

Chapter 3: Aims and Objectives

3.1 Rationale and Hypothesis:

TNF- α is an essential cytokine mediator for mounting inflammatory response and often found deregulated in cancers and inflammatory conditions. Increased levels of TNF- α had been observed in tumor microenvironment and in serum of patient suffering with chronic inflammatory conditions. TNF- α -induced NF- κ B pathway is one of the predominant proinflammatory pathway activated in these conditions. This pathway is regulated by ubiquitination where stimulus specific E3 ligases are recruited at distinct steps of the NF- κ B pathway which regulate their substrate stability and turnover, hence modulation of the pathway showing distinct outcome. Hundreds of target genes are activated by TNF- α but less is known about temporal expression of E3 induced by TNF- α and their role in regulation of NF- κ B pathway. Therefore, identification and characterization of TNF- α -induced E3 ligases may provide novel feedback regulators of this pathway which may play critical role in regulation of proinflammatory response.

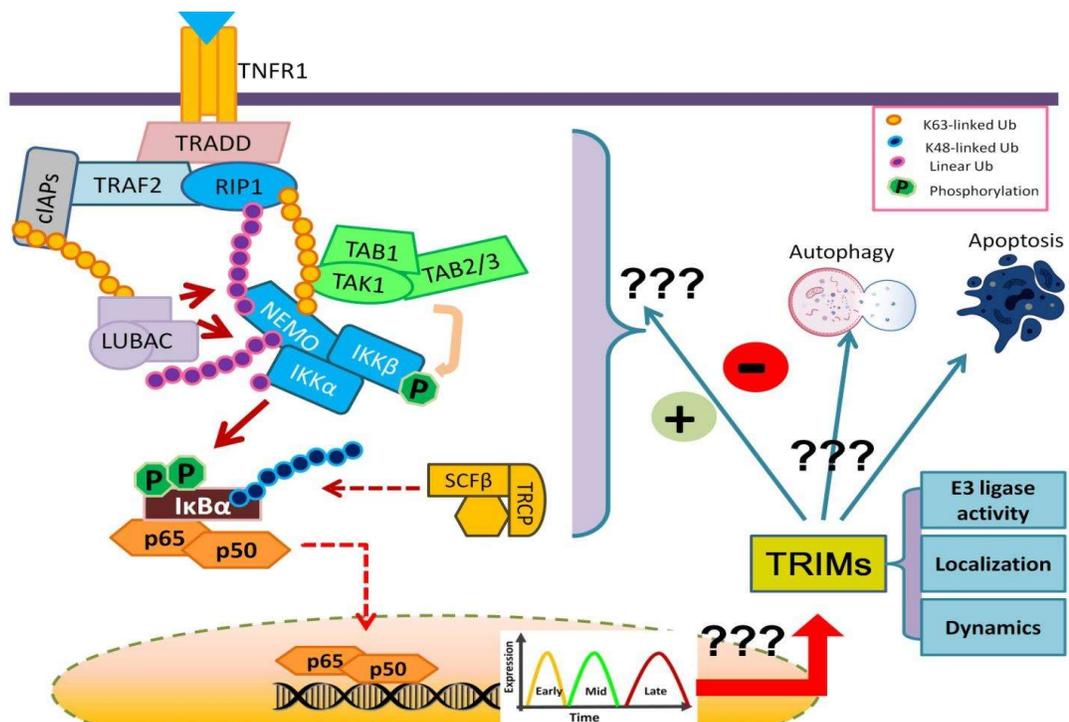


Figure 3.1 : *TNF- α -induced expression of TRIMs may regulate the NF- κ B pathway and cellular homeostasis by regulating autophagy and cell death.*

TRIM proteins are the largest family of RING E3 ligases involved in substrate modification, hence fate determination of their target pathways. Stimuli specific expression, stabilization and spatial translocation of these proteins play critical role in cellular homeostasis. Temporal expression of TNF- α -induced genes has been reported however expression of TRIMs is not known. Therefore, identification of TNF- α -induced temporal expression of TRIMs and their role in regulation of NF- κ B pathway and cellular homeostasis was proposed. To investigate these aspects the following objectives were designed:

3.2 Objectives of the study:

- 1.) Identification of TRIM family proteins involved in regulation of TNF- α mediated NF- κ B pathway**
- 2.) Study of spatiotemporal localization (in presence and absence of TNF- α) and E3 ligase activity of identified TRIMs**
- 3.) Study the molecular mechanism of identified TRIM mediated regulation of TNF- α -induced NF- κ B pathway**

The results obtained by following these objectives had been compiled in four chapters of this Ph.D. thesis.

Chapter 5 is the preliminary screening identifying TNF- α -induced TRIM expression and their role in feedback regulation of NF- κ B pathway. This chapter is a part of a manuscript we have communicated entitled ***"Novel DUB-like activity of TRIM15: RING E3 ligase; regulates TNF- α -induced NF- κ B pathway in a feedback manner"***.

Chapter 6 confirms that MID1/TRIM1 is a NF- κ B target gene negatively regulating TNF- α -induced NF- κ B pathway. MID2/TRIM1 is often downregulated in cancers and regulates expression of TRAF2 oncogene. Results and Conclusions of this chapter has been communicated as a manuscript entitled "***MID2/TRIM1; downregulated in cancers, transcriptionally inhibits TRAF2 oncogene and negatively regulates TNF- α -induced NF- κ B pathway***".

Chapter 7 demonstrates the role of TNF- α -induced 'Late' response gene; TRIM15 in negative regulation of NF- κ B pathway. It also provides clues about antagonistic effects of TRIM8 and TRIM15 in regulation of TNF- α -induced NF- κ B pathway and identifies novel DUB like activity of TRIM15 dependent on its PRY/SPRY domain. This chapter has been communicated as a manuscript entitled "***Novel DUB-like activity of TRIM15: RING E3 ligase; regulates TNF- α -induced NF- κ B pathway in a feedback manner***".

Chapter 8 identifies TRIM8 (previously identified positive regulator of NF- κ B) as a regulator of genotoxic stress induced gene and regulator of genotoxic stress induced NF- κ B, autophagy and cell death. The study has been published as manuscript entitled "***TRIM8 regulated autophagy modulates the level of cleaved Caspase-3 subunit to inhibit genotoxic stress induced cell death***". *Cellular Signalling*. 2018, DOI: 10.1016/j.cellsig.2018.04.003, (PMID: 29678622).