Bioengineering and biophysics of viral hemorrhagic fever

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ADDENDUM

Summary

Viral hemorrhagic fever (VHF) is an extremely infectious and life-threatening disease caused by a group of viruses including Ebola, Lassa, Dengue viruses. The number of viruses known to cause VHF is ever-increasing and its spread due to globalization and climate change lead to global distribution and burden. However, with limited effective cures or vaccines, we are not prepared to prevent the future virus outbreaks. Thus, further understanding of the pathogenesis and development of new tools are needed to guide the development of therapeutic and preventive strategies. Key viral targets in VHF include vascular endothelial cells and macrophages, the involvement of vascular endothelium and the perturbation of vascular barrier integrity has been recognized as a common pathological signature. In addition, the aberrant released cytokines by infected macrophages can in turn disrupt vascular permeability, as introduced in **Chapter 1**. Studies of VHF pathogenesis have been hindered due to the lack of suitable experimental models or analysis approaches; thus, the scope of this thesis is to develop bioanalytical, lab-on-chip and single cell assays to investigate VHF virus-induced changes in vascular biology and macrophage immunometabolism.

In **Chapter 2**, we reviewed microfluidic Organ Chip culture devices as a promising strategy for reproducing various viral disease phenotypes. Organ Chip has been applied to investigate some aspects of viral infection, including virus–host interactions, viral therapy-resistance evolution, and development of new antiviral therapeutics, as well as underlying pathogenesis. To mimic host–microbe interactions specifically, several Organ Chip platforms, e.g., liver, gut, nervous system, kidney, lung and microvessel (as described in this thesis) have been developed to study different viral pathogenesis. The capability to study virus-induced diseases in real time and at high resolution can open new avenues to uncover viral pathogenesis in a human-relevant environment and may eventually enable development of novel therapeutics and vaccines.

The development of models that mimic the structure and functions of human microvessels

is essential for studying the pathogenesis and therapeutic approaches for VHF disease-associated vasculopathy. In **Chapter 3**, we presented the first ever model for Ebola virus disease on a chip. The model recapitulates endothelial dysfunction associated with the Ebola hemorrhagic shock syndrome via Rho/ROCK signaling pathway activation and subsequent changes in actin stress fibers. In addition, our data showed that the Ebola glycoprotein plays a critical role in this process. Finally, we also demonstrated the applicability of this platform for pharmacological studies by studying the efficacy of two experimental molecular drug candidates, FX06 and melatonin, in phenotypic rescue.

As another example, in **Chapter 4** we developed the first Organ Chip model for Lassa Hemorrhagic syndrome that allows the modeling of Lassa-induced vascular phenotypes and provides an *in vitro* platform for drug studies. Luminal infusion of Lassa VLPs leads to dramatic increase in vascular permeability in a viral load-dependent manner, as well as changes in actin stress fibers. In addition, FX06 is also able to suppress the Lassa-induced vascular integrity loss. These findings strengthen the concept that our platform can be applied to study VHF-associated microvascular pathophysiology and carry out preclinical drugs evaluation.

The damage to vascular endothelial cells by NS1 has been speculated to be involved in the pathogenesis of Dengue virus disease. In **Chapter 5**, two microfluidics-based approaches, namely microvessel-on-a-chip and acoustic force spectroscopy were applied to study the direct contribution of Dengue NS1 to the vascular permeability and the alterations of viscoelastic properties of endothelial cells, respectively. Results show that NS1 led to a dramatic increase in the permeability of the engineered microvessels, involving VE-cadherin and F-actin stress fibers reorganization accompanied by increased hyaluronan biosynthesis, as well as significant decrease in the stiffness of endothelial cells. Overall, we discovered that NS1–mediated mechanical alterations is a key element of Dengue virus disease.

Besides application of organ and lab on a chip technologies to probe mechanical changes induced by VHF, metabolomics may deliver an informative readout of endothelial cells as

well as macrophages. In **Chapter 6**, we assessed the metabolic alterations of primary human endothelial cells, M1 and M2 macrophages upon exposure to Ebola VLP. In order to achieve this, a direct infusion mass spectrometry-based untargeted cellular metabolomic approach was applied. Metabolic analysis demonstrated that Ebola VLP exposure broke the metabolic homeostasis of the three cell types in a cell specific manner and significantly affected fatty acid-, steroid-, and amino acid–related metabolism pathways.

M1/M2 are the two major and opposing activities of macrophages, the metabolic differences are crucial for their distinct functional properties. However, such insights heavily relied on bulk measurements due to technical limitations. **Chapter 7** takes a first step in developing the ability to directly measure metabolic profiles of M1 and M2 macrophages on the single-cell level. The live single-cell mass spectrometry based metabolomic profiling coupled with a machine learning data analysis approach was developed and applied here for the first time. Our results demonstrate unique metabolic signatures of M1 and M2 respectively and reveal different levels of fatty acyls, glycerophospholipids, and sterol lipids in macrophage subtypes. This methodology succeeded in leveraging these metabolic signatures to classify each phenotype with a high degree of selectivity and sensitivity.

In conclusion, as illustrated in this thesis, the organ chip and high-resolution single cell analysis platforms developed here provide VHF research with fundamentally new approaches for the evaluation of experimental therapeutic strategies. We also discovered that VHF is a disease of mechanics and mechanical readouts can serve as physical biomarkers for disease analysis. Furthermore, we showed that chemical biomarkers are informative here, and direct infusion mass spectrometry can reveal interesting features of host cell response to the viral pathogens. The proof-of-concept single cell study will hopefully open new avenues towards future research which may reveal the pathogen induced re-shaping of immunometabolic landscape of heterogenous macrophage populations.