Host-directed therapy for intracellular bacterial Infections

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Table of Contents

Scope and outline of this thesis

In this thesis we describe our work towards identifying novel targets and candidate drugs for host directed therapy (HDT) as a novel therapeutic approach for drug-resistant bacterial infections, focusing on the major human pathogens *Mycobacterium tuberculosis (Mtb*) and *Salmonella enterica* serovar Typhimurium (*Stm*).

Chapter 1 provides an introduction to the background of our studies, including the global health problems caused by *Mtb* and *Stm* and the rise in drug resistance in these pathogens. The concept of HDT is introduced and recent studies in this field are summarized. Finally, technical aspects and limitations of the identification of novel chemical compounds and host targets for HDT is discussed. The studies discussed in **Chapter 1** demonstrate the highly significant need for alternative and additional therapies to combat bacterial drug resistance, as well as the challenges that accompany identification of drugs, key host-targets and the subsequent development of therapies.

In **Chapter 2**, we describe novel techniques we developed, that allowed us to perform large-scale chemical compound and RNAi screens to identify human HDT targets and candidate drugs, using fluorescence-based human *Mtb* and *Stm* infection models. We describe a new flow cytometry-based screening assay that allows fast, medium-throughput screening and faithfully reproduces results from classical colony forming unit (CFU) assays, which can take weeks in case of *Mtb*, in contrast to the three days assay system we report here. Importantly, the assay also confirms published HDT compound data from literature. In addition, we propose and validate the MelJuSo cell line as a novel human phagocytic model for *Mtb* infection.

The novel screening assay and infection models are then applied for a large scale screening of a Library Of Pharmacologically Active Compounds (LOPAC) in **Chapter 3**. Here we report on the identification of a number of highly active HDT compounds for both *Stm* and *Mtb*. Furthermore, we also describe a newly developed *in silico* prediction model for analysis of large chemical screens. This model is then applied to identify novel compounds based on target profiles derived from compounds from the LOPAC library. Both the library screening as well as the *in silico* model identified inhibitors of receptor tyrosine kinases (RTKs) as novel drug candidates for TB HDT and proposed Dovitinib, AT9283 and ENMD-2076 as candidates for drug-repurposing.

The novel screening assay from **Chapter 2** is used for a chemical screen in **Chapter 4**. Based upon our previous identification of H-89 as a host-directed inhibitor of *Stm* and *Mtb*, we here report the screening of a novel library of H-89-analogue compounds, and identify lead compound 97i as a superior host-directed inhibitor of *Mtb* and *Stm*. Using kinase inhibition profiling and genetic silencing, we further identify PCTAIRE kinases as direct targets of 97i and regulators of intracellular survival of *Stm* and possibly *Mtb*.

An alternative approach to identification of HDT targets is reported in **Chapter 5.** Here, we employed a zebrafish TB model to uncover a role for DNA Damage-Regulated Autophagy Modulator (DRAM1) in regulating autophagy upon recognition of mycobacteria by TLRs. We demonstrate in zebrafish embryos that DRAM1 is up-regulated upon mycobacterial infection and, importantly, confirmed this mechanism in *Mtb*-infected primary human macrophages. We further show that signaling through the TLR-MYD88-NF-κB axis was responsible for the induction of DRAM1 and that autophagic encapsulation of mycobacteria was dependent on the cytosolic DNA sensor STING as well as the autophagy adaptor molecule p62. The TLR-MYD88-NF-κB-DRAM1 axis may therefore be exploited for HDT to induce autophagy-mediated control of intracellular (myco)bacteria.

In **Chapter 6**, the major findings of the above studies are summarized and discussed.