RNA-seq reads of Influenza A genome appended with DNA-expression vector sequences facilitating protein transcription (18s) in Covid19 patients from Wuhan and Hong Kong - very worrying as this is unlikely to be contamination

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Letter

Some viruses like Hepatitis C virus (HCV) have hijacked the human protein translation machinery [1], having by an ‘internal ribosome entry site (IRES) that can autonomously bind a 40S ribosomal subunit and accurately position it at the initiation codon. This binding involves both ribosomal protein and 18S ribosomal RNA (rRNA) interactions’ [2]. Expression vectors are designed to facilitate gene expression, by adding a promoter, a proper translation initiation sequence (a ribosomal binding site) and start/termination codon, followed by a transcription termination sequence.

5 patients from Wuhan - Illumina sequencing

Metagenome from the bronchoalveolar lavage fluid of 5 Covid19 [3–5] patients from Wuhan (Accid:PRJNA605983) shows co-infection (sequences in SI.flu.zip) with influenza A virus (3 out of 5 patients). More worryingly, these reads show the presence of 18s sequences that are found in expression vectors to facilitate protein transcription (Fig.), showing highest homology to a synthetic construct (H1N1 HA gene, Accid:KY199426.1) used in a ‘digital-to-biological converter for on-demand production of biologics’ [6]. Along with SARS-Cov2 infection, a few patients are also infected with Nipah [7]. Its inconceivable that a BSL-4 facility will have so many contamination. Furthermore, the sequencing was RNA-seq - while the expression vectors are DNA - and thus need to be transcribed to be seen in RNA-seq data.

8 patients from University of Hong Kong - Nanopore sequencing

Metagenome from the saliva, nasopharyngeal aspirate or throat swab of 8 Covid19 patients from Hong Kong (Accid:PRJNA608242) shows the same H1N1 reads followed by the 18s sequences found in the expression vector (sequences in SI.flu.zip) in 7 patients. The Human 45S rRNA precursor transcription initiation region (Accid:K01105.1) allows more efficient transcription of the viral gene [8] (Fig). These samples were collected in late Jan 2020.

Possible origin of the Hong Kong outbreak

One read (SRR11178053.500500) has the closest match to sequences added in 2020 from the US from various states (Fig 2) - the closest being from Oregon. These sequences (example MT303728,lineage=A(H1N1)pdm09) has been added in two studies - either ”2018-2019 Influenza Surveillance among DoD Populations” or ”Influenza Sequencing Activity group”.

References

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Figure 1: Reads showing the integration of the influenza gene to 18s from an expression vector:

(a) **Patient from Hong Kong** The small extension on the first line is 18s from the expression vector made to help human cells make proteins more efficiently. The 18s is at the exact end of the gene, so its very unlikely to be sequencing artifacts. (b) **Human 45S rRNA precursor transcription initiation region (Accid:K01105.1)** allows more efficient transcription of the viral gene.

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
✓	Influenza A virus (A/Oregon/06/2020(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	220	220	100%	9e-54	100.00%	MT303728.1
✓	Influenza A virus (A/swine/Iowa/A02479374/2020(H1N2)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT644590.1
✓	Influenza A virus (A/swine/Wisconsin/A02479384/2020(H1N2)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT644576.1
✓	Influenza A virus (A/Texas/9803/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT639869.1
✓	Influenza A virus (A/Texas/9799/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT639666.1
✓	Influenza A virus (A/England/9779/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT639660.1
✓	Influenza A virus (A/Colorado/9776/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT639657.1
✓	Influenza A virus (A/Washington/9300/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT638140.1
✓	Influenza A virus (A/Ohio/9268/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT638135.1
✓	Influenza A virus (A/Italy/9231/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT638116.1
✓	Influenza A virus (A/Germany/9212/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT638111.1
✓	Influenza A virus (A/Germany/9206/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT638110.1
✓	Influenza A virus (A/Belgium/9175/2019(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds	215	215	100%	4e-52	99.16%	MT638100.1

Figure 2: **Possible origin of the virus in HK** One read (SRR11178053.500500) has the closest match to sequences added in 2020 from the US from various states (Fig ??) - the closest being from Oregon.