SUMO Wrestling in the Airway Epithelium: Does It Regulate Thymic Stromal Lymphopoietin?

SUMOylation is a critical post-translational regulatory mechanism in health and disease (1). It involves the reversible binding of a SUMO (small ubiquitin-related modifier) peptide to a lysine residue in the target protein. Four different SUMO isoforms have been discovered: SUMO1, SUMO2, SUMO3, and SUMO4. The addition of SUMO peptide is mediated by an enzyme cascade that requires an activating enzyme E1, an E2-conjugating enzyme, and an E3 ligase (1). The protein target can be modified by adding a single SUMO moiety, multiple SUMOs, or SUMO chains. Also, specific SUMO-modified proteins can be deSUMOylated by a group of sentrin/SUMO-specific proteases (SENPs) (1). The conjugation of a SUMO moiety affects proteins by altering their activity, subcellular localization, or stability. Dysregulation of SUMOylation has been linked to many pathological situations (1). Interfering with the SUMOylation machinery could represent a novel therapeutic approach in managing inflammatory diseases, including airway diseases.

TSLP (thymic stromal lymphopoietin) is a member of the IL-2 family of cytokines, initially characterized as a pre–B cell growth factor (2). Human and mouse TSLP exert their biologic activities by binding to a high-affinity TSLPR (TSLP receptor) complex. This complex is a heterodimer of the TSLPR chain, which is closely related to the common receptor γ chain and IL-7Rα (IL-7 receptor-α) (2). Accumulating evidence indicates that TSLP is critical for initiating inflammation in allergic diseases, including asthma and atopic dermatitis (2). TSLP expression was increased in the lungs of mice with antigen-induced asthma, whereas TSLPR-deficient mice had substantially attenuated disease. Lung-specific expression of a TSLP transgene induced airway inflammation and hyperreactivity characterized by T-helper cell type 2 (Th2) cytokines. TSLP expression was increased in asthmatic airways and correlated with disease severity (3). TSLP is produced mainly by epithelial cells of the lung, the intestine, keratinocytes, immune cells, and various structural cells including smooth muscle and cancer cells (2, 4). Mechanistically, TSLP controls type 2 inflammation via activation of dendritic cells (DCs), mast cells, and group 2 innate lymphoid cells (ILC2). TSLP induces the recruitment of Th2 cells and the induction of Th2 differentiation, DC production of chemokines CCL17 and CCL22, and induction of OX40 ligand, respectively (2). These studies and others have collectively rationalized targeting TSLP in allergic inflammatory diseases.

Two variants (short and long) of human TSLP have been identified. sfTSLP (short isoform TSLP) consists of 63 (or 60) amino acids, as opposed to 159 amino acids for lfTSLP (long isoform TSLP) (5, 6). These two isoforms are regulated by distinct gene promoters rather than alternative transcript splicing. sfTSLP shares the same amino acid sequence at the C-terminal domain but lacks the N-terminal domain of lfTSLP. The functional activity of lfTSLP is regulated by post-translational modifications (7). The proprotein convertase enzymes cleave lfTSLP between residues 130 and 131, generating a heterodimeric unstable form of TSLP. The carboxypeptidase N then digests 6 amino acids from the C-terminus of lfTSLP to produce a more stable dimerized form: TSLP (amino acids 29–124) and TSLP (amino acids 131–159). The stable dimerized TSLP is present in diseased and healthy conditions, suggesting a homeostatic function (7). Interestingly, the truncated heterodimeric unstable TSLP strongly activates myeloid DCs and ILC2 compared with mature lfTSLP, thus driving type 2 inflammation (7). Studies suggest that overproduction of lfTSLP may be a key trigger for amplifying type 2 inflammation in human disease (7) but raise questions of whether other mechanisms are involved in the increased expression of lfTSLP in type 2 inflammatory disorders.

In this issue of the *Journal*, Liang and colleagues (pp. 648–660) demonstrate that SUMOylation plays a critical role in the expression of lfTSLP in airway epithelial cells (8). They show enhanced SUMOylation in the airway epithelium using a chronic house dust mite (HDM) mouse model of allergic asthma. Treatment with the SUMOylation inhibitor 2-D08 downregulates BAL eosinophil and neutrophil concentrations, mucus cell hyperplasia, serum HDM-specific IgE, and airway hyperreactivity. Interestingly, among the alarmins known to be produced on allergen challenge (2), only epithelial TSLP protein expression was reduced on *in vivo* treatment with the SUMOylation inhibitor. *In vitro*, SUMOylation inhibition with 2-D08 reduced lfTSLP protein expression in human bronchial epithelial (HBE) cells without affecting the mRNA concentrations of either lfTSLP or sfTSLP. Mechanistically, SUMOylation E3 ligase CBX4 (chromobox 4) (1) silencing in HBE cells downregulates lfTSLP protein expression, without affecting its mRNA concentration. Furthermore, the ectopic overexpression of wild-type or CBX4 chromodomain mutant, but not SIM1/2 (SUMO-interacting motifs) mutant, increases lfTSLP protein expression in HBE cells.

Moreover, the effect of CBX4-mediated lfTSLP protein expression did not involve mRNA stability. Still, it implicates enhanced mRNA translation, as CBX4 silencing shifts lfTSLP mRNA translation from the active polyribosomes to the translation dormant complexes. The authors went on to elegantly show that CBX4 induces the RNA-binding protein MEX-3B (Mex-3 RNA binding family member B) that interacts with the 5′ untranslated region of TSLP mRNA, via the K-homology–type RNA recognition domain, resulting in enhanced translation in HBE cells. Finally, they found that the general transcription factor TFII-I (transcription factor II-I) binds the MEX-3B promoter and that CBX4-mediated SUMOylation of TFII-I enhanced its transcriptional activity of MEX-3B.

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This study raises many questions, and some limitations should be considered. First, given the inverse correlation between TSLP concentration and steroid-insensitive asthma (9), whether this pathway is dysregulated in severe asthma needs to be investigated. Second, assuming SUMOylation's role in many aspects of cellular function, such as trafficking and signal transduction (1), the study could have been strengthened by demonstrating the role of the SUMOylation pathway in dampening lfTSLP release and function. This functional aspect is relevant, as TSLP activates target cells such as DCs and ILC2, considered essential in initiating inflammatory responses in allergic diseases (2). Third, the signaling pathways of lfTSLP inducers such as Toll-like receptor ligands (i.e., polyinosinic:polycytidylic acid and flagellin) and proinflammatory cytokines (IL-1, IL-4, IL-13, and TNF) are regulated by SUMOylation (1, 2, 10). For example, TNF signaling depends on the SUMO E3 ligase activity of PIAS1 (protein inhibitor of activated STAT 1) and/or IKKα (IκB kinase α) (11). The authors demonstrate strong expression of PIAS1 in HBE after HDM stimulation, but silencing of PIAS1 did not affect either the protein or the mRNA expression of lfTSLP, which needs further investigation. Also, a previous study showed that the transcription of TSLP and secretion are enhanced via the PAR2 (protease-activated receptor-2)/ORAI1 (calcium release-activated calcium modulator 1) signaling pathway (12). The cross-talk between PAR2 and SUMOylation in mediating TSLP protein expression is unclear.

In conclusion, this study highlights the role of post-translational modification on the expression and functional activity of lfTSLP protein, which may pave the way for specific strategies targeting lfTSLP without affecting sfTSLP, considered to have antidefense properties (13). ■

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