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904. SARS-CoV-2 EVOLUTION IN A LONG-TERM PATIENT

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Keywords: *Long-Term Infection. WGS. Spike Variants.*

Case report: A 82 years old female patient was admitted to a hospital in March of 2020 and had tested positive for SARS-CoV-2 RT-PCR on a nasopharyngeal swab during 8 months. Her medical history referred a treatment with remdesivir as the only treatment strategy. Twelve respiratory samples were taken between the 24th of March and 16th of November 2020 and sent to the Laboratory of Influenza and Respiratory Viruses at the National Center for Microbiology for virological analysis. RT-PCR targeting E gene was used to detect the viral RNA in the clinical samples. Ct values obtained ranged from 16 to 26. All 12 samples were successfully sequenced using whole-genome sequencing methods (WGS). Viral RNA amplification was performed by SISPA RT–PCR method followed by a PCR using an equimolar mixture of ARTIC n-CoV-2019 v3 pool of primers. Libraries were performed according to Nextera XT library preparation kit instructions. According to WGS, all the sequences passed quality control status. In 9 of 12 samples the coverage was superior to 85% of the genome with a mean coverage between 3581x-9329x. Additionally, a single genome amplification of the S gene was performed, and Sanger sequencing confirmed mutations in these gene and complete the gaps in the whole original genome accurately. All the sequences were classified as B.1 lineage despite the circulation of novels lineages in the course of the pandemic. The sequence analysis illustrates a gradual increasing of changes over time by comparisons with Wuhan-1 reference sequence (NC_045512). In the first respiratory sample, the virus presented 2 AA changes with respect to SARS-CoV-2 isolate Wuhan-Hu-1. However, the complete sequence from sample taken after 8 months of follow up, showed 22 AA changes in the whole genome, and particularly in the S gene a total of 7 AA changes was identified. One of the most relevant changes is detected in the relevant position 484 (E484Q) due to the three variants of concern currently proposed, the presence of a lysine (K) has been described as an escape mutation from neutralizing antibodies. Finally, to rule out the possibility of re-infection over the time, the sequence profiles were analyzed for detecting each nucleotide change and studding the frequency of minority variants in all the sequences. The analysis revealed the presence of a total of 6 nucleotide changes, some of them not very common, present in all the sequences over the months.

991. A NOVEL HUMAN LUNG TISSUE MODEL FOR IDENTIFICATION OF SASRS-CoV-2 ENTRY INHIBITORS

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Keywords: *SARS-CoV-2. Antivirals. Tissue models.*

Introduction: No effective drugs against SARS-CoV-2 infection are available. Screening of therapeutic candidates is primarily performed using immortalized cell lines. However, primary cell targets might show intrinsic differences in the expression profile of relevant host proteins that could significantly affect the activity and potency of antivirals. Thus, the development of more physiological models for antiviral drug screening are urgently needed.

Material and methods: We developed a human lung tissue (HLT) model using lung tissues from thoracic surgical resections. Tissue was immediately digested before experiment set up. Cell populations and expression of ACE2 and CD147 were characterized by FACS, and cell targets for SARS-CoV-2 were identified using a VSV*ΔG(GFP)-S pseudotyped virus. 39 repurposing drugs previously identified by in silico models as potential viral entry inhibitors were tested using a VSV*ΔG(Luc)-S virus. Cytotoxic concentration (CC50) and inhibitory concentration (IC5O) values were calculated using a non-linear regression dose-response curve and were compared to drug activity in VeroE6 cells.

Results: Alveolar type II (AT-II) cells, the main cell target for SARS-CoV-2 infection in lungs, were identified within a fraction of cells characterized by CD45-, CD31-, EpCAM+ and HLA-DR+, (~0.01-0.5% of viable cells). Using a VSV*ΔG(GFP)-S virus we showed that viral entry was occurring in cells compatible with an AT-II phenotype, and infection

was efficiently blocked with anti-ACE2 and anti-CD147 antibody. Despite low and variable numbers of AT-II targets, antiviral assays using VSV*ΔG(Luc)-S were highly sensitive and reproducible (CV of 16%). Within drugs showing some antiviral effect, we found that 37.5% of the tested compounds had discordant results between HLT and VeroE6 cells; 31.25% of drugs showed some antiviral effect in HLT but no activity in VeroE6, and 6.2% showed only antiviral effect in VeroE6. Cepharantine (IC50 = 6 μM, CC50 = 13.8 μM) and Ergoloid (IC50 = 7.7 μM, CC50 = 87.7 μM) were identified as the most active entry inhibitors in HLT cells.

Conclusions: The HLT model is reproducible and highly relevant for SARS-CoV-2 antiviral testing in a physiological system that recapitulates main antiviral activities observed in cell models, an offers the identification of new compounds missed by conventional systems. Importantly, we identified repurposing drugs against SARS-CoV-2 with potential for clinical testing.

1039. EMERGENCE OF ADAPTIVE MUTATIONS OF THE SPIKE IN SARS-CoV-2

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Keywords: *SARS-CoV-2. Mutation. Spike.*

Surveillance of mutants of SARS-CoV-2 aims to monitor the appearance of new variants that could potentially change the biological properties of the virus. During the first year of SARS-CoV-2 evolution, different variants have been detected with different degrees of biological impact on the virus and also on the epidemic. We have detected two mutations of the spike protein of SARS-CoV-2 that have appeared independently multiple times in different genetic backgrounds and hosts, possibly indicating they could increase viral fitness. Interestingly, when both mutations appeared together, the genotype increased in frequency more than the individual mutants. This variant that we call Variant of Interest 1 and includes 12 other single nucleotide polymorphisms but no deletions with respect to the reference genome. VOI.1 appeared after the First epidemic Wave in Spain, and subsequently migrated and increased in frequency in 8 countries. VOI.1 includes a cluster of sequences that have acquired the concerning mutation E484K, which could result in antibody escape. We explored if these two mutations confer a greater capacity to the virus to produce more particles. Unexpectedly, we find a reduction in infectivity of this variant versus the 20EU1 in two different cell lines. However, clinical results differ slightly and we found that individuals infected with VOI.1 have similar levels of viral RNA in nasopharyngeal swabs than patients infected with 20EU1 variants, both of them significantly higher than non 20EU1 lineages. Finally, in order to assess the risk of VOI.1 because of antibody escape, we explored the impact on immunogenicity. A moderate but significant reduction in sensitivity to neutralization by sera from convalescent donors obtained from the early period of the pandemic was observed. Overall we could detect and monitor the spread of a variant of interest which could pose a potential risk. The expansion of such variants has allowed the acquisition of another potentially risky mutation, present in other variants of concern, which could pose an additional threat.