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Ubiquitin-like protein modifications in prostate and breast cancer

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1. ABSTRACT

Post-translational modifications by ubiquitin-like proteins have been implicated in the regulation of diverse cellular processes, including nuclear transport, transcription regulation, stress response and DNA repair. Ubiquitination is well characterized for its roles in regulating these cellular processes. As a newly identified member of ubiquitin-like proteins, the small ubiquitin-like modifier (SUMO) has received a great deal of attention for its functions distinct from ubiquitin. In particular, alterations of SUMO conjugation or sumoylation have been implicated in several human diseases, including cancer. Although little is known about the underlying mechanism of sumoylation-associated tumorigenesis, the modulation of nuclear receptor (NR)-mediated signaling pathways is likely to play a role in this aspect. NRs are a family of ligand dependent transcription factors which control cell growth and differentiation in many cell types, as well as during the development of cancer. In this review, we will discuss some basic aspects of sumoylation and how sumoylation modulates the NR-mediated gene expression, focusing on androgen receptor (AR) and estrogen receptor (ER), a key player in progression of prostate or breast cancer.

2. INTRODUCTION

It is well known that post-translational modifications govern a key mechanism of proteins' function by altering their activity, turnover, localization and interactions with other proteins after their synthesis. These modifications include those modified by various molecules, such as phosphate, acetate, lipids, and sugars and even by attachment of other polypeptides; modification of ubiquitin represents one of the best characterized examples of a polypeptide modifier, which targets proteins for degradation by the 26S proteasome. Recently, a new type of protein modification has been identified, the small ubiquitin-like modifier (SUMO). Both ubiquitin and SUMO belong to members of the ubiquitin-like protein (UBLs) family. The role of ubiquitination in cancer has been well documented (1, 2). Recent evidence has suggested that like ubiquitination, sumoylation could also play a role in cancer but possibly through different mechanisms. In this review, we will discuss general functions of SUMO modifications and its potential roles in cancer, with emphasis on prostate and breast cancer. To better understand how sumoylation affects cellular pathways, we will briefly discuss biochemical events of

sumoylation, as well as the enzymes required for sumoylation. For a general description of the sumoylation pathway and regulations, we recommend these excellent reviews for further readings (2-7).

3. THE STRUCTURE OF SUMO ISOFORMS

SUMO, a 10 kDa protein, is structurally related to ubiquitin. NMR studies have shown that SUMO-1 has a tertiary structure similar to ubiquitin, although they possess only limited sequence identity (~18%) (8, 9). Both SUMO and ubiquitin share a characteristic tightly packed ßßaßßaß fold, and a C-terminal di-glycine motif. However, SUMO is distinguished by a long and flexible N-terminal extension of up to 22 residues that is not present in ubiquitin, which provides an additional interface for possible protein interactions. In addition, the distribution of charged residues on the surface of SUMO is very different from that of ubiquitin. These differences indicate that they interact specifically with distinct enzymes and substrates. Unlike the sole ubiquitin, multiple isoforms of SUMO are found in the cell. To date, four SUMO isoforms termed SUMO-1, SUMO-2, SUMO-3, and SUMO-4 have been identified, which share high sequence and structural similarity (2, 10). For example, the sequence identity between SUMO-2 and SUMO-3 is 98%; ~ 86% for SUMO-4 and closely related SUMO-2/SUMO-3 and about ~50% for SUMO-1 and SUMO-2/SUMO-3. Interestingly, the sequences of the N-terminal extension portion absent in ubiquitin are different for the distinct SUMO isoforms, which may account for the selective modification by specific SUMO isoforms and function difference of distinct SUMO isoforms. The diversity of the SUMO isoforms, in comparison with the single member ubiquitin, suggests different roles and an additional level of complexity in modulating diverse cellular pathways (11).

SUMO-1, also known as Ubl1, Sentrin, GMP1 or Smt3, is the first identified isoform. Therefore, our understanding regarding SUMO function mostly comes from studies of this isoform. Despite their similarities, SUMO-1 differs from the other three isoforms, in regards that it lacks a conserved SUMO site, a requirement for SUMO to form a covalent linkage to another SUMO molecule (12). Thus, SUMO-1 can generally only form a monomer whereas other SUMO isoforms can form both a monomer and a polymer chain (13, 14). This difference may imply that these SUMO isoforms have a different role in modulating cellular pathways or their expression might be regulated differently (11). In contrast to ubiquitin, for which the formation of polymeric chains is critical, monomeric SUMO provides all essential functions in yeast (15). However, a mutant form of SUMO-3 lacking the conserved SUMO sites, can only be monomerically conjugated to target proteins, and interestingly, has an opposite effect on Aβ generation to that by wild type SUMO-3 (11). Thus, it is possible that polymerization or other post-translational modifications of SUMO may add more complexity to the function of SUMO in mammalian cells.

4. SUMOYLATION PATHWAY AND ITS COMPONENTS

The sumoylation cycle is strikingly similar to the ubiquitination cycle, presumably due to the nature of their modifications. For instance, both ubiquitin and SUMO are synthesized as inactive precursors at the beginning of their respective cycles. With the help of the specific protease to make the carboxyl-terminal double-glycine motif available for conjugation, mature ubiquitin or SUMO undergoes a three-step conjugation process. Both pathways require the activating enzyme E1, the conjugating enzyme E2 and the ligase E3. However, each pathway requires its own unique set of enzymes (3).

In contrast to the single subunit ubiquitin E1, the SUMO E1 enzyme is a heterodimer containing Aos1 and Uba2 subunits which perform separate adenylation and thioesterification functions (16). Furthermore, these two subunits are regulated differently. For instance, Uba2 levels remain roughly constant whereas Aos1 levels increase during the S phase in accordance with the observed peak concentrations of some SUMO-1-conjugated species during the S phase (17). This suggests that changes in Aos1 abundance may influence the sumoylation pathway and, thus, provide an additional level of differential control compared with the single ubiquitin E1 enzyme. Recently, Gam1, a protein encoded by an avian adenovirus was found to be able to inhibit the activity of the SUMO E1 activating enzyme by blocking the formation of the E1-SUMO thioester complex, thus blocking sumoylation (18). This finding suggests that the sumoylation pathway is a target for viruses, highlighting the significance of sumoylation in maintaining cellular functions.

Unlike the large number of ubiquitin E2 enzymes, The SUMO E2 conjugating enzyme, Ubc9, is the sole enzyme required for sumoylation. Despite its sequence similarity and tertiary structure with the core domain of ubiquitin E2 enzymes, it is specific for SUMO, catalyzing the transfer of activated SUMO isoforms to the target protein. This specificity of Ubc9 for SUMO is partially attributed to the Aos1/Uba2 heterodimer which binds SUMO concurrently with Ubc9 (19). On the other hand, a consensus SUMO site has been identified consisting of the sequence ψKXE, where ψ is a large hydrophobic amino acid and K is the site of SUMO conjugation (20). Direct recognition of this consensus motif by the Ubc9 active site makes recombinant E1, E2, and SUMO sufficient for ATP-dependent SUMO modification of substrates in vitro (21). Mutations in the amino acid region 126-135 of Ubc9 significantly affect the conjugation of SUMO1 to target proteins, such as p53 (22), whereas a mutation in which Cys93 is replaced by alanine (C93A) or by serine (C93S) exhibits a dominant inhibitory effect on the endogenous Ubc9 (23, 24). Thus, the dominant-negative mutant of Ubc9 (Ubc9-DN) is frequently used as a research tool to study the cellular function(s) of SUMO.

Since E1 and E2 are sufficient to catalyze SUMO conjugation in vitro, these early studies led to the initial doubt about the existence of SUMO E3 ligases. However,

since identification of the yeast Siz1 as an E3-like factor in the sumoylation pathway (25), several mammalian E3 ligases have been subsequently identified. They are protein inhibitors of the activated STAT (PIAS) family, the RanBP2/Nup358, and the polycomb group protein Pc2 (26-28). Among them only the siz/PIAS family is similar to RING-domain containing ubiquitin E3s; the rest functions as adaptors. All of these proteins can interact with both Ubc9 and the SUMO substrate, and thus, enhance the rate of substrate modification. Furthermore, they can be sumoylated themselves and many of them have SUMO-independent functions as well. SUMO E3 ligases target diverse proteins with no seemingly common features. In addition, their localization to specific subcellular complexes may suggest that their functional specificity is defined in a topographical fashion, rather than by common feature substrate terms. For instance, siz/PIAS proteins are found in the nucleoplasm and the nuclear bodies, RanBP1/Nup358 is associated with the nuclear pore complex, whereas the Pc2 group of proteins is found in a subnuclear structure, called the Polycomb body (28).

Modification of a protein by SUMO is reversible and dynamic. Both processing of immature SUMO and cleavage of SUMO from substrates are mediated by de-conjugating enzymes. The first enzymes identified to have SUMO de-conjugating activity were the yeast proteins, Ulp1 and Ulp2 (29, 30). To date, four mammalian SUMO-specific proteases, SENP1, SENP2, SENP3 and SENP6, have been identified (10, 31-33). The activity of isopeptidases seems to be highly active in the cells, which may explain why only small portion of the total proteins is sumoylated in most cases. Similar to SUMO E3 ligases, isopeptidases have distinct subcellular localizations. For instance, SENP1 has been localized to the nucleoplasm and nuclear bodies; SENP2 has been found at the nuclear pore; SENP3 localized to the nucleolus, and SENP6 lives in cytoplasm (10, 31, 33-35). Therefore, SUMO de-conjugation enzymes are also believed to contribute to the substrate specificity.

5. FUNCTION OF SUMO

The identification of the first SUMO-modified protein RanGAP1 opened the door of SUMO studies. An astonishing number of new SUMO substrates have been identified at a rapid pace over the past years as a result of recent proteomic (36) and bioinformatic (37) efforts. Most of them are nuclear proteins which are involved in a variety of cellular pathways such as transcription regulation, DNA repair, genomic integrity and the formation of nuclear bodies.

Sumoylation can serve as an addressing tag for nuclear trafficking. Many nuclear proteins shuffle between the cytoplasm and the nucleus, and this nucleocytoplasmic transport is influenced by sumoylation. The first identified SUMO-modified protein RanGAP represents the best example of a nuclear trafficking event. Unmodified RanGAP is located in cytoplasm, whereas SUMO-modified RanGAP is connected with the nuclear pore (10, 38). Sumoylation of RanGAP1 at K526 is required for nucleocytoplasmic transport of RanGAP1, and for its association with Nup358 (RanBP2) at the nuclear pore complex (38, 39). It has been shown that the nuclear import of a number of proteins depends on their modification with SUMO (40-43).

Sumoylation is also required for the formation of subnuclear structures, such as the nuclear speckles and the acute promyelocytic leukemia protein (PML) nuclear bodies (NB) (44-46). Sumoylation of PML is required for the formation of these nuclear domains as they are absent from $Pml^{-/-}$ cells transfected with a PML gene carrying mutations eliminating the sumoylation sites (45, 47). Mutation of the SUMO acceptor lysines in PML or overexpression of a SUMO protease causes nuclear body components such as CBP or Sp100 to relocalize in the nucleus (45, 48). These findings suggest that SUMO-modified PML supports some protein-protein interactions important for assembly or stability of this subnuclear domain. SUMO modification, in addition, has been linked to the redistribution of proteins in the nucleus. For instance, sumoylation DNA topoisomerase I (topo I) is associated with its nucleolar delocalization in response to topo I inhibitors (24, 49). Additionally, different SUMO isoforms can have different roles in protein localization. Attachment of SUMO-1 or SUMO-3 to the transcription factor SATB2 by gene fusion result in different patterns of subnuclear localization (50).

Another important role for SUMO is regulation of gene expression. Transcriptional factors and transcriptional co-regulators represent a particularly large subgroup of cellular SUMO target proteins, highlighting the significance of SUMO in regulating transcriptional processes (51). With few exceptions of proteins (52-54), sumoylation of transcription factors predominantly suppresses their transcriptional activation potency, such as the AP-2 transcription family (55), and the transcriptional coactivator p300 (56). The mechanisms of the SUMO-induced repressive state may be related with the finding that the consensus SUMO ψKxE motif is often localized within a negative regulatory domain of the transcription factor, and thus, the transcription factor may redistribute away from the transcriptional complexes with DNA (57), interact with co-repressors (58) or co-activators or subject to the SUMO-dependent modulation of chromatin structure and function (59). Many oncoproteins and tumor suppressors are transcriptional factors (e.g., c-Jun, p53), and their activities are modulated through sumoylation (53, 60).

It has long been recognized that sumoylation can also antagonize ubiquitination, which usually targets proteins for proteasomal degradation, thus, enhancing protein stability. The competition with ubiquitin for the same lysine residue of the target protein is believed to be attributable to this antagonizing function. For instance, IκBα is an inhibitor for NFκB, which prevents NFκB from entering the nucleus. Sumoylation of IκBα at K21, the same residue targeted for ubiquitination, protects IκBα from degradation, thus inhibiting NFκB activation (61). Similarly, sumoylation has also been shown to increase the

stability for proteins, such as the Smad4, Huntingtin, and the hypoxia-inducible factor 1α (HIF-1α) (62-64). Recent evidence also suggests that sumoylation may modulate protein-protein interactions by serving as an adaptor (2). For instance, several proteins have been shown to bind noncovalently to SUMO and at least one SUMO binding motif has been identified (65, 66). Therefore, via these interactions sumoylation may have broader effects on the cellular pathways.

Finally, more recent evidence suggests that SUMO plays an important role in DNA repair and genome integrity because it can act on the interface between the DNA replication, recombination, and repair processes. Regulation of DNA repair by SUMO involves several DNA repair enzymes, such as PCNA. For instance, PCNA acts as a molecular switch which, in its SUMO-modified form and during normal DNA replication, stimulates the error prone DNA polymerase zeta (ζ); and is able to overcome the replication fork blocks caused by refractory DNA structures (67). During DNA damage PCNA may be poly-ubiquitinated, inducing the RAD6-dependent error-free DNA repair (67), monoubiquitinated, mediating repair by damage tolerant DNA polymerases η and ζ (67) or sumoylated, inducing either error-prone repair as with monoubiquitination (67) or physical interaction with Srs2p/Hrp5, a helicase that disrupts Rad51 nucleoprotein filaments and thus, preventing recombination repair (68, 69). Therefore, PCNA sumoylation seems to be a guarding mechanism that prevents unwanted RAD51 recombination during replication, channeling DNA damage into the RAD6 post-replicative lesion bypasses. The role of PCNA modifications in mammalian cells is currently under investigation (70). Other SUMO targets implicated in DNA damage response include the thymine-DNA glycosylase enzyme, which is involved in base excision repair (71), the global genomic repair damage recognition factor xeroderma pigmentosum group C (XPC) (72, 73), the translin-associated factor X protein (TRAX), the structural maintenance of chromosomes protein 6 (SMC6) (74), and NF-κB essential modulator (NEMO) (43). Through regulation of these enzymes, SUMO may allow for proper responses to genotoxic stress from DNA damaging agents.

6. POTENTIAL ROLE FOR SUMOYLATION IN CANCER

Sumoylation modulates functions of cellular regulatory proteins, including oncoproteins, tumor suppressors, and cell cycle regulators, as well as enzymes involved in DNA repair and apoptosis, and thus, plays a key role in the control of cell growth and differentiation. Therefore, alterations of protein sumoylation and de-sumoylation may ultimately affect cancer development.

6.1. Deregulation of sumoylation genes and its effect on tumor growth

There is accumulating evidence that the activity, levels, and/or localization of several tumor suppressors, oncoproteins or cell cycle regulators are influenced by SUMO modification. For instance, the transactivation activity of p53 is critical for its role as a tumor suppressor and sumoylation affects its activity (53, 60), despite the controversy over the p53 activity (75). It was shown later that co-expression of SUMO and E3 ligase (PIASxβ) increases sumoylation of p53, and decreases the transactivation activity of p53 (76). Furthermore, although sumoylation appears to cause overall transcriptional repression, there are a few instances in which sumoylation increases the transcriptional activity of oncoproteins (77), suppresses transcriptional repressors (54, 78) or inhibitors of cell cycle progression (79).

An increasing number of reports indicate that sumoylation enzymes are upregulated in human malignancies. For instance, Ubc9 mRNA was found overexpressed in lung adenocarcinoma by microarray analysis (79) and in matched paired samples of ovarian carcinoma vs. normal ovarian epithelium patient specimens by semi-quantitative RT-PCR analysis (80). Alterations of sumoylation enzymes have been reported in several cancers. For instance, Increased PIAS3 expression is reported in 100/103 samples examined in a variety of human cancers, including lung, breast, prostate, colon-rectum, and brain tumors (81). These findings demonstrate that alterations in the expression level of E3 ligase are associated with cell cycle deregulation, and possibly linked to cancer. In human hepatocellular carcinomas (HCCs), the low survival subclass displayed higher expression of genes such as *uba2* and SUMO2, suggesting an etiological involvement of these processes in accelerating the progression of HCC (82). In gastric cancer cell lines BGC-823 cells, ATRA augments sumoylation of RARα, which has higher stability than its non-sumoylated RARα (83).

Moreover, a xenograft mouse model with a dominant negative mutant of Ubc9 (Ubc9-DN) and wild-type Ubc9 (Ubc9-WT) in the MCF-7 human breast tumor cells indicated that Ubc9-WT expressing tumors grew larger than the vector control, whereas Ubc9-DN expressing tumors were much smaller when compared with those from the vector control or Ub9-WT. Microarray analysis of gene expression profiling of Ubc9-DN, as compared with Ubc9-WT cells, showed that the expression of *bcl-2* proto-oncogene was significantly decreased in the Ubc9-DN cells, and was subsequently confirmed by semi-quantitative RT-PCR and Western blot analysis (80). Moreover, Ubc9 appears to also affect the subcellular redistribution of Daxx (84), a Fas-binding protein that is involved in the Fas-mediated apoptosis pathway (85). Subsequent studies demonstrate a higher rate of apoptosis and poor survival for the MCF-7 cells expressing Ubc9-DN (80), implying that Ubc9 may play a role in breast tumorigenesis in part through regulation of *bcl-2* and Daxx.

6.2 Sumoylation of nuclear receptors

The cell nucleus appears to be the main place for sumoylation presumably because the majority of SUMO substrates are nuclear proteins, among which are the nuclear receptors (NRs). NRs are a large family of transcription factors that regulate development, homeostasis, proliferation and differentiation. Thus, abnormal expression and dysfunction of NRs are directly related to the development of cancer, including prostate and

breast cancer. The NR superfamily members conserve the common functional domains including the N-terminal domain (the activation function region) (NTD), DNA binding domain (DBD), hinge region and C-terminal ligand-binding domain (LBD). The LBD responds to binding of the hormone ligand, while the DBD directs the receptors to bind specific DNA sequences as monomers, homodimers, or heterodimers (86). The NTD and LBD interact with other transcriptional cofactors, triggering ligand-regulated and ligand-independent effects on gene transcription. Of interest, the NR superfamily members, including androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), progesterone receptor (PR) and peroxisome proliferator-activated receptor gamma (PPARγ) are subjected to SUMO modification (87-91). Sumoylation of NRs may alter the dynamics of the NR-mediated transcription complexes, and thus, lead to expression of different sets of genes. Hence, sumoylation of these specific NRs, in particular, AR and ER, affects their transcription regulation, signal transduction and thus, may play a significant role in prostate and breast cancer. Understanding the mechanism of regulation of NRs by SUMO may help to find a new and more efficient approach to diagnosis and cancer treatment, as well as providing the basis for new drug development for cancer therapy.

6.3. AR

AR plays an important role in development, male sexual differentiation, and prostate cellular proliferation (92). AR has the common modular structure of other NR superfamily members, including the N-terminal transactivation domain, the central DNA-binding domain, and the C-terminal ligand-binding domain (92, 93). In the absence of ligands, AR is predominantly found in the cytoplasm in an inactive state. Upon binding to the ligand, AR undergoes a series of changes, including conformational change, translocation from the cytoplasm to the nucleus, and binding to an androgen response element (ARE) in the promoter regions of target genes to regulate their transcription. In addition, like other NRs, the activities of AR are subjected to modulation by a large number of co-regulators (co-activators and co-repressors) (93-95). It is becoming increasingly clear that AR co-activators connect various biological processes with AR-mediated transcription in a cohesive communication network. Therefore, SUMO may exert its influence on AR function through direct modification of AR and AR co-regulator (co-activator or co-repressor), alter the association of this receptor with other transcriptional co-regulators, and change its DNA binding ability and stability.

6.3.1. SUMO modification inhibits the transcription activity of AR

Two sumoylation sites were found in the N-terminal domain of human AR, K386 and K520 (96). Mutation of these residues increases the transactivation ability of AR, indicating that sumoylation negatively regulates the AR activity. Intriguingly, the sumoylation sites in AR are identical to the negative motifs in GR, which have recently been shown to restrict the transcriptional synergy of these receptors on promoters harboring multiple glucocorticoid response elements (97). In line with this, disruption of the SUMO-1 site in AR enhances its transcription on promoters with more than one hormone response elements (96). The synergy control motifs are identical with the sumoylation consensus sequence, and they can be found in negative regulatory regions of many, otherwise unrelated, transcription factors (97), suggesting that sumoylation can act as a general mechanism of activity control. Hence, sumoylation of AR affects AR transcriptional activity dependent on the promoter elements present in AR target genes, implying that sumoylation blocks the synergistic response of multiple AR binding sites in a promoter.

Furthermore, K386, but not K520, appears to play an important role in this synergy control of the receptor on multiple hormone response elements (98). However, these effects on synergy control clearly depend on the nature of the response elements. For instance, the K386 mutation does not increase the androgen response, if selective androgen response elements carrying direct repeats of 5'-TGTTCT-3'-like sequences are tested. In contrast, point mutations changing the direct-repeat elements into inverted-repeat elements restore the effects of the K386 mutation on synergy control. Thus, AR sumoylation might have a differential function in synergy control, with regard to the conformation of the AR dimer bound to DNA (98). The sumoylated core motif in AR is also present in the N-terminal domains of GR, MR, and PR, suggesting that sumoylation, like acetylation modification, may have a general mechanism for regulation of NR functions (96).

6.3.2. Sumoylation of the AR co-regulators

The biological function of AR at a great degree relies on its co-regulators (co-activators and co-regulators). AR co-activators serve as adapters between the receptor and the general transcription machinery, and enhance AR's ability to activate transcription. Meanwhile, the histone acetyltransferase activity of these co-activators also overcome the repressive effect of chromatin structure on transcription (99). The expression of AR co-activators in prostate cancer tissues closely correlates with AR expression, prostate cancer progression and recurrence (100). Inhibition of AR co-activators binding may be a promising approach for the treatment of prostate caner. AR co-repressors can inhibit AR-dependent transcription by recruiting histone deacetylases (HDACs) in a repression complex (93, 95). Thus, promoting the recruitment of a transcriptional co-repressor to AR will also contribute to prostate cancer treatment.

In this context, four AR co-regulators, SRC-1, SRC-2/GRIP1, p300, and HDAC1, have been found to be sumoylated (56, 87, 101, 102). SRC-1 has five sumoylation sites, and two major sites are localized to the NR box situated in the NR interacting region 1 (87). Sumoylation can increase the interaction of SRC-1 with the progesterone receptor. Two residues located in the nuclear receptor interacting region of SRC-2/GRIP1 are found to be sumoylated (102). Substitution at these two sumoylation sites could attenuate the activity of SRC-2/GRIP1 on AR-dependent transcription. Meanwhile, substitutions of the

conserved SUMO sites in GRIP1 alter subcellular distribution or localization of GRIP1 in relation to AR, suggesting that sumoylation sites in the NID of GRIP play a role in targeting or recruitment of the co-activator to AR-containing domains. Two sumoylated sites located in the CRD1 domain of P300 are required for its transcriptional-repression function. Mutations that reduce SUMO modification increase p300-mediated transcriptional activity (56). HDAC1 is also a substrate for SUMO(101). Mutation of two sumoylation sites of HDAC1 greatly reduces HDAC1-mediated transcriptional repression (101). Recently, a PIAS-like protein, hZimp10, a novel AR co-activator, whose expression in human prostate cancer cells increases the transcriptional activity of AR, is found to co-localize with AR and SUMO-1 at the replication foci (103). Studies using sumoylation deficient AR mutants suggest that the augmentation of AR activity by hZimp10 is dependent on the sumoylation of the receptor (103).

In addition, one of the SUMO-specific proteases, SENP1, profoundly enhances AR-dependent transcription (104). The effect of SENP1 on AR-dependent transcription is mediated mostly through desumoylation of HDAC1. SENP1 could overcome the HDAC1 repressive function and reduce HDAC1 deacetylase activity (104). These results support a role for SENP1 as a novel activator of AR-dependent transcription through desumoylation of HDAC1. Moreover, enzymes involved in SUMO modification also act as AR-dependent transcription co-regulators independent of their ability to catalyze SUMO conjugation, including E2 Ubc9 and E3 PIAS proteins, which will be discussed in more detail below.

6.3.3. SUMO modification dynamically regulate AR-dependent gene expression

SUMO modification is a reversible process in vivo. Several lines of evidence indicate that SUMO modification affects AR-dependent gene expression in a reversible and dynamic manner. In most cases, only small portion of sumoylated AR forms is detected in the cell transient transfected with SUMO-1, suggesting that the modification is transient and that there is a dynamic equilibrium between SUMO-1-conjugated and unconjugated receptor forms. Thus, sumoylation is likely to represent a mechanism for a rapid and reversible attenuation of AR function in distinct promoter contexts (96). In contrast to transcription factors, such as AR, p53, c-Myb and c-Jun, among which mutation of the SUMO sites is associated with the enhancement of their transcriptional activity (96, 105), mutation of the SUMO sites in GRIP1 attenuates their activity on AR-dependent transcription (102). It is possible that SUMO-1 modifications of AR and GRIP1 occur at different stages of AR-transcription complex formation to serve distinct roles in gene activation. It has been shown that ERα and co-activators assemble on target promoters in a sequential and fast cycling fashion (106). Thus, it is likely that reversible and highly dynamic characteristics of sumoylation provide an important means to regulate assembly and disassembly of AR transcriptional complexes (102). Moreover, as discussed above, SENP1, as a SUMO de-conjugation enzyme, is also involved in the regulation of AR-dependent transcription regulation. Together, these results suggest that SUMO regulates AR functions in a dynamic manner.

6.3.4. Effect of sumoylation enzymes on the AR-mediated transcription

Several sumoylation enzymes have been shown to interact with AR, affecting the AR-mediated transcription independent of their SUMO catalyzing activities. For instance, Ubc9, the unique SUMO conjugation enzyme, interacts with AR (96). The N-terminal half of the AR hinge region containing the nuclear localization signal (NLS) is essential for this interaction. Deletion of this part of the NLS, which does not completely prevent the transfer of AR to the nucleus, abolishes the AR-Ubc9 interaction and attenuates the transcriptional response to co-transfected Ubc9. However, Ubc9-DN does not influence its capability to stimulate AR-dependent transactivation (96). Similar to Ubc9, the protein inhibitor of activated STAT (PIAS) family proteins, including PIAS1, PIAS3, PIASxα, PIASxβ, and PIASy, as SUMO E3 ligase, regulate AR-mediated transcription dependent or independent of their E3 activity. AR-dependent transcription is enhanced by PIAS proteins without sumoylation of the receptor. It is possible that RING finger-like domain of PIAS proteins may recruit Ubc9 to the AR and stimulate the co-activator activity of Ubc9 toward the receptor. PIAS1 and PIASxα appear to repress AR-dependent transcription depending on the ectopic expression of SUMO-1 and their RING finger-like domain, and thus, modulation of AR-dependent transactivation by these two E3 ligases, at least in part, can be attributed to their SUMO-E3 activity toward AR (89). In prostate cancer cells, however, PIAS1 enhances the transcriptional activity of AR, but PIASy acts as a potent inhibitor of AR (107) and PIASy -mediated AR repression seems to be independent of sumoylation (108). A mutant PIASy , defective in promoting sumoylation, retains the ability to repress AR transcription. In addition, mutation of all the known sumoylation sites of AR does not affect the transrepression activity of PIASy on AR. It is likely that PIASy may repress AR by recruiting histone deacetylases, independent of its SUMO ligase activity (108).

6.3.5. Association of sumoylation alterations with prostate cancer

The enhanced activity of AR is essential for cancer cell growth, even in the androgen-refractory prostate cancer (92, 109). Although the mechanism underlying the regulation of AR activity and cancer cell growth is still not fully understood, cross talk between AR and growth factor-stimulated signal transduction pathways have been proposed as possible mechanisms to facilitate AR translocation and activity (92, 109, 110). Expression levels of some co-regulators of AR can be altered in the progression of prostate cancer, suggesting that they may be involved in the promotion or progression of prostate cancer through the regulation of AR activity (111). SUMO modification not only affects the AR activity by direct modifications of AR and its co-regulators as mentioned above, but also interferes with the interaction of the AR signaling pathway. STAT3-mediated signaling pathways can be inhibited by PIAS3 through promoting sumoylation

of important nuclear proteins. PIAS3 is an androgen-dependent gene and acts a negative regulator of AR signaling in a prostate cancer cell line (112). Over-expression of PIAS3 can induce apoptosis in prostate cancer cell lines both in vitro and in vivo (81). Moreover, it is also possible that SUMO interrupts the AR-related signal transduction associated with prostate cell growth by competing the same lysine with other posttranslational modifications, including acetylation and ubiquitination. Therefore, understanding the details of the interactions between PIAS3 and AR would provide a basis for new drug development for prostate cancer. It has been shown that, SENP1 functions as a strong activator of AR to markedly enhance AR-dependent transcription (104). The SENP1 mRNA is increased in prostate cancer cells, but not in normal prostate tissues (104), further suggesting a role of sumoylation in prostate cancer.

It is worth mentioning that a CAG repeat encoding a polyglutamine (polyQ) tract is found in the N-terminus of AR; AR transactivation is significantly affected by the polyQ deletion. It was demonstrated that the deletion of the polyQ tract results in an increased AR-transactivation capacity (113). It is possible that a shorter polyQ tract brings the AR a more accessible or stable surface for AR-interacting proteins, like SRC-1 or the AR-LBD (113). Although no effect of the presence or absence of the polyQ tract on sumoylation of the AR was observed, it is still possible that SUMO could involve the regulation of AR through polyQ context along with AR co-regulators because sumoylation has been implicated in neuronal diseases involving polyQ containing proteins (11, 63). Clearly, more studies are required to determine the direct connection between overexpression of sumoylation enzymes and pathogenesis of prostate cancer, and the effect of SUMO regulation on AR activity, in order to provide a new insight into prostate cancer development and treatment.

6.4. ER

Estrogen has crucial roles in the normal physiology of the mammary gland, as well as breast cancer development through binding to its receptors ERα and ERβ, which are ligand-dependent transcription factors. ER undergoes different types of post-translational modifications, and regulates transcription of the downstream genes. Transcription activities of ER can be regulated by interacting proteins such as co-activators and kinases as well as ligand-binding. As for AR, SUMO regulates ER transcription activity by direct modification of ER and its co-regulators, thus, affecting gene transcriptions. Even though the exact mechanism underlying the regulation of ER transcriptional activation by SUMO is still unclear, SUMO modification of ER and its co-regulators may be regarded as a new mechanism of modulating ER-mediated processes in both normal and cancer cells.

6.4.1. Sumoylation of ERa positively regulates ER-mediated transcriptional activity

Until recently, ERα was identified as a new target for SUMO-1 modification in vivo and in vitro (114). ERα is sumoylated at conserved lysine residues within the hinge region. Like AR, ERα sumoylation occurs strictly in the presence of hormone, suggesting that hormone binding, and perhaps the subsequent altering of receptor conformation, is essential for its interaction with components of the SUMO modification machinery. The ability of ligands to regulate ERα?sumoylation may also be in part due to the subcellular distribution of ERα. For instance, the non-ligand-bound form of ERα is distributed throughout the nucleoplasm, while the ligand-bound form of ERα is redistributed in discrete punctuate structures, which can regulate or facilitate the interaction of ERα with the sumoylation machinery. Unlike AR, in which SUMO modification exerts an inhibitory role in regards to receptor transcription activity, SUMO appears to stimulate ERα-dependent transcription (114). Intriguingly, mutations that prevent SUMO modification attenuate ERα-induced transcription without influencing ERα cellular localization (114).

6.4.2. SUMO modification of ER co-regulators

As for AR-mediated gene regulation, function of ER can be modulated by associations with a number of co-regulators. In particular, ER shares several co-regulators with those of AR, such as GRIP1, SRC1 and HDAC1 (87, 101, 102), which are also subjected to sumoylation. Sumoylation of these co-regulators by overexpression of SUMO-1 induces the nuclear receptor-mediated gene expression. For instance, sumoylation of SRC-1 increases the interactions of progesterone receptor (PR) with SRC-1 and thus, prolong SRC-1 retention in the nucleus (87). Because SRC-1 interacts with a variety of nuclear receptors and regulates signaling pathways mediated by the nuclear receptors (115), induction of SRC-1 activity by sumoylation is assumed to have a profound effect on expression of the nuclear receptor-mediated genes, including those ER-meditated genes (87). In addition, transcriptional activation/repression involves alteration of chromatin structure within promoters of target genes due to changes in histone modifications which may provide the platform for association with other factors (116, 117). Modification of HDAC can definitely have an impact on transcriptional consequence. Furthermore, phosphorylation of ERβ facilitates the recruitment of SRC-1 in a ligand independent manner (118). It is not clear whether sumoylation of SRC-1 will enhance this recruitment. Taken together, the modification of ER co-regulators certainly adds another level of complexity to the role of SUMO in ER-dependent transcription regulation.

6.4.3. Effect of sumoylation enzymes on the ER-mediated transcription

Similar to AR, enzymes involved in the SUMO conjugation pathway regulate ER-dependent transcription in a SUMO-independent manner. Ubc9 and PIAS1, SUMO conjugating enzyme and ligase, respectively, markedly interact with ERα in a ligand-dependent manner. These proteins mainly interact with the DNA-binding and ligand-binding domains of ERα. Overexpression of Ubc9 or PIAS1 increases ERα-mediated transcriptional activities in COS-1 cells in a dose-dependent manner, indicating that both Ubc9 and PIAS1 function as co-activators of ERα.

Paradoxically, the sumoylation-defective mutant Ubc9 is still able to enhance ERα-dependent transcriptional activities. These findings suggest that the ability of co-activators and the sumoylation capacities of Ubc9 and PIAS1 are separable and distinct (119).

6.4.4. Association of sumoylation with breast cancer

ERs are ligand-regulated transcription factors that play critical roles in the development and progression of breast cancer by regulating target genes involved in cell growth and proliferation. Although information on sumoylation-dependent regulation of ER function is limited, it is clear that ERα and its co-regulators are substrates for SUMO, and sumoylation of ERα and its coregulators affects expression of the downstream genes (114). Therefore, sumoylation of ER and its co-regulators at least in part could contribute to the development of breast cancer. Additionally, experiments with the intervention of Ubc9 indicates that Ubc9 affects breast tumor growth in the xenograft mouse model, further supporting the notion that sumoylation plays a role in breast cancer development (80). Finally, the increased expression levels of PIAS3 detected in breast tumors further suggest alterations of sumoylation in these tumors (81).

7. PERSPECTIVE

It has been well documented that deregulation of ubiquitination can lead to human malignancies. Recent evidence has suggested that sumoylation is also linked to the pathogenesis of a variety of disorders including cancer possibly through different mechanisms. Although sumoylation can impact cellular pathways by various mechanisms, the most significant one appears to be the regulation of gene expression. In particular, in the case of prostate and breast cancer, sumoylation could affect these diseases by targeting NRs such as AR and ER. Moreover, despite the direct effect on the NR-mediated gene expression by SUMO, it appears that enzymes required for sumoylation can also affect gene transcriptions independent of their capabilities to catalyze the sumoylation cycle. Thus, mechanisms underlying the effect of sumoylation of target proteins, or enzymes required for sumoylation on the development of cancer could be more complex than we previously thought. Given the possible roles of sumoylation enzymes in cancer, they could be potential therapeutic targets. Despite the concern with their ubiquitous expression, upregulation of some enzymes such as Ubc9 and E3 ligases in tumor tissues might provide a certain degree of selectivity against tumor over normal cells. Once we know better molecular mechanisms by which sumoylation affects tumorigenesis, we will be at a better position for designing a sumoylation-based therapeutic strategy for treatment of prostate and breast cancer.

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