ABSTRACT

The proinflammatory and immunomodulatory cytokine TNF-α is essential for several physiological functions including anti-pathogen response, lipid metabolism and apoptosis. TNF-α activates the NF-κB pathway and promotes inflammatory response essential for survival, host defence and injury repair. Elevated levels of TNF-α have also been reported in tumor microenvironment, systemic inflammation and in geriatric syndromes. Similarly dysregualtion of TNF-α-induced NF-κB pathway is often observed in pathological conditions like cancer, neurodegeneration and metabolic disorders.

Ubiquitination is a major post translational modification involved in regulation of TNF-α-induced NF-κB pathway. Terminal enzymes of ubiquitination process: E3 ligases are involved in identification of substrate, topology of ubiquitin chain and its fate determination. Several E3 ligases are recruited at distinct steps of TNF-α-induced NF-κB pathway which may positively/ negatively regulate the pathway. Moreover, TNF-α-induced NF-κB pathway promotes temporal expression of functionally correlated genes and regulates various cellular functions. However if this pathway regulate expression of E3 ligases that are involved in feedback regulation is not well understood. TRIM family proteins are a major subclass of RING domain containing E3 ligases. TRIMs are dynamic in nature and regulate several cellular processes including autophagy, innate immune response, cell survival and death. The current study systematically investigated the role of TNF-α-induced TRIMs in regulation of NF-κB pathway, autophagy and cell death.

In the first part of the study a two step screening identifies 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 regulating TNF-α-induced NF-κB pathway. Interestingly, most of the late response TRIMs inhibited the NF-κB pathway suggesting they may have crucial role in control of the pro-inflammatory signalling.

Further the study characterized the role TNF-α-induced gene TRIM1/MID2 in regulation of TNF-α-induced NF-κB pathway. Interestingly, TNF-α not only promotes the mRNA expression of TRIM1 but also stabilizes it turnover and alters its dynamics. Biochemical studies identified that its turnover is regulated by both autophagy and UPS pathway. TRIM1 acts as an E3 ligases and its RING domain indispensible for inhibition of NF-κB pathway. The study further confirms that TRIM1 transcriptionally inhibits TRAF2; a known NF-κB inducing oncogene often found upregulated in several solid tumors. Exploration of cancer datasets using different web servers also revealed that TRIM1 is downregulated in many cancer tissues compared to control and inverse correlation between TRIM1-TRAF2 were also observed in same 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 is predominantly observed in nucleus but also found as distinct punctate structure in cytosol. TRIM15 strongly inhibits TNF-α-induced NF-κB activation independent of its RING E3 ligase domain. Surprisingly, its expression show decreased K63 linked ubiquitination in both cytosolic and nuclear fractions. Moreover, the observed DUB-like activity was attributed to its PRY/SPRY domain which is also critical for inhibition of NF-κB pathway. Further TRIM15 interacts with TAK1 and inhibits its K63 linked ubiquitination. Furthermore, it was identified that TRIM15 interacts with another TRIM family member TRIM8 which is a known positive regulator of TNF-α-induced NF-κB pathway. TRIM15 antagonizes TRIM8 enhanced NF-κB activation and suggests that heterodimerazation of TRIMs may have regulatory role in NF-κB pathway.

Exploration of TRIM15 interacting partner TRIM8 showed an interesting effect on genotoxic stress induced cell survival. TRIM8 expression was induced by

etoposide treatment which also stabilized its turnover suggesting its possible role in DDR. The study further confirmed that TRIM8 enhances expression of autophagy and DDR regulator p62/SQSTM1 and promotes autophagy in RING domain dependent manner. TRIM8 promotes lysosomal biogenesis and autophagy flux during genotoxic stress and plays a cytoprotective role. Furthermore, TRIM8 interacted with XIAP and stabilized its expression in etoposide treated cells. It was also identified that TRIM8 forms a complex with XIAP and Caspase-3 and promotes autophagy mediated degradation of active caspase-3 hence survival under genotoxic stress. These results indicate potential role of TRIM8 in chemoresistance as elevated TRIM8 levels in cancer cells may provide them survival advantage.

Overall the study identified novel TNF-α-induced late response TRIMs as negative 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. This study will help define the role of these TRIMs in different pathological conditions where TNF-α-induced NF-κB pathway is involved, specifically chronic inflammatory conditions and cancers.