SUMOylation: An Important Post-translational Modification *in Vivo*

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Abstract Post-translational modification by the small ubiquitin-like modifer (SUMO) is emerging as a defining feature of eukaryotic cells. SUMO has been shown to covalently modify a large number of proteins, reversible modification by SUMO regulates nucleocytoplasmic translocalization, protein-DNA binding activity, protein-protein interaction, transcriptional regulation, DNA repair, and genome organization. SUMOylation also plays pivotal roles in human diseases such as cancer and neurodegenerative disorder, elucidating the biological significance of the protein SUMOylation and their roles in these diseases will provide powerful strategy for developing new therapeutic reagents.

Key words SUMO; SUMOylation; post-translational modification

Small ubiquitin-like modifer (SUMO) is one of ubiquitin-like proteins which was originally found to be covalently linked to the GTPase activating protein RanGAP1 [1]. Although shares 18% sequence identity with ubiquitin and mechanistic similirities, SUMOylation doesn't promote the degradation of proteins but instead alters a number of different functional parameters of proteins [2]. These parameters include but not limited to properties such as nucleocytoplasmic translocalization, protein partnering, protein-DNA binding and/or transactivation functions of transcription factors [3]. To date, more than 100 SUMO target proteins have been reported, of which a significant number are either transcription factors or other proteins involved in DNA transactions [4]. Among the systems that modify protein structure, SUMO had already become one of the best-studied examples of post-translational modification. In this review, we will focus on research progresses in the understanding of the mechanism of SUMOylation, SUMO function and regulation.

1 SUMO and SUMOylation

In lower eukaryotes (yeast and flies), only one SUMO gene is expressed, whereas mammals express four (SUMO 1– SUMO 4) and plants might even contains up to eight SUMO genes [5]. Mature SUMO2, SUMO3 and SUMO4 are nearly identical but differ substantially from SUMO1 (Fig.1) [6-8]. SUMO1 exists in the conjugated form, whereas the SUMO2/SUMO3 form poly-SUMO chains by conjunction to Lys11, and exist primarily as free proteins that are subject to rapid conjugation after cellular stress [9]. The recently described SUMO4 has a restricted pattern of expression with highest levels reported in the kidney, it lacks posttranscriptional processing and the capability to form covalent interactions with substrates [10].

Modification of proteins by SUMO is dynamic and reversible. In an enzymatic pathway, SUMO covalently attached to target proteins to finish SUMOylation process. On the contrary, SUMO-specific proteases (SENPs) participate in the processing of de-SUMOylation that remove SUMO conjugates from substrates. SUMOylation is analogous to ubiquitination, with the sequential participation of the E1-activation enzyme, the E2-conjugating enzyme (Ubc9), and E3 ligases [11]. The precursor of SUMO is cleaved by SUMO-specific proteases to reveal the C-terminal glycine residue that is linked to lysine side chains in target proteins. SUMO activating enzyme is a heterodimer containing SAE1 and SAE2 subunits, in an ATP-dependent reaction, the C-terminal carboxyl group of mature SUMO forms a

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SUM01    MSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMN
SUM02    MADE--KP-KEGVKTENN-DHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCE-------
SUM03    MSEE--KP-KEGVKTEN--DHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCERQGLSMR
SUM04    MANE--KP-TEEVKTENN-NHINLKVAGQDGSVVQFKIKRQTPLSKLMKAYCEPRGLSVK

SUM01    SLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGG|HSTV-------
SUM02    --R--------------QLEMEDEDTIDVFQQQTGG|VY---------
SUM03    QIRFRFDGQPINETDTPAQLEMEDEDTIDVFQQQTGG|VPESSLAGHSF
SUM04    QIRFRFGGQPISGTDKPAQLEMEDEDTIDVFQQPTGG|VY---------
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Fig.1 Amino acid sequence alignments of four SUMO homologs from human[6]

The SUMO acceptor lysine in this motif is printed in bold. The site of cleavage to produce the mature proteins with C-terminal glycine-glycine residues is indicated by "|".

thioster bond with the sulphydryl group of a cysteine residue in SAE2. Then, the activated SUMO is transterified from SAE2 to cysteine 93 of SUMO conjugating enzyme Ubc9. Ubc9 can recognize the substrate and directly transfer the activated SUMO by the formation of an isopeptide bond between the C-terminal carboxyl group of SUMO and the ε-amino group of lysine in the substrate protein which have ΨKxE (where ψ is a large hydrophobic residue and x is any residue) consensus motif [12]. However, not all proteins with this motif undergo SUMOylation, and sites not confirming to this motif can still be conjugated to SUMO [13].

Several SUMO E3 ligases have been identified that promote transfer of SUMO from the E2 to specific substrates. Although not required for SUMOylation *in vitro*, SUMO E3 ligases may be important in regulating substrate selection *in vivo* [14]. To date, three unrelated proteins have been found to have SUMO E3 ligase activity, they are RanBP2, the PIAS proeins, and the polycomb group protein Pc2 [15]. These three E3 classes have distinct subcellular localizations and mediate the modification of specific substrates.

Besides this covalent interaction, there exists a non-covalent interaction between SUMO and substrate proteins that contain SUMO-interacting motif (SIMs) [16]. It seems possible that SIM-mediated SUMO binding may replace many of the functional aspects of covalent SUMO linkage to substrates.

2 SUMOylation and Other Posttranslational Modification

Lysine residues act as acceptors not only for SUMO modification but also for other posttranslational modification (ubiquitylation and acetylation). The cross-talk between these posttranslational modificaton and SUMOylation pathways may be extensive and of fundamental importance in signal transduction as well as signal-dependent regulation of protein activity [17]. SUMO modification was found to antagonize IκBa ubiquitinaiton and protect it from degradation: In unstimulated cells, the NF-κB transcription factor is maintained in an inactive state by IκBa inhibitor protein. In response to activation signals, IκBa undergoes polyubiquitination at lysines 21 and 22, targeting the protein for proteasome-mediated degradation and releasing the active NF-κB. However, IκBa, which shuttles between the nucleus and cytoplasm, is also modified by SUMO1 on lysine21, thus blocking ubiquitination and stabilizing the protein [18]. As indicated above, NEMO/IKKγ PCNA are also subject to ubiquitination and SUMOylation on the same lysine residue [4].

Other post-translational modifications that modify other residues such as phosphorylation also have important regulatory function for SUMOylation. For example, the phosphorylation of the promyelocytic leukaemia (PML) during mitosis correlates with PML de-SUMOylation and the dispersion of the promyelocytic leukaemia nuclear body (PML NBs), the integrity of the PML NBs is dependent on the SUMOylation of their principal component [19]. Beiside this, phosphorylation had been suggested to regulate SUMO modification of tumor suppressor protein p53 and protooncogene c-Jun [2].

3 SUMOylation and Protein Targeting

SUMOylation has an important function both in

nucleocytoplasmic trafficking and nuclear export. The first identified SUMO substrate RanGAP is a small GTPase activiating protein that plays an important role in nuclear import. Unmodified RanGAP is cytoplasmic, wherease SUMO-modifed RanGAP is associated with the nuclear pore complex (NPC) where SUMO facilitate interaction with RanBP2, a component of the nuclear pore and SUMO E3 ligase [20]. Others well-characterized examples of SUMO-dependent changes in nuclear targeting including adenovirus E1B 55-kDa protein, the tumor suppressor PML, the IκB kinase regulator NEMO, pancreatic duodenal homeobox1(Pdx1), and the Fanconi anemia protein FANCD2 [4]. Most SUMO-modified proteins reside in the nucleus but cytosolic SUMO targets have also been identified. Nuclear SUMOylation of *Dictyostelium discoideum* Mek1 is essential for the translocation of Mek1 to the cytoplasmic cortex [21].

4 SUMOylation and Transcription

Among of more than 100 proteins substrates for the SUMO system, nearly one-third of the identified target proteins are putative transcriptional regulators. Attachment of SUMO appears to repress the activity of these transcriptional regulators [22]. The major site of SUMO modification in these substrate proteins lies with in an inhibitory domain, bringing SUMO to the promoter via a modified transcription factor is often sufficient for repression [23]. The transcription factor Sp3 has been shown to be SUMOylated in vivo and removal of SUMO by mutation of Sp3 acceptor lysines or contransfection with a SUMO protease dramatically increased transcriptional activity of Sp3[24]. Other transcription factors that can be repressed by SUMO including C/EBPα proteins, Smad4, Elk-1, c-Myb, STAT1, AP1, AP2, androgen receptor (AR), glucocorticoid receptor (GR), and steroid hormone receptor [25-27]. In a limited number of instances, SUMO modifation correlated with increased transcriptional activities, that is heat shock transcripton factors HSF1, HSF2 and the β-catenin-activated factor Tcf-4[9].

5 SUMOylation and Virus

A growing list of viral proteins was found to be covalently modified by SUMO, viruses exploit SUMOylation to facilitate its infection in two different ways: a viral protein interferences with the SUMOylation of host proteins, and/or a viral protein that needs to be SUMOylated in order to exert its function [28].

The IE1 protein of human cytomegalovirus is the first viral protein found to utilize the first way to interferences with the SUMOylation of PML [29]. It has found the modification of PML by SUMO is essential to form NBs, and essential for the maintenance of their integrity. The activity of IE1 in the regulation of transcription directly correlated with its ability to bind to and prevent SUMOylation of PML, thereby disrupting PML NBs. There are some different mechanisms with which virus use to prevent the SUMOylation of host proteins, human simplex virus ICP0 protein counteract the host's SUMOylation by enhancing de-SUMOylation, EBV Zta was shown to be modified by SUMO and outcompeted PML for a limited amount of intracellular SUMO to disperse PML NBs [30].

Some viral proteins exploit the host's SUMOylation because they need to be SUMO-modified in order to localize in infected host cells and exert their functions. One of the most recent examples reported is the Kaposi's sarcoma-associated herpesvirus' (KSHV) early lytic-cycle, KSHV basic leucine zipper protein (K-bZIP). K-bZIP repression activity correlates with its ability to be SUMOylated and to recruit Ubc9 to specific viral target promoters, exerting this protein's transcriptional repression activity [31].

6 SUMO and Cancer Development

A number of products of oncogenes and tumor suppressor genes, including p53, MDM2, PML, c-Myb, c-Jun/c-fos, pRB and KAI1, undergo SUMOylation [32]. The alteration of activity, level, and/or localization of these genes has great relationship with tumorigenesis and development of abnormalities. The tumor suppressor p53, which is mutated in over 50% and inactivated in a further 20% of human cancers, is regulated by SUMO-1 modification that affecting its stability, transcriptional activity, and ability to induce cell cycle arrest and apoptosis [33]. SUMO regulates p53 in direct way by modification of p53 as well as in indirect way

via modulating two pivotal upstream regulators of p53: Mdm2 and $p14^{ARF}$ [34]. However, under which physiological conditions p53 becomes SUMOylated and the significance of SUMOylation on p53 function needs to be further investigated.

Besides SUMO, alterations of SUMOylation enzymes also have been reported in several human cancers. For instance, increased expression of PIAS3, a SUMO-E3 ligase, was reported in brain, lung, breast, prostate and colon-rectum cancers [35]. There are accumulating reports indicate that SUMO E2 ligase enzyme Ubc9 was found overexpressed in lung adenocarcinoma and ovarian carcinoma [36]. As the unique conjugating enzyme, Ubc9 also is proposed as a potential clinical marker for some specific human abnormality diagnosis as well as a good candidate for drug target to strengthen or block SUMOylation through changing Ubc9 activity.

Given that SUMOylation plays pivotal roles in tumorigenesis and modification of many transcription factors related to development of abnormalities, the alteration of SUMOylation and de-SUMOylation often causes a defect to the cells resulting in development of cancer. Therefore, understanding and elucidating the biological significance of the protein SUMOylation and their roles in various stages of caner development, will provide powerful strategy for developing new therapeutic reagents.

7 SUMO Enhance Expression and Solubility of Fusion Protein in *E.coli*

Recent research had revealed that SUMO can works as fusion tags suited for difficult-to-express proteins [37]. Compared with other fusion tags, SUMO fusions not only enhanced expression and solubility of recombinant proteins most dramatically, but conveniently and efficiently generate native N-terminal amino acids cleaved by the natural highly specific SUMO protease.

8 Conclusions and Future Perspectives

Utilizing broad proteomics approaches, more and more proteins had been verified can be modified by SUMO, in which SUMO modifications are further found to be associated with cell cycle, DNA repair and replication, maintenance of genome integrity, and RNA metabolism [38]. Investigations of the regulation and function of SUMO modification of proteins will show us more unexpected diversity of physiological processes modified by this small molecular.

Since the important role of SUMO modification in these biological processes, alterations in SUMOylation process of specific substrates are associated with cancer or other functional disorder [2]. Further studies of the SUMO function and mechanism in diseased tissues and in animal models will determine the role of SUMO modification in these pathological states. Beside this, dozens of viral proteins were discovered to be modified by SUMO which distributed the following families: herpesviridae (human cytomegalovirus, Kaposi's sarcoma-associated herpesvirus, Epstein-Barr virus, Herpes simplex virus1), papillomaviridae (human papillomavirus), retroviridae (human immunodeficiency virus type 1, moloney murine leukemia virus), adenoviridae (avian adenovirus), coronaviridae (Cov-SARS), poxviridae (vaccinia virus), orthomyxoviridae (influenza virus), and flaviviridae (dengue virus type 2). To better understand the mechanisms that virus utilize SUMOylation for viral benefit may shed a new light on antiviral therapy.

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SUMO 化：一种重要的体内翻译后蛋白质修饰系统

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摘要　靶蛋白被小泛素相关修饰物(small ubiquitin-related modifier, SUMO)修饰已经成为真核细胞特有的翻译后蛋白质修饰标志之一。SUMO与靶蛋白之间这种可逆的共价连接，在核质运输、DNA与蛋白质结合活性、蛋白质之间相互作用、转录调控、DNA修复以及维持基因组稳定等方面均发挥着重要的调节作用。在许多人类疾病如癌症和神经退化性疾病中，SUMO化修饰作用对疾病的发生与发展起着极为重要的作用。阐明SUMO化修饰在这些疾病中的功能，将为疾病的治疗开辟一条崭新的思路。

关键词　SUMO; SUMOylation; 翻译后蛋白质修饰

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