SARS-CoV-2 and HCV infection and antiviral treatment monitored by multimodal imaging

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Background
A common feature among positive strand viruses is that they alter cellular membranes to generate replication complexes. Although the origin, nature and structure of these membranous compartments are not identical, they constitute a characteristic feature of these viruses and are observed in yeast, plants and higher eukaryote (+)-strand RNA viruses (3).

HCV infection provokes a rearrangement of intracellular membranes, designated membranous web (MW). This term referred to compact vesicle accumulations embedded into a membranous matrix (2).

In SARS-CoV-2, the expression of the viral proteins results also in a profound remodeling of the infected cell cytoplasm, with the characteristic membranous compartment, generically denominated viral replication organelle (VRO). One of the salient characteristics of the coronavirus VRO is the presence of double-membrane vesicles (DMVs), where various nsp and double-stranded RNA (dsRNA) have been shown to colocalize and where active RNA-dependent RNA synthesis has been shown to occur. Thus, DMVs are the structures where coronavirus RNA replication is thought to occur. While the nature and origin of the membranes may differ, DMVs are also the putative RNA replication organelle for hepatitis C virus (HCV).

In our research we study the morphology of the membranous rearrangements induced by HCV and SARS-CoV-2 infection in near-native conditions (1).
These infection alterations in HCV could be reverted by the clinically approved direct-acting antivirals (DAAs) for the treatment of chronic HCV infection. The availability of DAA drugs against HCV provides a unique opportunity to revert this process and to define the ultrastructural events that follow viral replication blockade short after antiviral treatment (4).

Methods
In this study we have performed infrared microscopy, confocal immunofluorescence and correlative cryogenic light-soft X-ray tomography (CLXT) in the water window photon energy range to investigate in whole, unstained cells, the morphology of the membranous rearrangements induced by HCV and SARS-CoV-2 infection and after antiviral treatments in near-native conditions.

Results
Our results compare the HCV and SARS-CoV2 replicating structures. SARS-CoV-2 infected cells display DMV structures similar to those found in other coronaviruses or hepatitis C virus infection. Our studies provide a wider cellular context in which these membranous alterations occur and point at the formation of compact perinuclear structures where viral antigens are concentrated by constriction within intermediate filaments, as determined by confocal microscopy. This perinuclear structure is formed by a tightly juxtaposed tubular membranous network reminiscent of a highly modified endoplasmic reticulum. This structure is virtually devoid of normal mitochondria and adjacent mitochondria display clear ultrastructural signs of stress. Finally, late stages of the infection indicate deformation of the cell nucleus in areas close to the viral factory and an overall cytoplasmic retraction of the infected cell.

Analysis of DAA-treated HCV replicons indicate that most viral antigens and RNA are eliminated within the first 48 hours of treatment. CLXT studies confirmed the rapid elimination of the viral machinery, and the concurrent appearance of large endo-lysosomes and multivesicular bodies, suggesting a major role for this recycling machinery in the elimination of HCV-induced membranous compartments (5). A general survey of control cells and HCV replicons indicates that HCV-induced membranous alterations are no longer visible after 24 hours of treatment and that a substantial fraction of NS5A, a viral component of the replicase is located in pleomorphic, high-absorption contrast organelles in DAA-treated cells.

Conclusions
Overall, our cryo-SXT data provide an additional piece of the puzzle in building a precise map of the ultrastructure of SARS-CoV-2-infected cells by providing insight into the overall structure of the viral replication organelle and the cellular context within which these changes occur.
Our results suggest that HCV replication compartment is constantly recycled by the endo-lysosomal system and that this equilibrium is unbalanced by DAA treatment, resulting in a transient activation of the endo-lysosomal system to achieve rapid viral machinery removal. Our results also constitute a proof of concept for the use of cryo-SXT at ALBA synchrotron and at lab-scale soft X-ray microscope (SXM) as a platform that enables determining the potential impact of candidate compounds on the ultrastructure of the cell that may assist drug development at a preclinical level.

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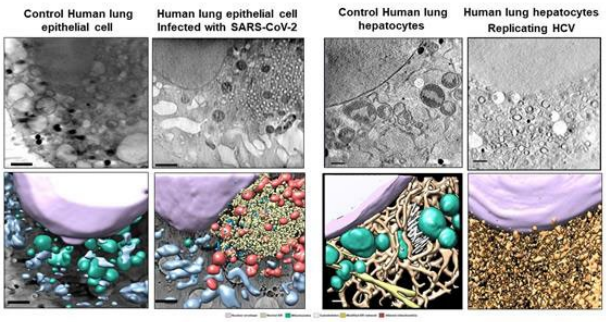
Graphic:



Keywords:

SARS-CoV-2, HCV, DAA, cryo-SXT,
cryogenic-light-soft-X-ray-tomography(CLXT)

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