Post-translational modifiers of the small ubiquitin-like modifier protein (SUMO) family have emerged as key regulators of protein function and localization. SUMO modification is a dynamic process, catalyzed by SUMO-specific E1, E2 and E3s and reversed by a family of SUMO-specific proteases (SENP). Although six human SENPs have been identified, each with different cellular locations and substrate specificities, the precise roles of SENPs in cellular processes involved has not been well-defined. This brief review will focus on recent advances about the identified targets of SENP1 and its potential role in tumorigenesis.

Conjugation of the small ubiquitin-related modifier protein (SUMO) to a large number of substrates has been shown to regulate protein localization and function. There are three SUMO family members, which slightly vary in length: SUMO1 is 101 amino acids, SUMO2 is 103 amino acids and SUMO3 is 95 amino acids. SUMO2 and SUMO3 are more closely related to each other (93.6% identity in 94-residue overlap) compared with SUMO1 (52.4% identity in 84-residue overlap). SUMO homologues have been detected from Arabidopsis thaliana to Homo sapiens, suggesting that SUMOylation is an evolutionarily conserved cellular process.

There are remarkable similarities between ubiquitination and SUMOylation pathways. However, the activating (E1), conjugating (E2) and ligating (E3) enzymes involved in SUMOylation are entirely distinct from those involved in ubiquitination. The E1 for SUMO is composed of two subunits, Aos1 and Uba2. In contrast to the large number of E2s used in the ubiquitination pathway, Ubc9 is the only SUMO-conjugating enzyme. E3 ligase can facilitate SUMO conjugation to catalyze the transfer of SUMO from UBC9 to a substrate. There are more than eight SUMO E3 ligases reported. These E3 ligases can be subdivided into three types. The first type is composed of the PIAS (protein inhibitor of the activator of STAT) family of proteins with an SP-Ring motif. The second type is Pc2, which is a component of polycomb protein complexes.

SUMOylation can be reversed by a family of SUMO-specific proteases (SENP). Two yeast SUMO-specific proteases, Ulp1 and Ulp2/Smt4, have been first characterized. Both enzymes can deconjugate SUMO/Smt3 from modified proteins and process SUMO/Smt3 precursors to the mature form with the C-terminal diglycine. Sequence comparisons predicted a ~200 amino acid protease fold, which defines this group of enzymes (C48 cysteine proteases). In mammalian cells, six SUMO-specific proteases (SENP) have been reported. The six members of SENP family can be divided into three subfamilies on the basis of their sequence homology, cellular location, and substrate specificity. The first subfamily consists of SENP1 and SENP2, which have broad substrate specificity. SENP1 is a nuclear protease that deconjugates a large number of sumoylated proteins. SENP2 is a nuclear envelope-associated protease that, when overexpressed, appears to have an activity similar to that of SENP1. There is a spliced isoform of mouse SENP2, called SuPr1, which could alter the activity of nuclear POD-associated proteins, such as CBP (CREB binding protein, CBP) and function and potentially novel mechanisms for the regulation of c-Jun-dependent transcriptional activities. The second subfamily consists of SENP3 and SENP5, both of which are nucleolar proteins with preferences for SUMO-2/3. The third subfamily consists of SENP6 and SENP7, which have an extra loop in their catalytic domain. Although SENP family have shown the activity for SUMO process and deconjugation, their precise physiological role in reversing SUMOylation has not been well-defined.

This review focuses on SUMO-specific protease 1 (SENP1) and the cellular processes involved, in particular, on the potential role of SENP1 in tumorigenesis.

**SENP1, Androgen Receptor Signaling and Prostate Cancer**

We initially performed a functional screening to determine which member of SENP family regulates the cellular processes involved by SUMOylated targets. Androgen receptor (AR) signaling is the first system to be chosen for this purpose. AR is a ligand-regulated transcription factor belonging to the nuclear receptor superfamily. It mediates the effects of androgen on the regulation of cell growth, differentiation, and maintenance of male reproductive functions. AR also plays an important role in prostate cancer development. Interestingly, AR and four of its co-regulators, SRC-1, SRC-2, p300 and HDAC1, are conjugated by SUMO.6
Above features make AR signaling as an attractive model to study the regulatory function of desumoylation. Thus, we designed a functional screen strategy to survey the member of SENPs family that involves in AR signaling. Among the members of SENPs family, only SENP1 specifically and dramatically enhance AR transcriptional activity. SENP1's ability to enhance AR-dependent transcription is mediated through desumoylation of histone deacetylase 1 (HDAC1), thereby reducing its deacetylase activity and repressive activity to AR-dependent transcription.\(^6\)\(^7\) As SENP1 activation in AR signaling is dependent on the present of androgen, we reasoned that androgen would be a regulator for SENP1 activity in this regulation process. Indeed, exposure of androgen enhances the SENP1 transcription in LNCaP cells selectively. This androgen-mediated augmentation of SENP1 is absent with co-administration of the androgen receptor antagonist bicalutamide or in AR-negative prostate cancer PC-3 cells, indicating an AR-dependent event. We further identified the specific androgen response element (ARE) on the promoter of SENP1 gene that are required for SENP1 induction by androgen.\(^7\)\(^8\)

We have determined that SENP1 can enhance androgen-induced AR activity, and SENP1 expression can be reversely induced by androgen-AR signaling and then further enhance AR activity by positive feedback. Such relationship between SENP1 and AR signaling prompted us to explore a potential role of SENP1 in prostate cancer development because AR is one of the most important factors in prostate cancer. In particular, we examined the expression of SENP1 in prostate cancer specimens. We found that SENP1 was overexpressed in more than 50% of the high-grade prostate intra-epithelial neoplasias (PIN) and prostate cancers in over 100 cases.\(^7\)

We further confirmed the overexpression of SENP1 plays an important role in the development of prostate cancer by using SENP1 transgenic mouse model. The 3-month old probasin-driven murine SENP1 transgenic mice showed the increased proliferation of prostate epithelial cells. Importantly, a significant increase of PIN-like structure formation was observed in SENP1 transgenic mice older than four months.\(^7\) These results strongly support a role of SENP1 in prostate tumorigenesis.

**SENP1, Erythropoiesis and HIF1α-Epo Signaling**

Yamaguchi et al. studied mice derived from an embryonic stem cell line with a retroviral vector that had been randomly inserted into the enhancer region on the *SENP1* gene.\(^9\) This reduced SENP1 transcription, causing the mice to die between E12.5 and E14.5, indicating that SENP1 plays an important role in development. However, no specific histological abnormalities were found in these mutant embryos, except the defect in the placenta.\(^9\)

As SENP1 expression is only reduced in Yamaguchi’s mouse model, we generated a mouse model with complete knockout of SENP1 to further delineate the contribution of SENP1 in development. We used similar strategy to generate this mouse model, which derived from an embryonic stem cell line with gene trapped vector that had been inserted into the open reading frame of mouse SENP1 gene at codon 310. This insertion resulted in a complete absence of transcripts that encode catalytic domain of SENP1 in SENP1-/- embryos.\(^10\) These SENP1-/- mice also showed embryonic lethal death from E13 to 15 days. However, a markedly decrease in the number of erythropoietic foci was observed in the fetal liver of our SENP1-/- mice, indicating that the activity of definitive erythropoiesis was greatly decreased. The inactivation of SENP1 causes the defect in definitive erythropoiesis, which might be a major contributor to the embryonic lethality of SENP1-/- mice. Furthermore, the deficiency in erythropoiesis was confirmed to occur at the committed erythroid colony-forming unit (CFU-E) stage (Ter-119+ cell), which was significantly decreased in SENP1-/- mice due to apoptosis.\(^10\)

The phenotype of deficiency in erythropoiesis in SENP1-/- mice offers a fascinating opportunity to study the role of SENP1 in the regulation of hypoxia-inducing factor-1α-erythropoietin (HIF1α-Epo) pathway, a major cell signaling involving in hypoxia-induced erythropoiesis. The expression of Epo, a crucial factor for erythroid or CFU-E survival, has been demonstrated significant decrease in the fetal liver of SENP1-/- mice. The characteristics of embryonic anemia in SENP1-/- mice are similar to those in Epo-null mice,\(^11\) indicating that Epo deficiency in the fetal liver of SENP1-/- mice might be the molecular mechanism that account for the erythroid apoptosis. Indeed, we proved this mechanism by addition of Epo that efficiently increased survival and decreased apoptosis of erythrocytes in SENP1-/- mice.\(^10\)

HIF1α, a member of the bHLH-PAS family of proteins, can bind to canonical DNA sequences (hypoxia-regulated elements, or HREs) in the promoters or enhancers of target genes.\(^12\) Epo is one of the target genes of HIF1α. We have confirmed that SENP1 regulates Epo expression dependent on HIF1α. SENP1 can de-conjugate SUMOylated HIF1α, a modification induced by hypoxia. Interestingly, the de-SUMOylation activity of SENP1 is essential for SENP1 to regulate Epo transcription, indicating that this regulation is through de-conjugating SUMOylated HIF1α.\(^10\)

Hypoxia is the established inducer of HIF1α,\(^13\) stabilization\(^14\) and Epo expression.\(^12,15\) Iyer et al. (1998)\(^16\) reported the expression of HIF1α during mouse embryonic development: at E8.5 day, HIF1α protein level was considerably low, however, at E9.5–E12.5 days, HIF1α expression was enhanced by over 10- to 20-fold in comparison to E8.5 day. This period coincides with the time when the mouse embryo is mostly under hypoxia. The expression of HIF1α under hypoxia is also much shorter in SENP1-/- MEF cells than in wild-type cells.\(^10\) These results reveal an important role of SENP1 in maintaining hypoxia-induced stabilization of HIF1α protein.

These findings indicate the potential role of SENP1 in regulating tumor angiogenesis. Tumor cells expansion relies on nutrition supply. Oxygen limitation is central in controlling angiogenesis, glucose metabolism, survival and tumor spread. These pleiotropic actions are orchestrated by HIF1α, which is a master transcriptional factor in hypoxia signaling. One of the key target genes in this hypoxia-HIF1α process is vascular epithelial growth factor (VEGF), an angiogenic factor. We found that the expression of VEGF response to hypoxia is significantly decreased in SENP1-/- mice. The half-life of HIF1α protein under hypoxia condition is also much shorter in SENP1-/- MEF cells than in wild-type cells.\(^10\) These results reveal an important role of SENP1 in maintaining hypoxia-induced stabilization of HIF1α protein.

**SUMOylation and Protein Stability**

The defect in hypoxia-HIF1α signaling in SENP1-/- mice also offers an opportunity to investigate the issue about the role of
SUMOylation in protein stability. This is because we observed the descending of HIF1α protein stability in SENP1 mutant cells. It is well-accepted that SUMOylation can stabilize target proteins because SUMO can conjugate the same lysine sites on target proteins as ubiquitin do and thus against ubiquitination (occupied mechanism). However, SENP1 de-conjugates SUMOylated HIF1α, which is essential for hypoxia-induced stabilization of HIF1α. This suggests that SUMOylation may promote HIF1α degradation. If being the case, the hypoxia-induced SUMOylated HIF1α should be easily to be detected in SENP1-/- MEF cells and also greatly accumulated when cells are treated with MG132, a proteasome inhibitor. Indeed, the result from SENP1 mutant cells is in consistent with that expected.

Furthermore, the hypoxia-induced accumulation of SUMOylated HIF1α is very difficult to detect in SENP1 wild-type cells, even in the cells treated with MG132. These results suggest that SENP1 normally de-conjugate most hypoxia-induced SUMOylated HIF1α and then stabilize it. SUMOylation of HIF1α is degraded under hypoxia in the proteasome-dependent manner. Although ubiquitin-conjugated sites on HIF1α protein have not been precisely defined, the reported ubiquitin-conjugated region of HIF1α indicates that at least the lysine 391 and 477, two SUMOylation sites on HIF1α protein, are not the major sites for ubiquitination. Actually, HIF1α SUMOylation can induce its ubiquitination. VHL have been identified as a E3 ligase for ubiquitination of SUMOylated HIF1α. Thus, SUMOylation can provide an alternative signal for binding of HIF1α to VHL E3 ligase for ubiquitination, allowing for proteasomal degradation of SUMOylated HIF1α to occur under hypoxia.

There are at least three mechanisms that control HIF1α SUMOylation. The first is that hypoxia induces HIF1α SUMOylation, which has already been reported by Bae et al. in 2004. This mechanism should be mediated by an un-defined E3 ligase. The second is that SENP1 de-SUMOlate HIF1α. The third is that SUMOylated HIF1α would undergo degradation through proteasome-dependent manner. In these HIF1α regulation mechanisms, SENP1 plays a crucial role in maintaining hypoxia-induced HIF1α stabilization. Inactivation of SENP1 will significantly induce the accumulation of hypoxia-induced SUMOylated HIF1α, thus SUMOylated HIF1α undergoes VHL-dependent degradation by proteasome, and finally the expression and activity of HIF1α induced by hypoxia would decrease. SENP1 acts as a switch to decide the fate of SUMOylated HIF1α, to degrade or stabilize under hypoxia.

Concluding Remarks

We used different strategies to determine some targets of SENP1 and the cellular processes involved. As these targets are related to tumorigenesis, it indicates the role of SENP1 in cancer development. However, there are still lots of questions need to be addressed. For instance, what is the precise mechanism underlying the functions of SENP1 and its target proteins in tumorigenesis? Are there any other SENP1 targets involved tumorigenesis? What is the clinical significance of SENP1 in tumorigenesis? We currently use genetically modified cells and mice models to identify SENP1 targets and determine the molecular mechanisms, and also use tumor specimens to analyze the relationship between SENP1, its targets, and clinical features of tumors.

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