Vantage point: New insights into the old enteric fever treatment by rescuing the host cell ubiquitin-proteasome system.

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Typhoid fever is one of the leading bacterial infections especially in developing countries like India. *Salmonella spp*. are responsible for causing this infection. Naturally, human cells clear these bacterial cells through autophagy involving the ubiquitin-based modifications. However, *Salmonella* hijacks this system and de-ubiquitinates the bacterial aggregates in order to escape the autophagy through its deubiquitinating (DUB) enzyme called SseL. In this study, we designed a small molecule PROTAC to selectively degrade the bacterial DUB and potentially prevent the bacterial survival thus promoting autophagy. The PROTAC was designed using computational tools and is currently in synthesis, to be evaluated further.

Keywords: Typhoid, SseL DUB, PROTAC, ubiquitin, proteasome, salmonella.

Typhoid fever, also known as enteric fever, is caused by *Salmonella spp*. (gram negative bacilli) mostly through contaminated food and water intake. The bacterial cells specifically invade the intestinal M-cells (Microfold cells) and also the epithelial cells from the intestinal lumen side to cross the intestinal epithelial barrier to reach the host blood stream. M-cells belong to the lymphoid lineage that sample the gut antigens to present them to the immune cells. As a part of this process, usually the macrophages engulf these bacterial cells when they cross the intestinal epithelium. Once engulfed, the macrophages fuse the bacterium containing vacuoles (also known as phagosomes) with their lysosomes in order to degrade the bacterium. However, in the case of *Salmonella spp*., the bacteria containing phagosomes (also known as *Salmonella* containing vacuoles or SCVs) do not fuse with the lysosomes inside macrophages. The bacterium secretes effector proteins that help avoiding the fusion of SCVs with the host lysosomes (1).

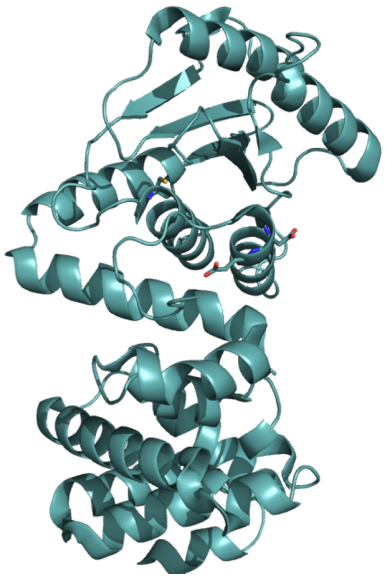

Figure 1. Structure of *Salmonella* SseL DUB downloaded from the protein data bank (PDB ID: 5HAF).

The bacterial effector proteins help escape the host defence system (2). One such effector protein is the SseL. The three--dimensional structure of SseL is shown in Figure 1. SseL is a deubiquitinating enzyme (DUB) secreted by the *Salmonella spp*.

The SseL de-ubiquitinates the bacterial proteins that have been targeted by the host ubiquitin-proteasome system. Due to the DUB activity of SseL, the bacterium escapes host cell ubiquitin-proteasome system as well as the autophagy. Thus, the bacterium gains control over the host cell. Eventually the macrophage undergoes apoptosis, also known as programmed cell death, which results in the survival of the bacterium and spread of the infection via the host blood throughout the body.

In this study, we hypothesize that by using a small molecule one can selectively target the *Salmonella* Ssel DUB for degradation by the host ubiquitin--proteasome system. The small molecule acts as a PROTAC (proteolysis targeting chimera) in which one part binds to the Ssel and the other part binds to the host ubiquitin E3-ligase enzyme such that the PROTAC brings the host ubiquitin ligase enzyme to the vicinity of Ssel DUB and thus ubiquitinating the Ssel DUB towards proteasomal degradation.

The structure of Ssel DUB (PDB ID: 5HAF) contains more than 10 α-helices and 4 β-strands of which 3 β-strands form a sheet. Followed by the structural analysis, ubiquitin molecule was modeled into the active site to identify the critical amino acids that contribute to the DUB activity. This molecular modeling was performed as described previously by Pruneda *et al.* (3).

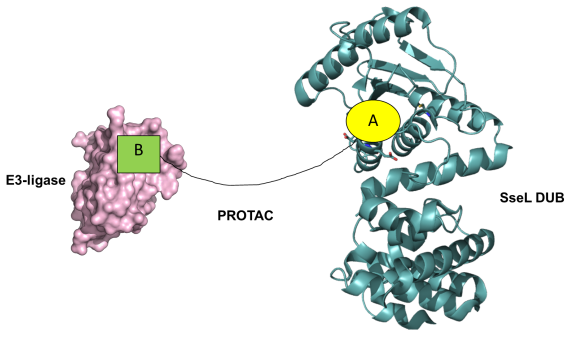

Figure 2. Proposed mechanism of the PROTAC molecule that targets the *Salmonella* SseL DUB to the host cell ubiquitin-proteasome system.

Based on the binding site analysis it was concluded that the DUB active site would be the most appropriate site for the PROTAC (TCABSE-120) design with reasonable binding affinity. Glutamic acid (E164), cysteine (C285) and aspartic acid (D163) were primarily targeted for the TCABSE-120 binding. The C-terminal arginine (R72) of the ubiquitin is a positively charged long side chain amino acid that was taken into consideration while designing TCABSE-120 for modeling. The proposed mechanism of TCABSE-120 binding is shown in Figure 2 bound to the SseL DUB and the ubiquitin E3-ligase. TCABSE-120 contains two parts, A and B. Part A binds in the active site of SseL DUB and part B binds to the ubiquitin E3-ligase. Both parts, A and B are connected by a hydrocarbon chain with few oxygen atoms strategically placed (structure is patent pending).

References

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Conflict of interest: This is an ongoing project currently at TCABS-E, Rajahmun -dry, India. The authors invite collaborations without any conflict of interest.