# ACTIVATION OF MONOCYTES AND CANCER CELLS BY INTERLEUKIN-13 (IL-13) AND ITS EFFECT ON CELLULAR FUNCTION

*A Thesis Submitted*
*In Partial Fulfilment of the Requirements*
*for the Award of the Degree of*

**DOCTOR OF PHILOSOPHY**

By

**Pritam Biswas**

**(Registration No.: NITD/PhD/BT/2016/00792)**

*Under the supervision of*

**Dr. Ashish Bhattacharjee**
Associate Professor
Department of Biotechnology

**DEPARTMENT OF BIOTECHNOLOGY**
**NATIONAL INSTITUTE OF TECHNOLOGY DURGAPUR**
Durgapur-713209, West Bengal, India.

September 2022

## *THESIS SUMMARY*

Th2 cytokine IL-4/IL-13 stimulates monocytes/macrophages towards alternative activation (showing M2 anti-inflammatory phenotype) leading to the up-regulation of some anti-inflammatory genes, including 15-lipoxygenase (15-LO) and monoamine oxidase A (MAO-A). IL-13/IL-4 exerts the effect via JAK/STAT signaling pathway in order to activate the monocyte/macrophage system. There are several Jaks (Janus kinases) which involved in this pathway, including Jak2 and Tyk2. On the other hand, different STATs, including STAT1, 3 and 6 play a pivotal role to facilitate the 15-LO and MAO-A gene induction in IL-13-stimulated primary human monocytes. 15-LO is a predominantly up-regulated gene product in human peripheral blood monocytes during the alternative activation process. 15-LO is identified as a lipid peroxidizing enzyme, which oxygenates polyunsaturated fatty acids (PUFAs) into the corresponding hydroperoxides. 15-LO converts arachidonic acid into 15-HpETE and linoleic acid into 13-HpODE, which are potent inflammatory mediators and promote apoptosis of cancer cells. These hydroperoxy fatty acids are endogenous ligands and activators of PPARγ transcription factor, which play a pivotal role in the apoptosis of cancer cells.

On the other hand, MAO-A is a catalytically active flavoenzyme which is located on the outer mitochondrial membrane and involved in the oxidative deamination of biogenic amines including serotonin, tyramine etc. to convert them into the corresponding aldehydes, and reactive oxygen species (ROS) is produced as a by-product. MAO-A is involved in neurodegenerative disorders, and MAO-A hyperactivity is associated with chronic depression and mental anxiety. Very recent studies demonstrated that MAO-A-induced ROS plays a crucial role in cancer cell apoptosis and autophagy.

In this present study, we demonstrated that 15-LO and MAO-A are co-induced in IL-13-activated primary human monocytes and A549 lung carcinoma cells. Moreover, we showed that STAT 1, 3 and 6 are crucial players for 15-LO and MAO-A gene induction in IL-13-stimulated monocytes and A549 lung carcinoma cells. Importantly, we revealed that MAO-A expression and activity (such as intracellular ROS generation) depends on 15-LO in monocytes and A549 cells after IL-13 stimulation. We further explored that 15-LO dependent MAO-A expression and function is regulated by PPARγ transcription factor in both monocytes and A549 cells stimulated by IL-13. Monocyte migration is a hallmark of inflammation, and we examined that IL-13 promotes monocyte migration through the

involvement of 15-LO and MAO-A. Furthermore, our data demonstrated that MAO-A-induced monocyte migration depends on 15-LO during alternative activation of monocytes.

Furthermore, we examined the IL-13-mediated apoptotic pathway in A549 lung cancer cells. Our data showed that IL-13 promotes apoptosis of A549 lung carcinoma cells through the involvement of 15-LO and MAO-A. Moreover, it is found that IL-13 induces cell cycle arrest at G0/G1 phase in A549 lung carcinoma cells. It is also observed that endogenous 13(S)HpODE (product of 15-LO) is produced in A549 cells after IL-13 treatment which has a considerable impact in the process of cancer cell apoptosis. Thus we scrutinized the effect of 13(S)HpODE on A549 cell viability and apoptosis. Our data indicated that 13(S)HpODE inhibits A549 cell viability and our flow cytometric analysis data revealed that 13(S)HpODE triggers A549 cell apoptosis. Our experimental data further demonstrated that 13(S)HpODE stimulates intracellular ROS production and p53 as well as p21 induction in A549 cells. As p53 is an effector protein of ROS, 13(S)HpODE-induced intracellular ROS production is correlated with p53 and p21 induction during A549 cell apoptosis. We further showed that 13(S)HpODE promotes apoptosis of HCT116 cells through the up-regulation of p53 and p21 expression.

This study also depicted the effect of 13(S)HpODE on CCF52 (GBM) cell apoptosis. Our observations in CCF52 cells established that the normal IL-13 signal pathway is impeded due to the presence of decoy receptor IL-13Rα2. These data thus revealed that IL-13 fails to induce 15-LO and MAO-A induction and ultimately had no inhibitory effect on the cell viability of CCF52 cells. In contrast, our observations demonstrated that 13(S)HpODE induces MAO-A expression in CCF52 cells. Moreover, the flowcytometric analysis revealed that 13(S)HpODE promotes apoptosis of CCF52 cells via the involvement of PPARγ transcription factor and MAO-A. Our results also showed that p53 and p21 induction is regulated by MAO-A in 13(S)HpODE-stimulated CCF52 glioblastoma cells.

Data further delineated that IL-13 stimulates p53 and p21 induction which is mediated through 15-LO and MAO-A in A549 cells. Moreover, we showed that p53 plays a crucial role in IL-13-stimulated A549 cell apoptosis, and p21 induction is regulated by p53. In addition we observed that PPARγ plays a vital role in apoptosis as well as in p53 and p21 expression in A549 cells in the presence of IL-13. Furthermore, we also revealed that IL-13-induced MAO-A mediated intracellular ROS is involved in A549 cell apoptosis. Our

results also showed that MAO-A-induced ROS contributes to p53 and p21 expression during IL-13-stimulated A549 cell apoptosis.

We validated our observations in case of an *in vivo* colon cancer tumorigenic study using syngeneic mice model and demonstrated that 13(S)HpODE significantly reduces solid tumor growth through the activation of apoptosis. These data thus confirmed the role of 13(S)HpODE as a potential anti-cancer agent both *in vitro* as well as *in vivo*.

In a nutshell, we demonstrated for the first time that 15-LO and MAO-A are co-induced, and MAO-A expression, as well as function, is directly regulated by 15-LO in IL-13-stimulated Monocytes and A549 cells. Our findings further demonstrate that the 15-LO-mediated expression and function of MAO-A have major implications for the resolution of inflammation. In addition, 15-LO>13(S)HpODE>PPAR-γ>MAO-A>ROS>p53 axis plays a pivotal role in the apoptosis of IL-13-activated A549 lung carcinoma cells.