

Redox-sensitive TP53INP1 SUMOylation as an oxidative stress sensor to activate TP53

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Oxidative stress-induced sumoylation of TP53INP1 (tumor protein p53-induced nuclear protein 1) is essential to enhance the TP53 response. Sumoylation of TP53INP1 on the K113 residue, which is mediated by protein inhibitor of activated STAT 3 (PIAS3) and chromo-box homolog 4 (CBX4) and removed by SUMO1/sentrin specific peptidase (SEN1, 2 and 6), favors its interaction with TP53 in the nucleus and enhances TP53-induced gene expression.

Commentary

Tumor protein p53-induced nuclear protein 1 (TP53INP1) is both a TP53 co-factor and a TP53 target gene and consequently its expression is induced in response to several physical and chemical stresses.^{1–3} TP53INP1 directly interacts with TP53 and also binds kinases such as homeodomain interacting protein kinase 2 (HIPK2) and protein kinase C delta (PKC δ), which modulate TP53 transcriptional activity by phosphorylation thereby creating a positive feedback loop between TP53 and TP53INP1.^{4,5} TP53INP1 expression is also modulated by the oncogenic microRNA miR-155, which is over-expressed in many cancers.⁷ Interestingly, TP53INP1 interacts with LC3 and ATG8-family proteins through the LC3-interacting region (LIR) to promote autophagy-dependent cell death. Several lines of evidence point to a tumor suppressive activity for TP53INP1; for example, *TP53INP1* knockout mice have increased susceptibility to cancer.⁶ Moreover, *TP53INP1* expression is lost early during pancreatic cancer progression⁷ and restoring its function in pancreatic cancer cell

lines reduces their malignancy.⁷ The tumor suppressive function of TP53INP1 is strongly associated with its ability to enhance the antioxidant function of TP53 and TP53INP1-deficient cells accumulate more intracellular reactive oxygen species (ROS) than wild-type cells.⁸ As for any kind of stress, the response to oxidative stress involves rapid fine tuning of protein function, a process mainly achieved through post-translational modifications (PTMs) of key proteins. It is now recognized that PTMs mediated by ubiquitin family members are involved in all biological functions of the cell. Small ubiquitin-like modifier 1 (SUMO1) is an ubiquitin-like protein that mediates sumoylation of target proteins. In contrast to ubiquitylation, sumoylation modifies lysine residues included within a consensus sequence in a specific sumoylation site. A search for sumoylation sites in TP53INP1 revealed one potential site at lysine 113. After verifying that TP53INP1 is indeed sumoylated on this lysine residue, we explored the functional role of this PTM and specifically the TP53 response to oxidative stress.

Findings

TP53INP1 has been shown to be involved in 2 main cell survival/death processes: (1) TP53-dependent regulation of apoptosis and cell cycle arrest, and (2) TP53-independent regulation of the autophagic flux. First, we demonstrated that sumoylation of TP53INP1 is not involved in its autophagic functions. Next, using oxygen peroxide and the antioxidant molecule N-acetylcysteine (NAC) we showed that this specific PTM of TP53INP1 is

Keywords:

Abbreviations: PTMs, post-translational modifications; SUMO, small ubiquitin-like modifier; TP53INP1, tumor protein 53-induced nuclear protein 1.

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triggered by oxidative stress; NAC decreased TP53INP1 sumoylation whereas H₂O₂ treatment significantly increased it.

Using the K113R sumoylation-deficient mutant of TP53INP1 we showed the importance of this PTM for its function under oxidative stress conditions and in particular with regard to TP53-mediated regulation of gene expression and antiproliferative effects. Indeed, we showed that TP53INP1 sumoylation is necessary for regulation of cell death and cell cycle progression under basal conditions, and that induction of this PTM is required to trigger apoptosis under conditions of oxidative stress. Specifically, sumoylation of TP53INP1 is necessary for its association with TP53 in the nucleus and is essential to enhance the transcriptional activity of TP53 on target genes involved in apoptosis and cell cycle control.

Like any PTM, sumoylation is supported by a set of enzymes that mediate the addition of SUMO1 to the substrate, the SUMO ligases, and a set of enzymes that removes SUMO1 from the targeted protein, the SUMO hydrolases or desumoylases. Molecular studies of TP53INP1 sumoylation using a siRNA approach to knockdown gene expression revealed that PTM is mainly mediated by 2 E3 ligases, CBX4 and PIAS3, and that desumoylation is performed mainly by the SUMO specific protease, SENP6, possibly in conjunction with the secondary enzymes SENP1 and 2.

Discussion

TP53 is almost certainly one of the proteins that undergoes the widest variety of PTMs: it is phosphorylated, acetylated, methylated, ubiquitinated, neddylated, sumoylated, O-GlcNAcylated, and ADP-ribosylated.⁹ These modifications of TP53 can directly affect its activity, but they also

create a code that is read by reader proteins containing specific PTM recognition domains, which in turn modulate the function of TP53. Our findings show that not only TP53, but also some of its molecular partners, are tightly regulated by PTMs. For example, TP53INP1 has to be sumoylated in order to be an efficient inducer of the TP53-dependent response to oxidative stress although the underlying mechanism remains unclear at present. As sumoylation of TP53INP1 strongly enhances its interaction with TP53 in the nucleus, and because TP53 has no SUMO Interacting Motif (SIM), it is likely that a third intermediate containing at least one such motif forms the bridge between TP53 and sumoylated TP53INP1. This intermediate, presumably one of the 931 known TP53 interacting proteins (geneCards data), would reside in the nucleus to favor interaction with both partners.

It is interesting to consider sumoylation of TP53INP1 as a possible mechanism for oxidative stress-specific regulation of the TP53-TP53INP1 axis. Indeed, SUMO1 activating and conjugating enzymes, as well as desumoylating enzymes, have redox-sensitive catalytic sites. Importantly, the effect of oxidative stress on SUMO conjugation is dose-dependent; thus, low levels of ROS might prevent the SUMOylation of cellular substrates whereas higher concentrations might impair the deconjugation and lead to an accumulation of SUMO conjugates. Hence, depending on the intensity and duration of the oxidative stress, TP53INP1 might differentially modulate the response of TP53 with respect to high-stress response genes.

Moreover, our findings for TP53INP1 imply that other TP53-associated proteins may be subject to the same kind of PTM-dependent regulation of their functions. Indeed, it may be as interesting to study PTMs of TP53-associated proteins as to study modifications of TP53 itself. It is also possible that different types of PTM

regulate the function of TP53 and TP53-associated proteins depending on the type of stress.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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