SYNOPSIS

Synopsis of work done by Ms. Dipasree Hajra (SR No. 03-04-00-10-11-18-1-15842) for the award of Ph.D. degree in the Faculty of Science, Indian Institute of Science, Bangalore, India.

Thesis Title: Understanding the mechanism of host deacetylases SIRT1 and SIRT3 in the modulation of *Salmonella* pathogenesis

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Chapter 1

Introduction

Salmonella continues to be a threat to the human population by taking a toll on the lives of about 20,000 individuals globally per year. *Salmonella enterica* serovar Typhimurium is a facultative intracellular Gram-negative enteric pathogen, causing a wide array of infections ranging from self-limiting gastroenteritis to diarrhoea in humans. *Salmonella enterica* serovar Typhi cause systemic infection in humans with typhoidal symptoms. Recent reports reported 21 million typhoid cases and 93 million non-typhoidal cases year-round. The virulence of *Salmonella* is majorly regulated by two pathogenicity islands, namely, SPI-1 and SPI-2. It uses SPI-1 encoded T3SS and the effector proteins to invade host cells. Inside the macrophages, they reside within the *Salmonella*-containing vacuoles (SCV) by virtue of its SPI-2 effectors. Macrophages, dendritic cells, and neutrophils are responsible for successful dissemination throughout the body through the reticulo-endothelial system (RES).

Sirtuins are NAD+-dependent deacetylases that are present in all forms of life. *Saccharomyces cerevisiae* serve as the founder for the discovery of Sir2. Sirtuins comprise a conserved core catalytic domain that removes acetyl moiety from the lysine residues of proteins in the presence of NAD+ as a cofactor giving rise to 2'O-acetyl-ADP-ribose and free nicotinamide as products.

Host Sirtuins are one of the important modulators of host immuno-metabolic regulation. However, the role of Sirtuins in the modulation of the immune metabolism pertaining to Salmonellosis is largely unknown. Here, we investigated the role of two important Sirtuins, SIRT1 and SIRT3 in the modulation of *Salmonella* pathogenesis. We have shown that *Salmonella*-induced modulation of SIRT1 and SIRT3 is crucial for governing immune-metabolic switch in the host and this switch influences the metabolic profile of intracellular *Salmonella* (**Chapter 2**). Moreover, SIRT1 and SIRT3 regulate the mitochondrial bioenergetics and dynamics in the *S*. Typhimurium infection scenario. SIRT1 and SIRT3 mediated alteration of mitochondrial bioenergetics help in the maintenance of intracellular pH of both the host and the bacteria, thereby influencing the bacterial intracellular niche (**Chapter 3**). In *in vivo* model of mouse infection, SIRT1 and SIRT3 have been shown to regulate *Salmonella* dissemination in blood and other secondary organs. However, *O. splanchnicus* gut colonization reversed the *Salmonella*-associated dissemination in mice. (**Chapter 4**).

Chapter 2

***Salmonella*-induced SIRT1 and SIRT3 are crucial for maintaining the metabolic switch in bacteria and host for successful pathogenesis**

We have elucidated the role of SIRT1 and SIRT3 in mediating metabolic switch in *Salmonella* and its implication in establishing pathogenesis. Our study indicated the ability of the live *Salmonella* Typhimurium to differentially regulate the levels of SIRT1 and SIRT3 for maintaining the high glycolytic metabolism and low fatty acid metabolism in *Salmonella*. Upon SIRT1 or SIRT3 knockdown or inhibition, the metabolism in intracellular *Salmonella* switched to high fatty acid oxidation and low glycolysis. This switch led to decreased proliferation of *Salmonella* in the macrophages. Further, *Salmonella*-induced higher levels of SIRT1 and SIRT3 led to a skewed polarization state of the macrophages from a pro-inflammatory M1 state

toward an immunosuppressive M2 making it more conducive for the intracellular life of *Salmonella*. Alongside, immunological functions by modulating p65 NF-κB acetylation, SIRT1, and SIRT3 also skew *Salmonella*-induced host metabolic switch by regulating the acetylation status of HIF-1α and PDHA1. Interestingly, though knock-down of SIRT1 and SIRT3 attenuated *Salmonella* proliferation in macrophages, in *in vivo* mice-model of infection, inhibition of SIRT1 and SIRT3 led to more dissemination and higher organ burden which can be attributed to enhanced ROS and IL-6 production. Our study hence reports for the first time that *Salmonella* modulates SIRT1 and SIRT3 levels to maintain its own metabolism for successful pathogenesis.

Chapter 3

SIRT1 and SIRT3 mediated modulation of mitochondrial bioenergetics and dynamics during *Salmonella* infection in macrophages skews both host and bacterial intracytosolic pH

Mitochondria are considered to play a central role in the regulation of host cellular processes and host responses pertaining to bacterial infections. Numerous microbial pathogens modulate mitochondrial functions and dynamics to evade host immune responses. On the other hand, the elicited host immune responses are dependent on the overall mitochondrial bioenergetics and their function. Thus, mitochondrial health is a crucial determinant for the outcome of several bacterial infections. We have explored the role of host deacetylases SIRT1 and SIRT3 in the modulation of mitochondrial bioenergetics and dynamics pertaining to *Salmonella* infection. Here, we show that inhibition of SIRT1 or SIRT3 function either by shRNA-mediated knockdown or by the application of specific catalytic inhibitors leads to increased mitochondrial dysfunction in the *Salmonella*-infected macrophages. We have shown that inhibition of SIRT3 function leads to increased production of mitochondrial superoxide

generation in the infected macrophages. The increased mitochondrial ROS generation in the infected macrophages coincides with mitochondrial membrane hyperpolarization, increased proton leakage and respiration rate, and a decline in ATP production and ETC function. The mitostress profile suggested an overall increase in respiration parameters such as basal respiration, non-mitochondrial respiration, proton leakage and ATP-linked respiration along with an increase in Extracellular Acidification Rate (ECAR) upon both SIRT1 and SIRT3 inhibitor treatment. The mitochondrial bioenergetic alteration triggers increased acidification of the macrophage cytosolic pH which in turn skewed the intra-bacterial pH of the intracellular bacteria within the SIRT1 and SIRT3 knockdown and inhibitor-treated macrophages. Here, we show that loss or inhibition of SIRT1 or SIRT3 trigger increased acidification of the macrophage cytosol which consequently lead to a loss in intra-bacterial acidification together resulting in decreased SPI-2 gene expression. Alongside the decline in mitochondrial bioenergetics, the *S.* Typhimurium infected macrophages depict alteration in mitochondrial dynamics with increased mitochondrial fission and mitophagy alongside decreased mitochondrial fusion dynamics. Together, our results suggest the role of SIRT1 and SIRT3 in preserving the mitochondrial bioenergetics and dynamics in *S.* Typhimurium infection scenario and thereby influencing the bacterial intracellular niche.

Chapter 4

***Odoribacter splanchnicus* mitigates *Salmonella*-induced gut inflammation and its associated pathogenesis in mice**

The existence of the gut vascular barrier (GVB) acts as a protective barrier against the entry of microorganisms into systemic blood circulation. Enteric pathogens such as *Salmonella enterica* can cross the mucus and intestinal epithelial barrier, ultimately leading to GVB damage and systemic dissemination. The gut microbiota plays another major role in the maintenance of the

intestinal epithelial barrier by the production of several metabolites such as Short-chain fatty acids (SCFAs), indoles, and polyphenol metabolites that contribute to epithelial barrier integrity and turnover by regulating the expression of Tight-junction (TJ) genes. Our previous study reported an incidence of increased *Salmonella* dissemination in blood, liver, and spleen upon SIRT1 and SIRT3 inhibition owing to the increased inflammatory response. Here, we aimed to understand this exact mechanism of *Salmonella* dissemination and whether colonization with a gut commensal like *Odoribacter splanchnicus* could ameliorate the increased *Salmonella*-induced inflammation and in turn its pathogenic dissemination. *Odoribacter splanchnicus*, belonging to the order Bacteroidales, is a common, member of the human intestinal microbiota and its decreased abundance has been linked to different microbiota-associated diseases, such as non-alcoholic fatty liver disease, cystic fibrosis and inflammatory bowel disease (IBD). Our study depicts the capability of *O. splanchnicus* in reversing the *Salmonella*-associated pathogenesis in mice. *O.splanchnicus* gut colonization mitigates the *Salmonella*-associated epithelial tight junction disruption by restoring epithelial tight junction function and by preventing gut vascular barrier disruption, angiogenesis, and inflammatory response.

Conclusion and Significance

Salmonella-induced SIRT1 and SIRT3 together play a pivotal role in the maintenance of the intracellular replication niche of *Salmonella* Typhimurium by regulating both host and bacterial metabolism and via the modulation of mitochondrial bioenergetics and dynamics within the infected macrophages. In *in vivo* mice model of infection, inhibition of SIRT1 and SIRT3 led to more dissemination and higher organ burden which can be attributed to enhanced ROS and IL-6 production. *O.splanchnicus,* a gut commensal, gut colonization reverses the *Salmonella*-associated pathogenesis in mice by restoring epithelial tight junction function, inhibiting the inflammatory response, and preventing gut vascular barrier disruption.