

## Synopsis Seminar

Seminar Title	: Mechanistic insights on phagosome-lysosome fusion by 4-(Benzyloxy)phenol and its effect on intracellular mycobacteria in human macrophages
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Supervisor	: Prof. Rohan Dhiman
Venue	: Seminar Hall, Department of Life Science
Date and Time	: 05 Dec 2024 (16:00)
Abstract	: Drug-resistant tuberculosis (TB) outbreak has emerged as a global public health crisis. Therefore, new and innovative therapeutic options like host-directed therapies (HDTs) through novel modulators are urgently required to overcome the challenges associated with TB. In the present study, we have investigated the anti-mycobacterial effect of 4-(Benzyloxy)phenol (4-BOP). Cell-viability assay asserted that 50 $\mu$ M of 4-BOP was not cytotoxic to Phorbol 12-myristate 13-acetate (PMA) differentiated THP-1 (dTHP-1) cells. It was observed that 4-BOP activates p53 expression by hindering its association with KDM1A. Increased ROS, intracellular $Ca^{2+}$ , and phagosome-lysosome fusion were also observed upon 4-BOP treatment. We further demonstrate that 4-BOP-mediated enhanced ROS production is mediated by acetylation of p53. 4-BOP-mediated killing of intracellular mycobacteria was abolished in the presence of specific p53, ROS, $Ca^{2+}$ , and phagosome-lysosome fusion inhibitors like PFT- $\alpha$ , NAC, BAPTA-AM, and W7, respectively. Next, we dissected the immunomodulatory regulation of 4-BOP in various cytokines. We found that 4-BOP treatment increases IL-35 production in uninfected and mycobacterial-infected dTHP-1 cells, which regulates the phosphorylation of JAK1 and STAT3. While blocking JAK1/STAT3 activation with Baricitinib and Stattic reduced 4-BOP-induced ROS and $Ca^{2+}$ production, impairing phagosome-lysosome fusion and enhancing mycobacterial survival. Furthermore, siRNA-mediated silencing of IL-35 receptors (IL-12R $\beta$ 2 and gp130) disrupted JAK1/STAT3 signaling, reduced ROS- $Ca^{2+}$ -phagosome fusion and increased intracellular mycobacterial burden in 4-BOP-treated cells. Inhibition of p53 using PFT- $\alpha$ reduced IL-35 production and JAK1/STAT3 phosphorylation, indicating that IL-35 activation by 4-BOP is p53-dependent. These findings highlight the role of 4-BOP in regulating p53 to eliminate intracellular mycobacteria associated with IL-35-mediated phagosome-lysosome fusion.