

The tyrosine phosphatase Shp-2 is an important regulator of Toll-like receptors.

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Intro. Polymorphisms in SHP-2 gene were reported in patients with ulcerative colitis. How Shp-2 contributes to colitis is still unclear. Interestingly, intestinal epithelial cell (IEC)-specific deletion of *Shp-2* results in severe colitis in mice (*Shp-2*^{IEC-KO}). Notably, we found important alterations in the gut microbiota composition that preceded colitis. Antibiotherapy or epithelial KO of *Myd88* (an adaptor of Toll-like receptors) inhibits colitis in these mice suggesting that microbiota alterations contribute to colitis development. We then speculated that Shp-2 might be an important regulator of TLR signaling pathways.

Methods. We analyzed the possible activation of Shp-2 in IEC-6 cells exposed to TLR ligands. The contribution of Shp-2 was tested by using a specific allosteric inhibitor (SHP-099, 10 μ M). To identify the molecular mechanisms by which Shp-2 regulates TLR signaling, the APEX2 proximity assay was used, in combination with mass spectrometry.

Results. 1- Shp-2 is rapidly phosphorylated (Y542) upon stimulation of IEC-6 with TLR ligands including LPS (TLR4), flagellin (TLR5), poly IC (TLR3), Pam-2 (TLR2), CpG-B (TLR9). 2- Pre-treatment of cells with the Shp-2 inhibitor SHP099 inhibits ERK/MAPK and p65/NF κ B phosphorylation induced by LPS and CpG-B. 3- APEX2 proximity labeling identifies 33 proteins as Shp-2 putative interactors. When all Shp-2 neighbors are analyzed by "Pathway Enrichment Analysis" with the Reactome database, those pathways related to adaptive and innate immune system, as well as to positive regulation of NF κ B pathway signaling are among the most significant pathways associated with Shp-2. Of note, the top Shp-2 interactor is DHX9 (103,3 fold change), an helicase that senses CpG-B (TLR9 ligand) and which is thus critical for sensing viral DNA pathogens in order to trigger differential cytokine responses.

Conclusion. These data thus suggest that Shp-2 tightly regulates TLR/Myd88 signaling. Experiments are in progress to validate Shp-2 interactome upon TLR stimulation.