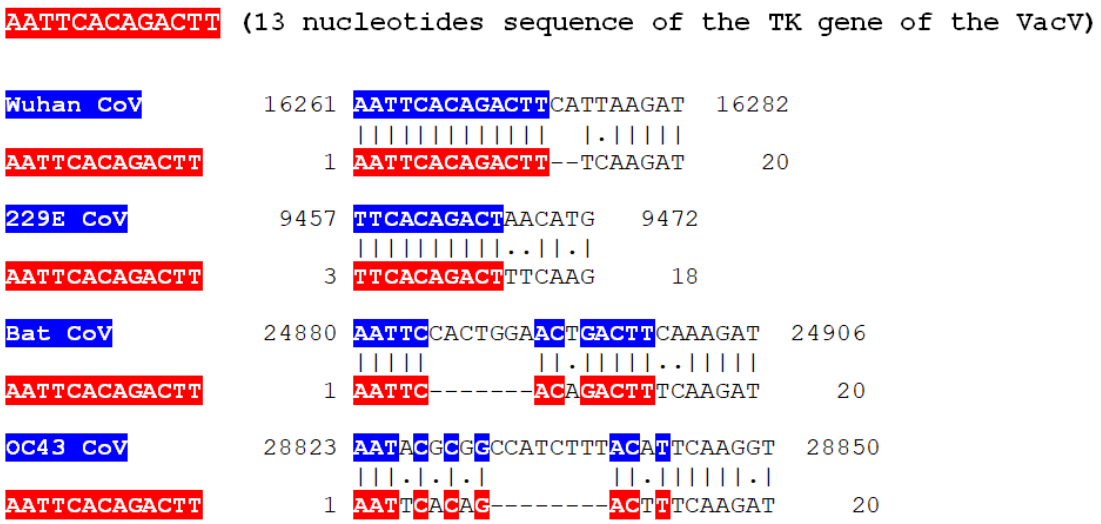

Fig 1. Sequence identity for Wuhan CoV and VacV

Segregation of the matching adjacent parts of that 13 nucleotides region from the severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1 clearly showed that the structure of that sequence was almost identical to the 23 nucleotides site of the VacV TK gene – AATTCACAGACT-T-TCA-AGAT-T-TTA (Tabel 1).

Tabel 1. Sequence similarity between Wuhan CoV and VacV TK

Wuhan CoV	AATTCACAGACT * TCA TTA AGAT G T ***
VacV TK	AATTCACAGACT T TCA *** AGAT * T TTA

There could be a possible trace of the thymidine kinase gene of the vaccinia virus in the Wuhan-Hu-1 and the USA/UNC_200189

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SAR-CoV-2 viruses resulted from homologous recombination between the adjacent TK segment of the VacV or its based vector/s with the coronavirus/es genome/s. Further research on such possibility is warranted.

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