ROBERT KOCH INSTITUT

Establishment of a Human Lung Organoid Method Platform for Infectious Disease Modelling

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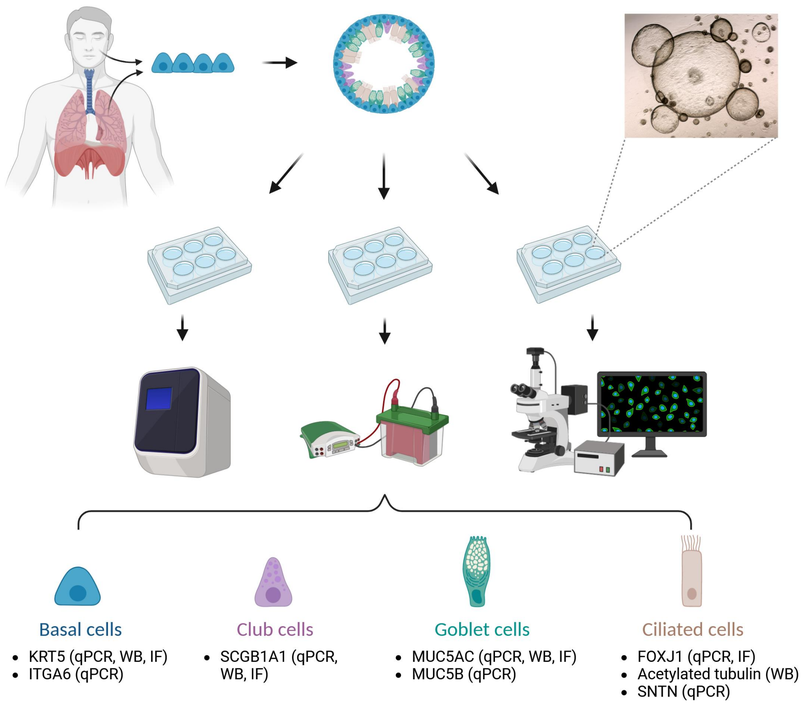
Establishment of an organoid platform at the Robert Koch-Institute (RKI)

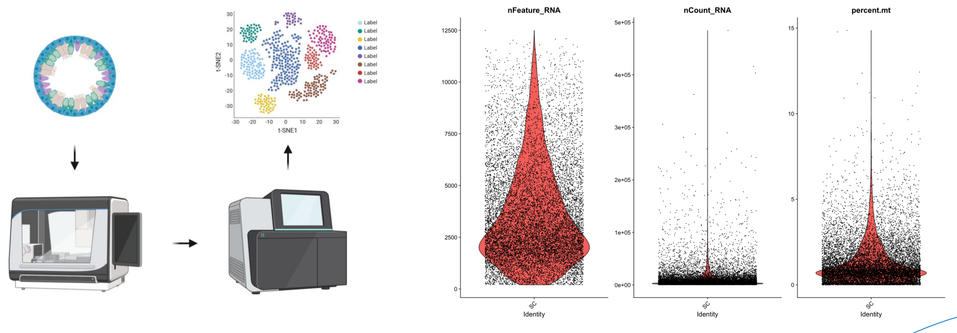


3D cell cultures have the advantage of a physiological cell composition compared to the standard monolayer cultures and thus enable broader possibilities as disease models for research. The RKI aims to implement an in-house organoid platform of multi-species origin for infectious disease research and risk assessment for the characterisation of novel and re-emerging pathogens. So far, lung organoids of different human origin, generated from primary adult stem cells (bronchial) and commercially available lung cells (nasal) have been established. Characterisation on mRNA and protein level revealed that they largely consist of cell types present under physiological conditions. Differences in specific cell proportions are most likely due to different physiological functions of the relevant source tissue.

Figure 1: Workflow of lung organoid generation from adult stem cells of nasal and tracheo-bronchiolar origin, and the downstream characterisation by qPCR, Western blot, and CLSM.

Ongoing and future projects

Single-cell RNA sequencing



- Determining the cell composition of organoids of different donors and tissue origin with available scSeq kits for quality control of the organoid platform
- Analysis of viral cell tropism, host cell response etc.

Figure 2: Quality control of first scSeq run of human lung organoids, data currently being analysed. Graphs (right) show proportion of transcripts mapping to mitochondrial genes. Good integrity of the cell membranes, thus high viability of cells could be achieved.

Organ-on-chip systems for disease modelling

Co-cultivation of organoids of different origin such as lung, liver, and intestine in a multi-organ chip for studying viral transmission within a 3D model system and the impact of immune cells.

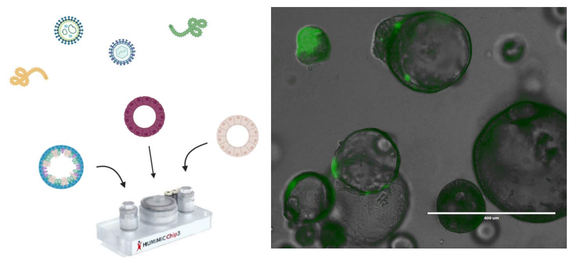
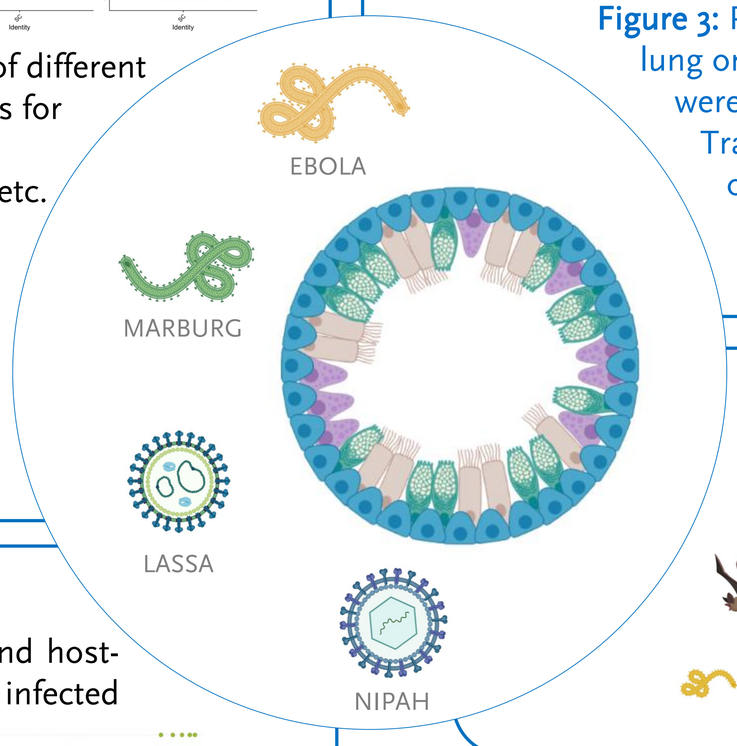
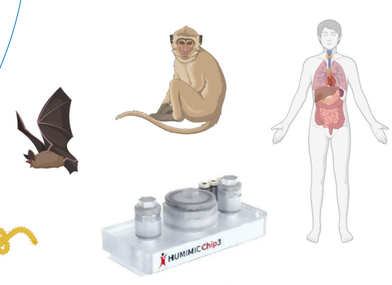


Figure 3: Proof for viral transmission on organ chips: lung organoids infected with Vaccinia-GFP reporter virus were co-cultured with naïve lung organoids. Transmission of newly produced viral particles from one chip compartment to the other is demonstrated via the detection of GFP-positive cells in previously non-infected organoids.



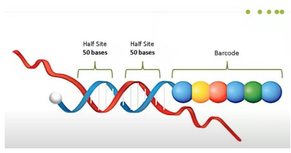
Multi-species organ chips



Interspecies viral transmission can be mimicked and studied using multi-organ chips supporting future risk assessment of highly-pathogenic viruses.

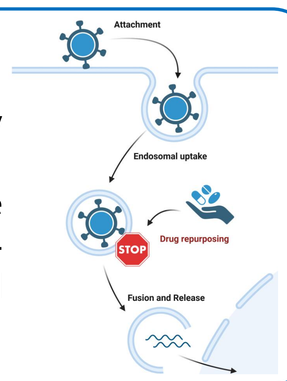
nanoString analysis

The differential expression of cellular anti-viral and host-response factors was analysed in lung organoids infected with EBOV, MARV, and NiV applying the nanoString technology. Preliminary data indicate a strong upregulation of CXCL10 for all three viruses.



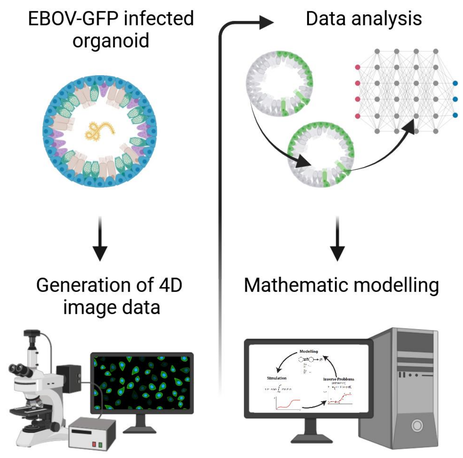
Anti-viral drug testing

Pharmacological intervention of viral replication by repurposing of clinically licensed drugs.
For example, itraconazole and fluoxetine mediate the alteration of cholesterol content in endo-lysosomal compartments, which blocks viral membrane fusion and thus, viral replication.



In silico modelling of viral replication using 4D live cell microscopy

Generation of 4D image data of EBOV-GFP-infected organoids being analysed with the help of deep learning neuronal network modelling for understanding the virus replication kinetics.



Federal Ministry of Health

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