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PIAS1 binds p300 and behaves as a coactivator or corepressor of the transcription factor c-Myb dependent on SUMO-status

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ABSTRACT

The PIAS proteins (Protein Inhibitor of Activated STATs) constitute a family of multifunctional nuclear proteins operating as SUMO E3 ligases and being involved in a multitude of interactions. They participate in a range of biological processes, also beyond their well-established role in the immune system and cytokine signalling. They act both as transcriptional corepressors and coactivators depending on the context. In the present work, we investigated mechanisms by which PIAS1 causes activation or repression of c-Myb dependent target genes. Analysis of global expression data shows that c-Myb and PIAS1 knockdowns affect a subset of common targets, but with a dual outcome consistent with a role of PIAS1 as either a corepressor or coactivator. Our mechanistic studies show that PIAS1 engages in a novel interaction with the acetyltransferase and coactivator p300. Interaction and ChIP analysis suggest a bridging function where PIAS1 enhances p300 recruitment to c-Myb-bound sites through interaction with both proteins. In addition, the E3 activity of PIAS1 enhances further its coactivation. Remarkably, the SUMO status of c-Myb had a decisive role, indicating a SUMO-dependent switch in the way PIAS1 affects c-Myb, either as a coactivator or corepressor. Removal of the two major SUMO-conjugation sites in c-Myb (2KR mutant), which enhances its activity significantly, turned PIAS1 into a corepressor. Also, p300 was less efficiently recruited to chromatin by c-Myb-2KR. We propose that PIAS1 acts as a "protein inhibitor of activated c-Myb" in the absence of SUMOylation while, in its presence, PIAS behaves as a "protein activator of repressed c-Myb".

1. Introduction

The PIAS (Protein Inhibitor of Activated STATs) proteins were originally identified by their role as negative regulators of cytokine signalling, inhibiting STAT transcription factors. The four members, PIAS1, PIAS2/PIASx, PIAS3, and PIAS4/PIASy, share several homologous domains, but have a variable C-terminal region (reviewed in [1–3]). The PIAS proteins act as SUMO E3 ligases, a function linked to their cysteine/histidine-rich SP-RING domain [1–4], which is functionally similar to the RING finger found in a subclass of ubiquitin E3 ligases [5]. PIAS proteins have a role in stabilizing the interaction between the SUMO-conjugating enzyme Ubc9 and the substrate protein [1]. PIAS proteins are also themselves SUMOylated in a RING finger-dependent manner [4] and they contain a SUMO interaction motif (SIM), making them capable of binding to other SUMOylated proteins [6].

The functions of PIAS proteins have been shown to be more diverse than being SUMO E3 ligases and negative regulators of STAT signalling. Even though SUMOylation of transcriptional regulators often leads to inhibition of their activity, PIAS proteins are known to act as both negative and positive regulators of transcription, in both SP-RING domain-dependent and -independent manners. PIAS1 is a good example of this. Its role as a negative regulator of transcription has been well documented [7–12]. Mechanistically, PIAS1 may lead to transcriptional repression due to a SUMO-dependent recruitment of corepressors, as exemplified by PIAS1-mediated SUMOylation of PPARγ [10]. PIAS1 may also repress transcription by changing the subcellular localization of the modified target protein, as observed for PIAS1-induced SUMOylation of p73 [9]. Furthermore, PIAS1 affects the nuclear mobility of FOXA1, and mutation of the FOXA1 SUMOylation sites slows down the mobility of the pioneer factor, further retarding the nuclear mobility of the androgen receptor (AR) [13]. For other target

Abbreviations: 2KR, double mutant of c-Myb (K503R + K527R); 6KR, multiple mutant of c-Myb with the following alterations (K428/442/445/471/480/485R); AR, androgen receptor; *BRD4*, member of the BET (bromodomain and extra terminal domain) family of genes; c-Myb, v-myb avian myeloblastosis viral oncogene homolog; *MYC*, gene encoding the c-Myc transcription factor; ChIP, chromatin immunoprecipitation; CoIP, co-immunoprecipitation; GST, glutathione S-transferase; HAT, histone acetyltransferase; hcM, human c-Myb; p300, E1A binding protein p300 or histone acetyltransferase p300; PAGE, polyacrylamide gel electrophoresis; PIAS, protein inhibitor of activated STAT; SIM, SUMO-interaction motifs; *SNAI1*, gene encoding the zinc finger protein and transcription factor SNAI1; SUMO, small ubiquitin-related modifier; *TCF4*, gene encoding the transcription factor 4; TF, transcription factor.

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proteins, PIAS1-mediated repression, caused by targeting to PML nuclear bodies, is independent of SUMOylation [7]. In the repression of STAT-1 and NFκB, PIAS1 was observed to block their DNA binding rather than to affect their SUMOylation status [8,14]. The E3 ligase activity of PIAS1 is, however, essential, indicating that SUMOylation of other proteins might be important for this repressive effect [15].

The functional diversity of PIAS1 is accentuated by it operating not only as a repressor but also as an activator of transcription. PIAS1 enhances the AR-mediated gene activation in prostate cancer cells, in contrast to PIAS4, which acts as a potent inhibitor of AR in prostate cancer cells (Gross 2001, 2004). Again, several mechanisms appear to be involved. SUMOylation of several nuclear receptors by PIAS1 has been demonstrated to enhance their transcriptional activity without altering their subnuclear localization [16–19]. In the case of AR and GR, the activation effect was dependent on both SP-RING and SIM domains in PIAS1 and the SUMOylation sites in the receptors [4,17,20]. The mechanism is not fully understood, but may involve reversing of a SUMO-mediated repressed state, as seen for Elk-1 activated by PIASx [21]. A dual function of PIAS1 was also observed in a recent study where PIAS1 depletion significantly influenced a subset of the AR-regulated genes by either activation or repression [22]. In general, the significance of the E3 ligase activity varies depending on the target substrate, but often the interaction seems to be of key importance.

The functional diversity of PIAS1 is also reflected in the range of biological phenomena in which PIAS1 has been implicated. In addition to its well-established role in the immune system and cytokine signalling [23,24], recent studies have revealed its importance in both DNA repair [25,26] and as a cell cycle regulator [9,11,27]. Moreover, aberrant expression of PIAS1 has been found associated with breast cancer [28,29] and prostate cancer [30]. In the latter case, PIAS1 was found to regulate a subset of androgen responsive genes involved in proliferation and tumour growth. PIAS1 has even been connected to functions as diverse as spatial learning [31], modulating insoluble mutant huntingtin protein accumulation [32], and epigenetic control of DNA methylation [33]. In the latter case, PIAS1 was found to operate as a key regulator of DNA methylation of hematopoietic stem cells required for their maintenance and lineage commitment [33]. This diversity of PIAS1 functions seems to be connected to its dual role both as a SUMO E3 ligase and an interaction partner of many proteins, in particular, transcription factors [1,3]. In this way, it may affect a broad range of processes and functions.

We have previously reported c-Myb to be one of the transcription factors interacting with PIAS1 [34]. The c-Myb factor plays a central role in the regulation of cell growth and differentiation of haematopoietic cells [35–38]. It operates as a regulator of stem and progenitor cells in bone marrow, as well as in colonic crypts and a neurogenic region of the adult brain [36]. Expression of c-Myb is elevated in several cancer types [36] and the factor has been linked to the phenomena known as oncogene addiction [39]. In line with the latter, c-Myb was found to have an important function in metastatic prostate cancer where it promotes tumour growth and regulates genes coding for proteins involved in the DNA repair pathway, which is associated with cancer recurrence [40,41]. Furthermore, c-Myb was found to cause enhanced motility and invasion and to be linked to the epithelial–mesenchymal transition [41].

The activity of c-Myb is controlled both by protein-protein interactions and by post-translational modifications. c-Myb is phosphorylated at several sites by different kinases including HIPK1 and HIPK2 [42–47], SUMOylated at two lysine residues in the C-terminal regulatory domain (CRD) [48–50] and acetylated by the coactivator CBP/p300 [51,52]. The transactivation potential of c-Myb is modulated not only by SUMO conjugation but also by non-covalent SUMO-binding [53].

In the present work, we investigated mechanisms of PIAS-mediated activation of c-Myb. We found that a novel interaction between PIAS1 and the coactivator p300 plays a role, suggesting a bridging function where PIAS1 enhances p300 recruitment to c-Myb-bound sites through interaction with both proteins. Chromatin immunoprecipitation (ChIP) experiments supported this model by showing enhanced recruitment of p300 to chromatin in the presence of PIAS1. Also, the E3 activity of PIAS1 plays a modulating role, enhancing further the coactivation effect. Remarkably, the SUMO status of c-Myb had a dominating role and removal of the two major SUMO-conjugation sites in c-Myb changed the effect of PIAS1 to that of a corepressor. In parallel, p300 was less efficiently recruited to chromatin by c-Myb. Our data suggest a SUMO-dependent switch in the way PIAS1 affects transcription factors, as shown here for c-Myb.

2. Materials and methods

2.1. Cell culture, transfection and luciferase assays

Three cell lines were used: CV-1 (ATCC ® CCL-70™ *Cercopithecus aethiops* kidney Normal), COS-1 (ATCC® CRL-1650™ *Cercopithecus aethiops* kidney), HEK-293 (ATCC® CRL-1573™ *Homo sapiens* embryonic kidney), and K562 (ATCC® CCL-243™ *Homo sapiens* bone marrow, chronic myelogenous leukemia). The nature of the latter was confirmed by cell line authentication (www.identicell.eu). The cells were grown and transiently transfected with the indicated plasmids as previously described [38,50]. Reporter assays in transiently transfected CV-1 and HEK-293 cells, stably transfected with a 5×Gal4-luciferase reporter, were performed in triplicate (24-well trays, 2×10^4 CV-1 cells/well or 3.2×10^4 HEK 293-c1 cells/well) using Luciferase Assay Reagent (Promega), each triplicate repeated in three independent experiments. K562 cells stably expressing tagged c-Myb variants (empty vector, 3xTY1-c-Myb, 3xFLAG-c-Myb, 3xFLAG-c-Myb-2KR) were selected by G418 (400 μg/ml) for 2 weeks before selecting single clones for one week. Stable expression was confirmed by Western blotting.

2.2. Plasmid constructs

The construct for expression of FLAG-tagged human PIAS1 was made by cloning of PIAS1 cDNA into the pCIneo-3xFlag vector. Mutations in the RING finger domain of human PIAS1 (amino acid C346S, C351S, H353A, C356S) were introduced using Quick-Change site-directed mutagenesis [34]. The pCMVβ-NHA-p300 was a kind gift from Prof. D. Livingston [54]. pCMVβ-p300-myc and pCMVβ-p300-myc HATmut (pCMVβ-p300.DY-myc), harboring an acetyltransferase-deficient D1399Y mutation, were obtained from Addgene. Constructs for expression of tagged c-Myb have been described [49,50,53].

The plasmid pCIneoB-GBD2-hcM-HA [1-640] N183A + N186A was made to encode a fusion of full length, HA-tagged c-Myb with the yeast Gal4 DNA-binding domain, harbouring two mutations in the DNA-binding domain of c-Myb, N183A + N186A, inactivating its DNA-binding properties [55]. A 2KR mutant version (harbouring K503R + K527R) of the same construct was also made. Other Gal-fusion constructs, pCIneoB-GDB2-hcM-HA [233-640] wild type and 2KR mutant, are described in [50], and similar constructs encoding amino acids 194 – 640 of c-Myb (pCIneoB-GBD2-hcM (194-640) wild type and 2KR mutant) were made by similar strategies. We also combined Gal4 elements of these plasmids with VP16 from pCIneo-R2R3-VP16 [56] to generate pCIneo-GBD2-VP16 encoding the Gal4 DNA-binding domain in fusion with the herpes simplex virus VP16 transactivation domain.

The acetylation-negative mutant of c-Myb (abbreviated 6KR) was obtained from Rein Aasland, University of Bergen. In this mutant, the following six lysine residues were mutated to arginine: K428, K442, K445, K471, K480 and K485 (numbering in human c-Myb). Five of these correspond to those previously reported [51,52] while K428 was identified as the sixth lysine which must be mutated to arginine to abolish all acetylation by p300 in vitro (Prakash Yalamanchili and Rein Aasland, pers. comm.).