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ABSTRACT

The small ubiquitin-related modification molecule (SUMO), one of the post-translational modification molecules, is involved in a variety of cellular functions where it regulates protein activity and stability, transcription, and cell cycling. Modulation of protein SUMOylation or deSUMOylation modification has been associated with regulation of carcinogenesis in breast cancer. In the dynamic processes of SUMOylation and deSUMOylation in a variety of cancers, SUMO proteases (SENPs), reverse SUMOylation by isopeptidase activity and SENPs are mostly elevated, and are related to poor patient prognosis. Although underlying mechanisms have been suggested for how SENPs participate in breast cancer tumorigenesis, such as through regulation of target protein transactivation, cancer cell survival, cell cycle, or other post-translational modification-related machinery recruitment, the effect of SENP isoform-specific inhibitors on the progression of breast cancer have not been well evaluated. This review will introduce the functions of SENP1 and SENP2 and the underlying signaling pathways in breast cancer for use in discovery of new biomarkers for diagnosis or therapeutic targets for treatment.

INTRODUCTION

Despite recent advances in diagnosis and treatment, breast cancer is the second leading cause of cancer-related mortality worldwide. In fact, the survival rate of women with breast cancer has improved, but the prognosis for patients with locally advanced or metastatic disease is still poor.

Most breast cancers are associated with three different receptor expressions,

including estrogen receptor-alpha ($ER\alpha$), progesterone receptor (PR), and overexpression of ERBB2/HER2 (1, 2). The molecular subtype of breast cancer based on receptor expression is helpful for prognosis and therapeutic response. Four common molecular subtypes are associated with breast cancers, Luminal A ($ER\alpha+$ /PgR+, HER2-), Luminal B ($ER\alpha+$ /PgR+, HER2+), HER2 ($ER\alpha-$ /PgR-, HER2+), and triple-negative (TN, $ER\alpha-$ /PgR-, HER2-) (2, 3). The luminal breast cancer subtypes are characterized by the expression of a set of $ER\alpha$ -related genes and represent approximately 75% of breast cancer patients in postmenopausal women (1). Among these four subtypes, HER2 and TNBC types show a poor prognosis due to p53 gene mutations (4). The receptor expression of these subtypes is well established and used to predict the prognosis and treatment of breast cancer patients, but little is known about the transcriptional mechanisms associated with the different breast cancer subtypes. This review will introduce the mechanisms of post-translational modifications responsible for breast cancer subtypes.

Transcriptional regulation of Estrogen Receptor in breast cancer

Estrogens are important for sexual and reproductive development, especially in women. But, estrogens have also been directly implicated in hormone-dependent cancers, including breast, endometrial, and ovarian cancers (5). ER positive breast cancer accounts for about 75% of all breast cancer subtypes, and estrogen binds to and activates two isoforms, $ER\alpha$ and β leading to physiological functions via a genomic or a non-genomic pathway (6). Between $ER\alpha$ and β , the $ER\alpha$ -mediated signaling pathway is known to be a major driver of breast cancer. Ligand binds to $ER\alpha$ causing $ER\alpha$ -

dependent transcription, therefore investigating coregulatory proteins that inhibit ER α -dependent transcription can be an important treatment for ER α -positive breast cancer. When the ligand binds to the receptor, the receptor undergoes a conformational change to create a new interface for the recruitment of coactivators and corepressors (6). The coactivators exist in large multiprotein complexes that regulate chromatin remodeling by changing histone-histone or histone-DNA interactions (7). Sequentially, these interactions mediate addition of histone posttranslational modification (7). Indeed, it has been reported that E2 promoted ER α -dependent transcription of the target genes by employing coactivators with histone acetyl transferase (8, 9). These coactivator interactions cause maximal growth of breast cancer cells via ER α -dependent expression of genes that promote cell proliferation (8, 9).

On the other hand, one of the corepressors, known as the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) mediates both stimulation and repression of E2-dependent ER α activity in a gene-selective manner (9, 10). The dual function of SMRT as a coactivator and a corepressor of ER α makes it difficult to predict if regulation of SMRT plays a role in pro- or anti-tumorigenesis in breast cancer. However, the role of SMRT in the regulation of ER α has been evaluated as that of a coactivator, since elevated nuclear expression of SMRT was associated with earlier tumor recurrence and this expression correlated with poor prognosis of breast cancer (11, 12). A recent study reported that SMRT induced E2-dependent cell proliferation by cell cycle regulation and apoptosis inhibition in ER α -positive breast cancer cells, but not in ER α -negative MDA-MB-231 cells (13). SMRT increased E2 α -dependent cell cycle molecules, including cyclin D1 to promote E2-dependent G1/S transition (13).

Consistent with this data, deletion of coactivators, such as SRC-2 or SRC-3 also inhibited E2-dependent cell proliferation of MCF-7 cells from G1 to S cell cycle (9, 14). Interestingly, Blackmore et al. recently reported that ER α signal mediated E2-dependent recruitment with the SMRT/SRC-3 complex in the cyclin D1 gene for the proliferation of breast cancer cells (13).

Another cell cycle regulatory molecule, histone deacetylase 3 (HDAC3) has been reported to be a mitotic regulator through histone deacetylation and mitotic spindle formation with several members of the HDAC3 corepressors, including NCoR, TBL1, and TBLR1 (15). Treatment with HDAC3 inhibitors inhibited cell proliferation via apoptosis (15) and the SMRT/NCoR complex was involved in regulation of HDAC3 activity as a coactivator in breast cancer cells (13).

Together, the various transcriptional regulators are involved in pro- or anti-tumorigenesis of breast cancer cells, so understanding the precise role of transcriptional regulators in breast cancer cell function is crucial to finding good treatments for these cancers.

SUMOylation and breast cancer

Most transcription factors are functionally regulated by post-translational modifications (PTMs), which are important to efficiently regulate cell functions in response to multiple extracellular stimuli or intracellular signals (16-19). Of PTMs, protein modification by a small ubiquitin-like modifier (SUMO) peptide on a lysine residue affects many different biological processes by regulating protein activity, transcriptional activity, protein stability, or localization change (Figure 1) (20-22). The SUMO modification dynamically happens by conjugation or deconjugation in a small

portion of a substrate at a certain time (17). The SUMOylation pathway is similar to that of ubiquitination by different set of enzymes. SUMO is activated in an ATP-dependent manner by an E1-activating enzyme that consists of a SUMO activating enzyme (SAE1) (as known as Aos1)-SAE2 (as known as Uba2) heterodimer. Activated SUMO is transferred to Ubc9, the E2-conjugating enzyme, and is subsequently attached to the ε amino group of specific residues in target proteins (18). Finally, studies have shown that E3 SUMO ligase-like protein inhibitors of activated STAT (PIAS)y conjugates activated SUMO to the target protein (18, 23).

On the other hand, deSUMOylation is mediated by the SUMO proteases (SENPs) and six SENPs have been identified in humans (17, 24). Each SENP shows different cellular location and substrate specificities (24). Among the six SENPs, SENP1 and SENP2 process all three SUMO isoforms (SUMO1, 2, and 3) and deSUMOylate both mono- and polymeric SUMOylated proteins (Figure 1A) (25). SUMO3 and SUMO5 process only SUMO2/3, whereas SENP6 and SENP7 display only hydrolase activity (25).

Interestingly, expression levels of protein SUMOylation affect normal cellular physiology and tumor formation (19, 26, 27). Indeed, hyper levels of SENP1 have been seen in thyroid adenocarcinoma and prostate cancer (28, 29), and SENP2 was shown to be important for the development of trophoblast stem cells through p53/Mdm2 regulation (30). In addition, SENP2 regulated activity of transcription factors by controlling PR, whereas inhibition of SENP2 activity reduced ER α -induced gene expression and breast cancer cell proliferation (31, 32).

These reports suggest that the regulation of target protein SUMOylation can be one of the key strategies for the treatment of breast cancers. This SUMOylation pathway

associated with various cancer cell functions is shown in Table 1.

SENP1 regulates Pin1 in breast cancer

Proline-directed protein phosphorylation (pSer/Thr-Pro) is a one of the important signaling pathways in diverse cellular processes, including cell proliferation and transformation. Pin1 is a peptidyl-prolyl cis/trans isomerase (PPIase) and the N-terminal WW domain of Pin1 can bind to specific pSer/Thr-Pro motifs, and subsequently bind to its substrates to induce proteins conformational changes (33-35). These Pin-1-induced protein conformational changes following phosphorylation regulate various cellular functions, particularly in cancer cells, including cell cycle regulation, transcription splicing, DNA damage responses, germ cell development, and neuronal survival (33, 34, 36, 37). In particular, Pin1 was highly detected in human cancers and its overexpression levels were correlated with poor clinical outcomes, whereas reduced Pin1 expression caused by genetic polymorphisms was associated with reduced cancer risk in humans (33, 34).

Pin1 regulates various proteins involved in oncogenes/growth enhancers and tumor suppression. Indeed, Pin1 activates oncogenes, including cyclin D1, NF-kB, c-Jun, c-fos, Raf-1, Stat3, Neu/ErbB2, Notch and Akt, but inactivates tumor suppressors, including FOXOs, PML, SMRT, Smad, Pin2/TRF1, Rb, and Fbw7 (33, 34). Pin1 and the regulatory signaling pathway of Pin1 has, therefore, been considered one of the best candidates for treating breast cancer.

Recently, overexpression of SENP1, one of the SUMO proteases, was shown in several human cancers including prostate, thyroid cancer, and breast cancer (29, 38, 39). Chen et al. reported that increased levels of Pin1 SUMOylation at Lys6 in the WW

domain downregulated Pin1 protein activity, whereas SENP1 regulated Pin1 SUMOylation to induce Pin activity in centrosome amplification and cell transformation. This group also showed correlation between the expression level of SENP1 and Pin1 in human breast cancer (39). The results suggest that increased levels of SENP1 activity are important for regulating Pin1 SUMOylation to increase oncogenesis in breast cancer, and this new SENP1-Pin1 module can be an attractive alternative target for anti-cancer therapies.

Role of Mel-18 on SENP1 function in hormone-dependent breast cancer treatment

As mentioned earlier, about 25% of breast cancer patients do not express the ER α -related genes, and so are resistant to anti-estrogen therapy and have a poor prognosis (1). Since ER α -negative breast cancer cells, such as HER2 (ER α -/PgR-, HER2+) or TN (ER α -/PgR-, HER2-) cells do not derive any benefit from conventional hormone therapy, attempts have been made to reverse the estrogen receptor (ESR)1 gene in these cells through epigenetic changes (40, 41).

Recently, Mel-18 was reported to modulate ESR1 gene transcription by altering the activity of SENP1 (42). Mel-18 is known as a polycomb group ring finger 2 and is structurally similar to Bmi-1 (43). In a previous report, Mel-18 was shown to function as a key epigenetic modulator of somatic stem cells and cancer cells (44). Functionally, Mel-18 binds to the promoter region of specific genes presenting a certain nucleotide sequence, 5'-GACTNGACT-3'. One of the targets was c-myc, and it was transcriptionally repressed by Mel-18 (43). Mel-18 deficiency showed enhancement of breast cancer stem cell activity, tumor angiogenesis, and epithelial-mesenchymal transition (45, 46). Therefore, the loss of Mel-18 is thought to be one of the causes of

aggressive breast cancer.

A recent report demonstrated that Mel-18 deficiency was associated with SUMOylation or deSUMOylation-dependent ESR1 and prostaglandin receptor (PGR) expression, rather than hormone-dependent phenotypes (42). The study showed that Mel-18 induced deSUMOylation of p53 and SP1, transcriptional factors of the ESR1 gene, by increasing ubiquitin-proteasomal degradation of SENP1. It caused the transactivation of ESR1, as well as PGR. Together, regulation of the SUMO-mediated hormone receptors by Mel-18 is important for breast cancer tumorigenesis, and Mel-18 can be used as a prognostic indicator in patients with resistance to hormone-therapy or triple negative breast cancer.

SENP2 regulates Estrogen Receptor α signaling in breast cancer

Similar to histone protein-related modifications, other posttranslational modifications such as phosphorylation, acetylation, and ubiquitination can affect ER α activity and stability (47). A new covalent protein modification, which is another SUMO modification (SUMO1) has been reported, and SUMO1 binds ER α and regulates ER α transcriptional activity (48). Sentis et al. reported that SUMO-E3 ligases (PIAS1 and PIAS3) mediated ER α -SUMO1 interaction in the presence of hormone and altered ER SUMOylation-mediated ER transcriptional activity (49). Since SUMOylation is reversible by SENPs, which cleave the isopeptide bonds between the glycine residue of SUMO and the lysine of the substrate protein, the role of SENP2 on ER α -SUMOylation was recently studied in breast cancer cells (32).

Unexpectedly, SENP2 was found to regulate estrogen signaling, independent of

deSUMOylase activity. Instead of regulating ER α -SUMOylation, SENP2 recruits HDAC3 in the repressor domain at the amino-terminal region and acts as an ER α -transcriptional corepressor (32). It has been reported that transcriptional regulators of ER α are recruited into a variety of promoters to alter the function of breast cancer cells (50, 51). For example, estrogen-activated ER α -receptor recruited the SMAR/HDAC3 complex into a PROS1 promoter to induce chromatin hypoacetylation (51).

Therefore, this suggests that ER α -mediated transcription of target genes is altered by its SUMOylation and regulation of ER α -SUMOylation may be an important strategy for breast cancer treatment. Since SENP2 regulates ER α -mediated cellular function by transrepression, rather than deSUMOylation activity, it may be important to evaluate the function of SUMOylation on the cellular function using SUMO mutants in breast cancer cells.

Perspective breast cancer treatment

Since the target protein SUMOylation is involved in regulation of carcinogenesis, SUMOylation modulators have been developed as promising anti-cancer drugs. In fact, the isopeptidase activity of SENPs is critical for the regulation of SUMOylation and SENPs expressions have been associated with a variety of cancers (28-32). Several inhibitors of SENPs have been developed to date, but the effect on cancer has not been well evaluated (52-56). Among the SENPs, only SENP1 inhibitors have been developed through chemical synthesis or natural product discovery, and evaluated for their effects on cancer. For example, triptolide, extracted from the Chinese herb Tripterygium

wilfrodii Hook F showed anti-tumor activity via down-regulation of SENP1 that inhibited the AR and c-Jun mediated transcription in prostate cancer (57). In a recent study, Momordin Ic, a natural pentacyclic triterpenoid, inhibited SENP1 enzyme activity *in vitro* and suppressed tumorigenesis in a xenograft PC3 tumor mouse model (58). This suggests that inhibitors of SENP1 may be developed, at least as important anti-cancer drugs for prostate cancer, and that studies on other cancer types, especially breast cancer, should proceed. Together, the discovery of isoform-selective potent SENP inhibitors will be important in validating the role of SENPs in tumorigenesis as a new therapeutic targets. In addition, the patho-physiological role of isoform-selective SENP inhibitors in breast cancer should be evaluated for therapeutic development.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. The scheme of SUMOylation and deSUMOylation pathway. (A) Protein SUMOylation is associated with a recycling system consisting of conjugation and deconjugation pathways. Both conjugation and deconjugation enzymes mediate the dynamic and reversible process of SUMOylation. The protein SUMOylation alters

protein activation, transcriptional activity, stability, and localization change. (B) SUMO proteins covalently modify certain residues of specific target substrates and change the function of these substrates. The conjugation pathway is mediated by SUMO E1, E2, E3 enzymes, whereas the deconjugation pathway is mediated by SUMOisopeptidase, SENPs.

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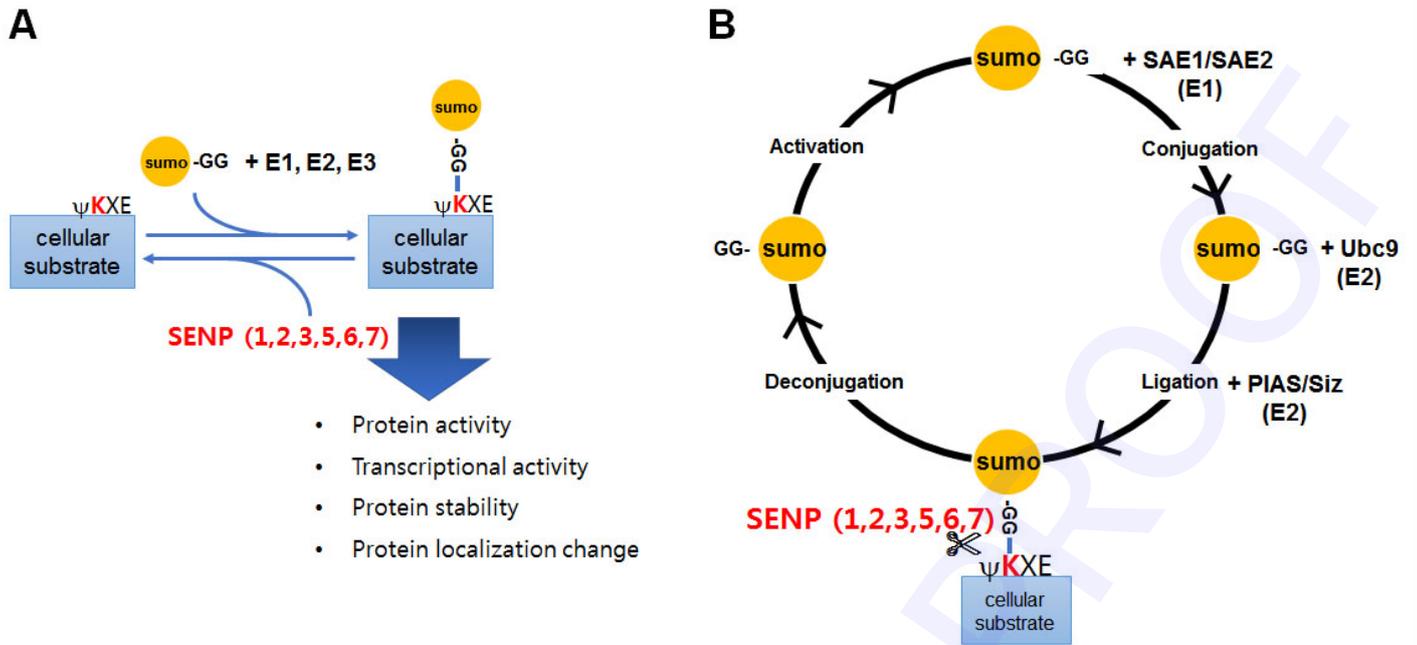


Fig. 1.

Table 1. SENP1 and SENP2-regulated SUMOylation targets associated with various cancer cell function

SUMO enzyme	Cancer types	Cell regulation	Tissue expression	Ref
SENP1	Thyroid	expressed in mitochondria	Up-regulated	(28)
	Prostate	upregulation of transcriptional activity of androgen receptors (ARs) and c-Jun, as well as cyclin D1 expression	Up-regulated	(29)
	Breast	down-regulates Pin1 to increase oncogenesis	Up-regulated	(39)
	Hepatocyte	development of multidrug resistance (MDR)	Up-regulated	(19)
	Pancreatic	regulates MMP-9 mediated metastasis	Up-regulated	(59)
SENP2	Hepatocyte	regulates β -catenin stability	Down-regulated	(60)
	Bladder	inhibits MMP13 expression	Down-regulated	(61)
	Bladder	suppress EMT of bladder cancer cell	Down-regulated	(62)
	Gastric	acts as a tumor suppressor by inducing deSUMOylation of NDRG2	Down-regulated	(63)
	Breast	Causes failure of hormone-dependent therapy via transcriptional repression of ER α	Unknown	(32)
	Breast	SENP2 (363-400) fragment is critical for TGF-b-induced cell migration	Unknown	(64)
	Breast	down-regulates FOXM1B through deSUMOylation activity	unknown	(65)