SYNOPSIS

Vast array of disorders and pathological conditions are characterized by inflammation. For examples allergy, asthma, autoimmune diseases, coeliac disease, glomerulonephritis, hepatitis, inflammatory bowel disease and preperfusion injury show inflammation. Additionally, metabolic disorders and several cancers also show chronic low grade inflammation aiding in the pathogenesis. Chronic inflammation induced by NF-κB activation leading to inflammatory milieu having high level TNF-α is observed in most inflammatory diseases and cancers. Moreover, the proinflammatory and immunomodulatory cytokine TNF-α is essential for several physiological functions including anti-pathogen response, lipid metabolism and apoptosis, hence targeting TNF-α has not shown promising results in treatment of these pathological conditions. ***Therefore, identification of novel regulators of TNF-α-induced inflammatory signalling may provide novel targetable proteins that may lead to improved therapeutics for these disease conditions.***

Posttranslational modification of proteins plays important role in modulation of NF-κB in different patho-physiological conditions. The ubiquitination and editing of ubiquitin chains of the targets at different steps of NF-κB is one of the major mechanism of modulation in a given patho-physiological conditions. E3 ligases are the terminal enzymes of ubiquitination process and known to recognize the target and determine the topology of ubiquitin on recruited substrate. Moreover, different types of E3 Ligases are recruited in specific patho-physiological conditions to modulate NF-κB pathway. Although the activation of TNF-α-induced NF-κB pathway by these E3 ligases has been widely studied, very less reports shows how this pathway is regulated in feedback manner. ***Therefore we focused on identification of novel feedback regulator of TNF-α-induced NF-κB pathway.***

Tripartite Motif containing proteins TRIMs are unique class of modular E3 ligases classified by presence of their signature motif composed of RING, B-

box and Coiled-coil domains. This largest family of RING domain containing E3 ligases are further classified in 11 sub-classes based on their variable c-terminal domain. ***TNF-α-induced NF-κB pathway promotes temporal expression of functionally correlated genes, however whether it also regulate expression of E3 ligases involved in feedback regulation of the pathway had not been investigated systematically.*** In the current study we systematically investigated the role of TNF-α-induced TRIM E3 ligases in regulation of NF-κB pathway, autophagy and cell death.

In our quest for novel feedback regulators of TNF-α-induced NF-κB pathway, we deployed a two step screening strategy that identified several TRIMs (TRIM1, 2, 3, 8, 9, 15, 16, 21, 31, 37, 38, 39, 41, 44, 46, 47 and 55) as 'late' response TNF-α-induced genes and most of these late response TRIMs inhibited the NF-κB pathway. Suggesting they may have crucial role in control of the pro-inflammatory signalling by feedback regulation of TNF-α-induced NF-κB pathway.

Further we characterized the role TNF-α-induced gene TRIM1/MID2 in regulation of TNF-α-induced NF-κB pathway. We found that TNF-α not only promotes the mRNA expression of TRIM1 but also stabilizes it turnover by both autophagy and UPS pathway and alters its dynamics. The study further confirmed that TRIM1 transcriptionally inhibits TRAF2; a known NF-κB inducing oncogene often found upregulated in several solid tumors. Additionally, exploration of cancer datasets using different web servers also revealed that TRIM1 is downregulated in many cancer tissues compared to control and existence of inverse correlation between TRIM1-TRAF2 cancer tissues. Suggesting, TRIM1 mediated inhibition of NF-κB pathway may have a tumor suppress role in cancers.

Next, the characterization of another TNF-α-induced gene TRIM15 revealed a novel DUB-like activity of TRIM family members. TRIM15 strongly inhibited

TNF-α-induced NF-κB pathway independent of its RING E3 ligase activity. Moreover, the observed DUB-like activity was attributed to its PRY/SPRY domain which is also critical for inhibition of NF-κB pathway. Interestingly, functional correlation between K63 linked ubiquitination and NF-κB activation was also observed. Further exploration revealed TRIM8 antagonizing function of TRIM15, and its interaction with TAK1 leading to inhibition of its K63 linked ubiquitination. Overall the study confirmed the role of TRIM15 as feedback negative regulator of NF-κB pathway with implication in chronic inflammatory conditions like psoriasis.

We went on to study TRIM15 interacting partner TRIM8 and identified an interesting effect on genotoxic stress induced cell survival. Genotoxic stress induced by etoposide treatment increased TRIM8 expression and stabilized its turnover. We found that TRIM8 enhances expression of autophagy and DDR regulator p62/SQSTM1 and promotes autophagy in RING domain dependent manner. TRIM8-induced autophagy flux under genotoxic stress was due to TRIM8-induced lysosomal biogenesis and also due to increased expression of autophagy adapter proteins p62/SQSTM1. Furthermore, TRIM8 promoted autophagy dependant cytoprotection under genotoxic stress. We identified that TRIM8 forms a complex with XIAP and Caspase-3 and promotes autophagy mediated degradation of active caspase-3 indicating potential role of TRIM8 in chemoresistance.

Overall the study identified novel TNF-α-induced late response TRIMs as feedback regulators of NF-κB pathway. Further, their molecular characterization suggests the recruitment of unique TRIMs at the different steps of TNF-α-induced NF-κB pathway beginning with TRIM1 at TRAF2 level, TRIM15 and TRIM8 at TAK1 in cytoplasm. The study also confirms that feedback regulatory mechanism independent of IκBα and A20 exists that may control signalling outcomes activated by TNF-α and biphasic NF-κB activation.

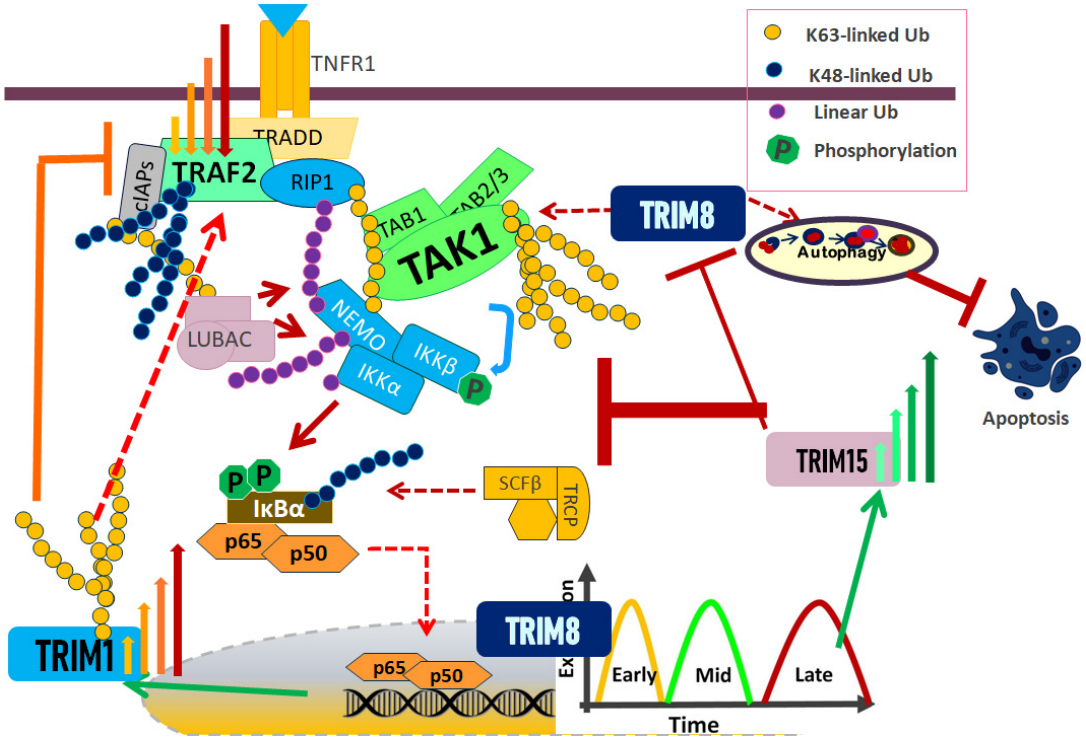

Figure 1: TRIMs: Novel feedback regulators of TNF-α-induced NF-κB pathway and their role in regulation of autophagy and cell death.

Moreover, identification of functional antagonism between TRIMs resulting in unique outcomes, including their role in regulation of autophagy-cell death crosstalk has also been identified in the study. Not only the study explores basic molecular aspects of TNF-α-induced NF-κB pathway but using open access public databases and web servers establishes the importance of these TRIMs in pathophysiological conditions like psoriasis and cancer. ***This study provides platform for further investigation of these TRIMs in different pathological conditions where TNF-α-induced NF-κB pathway is involved, specifically chronic inflammatory conditions and cancers.***