Chapter 2:

Review of Literature

2.1 Inflammation: A friend and a foe

2.1.1 Inflammation: The protective barrier and defense coordinator

Inflammation is a highly coordinated defense mechanism initiated by infection, tissue injury and tissue stress. Temporally, inflammation has been characterized as acute and chronic conditions. As an adaptive response, the recruitment of leukocytes and plasma proteins to the affected site results in cardinal signs of inflammation; Rubor (red discoloration), Calor (heat), Dolor (pain), Tumor (mass effect). Most of these symptoms arise due to vasodilation, increased vascular permeability and infiltration of white blood cells to site of infection or injury. Inflammation is multi-factorial process involving complex regulatory networks consisting of consists of inducers, sensors, mediators and effectors (Kemp et al., 2013; Medzhitov, 2008).

Acute inflammation resolves itself, but its auto-regulatory failure or persistence of inducer/stimuli may lead to chronic inflammation. Inflammation can be induced by stimuli either of exogenous like pathogen-associated molecular patterns (PAMPs), allergens, foreign bodies or endogenous origin like damage associated molecular patterns (DAMPs) (Grivennikov et al., 2010; Wertz and Dixit, 2010). Cells have array of sensors (TLRs, Inflammasomes) to detect these inducers and relay signals downstream for production of mediators and effectors. On the basis of their chemical properties they have been classified in seven groups: cytokines, chemokines, vasoactive amines, vasoactive peptides, proteolytic enzymes fragments of complement components and lipid mediators. These mediator act in concert to mount effective inflammatory response and engage the host immune system during infection and injury (Medzhitov, 2008). Tissue and cells of host are the effectors in any inflammatory response and their response to inflammatory mediators such as (IL1A and TNF-α) are specific in a given pathophysiological conditions. (Kemp et al., 2013; Medzhitov, 2008).

2.1.2 Inflammation: A double edged sword

Inflammation is a double edged sword; essential for innate and adaptive immunity and deleterious in autoimmune and chronic inflammatory conditions (Franks and Slansky, 2012). Inflammation serves as primary defense mechanisms and priming factor for tissue repair, regeneration and wound healing. Various transcription factors like Nuclear factor kappa B (NF-κB), Signal Transducer and Activator of Transcription 3 (STAT3), Interferon Regulatory Factors (IRFs) and Activator Protein-1 (AP-1) regulates expression of genes essential for mounting inflammatory response (Ahmed et al., 2015; Smale and Natoli, 2014). Dysregulation of inflammatory response is linked with several disease conditions including autoimmunity, neurodegeneration and cancer. Inflammation also plays critical role in tumorigenesis and affects all stages of cancer development from tumor initiation to metastatic dissemination of cells (Grivennikov et al., 2010; Hanahan and Weinberg, 2011; Taniguchi and Karin, 2018). Hence, it is essential to investigate these pro-inflammatory pathway and duality of functions in different pathophysiological conditions.

2.2 TNF-α: physiology, functions and signaling

2.2.1 TNF-α; a pleiotropic cytokine with immunomodulatory and inflammatory functions: A member for TNF super family

Tumor necrosis factor (TNF) superfamily (TNFSF) consists of 19 ligands and 29 receptors in humans. Additional three receptors have been identified in mice. LTα and TNF-α bind to TNFR1 and TNFR2 receptors and target wide variety of cells to promote inflammation. OX40L binding to OX40 receptor promotes expansion and accumulation of several effector T cells including T_H1, T_H2, T_H17 and cytotoxic T lymphocytes, whereas CD40 receptor-ligand pair promotes inflammatory cytokine production via activation and maturation of dendritic

cells, macrophages and B cells. Other members of TNFSF like LTα, LIGHT and TWEAK act on tissues and regulate tissue inflammation. Constitutive and inducible expression of TNFSF in lymphoid and non-lymphoid (epithelial cells, fibroblasts, smooth muscle cells, and endothelial) cells participate in pro and anti-inflammatory signaling. They target wide range of immune and non-immune cells which makes them the major regulators inflammation and immune response. Deregulation of these interactions and inflammatory signaling by these proteins may lead to inflammatory conditions and several autoimmune conditions (Holbrook et al., 2019; Kalliolias and Ivashkiv, 2016; Wajant et al., 2003).

TNF-α is primarily produced by macrophages and monocytes as 26 kDa type II transmembrane protein stable as homotrimeric form and as 19 kDa soluble forms upon the proteolytic cleavage by TNF-α converting enzyme (TACE). Soluble TNF-α is released as 51 kDa homotrimer and exerts its biological function upon binding to TNFSF receptors TNF-R1 and TNF-R2. TNF-α is a pleiotropic cytokine with proinflammatory and immunomodulatory functions and its functional duality is strongly associated with several pathophysiological conditions. Interestingly it stimulates both death and survival depending on context and its implication in several pathophysiological conditions (Holbrook et al., 2019; Kalliolias and Ivashkiv, 2016; Wajant et al., 2003) is well known.

Lymphotoxin and LIGHT are TNFSF cytokines with similar functions to those of TNF-α. They have been identified in regulation of development and homeostasis of lymphoid tissue and control immune cell functions by regulating T cell and APC responsiveness (Browning, 2008; Herro and Croft, 2016). TNF-α is physiologically important for normal response to infection against bacterial, viral and parasitic infections. Beyond its immune and inflammatory functions, TNF-α also plays an essential role in several

homeostatic functions. TNF-α is required for establishing lymphoid-organ architecture and formation of germinal-center formation, development of granulomas, and also induction of tissue repair (Kalliolias and Ivashkiv, 2016).

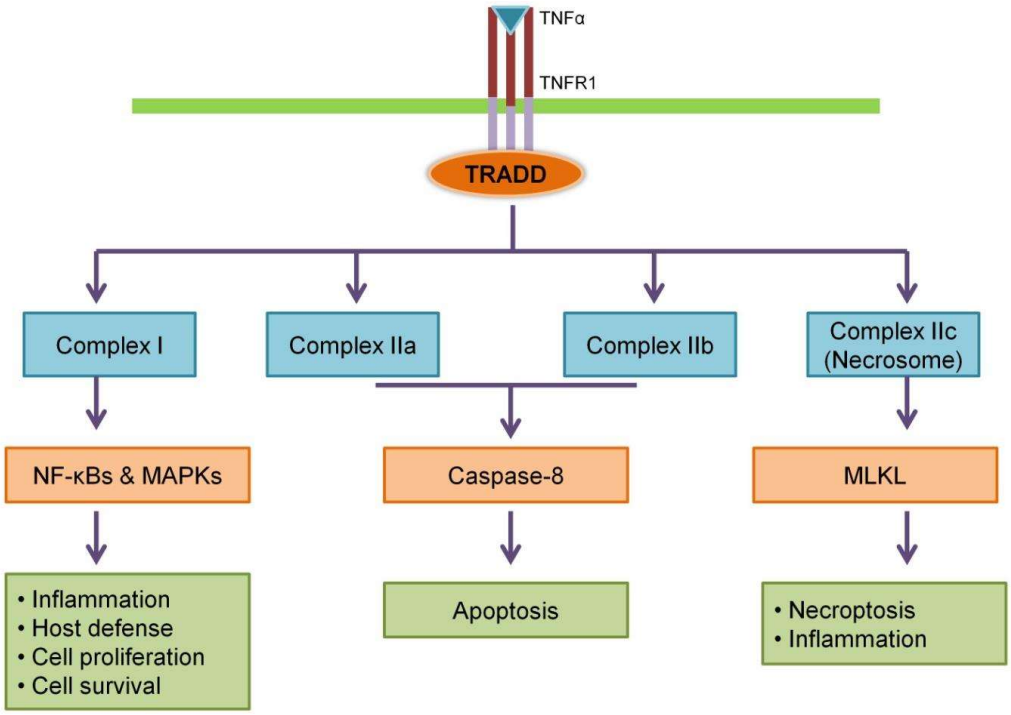

Figure 2.1: TNF-α signaling and its implication in pathophysiological conditions*. TNF-α binding to the death domain contain TNF-R1 receptor can activate distinct signaling modalities. Assembly of TRADD to the intercellular face of the receptor initially activates NF-κB and MAPKs through Complex I signaling leading to cell survival and inflammation. Alternatively, formation of Complex IIa, IIb (aka ripoptosome) leads to apoptosis, whereas Complex IIc (necrosome) can lead to necroptosis and inflammation.*

Its role in neuronal remyelination, cardiac remodeling and cartilage regeneration also indicate towards its physiological functions (Kalliolias and Ivashkiv, 2016). Physiologically, TNF-α regulates lipid metabolism in adipose tissue and protein catabolism in muscle (Garcia-Martinez et al., 1993; Ryden et al., 2004). During normal physiology, TNF-α protects against expansion of the

adipose tissue depots, increases lipolysis and free fatty acid production. Importantly tolerization of macrophages and apoptosis of inflammatory cells is also important physiological aspect of TNF-α signaling (Kalliolias and Ivashkiv, 2016).

2.2.2 TNF-α in disease manifestation

The level of TNF-α is high in many metabolic disorders like obesity and diabetes and responsible for obesity-related insulin resistance (Plomgaard et al., 2007). Insulin activated tyrosine phosphorylation of IRS-1 is required for the insulin signaling and it has been observed that TNF-α promotes the serine-threonine phosphorylation of IRS-1 and hence dampens the insulin signaling (Kanety et al., 1995).

Dysregulation of TNF-α-induced NF-κB pathways leads to several inflammatory and autoimmune diseases including rheumatoid arthritis, inflammatory bowel disease (IBD), ankylosing spondylitis, psoriasis, diseases of the central nervous system, cardiovascular disease and renal diseases. Notably, TNF-α targeting monoclonal antibodies have been approved for treatment of inflammatory diseases like rheumatoid arthritis (RA), IBD (Crohn's disease and ulcerative colitis), psoriasis, psoriatic arthritis, ankylosing spondylitis and juvenile idiopathic arthritis (JIA) further suggesting potential of TNF-α activated signaling in management of chronic inflammatory conditions (Bradley, 2008; Kalliolias and Ivashkiv, 2016).

TNF-α targeting therapeutics comes with a warning as reports have confirmed that usage of TNF-α targeting antibodies may lead to several adverse effects including opportunistic infections, latent tuberculosis reactivation, lupus-like symptoms, demyelination, psoriasis, sarcoidosis and hepatosplenic T-cell lymphomas (Kalliolias and Ivashkiv, 2016). Therefore, it is important to

understand the regulation of TNF-α-induced signaling and its fine tuning for better understanding of associated pathological conditions.

2.2.3 Dual role of TNF-α in cancer

TNF-α plays a dual role in cancer and its role may be different during the different stages of tumor progression. Reports showed that TNF-α promotes necrosis of tumors cells by altering tumor vasculature to compromise blood supply (Kalliolias and Ivashkiv, 2016). TNF-α also promotes cancer cell death by assembling RIP3 mediated large signaling complex thus promoting necroptosis in colon cancer (Moriwaki et al., 2015) and leukemia (Sawai, 2014). An aminopeptidase N (CD13) ligand fused TNF (NGR-TNF) reduced the tumor burden in lymphoma and melanoma patients (Curnis et al., 2000). Combinatorial action of IFN-γ and TNF-α has been shown to drive cancer cell senescence (Braumuller et al., 2013). Reports have confirmed the cytostatic effects of TNF-α on T47D breast cancer cells (Pusztai et al., 1993) and chemotherapy and radiotherapy sensitization by TNF-α in breast cancer cells (Wu et al., 2017). All these evidences suggest the tumor inhibitory roles of TNF-α in various cancers.

On the other hand, there exist evidences which show TNF-α promoted tumor cell proliferation or survival by activating transcription factors like NF-κB and AP-1. Both of these mechanisms contribute to survival and proliferation of cancer cells (Wajant, 2009; Waters et al., 2013). Reports have shown that TNF-α acting via a PKC-α- and AP-1-dependent pathway shows tumor promoting effect and TNF-α null mice show resistant to skin cancer (Arnott et al., 2002; Moore et al., 1999). TNF-α also promotes expression of matrix metalloproteinases (MMPs) and mediated epithelial to mesenchymal transition hence can directly affect migration and invasion ability of cancer cells and aid in tumor progression (Kalliolias and Ivashkiv, 2016; Wajant, 2009; Waters et al., 2013). Individual groups have confirmed that TNF-α promotes

cell migration ability of breast cancer cells (Wolczyk et al., 2016), colon cancer cells (Zhao and Zhang, 2018) and invasion of melanoma cells in vitro (Katerinaki et al., 2003).

These results suggest that TNF-α-induced signaling pathways and context dependent gene expression induced by TNF-α may lead to different outcomes and affect tumor progression. ***Therefore, systematic investigation of TNF-α-induced genes and their role in associated signaling pathway may provide critical evidences regarding the role of TNF-α in cancer pathogenesis.***

2.2.4 TNF-α activated signaling: Activation of proinflammatory transcription factors

Both the membrane bound and soluble TNF-α activates signaling pathways and their binding to TNFR1 and R2 receptors can have different implications. TNF-R1 is constitutively expressed in most tissues and acts as the key mediator of TNF-α mediated signaling (Croft and Siegel, 2017; Holbrook et al., 2019; Kalliolias and Ivashkiv, 2016; Wajant et al., 2003). TNF-α-TNFR1 mediated signaling activates two major transcription factors NF-κB and AP-1 thus promoting production of stress and inflammatory response factors (Kalliolias and Ivashkiv, 2016; Wajant et al., 2003).

TNF-R2 expression is highly regulated and specifically expressed on immune cells whereas signaling activated by TNFR2 is predominantly promotes cell survival and proliferation as it lacks the death domain. Moreover, TNF-TNFR2 activated signaling also promotes the reciprocal PI3K/Akt signaling pathway and recruits Etk to form the TNFR2–Etk–VEGFR2 (vascular endothelial growth factor receptor 2) complex which participates in cell adhesion, migration, survival, and proliferation (Zhang et al., 2003). TNF-TNFR2 can activate both STAT5 and NF-κB signaling pathways.

TNF-α is a prototypic activator of the NF-κB transcription factors; essential mediators for mounting pro-inflammatory response and cell survival (Hayden and Ghosh, 2008; Taniguchi and Karin, 2018; Wajant and Scheurich, 2011). Dysregulation of TNF-α-induced NF-κB pathway has been associated with many pathological conditions. For example high TNF-α levels and NF-κB activation has been observed in Chiron's diseases (Kyriakou et al., 2014; Maeda et al., 1992; Schreiber et al., 1998), psoriasis (Kyriakou et al., 2014; Lizzul et al., 2005) and rheumatoid arthritis patients (Thilagar et al., 2018). In these pathological conditions, anti-TNF-α antibody has also been approved for therapeutics suggesting the role of TNF-α -induced NF-κB pathway in chronic inflammatory conditions. These evidences further warrant investigation of TNF-α-induced NF-κB and proinflammatory axis for better understanding of these pathological conditions.

2.3 NF-κB: The proinflammatory mediator

2.3.1 NF-κB family of transcription factors: The master regulators of inflammatory response

The NF-κB or REL family of mammalian transcription factors comprises of five proteins, p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1), and p100/52 (NF-κB2). These transcription factors are active in their homo or heterodimeric forms and regulate activation of target genes by binding to their κB-binding sites present in their enhancer or promoter regions (Lecoq et al., 2017). Each member shares a conserved N-terminal REL homology domain (RHD) and a nuclear localization signal (NLS). The RHD domain has high amino acid similarity to the v-rel oncogene product from the Reticuloendotheliosis virus REV-T (Zhang et al., 2017b). N-terminal part of RHD is responsible for DNA binding specific to NF-κB, whereas the C-terminal is involved in homo and heterodimerization, and their interaction with IκBα proteins. Three members RelA, RelB, and c-Rel possess a transactivation domain (TAD) which is mainly

involved in protein-protein interactions. TAD domain of NF-κB heterodimers interacts with transcriptional regulatory proteins (CBP/p300) and general transcription factors (TAFII31, TFIIB, TFIID and TFIIH) and is essential for activation of target genes (Lecoq et al., 2017).

p105 and p100 also possess ankyrin repeats (AnkR) similar to the Inhibitors of NF-κB (IκB) proteins. p52 and p50 are the processed forms formed due to the proteolytic processing of C-terminal AnkR-containing, IκB-like regions of p100 and p105. The NF-κB heterodimers are retained in the cytosol of unstimulated cells by their interaction of IκB proteins (Zhang et al., 2017b). Stimuli induced degradation of these inhibitor proteins unmasks the NLS of NF-κB heterodimers, hence promotes their nuclear translocation. The activation of NF-κB signaling has been classified in two distinct pathways: canonical NF-κB activation (IKK complex dependent) and non-canonical NF-κB activation (NIK dependent). Activated NF-κB leads to distinct response depending on dimeric composition of NF-κB, cell type, time and context.

Optimal activation of NF-κB pathway is critical for organismal survival and fitness. Numerous target genes are activated by variety of stimuli through NF-κB pathway and their act in concert leads to discrete physiological response. p65-p50 heterodimers are the most abundant Rel dimers found the almost all cell types. Other Rel dimers like p65/p65, p65/c-Rel, p65/p52, c-Rel/c-Rel, p52/c-Rel, p50/c-Rel, p50/p50, RelB/p50, and RelB/p52 have been identified in limited subsets of cells. Moreover, Rel dimers lacking TAD domains also bind to DNA and found to inhibit transcription of target genes.

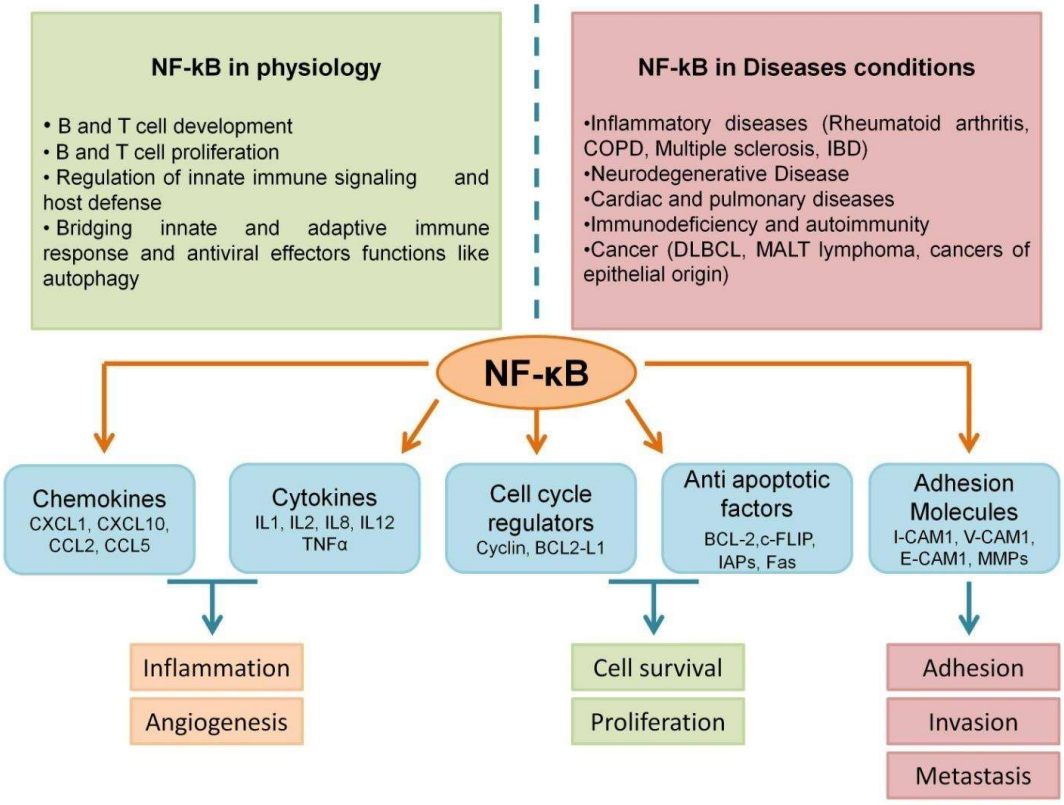

Figure 2.2: NF-κB in pathophysiological conditions. *NF-κB activates transcription of target genes involved in regulation distinct cellular functions and physiological processes. Context dependent activation of hundreds of target genes fall in different functional categories regulating inflammation and angiogenesis, cell survival and proliferation and adhesion, and adhesion, invasion and metastasis. NF-κB activation is critical for development of lymphoid organ, hence humoral immune system. Dysregulated NF-κB pathway has been found responsible for variety of diseases including neurodegeneration and cancer.*

2.3.2 TNF-α-induced NF-κB pathway: Activation and Regulation

TNF-α binding to its receptor TNF-R1 leads to rapid assembly of adapter proteins TRADD, TRAF2 & 5 and receptor interacting protein 1(RIP1) and E3 ubiquitin ligases cIAP1 and cIAP2 at the cytosolic face of the receptor. Initially, TRAF2 is recruited to the N-terminal TRAF-binding domain of the TRADD

through its C-terminal TRAF domain (Hayden and Ghosh, 2008; Wertz and Dixit, 2010). TRAF2 recruits RIP1 to the TRADD complex and promotes Lysine (K) 63 linked ubiquitination of RIP1 (Alvarez et al., 2010). TRAF2 also recruits E3 ligases cIAP1 and 2 to the membrane bound TNFR1 complex, which also contributes to RIP1 ubiquitination. Membrane bound ubiquitinated RIP1 acts as a recruitment platform for the TAB2, TAB3 and TGFβ (transforming growth factor β)-activated kinase-1 (TAK1) through their ubiquitin binding domains (UBDs) (Wajant and Scheurich, 2011). Importantly, the kinase activity of RIP1 is not required for activation of TNF-α induced NF-κB or p38 MAP kinase pathway, suggesting it primarily acts as an assembly hub rather that an activating kinase. Once assembled on the K-63 Ub chains of RIP1, TAK1 is ubiquitinated at lysine 158 by TRAF2. TAK1 ubiquitination promotes conformational changes leading to its own phosphorylation at Thr178, Thr184, Thr187, and Ser192 positions. This ubiquitination induced phosphorylation is critical for its own kinase activity required for IKK complex activation by phosphorylation (Fan et al., 2010; Wajant and Scheurich, 2011). The other kinase complex IKK, composed of IKKα, IKKβ and regulatory IKKγ/NEMO is recruited to the K-63 ubiquitinated RIP1 in a TRAF2 dependent manner. The presence of both TRAF2 and RIP1 is indispensable for the kinase activity of IKK complex (Devin et al., 2000). Ubiquitination of RIP1 on Lysine 377 residue is critical for recruitment of both IKK and TAB-TAK complex at TNF-R1 (Wajant and Scheurich, 2011).

cIAPs catalyze K11-, K48- and K63-linked ubiquitin chains to different components of TNF-R1 complex including K11-linked ubiquitination of RIP1. The E3 ligase activity of cIAPs is required for HOIL-1 (LUBAC component) recruitment to the TNF-R1 complex (Haas et al., 2009).

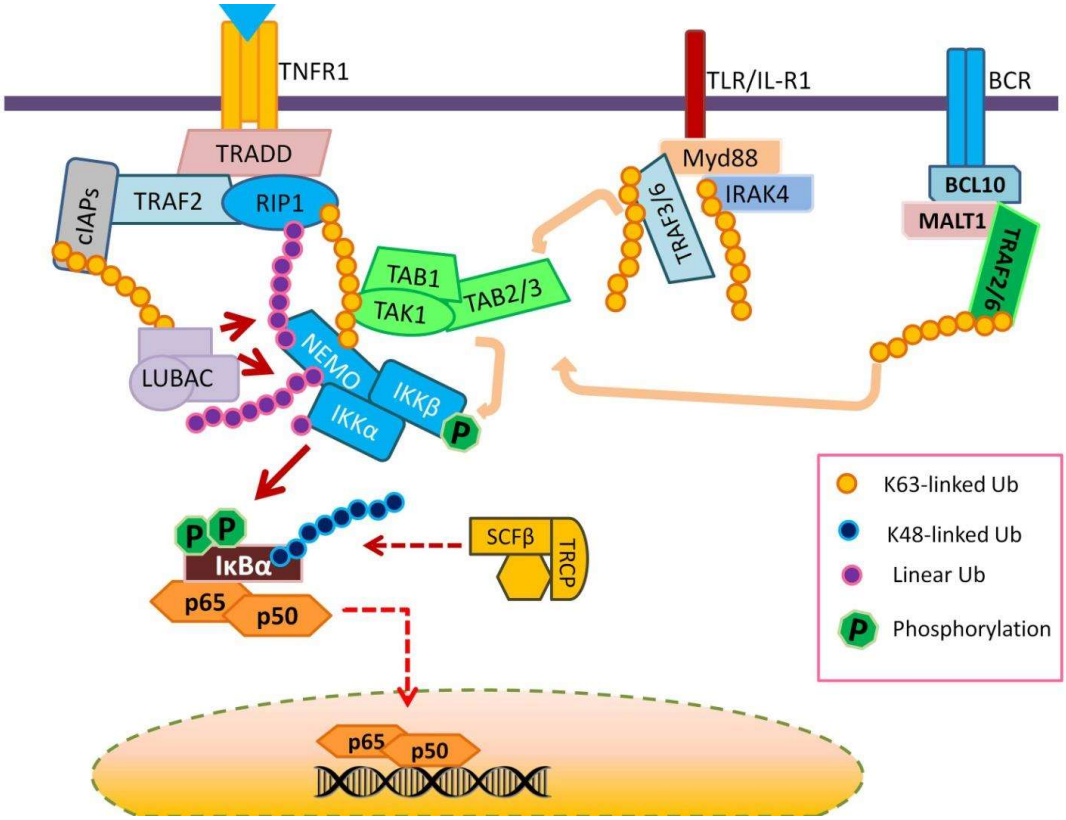

Figure 2.3: Regulation of TNF-α-induced NF-κB pathway by ubiquitination. *Activation of NF-κB pathway by TNF-α, TLR and TCR converges on the IKK complex through discrete adapter complexes like TRADD-TRAF2, Myd88-IRAK2 or BCL10-MALT1. Activated IKK complex primes degradation of IκBα by promoting its phosphorylation which serves as a maker for its ubiquitination mediated degradation through UPS.*

Consequently, LUBAC (Composed of SHARPIN, HOIL-1L, and HOIP) promotes the complex stability by promoting linear ubiquitination of NEMO, RIPK1, and TRADD of the TNF-R1 complex and also enhances NEMO recruitment to TNF-R1 (Haas et al., 2009; Spit et al., 2019). LUBAC is also required for linear polyubiquitination of NEMO occurs at Lysine 285 and Lysine 309 (Tokunaga et al., 2009). Moreover reports also show that linear ubiquitination of NEMO at these positions are also important for activating genotoxic stress induced NF-κB activation (Niu et al., 2011).

Assembly of TAB-TAK and IKK complex on the TNF-R1 complex leads to series of phosphorylation events. Initially TAK1 mediated Ser177 phosphorylation of IKK complex subunit IKKβ promotes its autophosphorylation at Ser181. This potentiates IKKβ kinase activity for substrates including NF-κB inhibitor alpha (IκBα) (Hayden and Ghosh, 2008). IκBα phosphorylation at Ser32 and Ser36 leads to its identification and ubiquitination by SCF-ubiquitin E3 ligase complex which in turn promotes its degradation by ubiquitin proteasome system (UPS) (Hayden and Ghosh, 2008; Wertz and Dixit, 2010). Degradation of IκBα exposes the nuclear localization signal of NF-κB heterodimer consequently promoting its nuclear translocation by importing isoforms alpha 3 and 4 (Fagerlund et al., 2005). The NF-κB heterodimers bind to the target DNA sequence and activates transcription of hundreds of target genes which may play critical role in mounting inflammatory response, cell survival and proliferation, depending on the context.

These mechanisms clearly suggest involvement of several E3 ligases and their sequential action in activation of the NF-κB pathway, but whether E3 ligases also inhibit the pathway in various pathophysiological conditions is not known.

2.3.3 Oscillatory NF-κB activation and temporal gene expression in response to TNF-α

The activation of TNF-α-induced NF-κB can be monophasic, where NF-κB translocation to nucleus takes place and leads to re-synthesis of inhibitory IκBα protein, consequently, leading the redistribution of NF-κB heterodimers back to cytoplasm. Conversely, constitutive presence of TNF-α leads to oscillatory activation of NF-κB due to prolonged IKK complex activation resulting in continued proteolytic degradation of IκBα and several rounds of NF-κB heterodimer nucleo-cytoplasmic translocations. Both of the mechanisms depend on the IκBα transcription which is a NF-κB target gene

(Nelson et al., 2004; Tian et al., 2005). These two distinct mechanisms are also known as biphasic NF-κB response. Moreover several studies focusing the biphasic response has suggested that the outcome or functional consequence may depend on the number, period, and amplitude of oscillations (Nelson et al., 2004). Another NF-κB target gene TNFAIP3 (aka A20) has been found to regulate the NF-κB oscillations (Werner et al., 2008; Zambrano et al., 2016).

Interestingly, these feedback regulators IκBα and A20 determine the duration of first and the second phase (Werner et al., 2008). It had been observed that these oscillations translates into functionally related proteins suggesting NF-κB oscillations may have critical role in execution of TNF-α-induced effectors response (Zambrano et al., 2016).

TNF-α-induced NF-κB activates expression of several genes including auto regulatory genes involved in fate determination of pathway. Temporal expression of TNF-α-induced genes in response to biphasic activation has provided three distinct classes of genes showing expression peaks at 1, 3 and 6 hours, termed as 'Early', 'Mid' and 'Late' response genes (Tian et al., 2005).

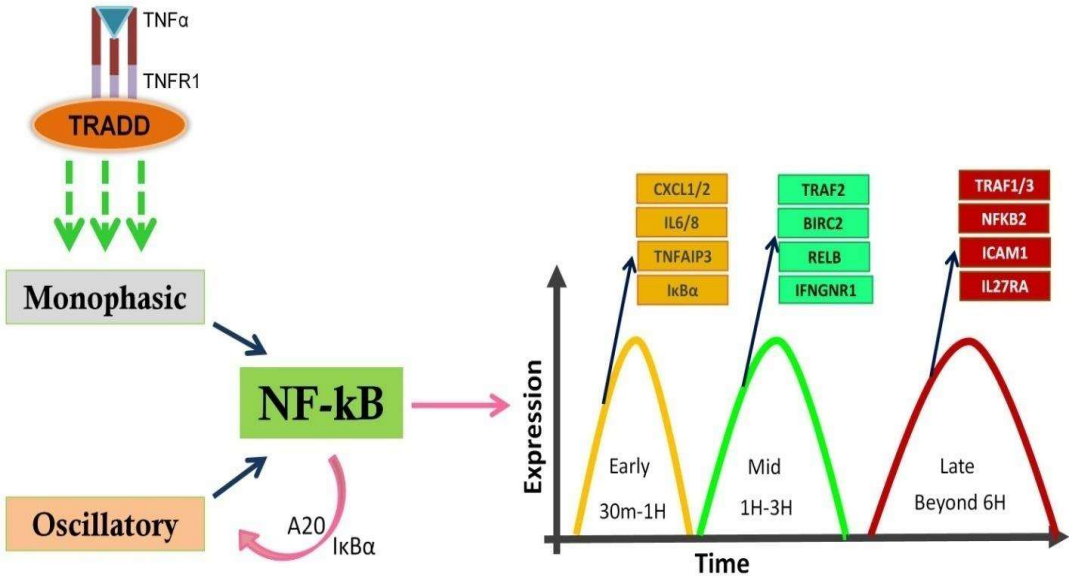

Figure 2.4: Oscillations in NF-κB activation and temporal gene expression induced by NF-κB. *Depending on the temporal expression pattern TNF-α-induced NF-κB target genes are categorized in three distinct classes; early, mid and late response genes. Early response genes TNFAIP3/A20 and IκBα are feedback regulators of the pathway results in cytosolic relocalization of NF-κB heterodimers whereas persistence of stimuli causes another cycle of NF-κB translocation. These two phases of pathway activation creates an oscillation of NF-κB nuclear translocation.*

Among them the 'Early' response genes show molecular functions related to cytokine, chemokines signaling, 'Mid' response genes show sugar and protein binding, whereas the late response genes show peptide transporter activity and protein binding functions (Tian et al., 2005). Moreover, both feedback inhibitory proteins IκBα and A20 are 'Early' response genes and less is known about the possible role of 'Late' response genes in feedback regulation of TNF-α-induced NF-κB pathway.

Therefore, it further warrants investigation of TNF-α-induced 'Late' response genes and its possible role in feedback regulation of the pathway and control of chronic inflammation.

2.4 Ubiquitination: mechanism, physiology and functions

2.4.1 Ubiquitination: Adding diversity to the limited genome

Chemical changes of proteins at targeted sites after translation by enzymatic non-enzymatic processes are known as post translational modifications (PTMs). This may include addition of specific chemical moieties or processing to the polypeptide chains. PTMs play role in virtually all cellular processes by controlling protein structure, functions, stability degradation and assembly of complexes (Prabakaran et al., 2012; Walsh, 2006).

Addition of ubiquitin to the substrate lysine (K) residue by an enzyme catalyzed process is known as ubiquitination. Eukaryotic genomes contain multiple Ubiquitin genes. Ubiquitin (Ub) is a highly conserved 76 amino acid protein produced in metazoans by four genes. They are produced as either a fusion to ribosomal proteins (encoded by UBA52 and RPS27A) or as polyubiquitin cassettes (encoded by UBB and UBC). These fusion proteins are processed by deubiquitinases (DUBs) to produce mature free monomeric ubiquitin (Rape, 2018; Spit et al., 2019). Ubiquitin itself possess several additional modification sites. Ubiquitin possess seven lysine residues (K6, K11, K27, K29, K33, K48 and K63) (Komander and Rape, 2012; Prabakaran et al., 2012; Swatek and Komander, 2016). All these lysine residues can act as substrates for further addition of ubiquitin moieties leading to formation of specific or mixed type of ubiquitin chains. An eighth type of chain can form when ubiquitin is attached to the N-terminal methionine (M1) of a second ubiquitin, leading to M1 or linear ubiquitin linkage (Rape, 2018; Swatek and Komander, 2016; Zheng and Shabek, 2017). These polymeric ubiquitin chains adopt distinct conformations and can be recognized by unique assembly factors and effector proteins.

Some of these chain topologies has been ascribed specific functions such as K11 or K48, or K11/K48-branched polyubiquitin chains leads to proteasomal degradation of substrates, for example K48 linked chains promote degradation of substrates like IκBα, JMJ2A & 2B (Mallette and Richard, 2012), whereas, M1 or K63 linked polyubiquitin chains regulate the assembly of signaling complexes, sorting of proteins during autophagy and endocytosis (Rape, 2018). Linear ubiquitination of RIP1 and NEMO stabilizes the TRADD-TARF and IKK complex respectively (Spit et al., 2019; Tokunaga et al., 2009). K63 linked ubiquitination of TAK1 by TRIM8 promotes the activity of TAB-TAK complex (Li et al., 2011), whereas K63 ubiquitination of Beclin1 is required for

autophagy (Xu et al., 2016). Interestingly, other PTMs of ubiquitin like phosphorylation, phosphoribosylation, acetylation or deamidation of glutamine can also increase the complexity of the ubiquitin code and provide fine tuning of signaling cascades and pathophysiological response (Rape, 2018).

Many proteins share the ubiquitin fold and are conjugated to specific substrates. These proteins are collectively called as ubiquitin like modifier proteins (Ubls). Ubls like are NEDD8 and SUMO are universally present in eukaryotes, whereas FAT10, ISG15 and UFM1 are only present in higher eukaryotes like mammals and rodents (Pickart and Eddins, 2004; Walsh, 2006). The topology determined ubiquitin code has certainly expanded as the integration of other Ubl modifiers into Ub chains can gives rise to heterologous Ub chains and these atypical Ub chains may have specific functions (Ikeda and Dikic, 2008).

Linear/M1 linked ubiquitination of substrates are also emerging as critical regulator of signaling processes. Recent reports have shown linear ubiquitination as critical regulator of innate and adaptive immune response and critical for inflammatory and cell death signaling. Interestingly, HOIL, HOIP and SHARPIN composed linear ubiquitin chain assembly complex (LUBAC) is the only known E3 ligase complex reported to have linear ubiquitin ligase activity (Spit et al., 2019).

2.4.2 Mechanism of Ubiquitination

The transfer of ubiquitin to the substrate lysine requires sequential action of three enzymes, 1) Ubiquitin activating enzyme, 2) Ubiquitin conjugating enzyme and 3) Ubiquitin ligase. The first step is to activate the Ub by adenylation of its c terminal carboxyl group. Initially a high energy thioester bond is formed between cysteine of E1 activating enzyme and c terminal of

Ub. Human genome codes for two activating enzymes, UBA1 and its homolog UBA6. Predominantly UBA1 is responsible for the activation of ubiquitin in human cells, whereas UBA6 can activate Ub and Ubiquitin like protein (Ubl) FAT10. Activated ubiquitin is transferred from E1 to another cysteine residue of one of ~40 E2 ubiquitin-conjugating enzymes via a transthiolation reaction (Pickart and Eddins, 2004; Stewart et al., 2016).

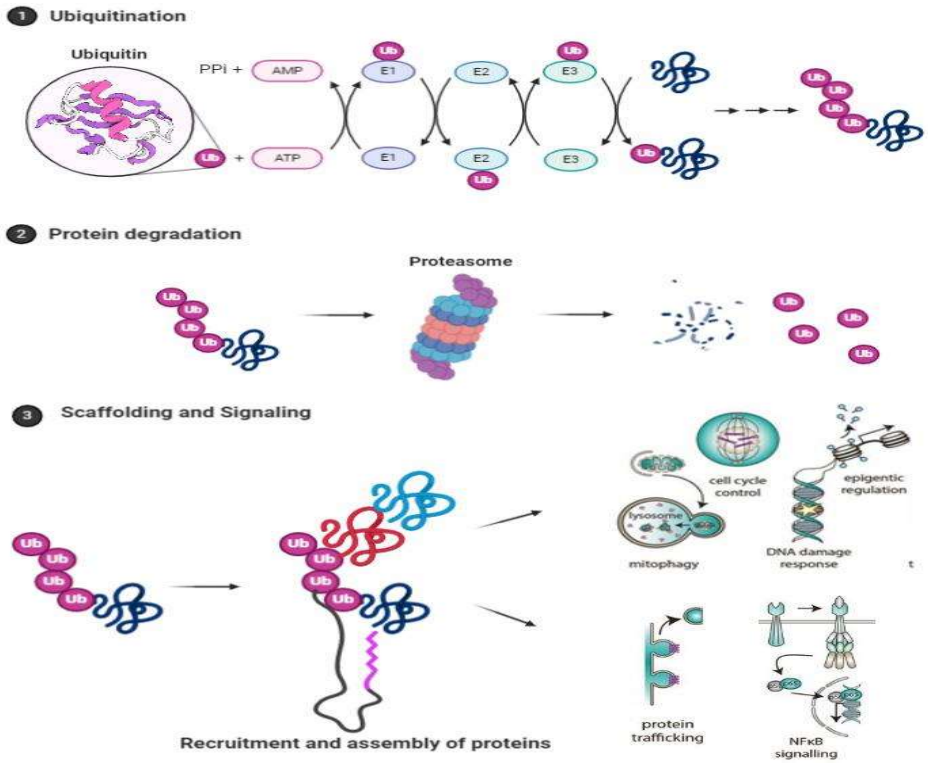

Figure 2.5: Mechanisms of Ubiquitination and outcomes. *The process of ubiquitination requires sequential action of E1 activating, E2 conjugating and E3 ligases. E3 ligases recognize the substrate and determine ubiquitin chain topology of the substrate hence determines their fate. Topology of ubiquitination determines target protein degradation or assembly of signaling components on the ubiquitinated proteins.*

Most E2 enzymes interact with either of the E1 and several other E3 enzymes, depending on the context. Most E2 enzymes function as a dimer and serve two basic functions: 1) Ub chain initiation and 2) Ub Chain elongation. More

importantly E2 enzymes dictate the topology of ubiquitin chain assembly on the substrate when they are in complex with RING type E3 ligases. E2 enzymes also transfer Ub to the substrate via a transthiolation reaction (Komander and Rape, 2012; Pickart and Eddins, 2004; Stewart et al., 2016; Zheng and Shabek, 2017). Third enzymes of the ubiquitination process are called E3 ligase. They may act as a bridge between E2 enzymes and substrate or directly transfer the Ub after receiving from E2 enzymes. The human genome codes for about ~1000 E3 ligases. Repeated action of E2-E3 enzyme pairs and combination of such pairs can lead to distinct Ub chain topology (Komander and Rape, 2012; Rape, 2018).

2.4.3 Ubiquitin E3 ligases

These proteins are responsible for the selection of target and some also determine the Ub topology (in care of HECT E3 ligases) of substrate; consequently, determines substrate fate and brings specificity to the pathway. E3 ligases may function as single peptide (like parkin) (Nguyen et al., 2016), homo/heterodimers (like MDM2/MDM4 or XIAP) or multimeric complex (like Cullin-RING-ligase complexes or the anaphase promoting complex/cyclosome). Based on their domain composition and mechanism involved in ubiquitination, E3 ligases are classified in three classes: the Really Interesting New Gene (RING) type E3s, the Homologous to the E6AP Carboxyl Terminus (HECT) type E3 ligases and RING-IBR-RING E3s (Zheng and Shabek, 2017).

2.4.3.1 HECT E3 ligases

HECT class of E3 ligases are composed of about 30 members. They promote the Ub transfer to the substrate in a two-step reaction: in the first step, they form a thioester-linked intermediate with activated Ub from the E2 in a transthiolation reaction on their catalytic cysteine (present in the C-terminal

lobe). In the second step, the Ub moiety is transferred to a lysine on the target substrate. They can promote substrate polyubiquitination and their c-terminal region is responsible for the determination of specific chain linkages on substrates, independent of E2 conjugating enzymes (Weber et al., 2019; Zheng and Shabek, 2017). For example, the founding member of the HECT E3s, E6AP (E6-associated protein), promotes K48-linked polyubiquitination of substrates, whereas Rsp5 which is a member of the NEDD4 family of HECTs, preferentially catalyze K63–linked substrate ubiquitination. Based on their N-terminal domain organization HECT E3 ligase have been sub-classified in to three families; NEDD4 family (9 members), HERC family (6 members) and 'others' (about 13 members) (Weber et al., 2019). Deregulated expression of these E3 ligases has been linked to cancers and neurological diseases (Weber et al., 2019).

2.4.3.2 RING-Between-RING E3 ligases

The RBR (RING between RING)-type E3s family are about 14 members. They are RING-HECT hybrids encompassing RING1, central zinc domain known as in-between RING (IBR) domain and RING2, sequentially. They also possess unique N- and/or C-terminal flanking domains involved in regulation of their E3 ligase activity. PARKIN is a classic example of RBR E3 ligases, essential for mitochondrial quality control by promoting mitophagy (Nguyen et al., 2016). It has been found mutated in Autosomal Recessive Juvenile Parkinsonism (AR-JP) (Kitada et al., 1998). HOIP is another notable RBR type E3 ligase; an integral part of LUBAC (Linear Ubiquitin Chain Assembly Complex) essential for regulating NF-κB pathway. Its mutation has been found to cause auto-inflammation, immunodeficiency, amylopectinosis, and lymphangiectasia in patients (Boisson et al., 2015).

2.4.3.3 RING E3 ligases

RING proteins are the largest family of E3 ligases with approximately 1000 predicted members of this family. All the members of this family possess the characteristic RING zinc finger domain. The RING domains coordinate Zn^{2+} in a cross brace arrangement which serves as a platform for E2 binding (Metzger et al., 2014). Unlike the HECT domain E3 ligases they lack the catalytic domain and majorly act as a bridge between the substrate and the E2 conjugating enzymes. E3 ligases are also important for reactivity enhancements of the partner E2. The vast number of these proteins and specific engagement with the E2 enzymes are critical for substrate ubiquitination (Deshaies and Joazeiro, 2009; Joazeiro and Weissman, 2000; Komander and Rape, 2012; Metzger et al., 2014; Zheng and Shabek, 2017). Stimuli specific expression, cellular localization and dynamics, self-modifying activity and combinatorial activity of partner E2 enzyme results in unique outcomes regulated by these enzymes. Mutation in E3 ligases have been linked with variety of diseases including cancers. Members including BRCA1, FBXO11 and VHL are known to be mutated in cancer.

2.5 Limiting inflammation: Ubiquitin dependent and independent inhibition of TNF-α-induced NF-κB pathway

The persistent activation of TNF-α-induced NF-κB pathway causes chronic inflammation and several pathological conditions, hence inhibition of this pathway is equally important in pathophysiological conditions. Like any other pathway it is regulated by various post-transcriptional and post-translational regulatory mechanisms.

Reversibility of ubiquitination provides the critical inhibitory arm to the TNF-α-induced NF-κB signaling. Several deubiquitinating enzymes (DUBs), their unique targets and chain specific ubiquitin removal activity are essential for

inhibition of TNF-α-induced NF-κB pathway. Their increasing numbers, unique and redundant substrates provide greater insight on restriction of TNF-α-induced proinflammatory signaling. ***DUBs involved in regulation of the pathway are listed in Table 1.***

DUB	Description	Mechanism of action
TNFAIP3/A20 (Ubiquitin Editing enzyme)	TNF-inducible OTU domain containing DBU Polymorphisms or mutations of associated with inflammatory diseases such as RA, SLE, psoriasis, and IBD Mutation and deletions are associated with various cancers including DLBCL, MALT and Hodgkin Lymphoma	Removes K63-linked chains from RIP1 and NEMO and conjugates K48-linked chains on RIP1, UbcH5c and Ubc13 Promotes proteasomal degradation of RIP1 Binds to linear ubiquitin chains and antagonizes NEMO and E2 ubiquitin-conjugating enzyme binding
Cezanne	TNF-inducible OTU domain containing DBU	Cleaves K11 linked ubiquitin chains from RIPK1
OTULIN	OTU domain containing DBU Regulated by phosphorylation (Tyr56 phosphorylation inhibits its activity)	Removes linear ubiquitin chains from RIPK1 and NEMO Interacts with HOIP and regulates LUBAC's E3 ligase-activity
CYLD	USP family DUB CYLD mutations is responsible for predisposition to cylindromatosis	Primarily removes K63-linked ubiquitin chains and secondarily linear ubiquitin chains from NEMO, TRAF2 and TAK1
USP11 and USP15	USP family DUB	Prevents proteasomal degradation of IκBα by removing its K48-linked ubiquitin chains
USP4 and USP21	USP family DUB	Remove K63-linked ubiquitin chains from TRAF2, RIPK1

		and TAK1
USP31	USP family DUB	Removes K63 linked chains from TRAF2 and removes ubiquitin chains from p65 (modulates in transcriptional activity)
MCPIP1	TNF-inducible unclassified DUB with RNase or DUB activity Its RNase activity promotes Degradation of inflammatory mRNAs	Removes K63 linked chains from TRAF2 and RIPK1 and K48 linked chains from IκBα

Table 1: DUBs involved in regulation of TNF-α-induced NF-κB pathway.

Interestingly, ITCH is the only known E3 ligases targeting TAK1 for proteasomal degradation involved in inhibition of NF-κB pathway. Moreover it acts in concert with DUB CYLD to modify TAK1 and promotes its degradation (Afonina et al., 2017; Kalliolias and Ivashkiv, 2016). Suggesting novel E3 ligases may be involved in negative regulation of TNF-α-induced NF-κB pathway and function as inflammatory response.

2.6 Tripartite Motif containing Proteins (TRIMs)

Tripartite motif containing proteins (TRIMs) are member of RING family of ubiquitin E3 ligases (Meroni and Diez-Roux, 2005; Reymond et al., 2001). 75 members of this protein family share the unique N-terminal Tripartite Motif composed of RING, one or two B-Box and Coiled coil domain (Meroni and Diez-Roux, 2005; Reymond et al., 2001; Sardiello et al., 2008). They also possess additional c terminal domains of various length and compositions and the TRIM family proteins are further sub-classified based on their c-terminal domain composition. Majority of TRIMs possess PRY or/and SPRY domain in their C-terminal region. C-terminal subgroup One Signature (COS), fibronectin type 3 (FN3), plant homeodomain (PHD), bromodomain (BR), bromodomain, filamin type Ig (FIL), NCL-1, HT2A and LIN-41 (NHL) repeats, meprin and TRAF

homology (MATH), ADP ribosylation factor-like (ARF) and transmembrane domains (TMs) are among the additional C-terminal domain possessed by members of TRIM proteins (Hatakeyama, 2017; Sardiello et al., 2008; Tomar and Singh, 2015).

These proteins are highly modular in nature and specific functions are ascribed to each domain. RING and B-box domain of TRIM proteins may function as E3 ligases domain whereas the coiled coil (CC) domain of TRIM proteins show homo and heteromeric interactions leading to oligomers and complexes of higher order structures (Li et al., 2014; Reymond et al., 2001). The B30.2 domain (also known as RFP-like or PRY/SPRY) is defined by the presence of highly conserved sequence motifs (LDP, WEVE, and LDYE). Proteins containing PRY/SPRY domain have been shown to have functions related to innate immune response (D'Cruz et al., 2013). Recent evidences also suggest that TRIMs can assemble novel signalosomes in different pathophysiological conditions (Kimura et al., 2015; Kimura et al., 2016b; Kumar et al., 2017; Mandell et al., 2014; Rajsbaum et al., 2014b; Tan et al., 2017).

2.6.1 RING Domain

40-60 residues RING-domain is a special type of Zn-finger that binds two atoms of zinc, involved in mediating protein-protein and protein-DNA interactions (Borden, 1998). Based on the different cysteine/histidine pattern RING domains have two variants; the C3HC4-type and a C3H2C3-type (Esposito et al., 2017). This protein interaction domain is implicated in various cellular functions including the intrinsic E3 ubiquitin-protein ligase activity (Joazeiro and Weissman, 2000). The RING domain mediates the substrate interaction with the appropriate E2 conjugation enzymes and promotes ubiquitination by facilitating the direct transfer of ubiquitin from E2 enzymes to lysine residues on the target (Deshaies and Joazeiro, 2009; Joazeiro and Weissman, 2000). Several transcription factors including HLTF, TIF1β/TRIM28,

PML/TRIM19, NFX1 and NFXL1 contain the RING domain which mediates their interaction with DNA (https://www.genenames.org/data/genegroup/#!/group/58).

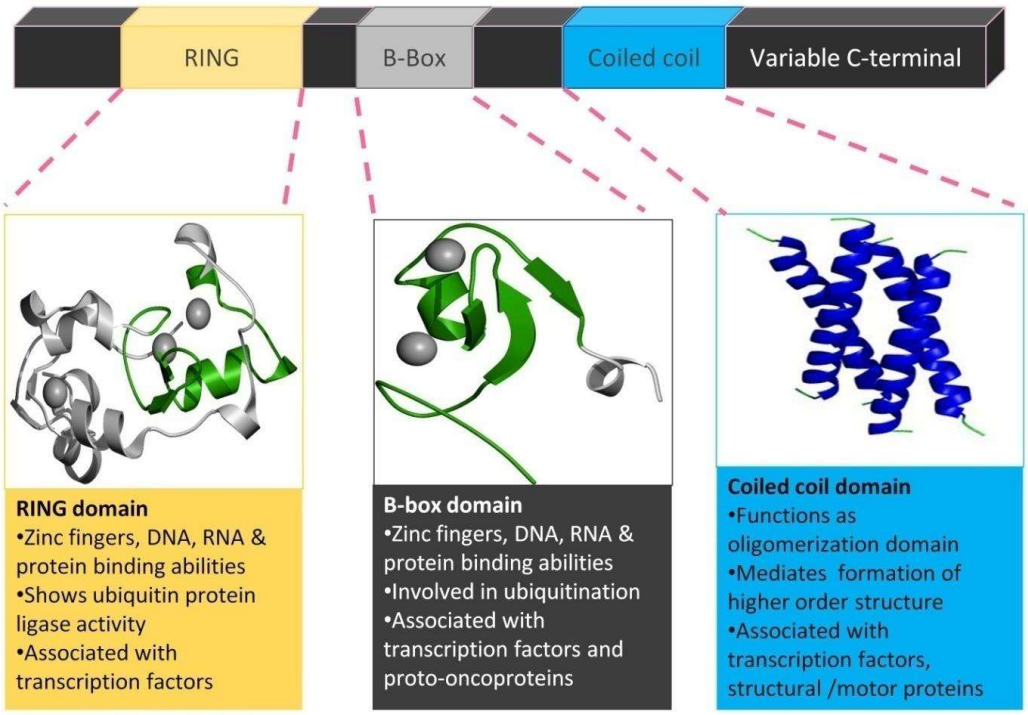

Figure 2.6: Domain architecture of TRIM proteins. *The tripartite motif of these proteins is composed of RING, B-box and coiled coil domain. Each domain has specific functions attributed to them. The C-terminal region comprises additional domains which add diversity to their cellular localization and functions.*

2.6.2 B-Box Domain

Most TRIM family proteins contain either one or two B-Box domains. Both the B-Box domains are rich in cysteine/histidine amino acids and are similar to RING domain. The B-box1 domain is composed of a 50–60 amino acid zinc-binding consensus sequence of [C5(C/H)H2] whereas, the B-box2 domains have 35–45 amino acid and a consensus sequence of [CHC(C/D/E)C2H(C/H)].

B-box1 domain precedes the B-box2 domain in TRIM proteins having two B-box domain but single B-box domain TRIM proteins have the B-box2 domain (Esposito et al., 2017). These domains are involved in protein-protein interaction and also been shown to possess E3 ligase activity (Borden, 1998). B-box domain show weaker E3 ligase activity compared to RING domain but studies have also found that B-box domain of TRIM16 (lacks RING domain) show stronger E3 ligase activity (Bell et al., 2012).

2.6.3 Coiled-coils (CC) Domain

CC-domains are formed of two or more a-helices wrapped around each other with a superhelical twist. The CC domain is highly conserved from viruses to plants and mammals and normally comprised of two and six helices arranged either parallel or antiparallel to each other (Sanchez et al., 2014). The main function of CC domain is oligomerization and various proteins containing CC domain can form homo or heterotypic oligomers. They are found in variety of proteins including structural proteins, transcription factors and motor proteins. The coiled coil domain of TRIM proteins mediate their homo and heteromeric interaction and also help in binding substrates for their E3 ubiquitin ligase activity (Esposito et al., 2017; Sanchez et al., 2014; Truebestein and Leonard, 2016).

2.7 TRIMs: Dynamic subcellular spatial localization

TRIM proteins are highly dynamic in nature and often found to be shuttling between different cellular compartments. Normally they are found as discrete cytoplasmic or nuclear structures. Many TRIM proteins forms ‘cytoplasmic bodies’, whereas TRIM8, 19, 30 and 32 are found in nucleus and forms "nuclear bodies" (Reymond et al., 2001).

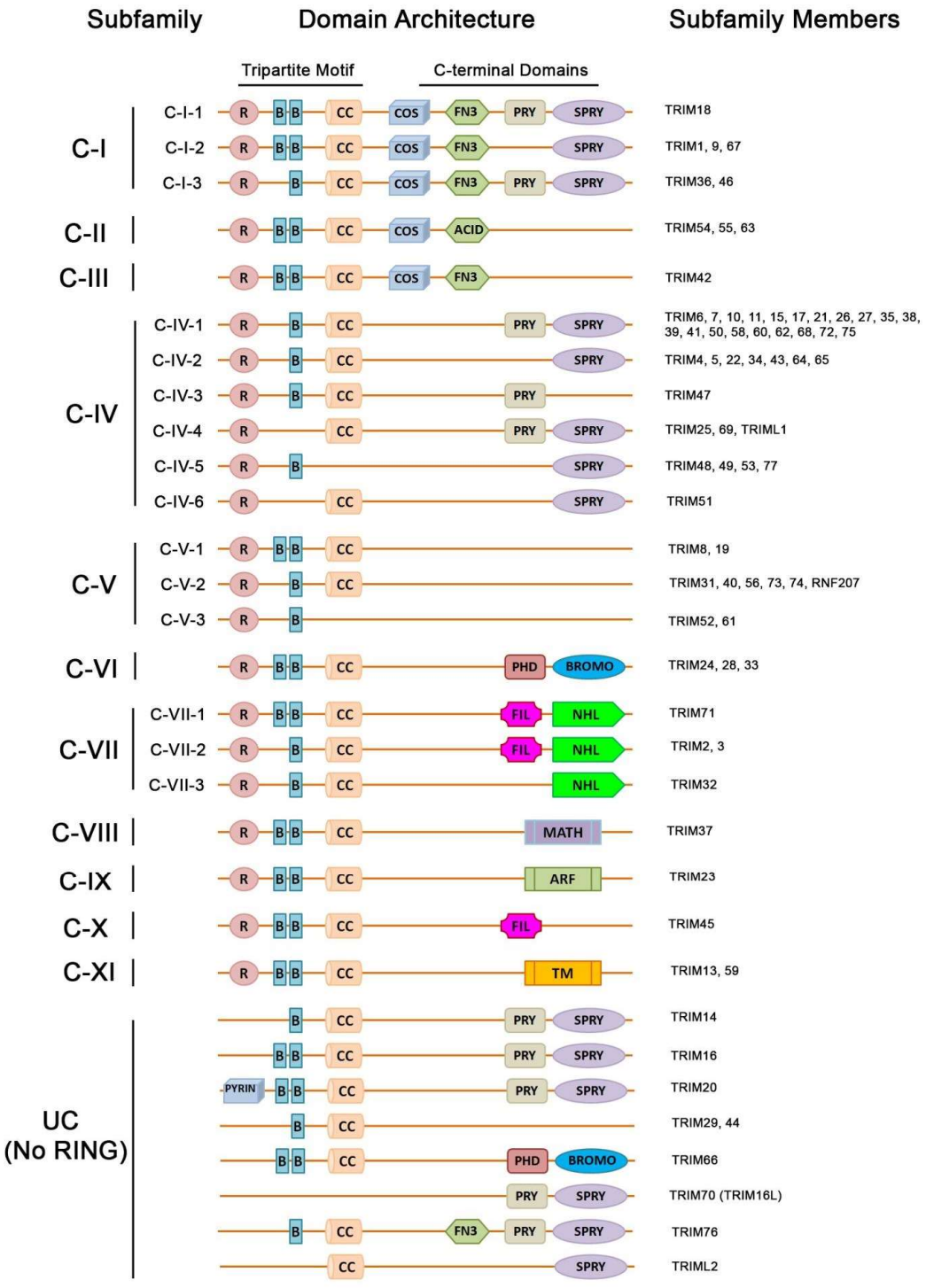

Figure 2.7: Classification of TRIM proteins and their schematic representation. *Based on their C-terminal domains TRIM family proteins have been classified in 11 classes.*

Attributed to their COS domain, TRIM1, 2, 3 and 18 form filamentous structures (Cainarca et al., 1999; Reymond et al., 2001). Interestingly, TRIM13 is localized at the outer ER membrane (Ji et al., 2019; Tomar et al., 2012a), TRIM4 transiently interacts with mitochondria whereas TRIM25 translocate to mitochondria in influenza A virus infected cells (Ohman et al., 2009). Several other TRIMs also show redistribution in cytosol during infection (Versteeg et al., 2013). Reports from our lab has shown that TNF-α promotes temporal translocation of TRIM8 from nucleus to cytoplasm which is critical for its role in regulation of TNF-α-induced NF-κB activation (Tomar et al., 2012b). These evidences suggest that TRIMs are not limited to individual cellular compartments and their shuttling and dynamic interaction with cellular compartments may play critical role in regulation of various processes, ultimately cellular homeostasis.

2.8 Stimuli and stress induced TRIMs and their role in cellular homeostasis

2.8.1 Induction of TRIMs by type I and type II cytokines:

In monocyte derived macrophages (MDM) both type I and II IFN up-regulated the expression of 19/PML, 20/MEFV, 21, 22, 25, 56 and 69, whereas type I IFN additionally up-regulated the expression of TRIM5, 6, 14, 26, 31, 34, 35, 38, and 58. In peripheral blood lymphocytes (PBL) type I IFN induced expression of TRIM5, 6, 14, 19/PML, 20/MEFV, 21, 22, 25, 26, 31, 34, 35, 38 and 56, whereas type II up-regulated expression of TRIM19/PML, 20/MEFV, 21, 22, 26, 56 and 69 (Carthagena et al., 2009). Though reports by other groups also show high degree of overlap with TRIMs expression to type I and II IFNs but studies correlating gene expression with protein expression are still lacking.

Interferon-gamma (IFN-γ), belongs to type-II IFN, is a pleiotropic molecule which is known to have versatile functions. It is known to have

antiproliferative, pro-apoptotic and antitumor functions. TRIM1, TRIM8, TRIM20, TRIM21, TRIM22, and TRIM65 have been identifies as regulator of IFN-γ induced autophagy. Interestingly TRIM20, TRIM21 and TRIM22 are also up-regulated by IFN-γ further establishing functional correlation of IFN induced TRIM genes (Kimura et al., 2015).

Functional correlation of many of these IFN-inducible proteins has been established. For example IFN-β induced PML/TRIM19 inhibits VSV and influenza virus replication in human monocytic cell line U937 (Chelbi-Alix et al., 1998). EFP/TRIM25 is also induced by both type I and type II IFNs (Carthagena et al., 2009) acting as a positive regulator of RIG-I mediated activation of interferon regulatory factor-3 (IRF-3) and NF-κB. It has also been found as a tumor promoting factor regulating breast cancer cell proliferation (Ueyama et al., 2010), breast cancer metastasis (Walsh et al., 2017), endometrial cancer cell proliferation (Sato et al., 2018) and Myc-overexpressing human cancers (Zhang et al., 2019) by various mechanisms and targeting specific substrates. Many of these IFN induced TRIMs have been studies for their role in antiviral response but their implication of regulation of proinflammatory response and cellular homeostasis is not understood.

2.8.2 Expression of TRIMs by innate immune receptors:

Expression of TRIMs in THP-1 derived macrophages suggest that agonists for TLR receptors (TLR1/2, 3, 4, 5, 7, 8, 9) promotes expression of TRIM10, 15, 29, 31, 40, 42, 43, 48, 49, 50, 51, 60, 61, 64 and TRIM77, whereas the TLR6/2 agonist inhibited the expression of most of the TRIMs. Expression of these TRIMs was also induced by TNF-α (Jiang et al., 2017). TRIM13 expression is induced by TLR2 receptor agonist Pam_3CSK_4 (Huang and Baek, 2017) and various TLR agonists induces expression of TRIM38 (Zhao et al., 2012b) and TRIM30α (Shi et al., 2008).

2.9 TRIMs: Emerging regulators of crosstalk between TNF-α and TLR induced NF-κB pathway

Isolated reports have confirmed the role of TRIM proteins in regulation of TNF-α-induced NF-κB pathway. Reports from our lab and other suggest that individual members of TRIM family proteins regulate the TNF-α-induced NF-κB pathway at distinct steps by ubiquitin dependent and independent signaling.

TRIM8 has been identified as a positive regulator of TNF-α-induced NF-κB pathway, whereas TRIM13 and TRIM38 act as negative regulators (Hu et al., 2014; Li et al., 2011; Tomar and Singh, 2014; Tomar et al., 2012b). TRIM8 and TRIM38 acts at the TAB-TAK complex, whereas, TRIM13 acts at IKK complex to regulate the NF-κB pathway. TRIM8 and TRIM38 have antagonizing effects of TNF-α-induced NF-κB activity. TRIM8 promotes TAK1 ubiquitination for NF-κB activation, conversely TRIM38 destabilizes TAB2/3 complex and promotes lysosome-dependent degradation of TAB2 resulting in inhibition of NF-κB activity (Hu et al., 2014). Suggesting, Individual TRIM proteins are recruited at different steps of TNF-α-induced NF-κB pathway and regulates the NF-κB pathway.

TRIMs are also modulators of TLR induced NF-κB and inflammatory pathway. TLR activated TRIM proteins, TRIM30α, TRIM38 and TRIM32 restricts TLR induced NF-κB pathway and inflammatory response. TRIM38 acts as negative regulator of TLR induced signaling by promoting UPS mediated degradation of NAP1 (Zhao et al., 2012a), TNF-α receptor associated factor 6 (TRAF6) (Zhao et al., 2012b) and TRIF (Xue et al., 2012). TRIM30α promotes TAB2 and TAB3 degradation by UPS and consequently dampens NF-κB activation and inflammatory response. Interestingly, TRIM38 acts as E3 SUMO1 ligase for Mda5, Rig-I (Hu et al., 2017) and cGAS and Sting (Hu et al., 2016) antagonizes their K48-linked polyubiquitination and degradation, hence positively

regulates the cGAS- and RLR-mediated innate immune signaling (Hu et al., 2017). TRIM13 potentiates TLR-induced NF-κB activity by promoting K29-linked ubiquitination of TRAF6 (Huang and Baek, 2017), whereas brain specific TRIM9 is identified as negative regulator of NF-κB and resolves inflammatory response to protect from consequences of neuroinflammation (Shi et al., 2014; Zeng et al., 2019).

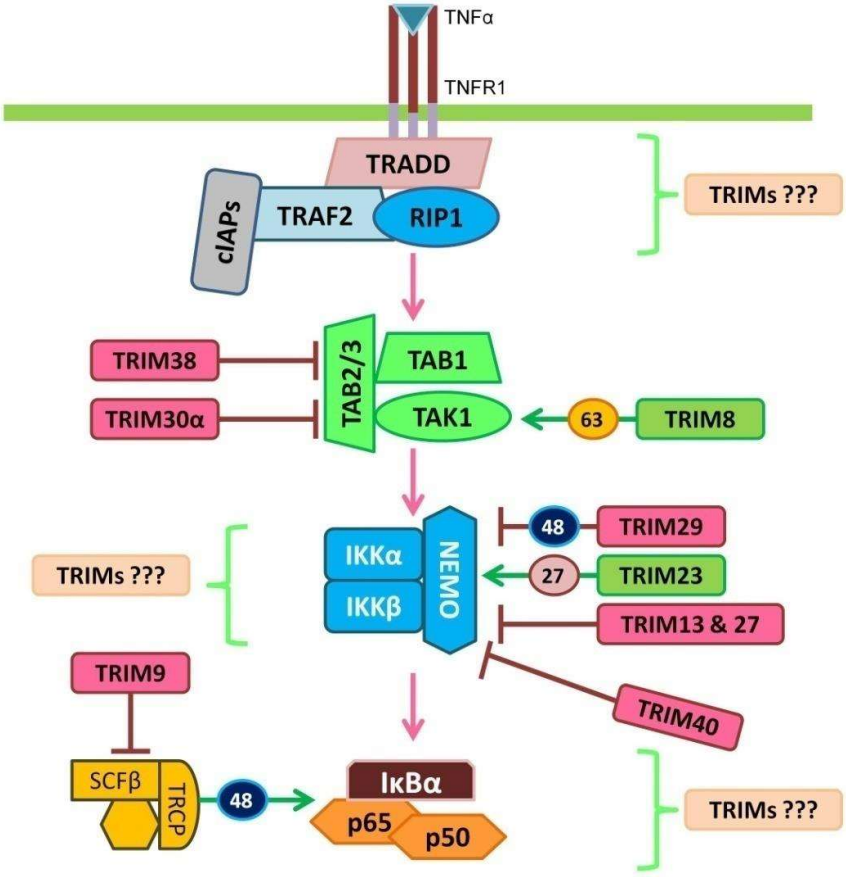

Figure 2.8: TRIM proteins in regulation of TNF-α-Induced NF-κB pathway. *TRIM proteins are recruited at discrete steps of NF-κB pathway and regulate the pathway in positive or negative manner.*

These evidences confirm the role of TRIM proteins in regulation of activation and resolution of inflammatory signaling activated by different mediators. It also suggests that TRIM family proteins might have evolved to regulate specific arms of innate immune signaling and may act in synergistic or

antagonistic manner for distinct arms of innate immunity. Moreover, it suggests that cells possess sophisticated regulatory mechanisms dependent on TRIM E3 ligases to enhance or dampen specific arms of innate immune response and control inflammation. ***The role of TNF-α and TLR induced TRIMs in regulation of NF-κB pathway, their substrate and mechanism remain less understood and needs to be investigated further. Furthermore the tissue specific expression of these proteins may have implication in tissue/organ specific pathophysiological conditions which needs to be further investigated.***

2.10 TRIMs: Emerging regulators of stimulus specific autophagy and cell death pathways

PRRs and IFNs activate autophagy as antipathogen effector. Moreover, autophagy is analogous to UPS in regulation of proteostasis and also regulates turnover of cellular organelle, hence play critical role in regulation of cellular homeostasis. Recent reports are also suggesting its role in selective degradation of proteins and its implication in modulating cellular processes. Several TRIM proteins have been identified as regulator of starvation-induced and basal autophagy (Mandell et al., 2014; Tomar et al., 2012a). TRIMs 6, 22 and 49 interacts with both ULK1 and Beclin-1, hence, serve as platforms to assemble autophagy machinery.

TRIM proteins interact with galectins to sense the damage to endomembranes and direct autophagy for their clearance. TRIM5a, TRIM6, TRIM17, TRIM20, TRIM22, TRIM23, and TRIM49 interact with both Galectin-3 and Galectin-8, whereas TRIM16 only interacts with Galectin-3 (Chauhan et al., 2016). The lysosomal damaging agent Leu-Leu-O-Me (LLOMe) and *Mycobacterium tuberculosis* induced endomembrane damage enhances association of TRIM16 and ATG16L1, leading to enhanced ubiquitination of ATG16L1. Ultimately promotes autophagic response to lysosomal damage and protect

cells against consequences of endomembrane damage and *Mycobacterium tuberculosis* infection (Chauhan et al., 2016).

Interesting crosstalk exist between autophagy and NF-κB pathway. Evidences have confirmed that they both can regulate each other under normal or unique pathophysiological conditions. Autophagy can control NF-κB by promoting degradation of IκBα in intestinal epithelial cells stimulated with TNF-α for long time; resulting in persistent activation of NF-κB pathway (Colleran et al., 2011). On the other hand NF-κB target genes Beclin-1 and p62/Sqstm1 can induce mitophagy to can restrict over-amplification of mitochondria mediate inflammatory response (Salminen et al., 2012; Zhong et al., 2016).

Type II IFN induced TRIM20 has been identified to regulate IFN-γ induce autophagy, it also regulates precision autophagy mediated degradation of inflammasome component NLRP3 and NLRP1 (Kimura et al., 2015). Other components of NLRP3 inflammasome, pro–caspase 1 is also know to interact with TRIM20 with its SPRY domain and inhibit processing of proIL-1β (Papin et al., 2007). TRIM21 induced by IFN-γ promotes autophagy mediated degradation of activated IRF3 and restricts type I IFN production (Kimura et al., 2015). TRIM5a and TRIM23 are found as regulator of virus induced autophagy and antiviral effectors (Sparrer and Gack, 2018).

Organelle stress response can also contribute to induction of autophagy. Interestingly, TRIM13 has been identified as ER stress stabilized protein which regulates ER stress induced autophagy and promotes translocation of caspase-8 to autophagosome during ER stress induced cells death (Tomar et al., 2013b; Tomar et al., 2012a).

These evidences point toward interesting cross-talk between TRIM proteins mediated NF-κB autophagy and inflammatory pathway in

regulation of cellular fitness and survival. It also warrants further investigation of this crosstalk to understand cellular homeostasis and involvement in pathophysiological conditions. Therefore, the investigation of TRIMs as regulators of NF-κB pathway and their implication in regulation of autophagy and cell death crosstalk would provide important clues about how cells maintain cellular fitness and curb various stresses.