Chapter 1:

Introduction

1.1 Inflammation: Essential but consequential

Inflammation is physiologically essential for host defense from infections and homeostatic response against tissue injury and stress. The complex process of inflammation requires the coordinated action of inducers (injury, infection), sensors (TLRs, NLRs), mediators (cytokines, chemokines) and effectors (immune cells). Majorly, it is a self-regulated process, but its dysregulation underlies several pathological conditions including chronic inflammatory diseases, neurodegeneration, and cancer. Recent statistics of global and regional burden of Inflammatory bowel disease (IBD) showed an increase of 85·1% in global prevalence cases of IBD indicating the gravity and increased threat of pathological conditions with inflammation (2020). Several biochemically distinct mediators including cytokines are produced initially to mount the inflammatory response and their levels, spectrum and temporal presence at the site of infection/injury can dictate the inflammatory response (Kemp et al., 2013; Medzhitov, 2008).

These cytokine mediators also function as a bridge between innate and adaptive immune system, consequently, their dysregulation is often observed in autoimmune and immunodeficiency disorders. These mediators activate a variety of transcription factors including NF-κB, IRFs and AP1 to primarily induced additional factors required for mounting effective inflammatory response (Croft and Siegel, 2017; Grivennikov et al., 2010; Kalliolias and Ivashkiv, 2016). ***Therefore, it is important to study the signaling pathways activated by these mediators to gain a better insight into the inflammatory axis in associated disease conditions.***

1.2 TNF-α induced signaling: role in health and diseases

TNF-α is a proinflammatory cytokine with immunomodulatory functions. It is physiologically essential for several processes like tissue regeneration, and

repair, development of lymphoid organ and tolerization of macrophages. It is majorly produced by immune cells; exerts its effect through two distinct receptors (TNF-R1 and TNF-R2). Its action through the TNF-R1 receptor can affect almost all cell types of the human body (Kalliolias and Ivashkiv, 2016; Wajant and Scheurich, 2011).

As a cytokine mediator, it serves an essential role in mounting an inflammatory response and host defense against pathogens. It induces the transcription of target genes by promoting the activation of NF-κB and AP1 transcription factors primarily involved in stress and inflammatory response (Wajant and Scheurich, 2011). Moreover, due to its crucial role in the regulation of various physiological processes, its dysregulation has often been associated with pathological conditions like diabetes, cancer, inflammatory and autoimmunity (Holbrook et al., 2019; Waters et al., 2013). The increasing number of evidences also suggests its complex role in cancer pathogenesis and progression of the disease. Therefore, investigation of TNF-α-induced signaling, its regulation, and characterization of its target genes can provide a better understanding of its role in pathophysiological conditions.

1.3 Ubiquitination: Determinants of substrate fate and signaling pathways

Target proteins can be modified by numerous post-translational modifications (PTMs) which can decide their fate. PTM by ubiquitination plays a diverse role in cellular homeostasis by meditating substrate stability and assembly of protein complexes. The sequential action of E1 activating enzyme, E2 conjugating enzymes, and E3 ligases adds ubiquitin chains on the substrate lysine residue and determines chain topology and length, hence substrate fate. Virtually all pathways including pro-inflammatory pathways are also regulated by ubiquitination (Komander and Rape, 2012; Rape, 2018; Zheng and Shabek, 2017).

There are about 1000 E3 ligases encoded by the human genome and these terminal enzymes of the ubiquitination pathway are responsible for substrate identification and in some cases ubiquitin transfer to the substrate. Many of these E3 ligases are recruited at different steps of various signaling pathways including TNF-α-induced NF-κB and can change the outcome of the pathway by altering the stability of their substrates (Holbrook et al., 2019; Taniguchi and Karin, 2018; Wajant and Scheurich, 2011). The temporal expression, stability, localization, and dynamics of these proteins may play a crucial role in the regulation of signaling pathways and associated physiological functions. Therefore, identification and characterization of novel E3 ligases regulating various pathways may provide critical information about pathophysiological conditions.

1.4 TNF-α-induced NF-κB pathway and its regulation by ubiquitination

Activation of NF-κB family transcription factors by variety of ligands regulates expression of hundreds of genes; temporally and contextually. The NF-κB target genes include, cytokine mediators, growth factors, cell cycle regulators which may act in concert to regulate inflammatory response and cellular homeostasis. Moreover, they also promote expression of self-regulatory genes that are critical for resolution of inflammatory response hence protects from adversities of long term activation of the pathway (Afonina et al., 2017; Taniguchi and Karin, 2018; Wertz and Dixit, 2010). ***Although the activation of the pathway has been studied extensively but its feedback regulation and control of chronic activation of the pathway is poorly understood.***

TNF-α activates the NF-κB pathway through a signaling cascades assembled at the intracellular face of the TNF-R1 receptor. Ubiquitination is critical for regulation of activation of NF-κB pathway. Recruitment of E3 ligases like TRAF2, cIAPs at the membrane bound TRADD-TRAF complex promotes ubiquitination of RIP1 and TRAF2 itself. The ubiquitinated RIP1 serves as

assembly platform for kinase complexes IKK and TAB-TAK complex. The activation of kinases leads to phosphorylation dependent ubiquitination of IκBα and release of NF-κB transcription factor. Importantly, the specific ubiquitin chain topology and individual substrates are critical for the efficient activation of the pathway (Hayden and Ghosh, 2008; Wajant and Scheurich, 2011; Wertz and Dixit, 2010). ***Although the activation of TNF-α-induced NF-κB pathway is investigated in great details but its control/inhibition by ubiquitin E3 ligases are not well investigated.***

TNF-α-induced gene expression shows interesting temporal pattern of functionally related genes. Most of these genes are involved in potentiating pro-inflammatory response and immunomodulation. Only IκBα and TNFAIP3/A20 are the known feedback regulators of the NF-κB pathway and whether E3 ligases are also involved in feedback regulation of TNF-α-induced NF-κB pathway is unknown. These feedback regulators also control the biphasic response of NF-κB activation by inhibiting the second wave of nuclear NF-κB translocation or creates oscillatory pattern of NF-κB translocation and gene expression in constitutive presence of TNF-α. Both IκBα and A20 are early response genes and whether late response genes can regulate the biphasic response is not known (Afonina et al., 2017; Nelson et al., 2004; Werner et al., 2008).

Moreover, the TNF-α-induced expression of E3 ligases and functional aspects are not well investigated. It is important to analyze the expression of E3 ligases by TNF-α-induced NF-κB pathway and their possible role in feedback regulation and regulation of inflammatory response for modulation of this pathway in a given pathophysiological conditions.

1.5 TRIMs; RING E3 ligases: The emerging regulator of innate and inflammatory pathways

Tripartite Motif containing proteins are the largest family of RING domain containing E3 ligases. The E3 ligase activity of these proteins is attributed to their RING domain. Depending on their C-terminal domain they have been further classified in 11 subfamilies (Hatakeyama, 2017; Tomar and Singh, 2015). These proteins are known to homo-heterodimerize through their coiled coil domain and help in assembly of novel signalosomes. These proteins perform diversity of functions. They have been identified as critical regulator and innate immune and antiviral response. These proteins had been identified as augmenter of innate immune signaling and also as autophagy modulators (Bell et al., 2012; Esposito et al., 2017; Li et al., 2014; Rajsbaum et al., 2014b). The role of IFN-induced TRIMs in regulation of autophagy shows their functional role as innate immune effectors (Mandell et al., 2014; Sparrer and Gack, 2018). Moreover, various reports from our group and others has shown that these proteins may regulate autophagy and cell death depending on stimuli and cellular background hence play critical role in cellular homeostasis and stress response. Some of these members also show regulatory role in TNF-α-induced NF-κB pathway (Li et al., 2011; Tomar and Singh, 2014, 2015; Tomar et al., 2012b).

Many of these proteins show stimuli specific expression, stabilization and localization to distinct cellular compartments (Tomar et al., 2013b; Tomar et al., 2012b). The systematic studies monitoring their expression, stability, localization and dynamics in presence of TNF-α has not been carried out.

This study systematically identified TNF-α-induced expression of TRIM E3 ligases and characterized their role in regulation of NF-κB pathway. Further characterization of these proteins revealed the step regulated by these proteins and substrate modification involved in NF-κB regulation.

The current study also identified an interesting nexus between genotoxic stress induced autophagy and cell death regulated by one member of this protein family and its cytoprotective role in stressed cells.