1. OBJECTIVE

Coronaviruses are enveloped, ssRNA viruses. Currently six human coronaviruses are known: HCoV-229E and HCoV-OC43 recognized already in 1960's, and four new pathogens: SARS-CoV (identified in 2003), HCoV-NL63 (identified in 2004), HCoV-HKU1 (identified 2005) and the newest one – HCoV-EMC (identified in 2012). Human coronaviruses are generally associated with respiratory tract infections, though some reports also suggest association of these pathogens with gastroenteritis. The severity of the disease depends on the viral species involved, though patient-related factors (e.g., age, general health status) are also of importance.

The current project is aimed to identify and characterize route of entry of human coronaviruses, including species associated with relatively mild disease (HCoV-NL63 and HCoV-HKU1) and highly pathogenic coronaviruses (SARS-CoV, HCoV-EMC). Human coronaviruses cause primarily infections of human respiratory tract, replicating in the respiratory tract epithelium, thus in order to appropriately study the subject, the research will be carried on using fully differentiated human airway epithelium – the three-dimensional *ex vivo* model that mimics the natural infection environment.

Particular goals of the current project:

- Decipher the mode of entry of human coronaviruses.
- Understand the intracellular trafficking of endosomal vesicles carrying coronaviruses.
- Delineate the transport of the nucleoprotein to the replication-transcription sites.

2. RESEARCH METHODOLOGY

Highlights

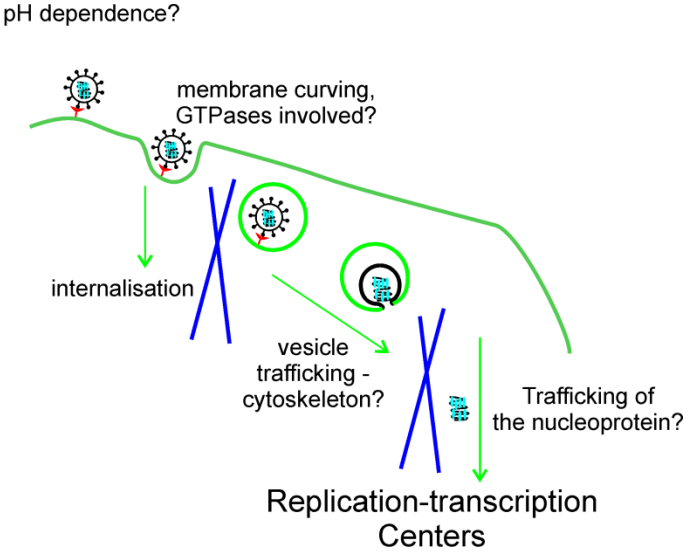
- *ex vivo* human epithelium cultures.
- real-time imaging.
- complete viruses.

Deatiled description:

1. The role of different endocytosis pathways during coronaviral infection (HCoV-NL63, HCoV-HKU1, MERS-CoV, SARS-CoV) will be determined using chemical inhibitors and synthetic siRNAs.
2. Influence of inhibition of different endocytosis pathways will be evaluated by assessment of replication inhibition, confocal microscopy and imaging of sub-cellular structures.
3. Co-localization of virions and endocytosis markers will be evaluated.
4. Mechanisms of cellular entry will be delineated using electron microscopy.
5. Analysis of coronaviral entry to living cells will be carried on using high-resolution confocal microscopy. Resulting data will allow to track the virus internalization in real-time mode.
6. Analysis of virus trafficking inside the cell will be conducted using confocal microscopy.
7. Interactions between the nucleoprotein and host's proteins will be deciphered.

3. EXPECTED IMPACT

The knowledge on modes of entry of coronaviruses is scarce and fragmented. The basic knowledge on entry of clinically relevant human coronaviruses into susceptible cells of the respiratory tract will be obtained. These results may also to some extent support the understanding of cell and tissue specificity of different coronaviruses and possibly also the stark differences in pathogenicity between different virus species (e.g. SARS-CoV and HCoV-NL63, both utilizing the same cellular receptor). Further, obtained results will also serve as a base for future R&D projects (e.g., development of novel drugs against new molecular targets).



Scientific output:

- Identification of endocytosis routes utilized by clinically relevant human coronaviruses NL63, HKU1, SARS-CoV and MERS-CoV.
- Mapping of intracellular trafficking of endocytic vesicles containing virions and nucleoprotein itself.
- Identification of new, potential targets for anti-coronaviral therapy.

Indicators:

- Scientific publications in leading scientific journals.
- Presentations on international meetings.