Multiple Functions of Protein Inhibitor of Activated STAT1 in Regulating Endothelial Cell Proliferation and Inflammation

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Protein modifications with small ubiquitin–like modifier (SUMO) have been found to play a key role in regulating the formation of atherosclerosis.\(^1\)-\(^3\) SUMO proteins covalently modify certain residues of specific target substrates and change the function of these substrates. It has been well established that the protein inhibitor of activated STAT (PIAS) family of proteins has not only SUMO E3 ligase activity, but also transrepression activity.\(^4\) Lerchenmüller et al\(^5\) have now reported a critical role for PIAS1 in regulating S100A6-mediated endothelial cell proliferation. In particular, they show that S100A6 induces PIAS1 expression and, consequently, increases the entry and progression of cell cycle in endothelial cells via inhibiting STAT1-mediated induction of IFITM1 (interferon-inducible transmembrane protein 1; Figure A). It has been reported that the direct inhibition of Thr55 phosphorylation of p53 induced by IFITM1 stabilizes p53 expression and upregulates p53-p21 expression, leading to cell cycle inhibition.\(^7\) Therefore, Lerchenmüller et al\(^5\) assume that this may be one of the regulatory mechanisms by which IFITM1 can inhibit endothelial cell proliferation. These data suggest that in response to vascular endothelial growth factor-A and subsequent S100A6 induction, PIAS1 plays a crucial role in regulating endothelial proliferation. In this editorial, the role of the PIAS family will be briefly reviewed by focusing on the regulation of endothelial functions, including inflammation, proliferation, and Kruppel like factor 2/endothelial nitric oxide synthase expression.

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The PIAS family of proteins contains (1) an SP-RING (Siz/PIAS-really interesting new gene) domain with SUMO E3 ligase activity; (2) an SAP (scaffold attachment factor-A/B, apoptotic chromatin-condensation inducer in the nucleus, and PIAS) domain, which leads to PIAS transcriptional repression activity; (3) a Pro-Ile-Asn-Ile-Thr motif domain, which leads to nuclear retention; and (4) a SUMO-interacting domain (Figure B).\(^6\) As we explained earlier, the PIAS family possesses 2 different molecular functions: SUMO E3 ligase and transcriptional repression activities. SUMOylation can regulate diverse cellular processes, including cell-cycle progression, genetic stability, intracellular trafficking, and transcription by altering localization or activity of the substrate.\(^10\)-\(^12\) Especially, it is well known that SUMO modification of transcription factors and cofactors induces transcription repression.\(^10\),\(^11\) It has been suggested that covalent attachment of SUMO provides a new interaction interface that mediates recruitment of transcriptional corepressors.\(^13\) For example, SUMOylation of transcriptional factor Elk-1 promotes recruitment of HDAC2 (histone deacetylase), which then induces histone deacetylation and transcriptional repression of the c-fos promoter.\(^14\) The possible contribution of REST corepressor 1 (RCOR1) in SUMO-mediated transcriptional repression has also been reported. RCOR1 plays as a corepressor by recruiting the RCOR1/KDM1 (histone lysine–specific demethylase)/HDAC1 and 2 complex to transcriptional factors and coordinately regulates deacetylation and demethylation of histone tails to generate a repressive chromatin structure.\(^5\) Ouyang et al have reported that RCOR1 binds directly and noncovalently to SUMO2 via its nonconsensus SUMO-interaction motif.\(^5\) This association is crucial for recruiting the RCOR1/KDM1/HDAC1/2 complex to various covalently SUMO-modified transcription factors, altering histone acetylation and methylation, and leading to transcriptional repression (Figure C).

These data suggest that PIAS family-mediated STAT1 SUMOylation can inhibit its transcriptional activity by recruiting corepressors. In this study by Lerchenmüller et al\(^5\) S100A6 increased PIAS1 expression, which inhibited both expression and tyrosine phosphorylation of STAT1 (Figure A). In this regard, there are several possible mechanisms that PIAS1 SUMO E3 ligase activity can regulate STAT1: (1) PIAS1 increases SUMO modification of transcription factors, which regulates STAT1 expression, and inhibits it, (2) PIAS1 increases STAT1 SUMOylation and inhibits STAT1 tyrosine phosphorylation, and (3) PIAS1-mediated STAT1 SUMOylation directly inhibits its transcriptional activity. However, the effect of STAT1 SUMOylation on STAT1 transcriptional activity is controversial. Although some data do not support a role of STAT1 SUMOylation in gene regulation,\(^15\),\(^16\) other studies indicate that the STAT1 SUMOylation affects STAT1 transcriptional activity, probably in a gene-dependent manner.\(^4\),\(^17\),\(^18\)

Of note, SUMO E3 ligase activity of PIASs can be regulated by their interaction with other molecules. It has been reported that the activated PKC\(\zeta\) kinase can associate with the catalytic site, the RING domain, of PIAS4, which forms the PIAS4/substrate complex by recruiting the cognate E2-conjugating enzyme to facilitate SUMO conjugation.\(^19\) Therefore, the association of PKC\(\zeta\) with PIAS4 may alter the structure and enzymatic activity of PIAS4. The C-terminus

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The kinase domain of PKCζ (amino acids 401–587) was shown to be a PIAS4-binding site, and deletion of the N-terminus autoinhibitory domain (amino acids 1–200) was shown to increase the PKCζ–PIAS4 association. Inhibiting the PKCζ–PIAS4 association by inducing the binding inhibitory fragment reduced PIAS4 SUMO E3 ligase activity, supporting the crucial role of the PKCζ–PIAS4 association in upregulating the PIAS4 SUMO E3 ligase activity.19 Because PKCζ-mediated PIAS4 phosphorylation could not be detected, in addition to PKCζ protein kinase activation, the subsequent release of the PKCζ N-terminus autoinhibitory domain is necessary for the PKCζ–PIAS4 association, which upregulates p53 SUMOylation and subsequent endothelial p53 nuclear export and apoptosis.19

It has also been reported that PIAS1 negatively regulates NF-κB (nuclear factor kappa-light chain-enhancer of activated B cells) and STAT1 transcriptional activation by directly interacting with NF-κB p65 and STAT1, respectively.4 HDACs associate and regulate PIAS family–induced transrepression activity against androgen receptor transcriptional activity, which is independent of its SUMO E3 ligase activity, suggesting the crucial role of HADC activity in transrepression activity induced by PIASs20,21 (Figure A). The crucial role of PIAS1 phosphorylation in regulating transrepression activity has also been reported4,22 (Figure A). Tumor necrosis factor–mediated activation of IkB kinase (IKK)α induces NF-κB transactivation through the canonical tumor necrosis factor–IKKα–NF-κB signaling pathway. Interestingly, IKKα can also negatively regulate this event by phosphorylating PIAS1 at the S90 residue as a negative feedback mechanism.22 In addition to IKKα, tumor necrosis factor–mediated activation of MAPK-activated protein kinase-2 can also phosphorylate PIAS1 at the S522 residue to enhance its transrepression activity on NF-κB. MAPK-activated protein kinase-2–mediated S522 phosphorylation of STAT1 expression (Post-translational modifications?) VEGF-A expression S100A6 expression PIAS1 expression STAT1 Y701 phosphorylation STAT1 SUMOylation STAT1 function IFITM1 expression p53-p21 expression Endothelial cell proliferation TNF NF-κB transcriptional activity (Negative feedback loop of TNF-induced endothelial cell inflammation) KLF2 eNOS expression PIAS1 N S90 P PINIT S522 P RLD AD ST/T CF AVEG-A expression PIAS1 expression SUMO E3 ligase SUMO E3 ligase aN-κB interacting domain N-κB interacting domain SAP domain scaffold attachment factor A and B; SIM, SUMO-interaction motif; S/T, serine/threonine rich region; TFs, transcriptional factors; TNF, tumor necrosis factor; and VEGF-A, vascular endothelial growth factor-A.

Figure. A, Multiple functions of PIAS1. B, The domain structure of PIAS1. PIAS1 S90 site lie in the NF-κB (nuclear factor kappa-light chain-enhancer of activated B cells) interacting region, whereas PIAS1 S522 site does not lie in any of PIAS1's functional domains involved in NF-κB binding or its SUMO E3 ligase activity (RLD) domain.6 Reprinted from Le et al7 with permission of the publisher. Copyright © 2013, Wolters Kluwer Health, Inc. C, Model of SUMO-dependent transcriptional repression by the RCOR1/KDM1/HDAC (histone deacetylase) corepressor complex.6 AD indicates highly acidic domain; eNOS, endothelial nitric oxide synthase; HDAC, histone deacetylase; IFITM1, interferon-induced transmembrane protein 1; IKK, IkB kinase; KDM1, lysine-specific demethylase 1; KLF2, Kruppel like factor 2; MK2, MAPK-activated protein kinase 2; PIAS1, protein inhibitor of activated STAT 1; PINIT, Pro-Ile-Asn-Ile-Thr motif; RCOR1, REST corepressor 1; RLD, RING-finger-like zinc-binding domain, protein–protein interactions, interacts with the SUMO conjugase Ubc9, sumoylation; SAP domain, scaffold attachment factor A and B; SIM, SUMO-interaction motif; S/T, serine/threonine rich region; TFs, transcriptional factors; TNF, tumor necrosis factor; and VEGF-A, vascular endothelial growth factor-A.
PIAS1 can also increase p53 SUMOylation and p53 nuclear export and, subsequently, inhibit Kruppel like factor 2–mediated endothelial nitric oxide synthase expression. In contrast to the case of the androgen receptor, the ability of IKKα to phosphorylate S90 PIAS1 and inhibit NF-κB activity in vivo is dependent on the SUMO ligase activity of PIAS1, but the precise role of PIAS1 SUMO E3 ligase activity in regulating Ser 90 PIAS1 phosphorylation remains unclear.

Lerchenmüller et al. have nicely shown the crucial role of PIAS1 in S100A6-mediated endothelial cell proliferation by inhibiting STAT1 expression and tyrosine phosphorylation. In addition, the possible contribution of STAT1-IFITM1–mediated p53 stabilization in inhibiting endothelial cell proliferation has been suggested. In this study, there was no change in NF-κB expression and activity after the depletion of S100A6 and subsequent inhibition of PIAS1. The specific role of PIAS1 in regulating STAT1 under vascular endothelial growth factor-A stimulation and subsequent S100A6 induction may be because of some unique post-translational modifications of PIAS1, which is probably different from Ser 90 or 522 phosphorylation after tumor necrosis factor stimulation. It is clear that we do not know the exact regulatory mechanisms and functional roles of the PIAS family of proteins under various different stimuli in endothelial cells. This study has opened a door to future studies of multiple functions of the PIAS family and how PIAS1 can regulate unique sets of gene expression mediated by different stimuli and upstream regulators.

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Disclosures

None.

References


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