COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by Ruth Serra-Moreno (University of Rochester), May 19, 2021

Title: *Membrane remodeling dynamics by SARS-CoV-2*

Ruth Serra-Moreno CIC Profile

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Transcript

Ruth Serra-Moreno:

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First of all, I want to thank you all for inviting us to participate in the lightning talks.

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I don't think that this virus needs introduction. I just want to bring to your attention the simplicity of this infectious agent. It consists only of an envelope or lipid bilayer that is embedded in several glycoproteins including the S or spike that binds to the ACE2 receptor on the human airway epithelial cells and that allows the virus to enter into the cells. Underneath this envelope there's a nucleocapsid that consists of a single strand of RNA around 30 kilobases long. That's the genome of the virus and it's wrapped in the N or nuclear acid protein.

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So similar to other coronaviruses that infect humans, SARS-CoV-2 is the result of a spillover event of a coronavirus infecting bats that was transmitted to an intermediate host and then to the human population.

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Exposure to viruses that infect other species is common. However in most of those occasions, the interactions lead to dead ends. On a few occasions, however, those interactions can result in a productive infection due to the failure of the species barrier containing the pathogen. If due to the growth in the human population, there is an overlap with the habitat of the natural host, this increases the likelihood of future potential exposure events that can set the stage for adaptation where the virus can accumulate mutations, undergo recombination events, and finally natural selection operates. An indication of such adaptation is the presence of human to human transmission where the interaction with the direct host is no longer required.

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So since the beginning of the century, three highly pathogenic coronaviruses have emerged in the human population: the original SARS Coronavirus one, MERS Coronavirus, and SARS Coronavirus 2. All of them are the result of spillover events of beta coronaviruses infecting bats that were transmitted to other mammals and then they finally reach humans. Something characteristic about these infections is that human to human transmission appears quite rapidly, which indicates that these viruses find an ideal cell environment for the replication in the natural, intermediate, and the final host.

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And we hypothesize that autophagy is one of those several processes that coronavirus is used for the replication since autophagy is highly conserved across mammals.

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So autophagy consists of the formation of double membrane vesicles also called autophagosomes for the collection of cargo such as misfolded proteins malfunctioning organelles that need to be delivered in the lysosome for their elimination. If we look at this schematic of the life cycle of coronaviruses, we can see that something peculiar in these infections is the active remodeling of the cellular membranes to generate double membrane vesicles or DMVs that work as viral replication factories. This is the location where the replication of the genome of the coronaviruses takes place. And the structure of these DMVs closely resembles that of autophagosomes which is why we hypothesize that coronaviruses, and in particular, SARS-CoV-2 may hijack the autophagy machinery as a source of these double membrane vesicles.

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So this NSF RAPID consists of two different objectives. For the sake of time, I'm only going to discuss Objective 2 which relates to this interaction between autophagy and coronaviruses.

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So for these experiments, we engineer a set of cell lines that express an autophagy marker LC3 fused with GFP so we can monitor autophagosome formation in autophagy flux. The cell lines that we engineer are the Vero E6 cell which is a prototype cell line for coronavirus research ACE2 expressed in Ferret lung cells, bat lung cells, and ACE2 expressed in human lung cells in particular the A549 cells.

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In addition to these cells we also acquired a library of the different open reading frames that are encoded in the SARS coronavirus genome so we could assess how individual expression of these genes affect autophagosome formation and autophagy flux. And we could see some candidates, such as the open reading frame 3a, 7a, the structural protein M, and the non-structural proteins 6 and 14. And this was very exciting because this indicates that these proteins are triggered in autophagy. However, we need to take this information with caution since these experiments were performed under conditions of transfection in the absence of the rest of the SARS-CoV-2 genome that may also help modulate the expression of these proteins.

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So the next step is to reconcile this information in the context of an infection. So while we were waiting on the BSL3 clearance and receiving the PPE, the personal protective equipment, we performed some of these assays using another coronavirus, a biosafety level two coronavirus that infects humans OC43, and also affects the respiratory tract.

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So the first thing that we did was assessing at what time post infection we are detecting already genome replication because by then, the remodeling of the solar membranes must have already happened. And we can see an exponential increase in the amount of copies of genomic RNA accumulated between four and eight hours post-infection. And this is consistent with the fact that we are already detecting the [novel virion?] particles eight hours post-infection.

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So the next thing that we did was infecting our engineer cells with OC43 at a multiplicity of infection of one and this means that each single cell is receiving one viral particle. And we performed time-lapse live imaging for eight hours to see if there were any fluctuations in autophagosome formation.

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So these are some representative images of this study where we had three different experimental conditions. The first one was cells treated with rapamycin on the top. Rapamycin is a drug that triggers autophagy and these allow us to use it as a positive control, mock treated cells in the middle, and then in the bottom we have the OC43 infected cells. The first thing that I want you to see is that at time zero with no exposure, with no stimuli, we are already detecting all the autophagosomes that are depicted by this [pante?], these shiny dots. This is normal and this is expected. Autophagy is very important for solar homeostasis and there's always going to be some degree of autophagy activation - STEM cells even more than others. The next thing that I want you to notice is that two hours after stimulating the cells with rapamycin, we see a significant increase in all the thousand formation even in the cell that already had a lot of autophagy going on but even more in the other one that we barely even saw in the previous picture. Over time, these autophagosomes increase not only in number but also they enlarge. They increase in size and then by eight hours post treatment, we start seeing a decline in autophagosome number which is an indication of the resolution of this pathway fusion with lysosomes will result in the elimination of these structures. In the mock treated cells we don't see much fluctuations in the number of autophagosomes over time. And then, in the OC43 infected cells we see increasing autophagosomes particularly at six hours post infection but unlike the cells treated with rapamycin, we don't see the enlargement in autophagosomes and the number seems to remain more steady.

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So these are the quantifications of the kinetic study for all the cells that we image and we quantify once again the mock treated cells no much fluctuation over time, rapamycin treated cells huge increase in autophagosomes, plateaued, and then resolution, and the OC43 infected cells, we see an increase in autophagosome formation, not as much as with rapamycin, but we don't see the enlargement of autophagosomes and the resolution of that pathway. This is encouraging. This may indicate that this particular coronavirus is somehow taking advantage of the autophagy machinery, but this could also be interpreted differently. We know that autophagy not only is important for cell homeostasis, it's also a very important antiviral defense mechanism. So at this point we are not sure if this increasing autophagosome is virus induced or is the cell responding to the infection.

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So one way that we can answer this question and I apologize because these experiments are ongoing this week and I don't have the data yet, but the one way to answer this question is to see if the replication machinery of the virus colocalizes with these autophagic structures. On one hand the replicates- the RNA dependent RNA polymerase but also the products of genome replication, the novel copies of genomic RNA biproducts of the replication like double-stranded RNA. The next thing that we need to do is repeat these experiments with SARS-CoV-2. We now have the biosafety clearance. We have the stock of viruses so we are ready to go. One question that we are very interested in answering is if we see differences among different variants of SARS-CoV-2, particularly those variants that are highly

transmittable like the U.K. variant and the South African variant. So stay tuned. I'll keep you posted on this.

And with this, I would like to thank those who have contributed to this work, especially my grad student Yuexuan Chen. She's a brilliant grad student, super motivated, very enthusiastic. She's the true leader of this project and it's a privilege having her in the lab so I couldn't be happier. The rest of the team Yuhang, Sydney, Jared, and Sergio who recently moved for a postdoctoral fellowship in Sweden and of course NSF for funding and support. And thank you. I'll be happy to take questions in the chat.