FEBS Letters 589 (2015) 1890–1896

journal homepage: www.FEBSLetters.org

Nuclear termination of STAT3 signaling through SIPAR (STAT3-Interacting Protein As a Repressor)-dependent recruitment of T cell tyrosine phosphatase TC-PTP

Fangli Ren [a], Yongtao Geng [b], Takayuki Minami [a], Ying Qiu [a], Yarui Feng [a], Chunxiao Liu [a], Juan Zhao [a], Yinyin Wang [a], Xuanzi Fan [a], Yangmeng Wang [a], Mengdi Li [a], Jun Li [c,*], Zhijie Chang [a,*]

[a] State Key Laboratory of Biomembrane and Membrane Biotechnology, School of Medicine, School of Life Sciences, Tsinghua University, Beijing 100084, China
[b] Structure Biology, Memorial Sloan Kettering Cancer Centre, New York 10065, USA
[c] Institute of Immunology, The Third Military Medical University, Chongqing 400038, China

ARTICLE INFO

Article history:
Received 9 February 2015
Revised 8 May 2015
Accepted 15 May 2015
Available online 27 May 2015

Edited by Lukas Huber

Keywords:
STAT3-Interacting Protein As a Repressor
STAT3
Dephosphorylation
Protein tyrosine phosphatase
T cell protein tyrosine phosphatase TC45

ABSTRACT

STAT3 is associated with embryo development and survival as well as proliferation and metastasis of tumor cells. In a previous study, we demonstrated that STAT3-Interacting Protein As a Repressor (SIPAR) enhances the dephosphorylation of STAT3 and negatively regulates its activity. However, it remains unclear how SIPAR inhibits phosphorylation of STAT3. Here we demonstrate that SIPAR directly interacts with T cell protein tyrosine phosphatase TC45 and enhances its association with STAT3. This interaction triggers an accelerated dephosphorylation process for STAT3. Furthermore, SIPAR inhibits the transcriptional activity of STAT3 in wild-type MEF cells but not in TC45 null MEF cells. These results suggest that SIPAR terminates the activation of STAT3 through a dephosphorylation process that is dependent upon interaction with TC45 in the nucleus.

1. Introduction

STAT3 is a transcription factor that participates in embryo development and many cellular processes [1]. Like other members of the STAT family, STAT3 is tyrosine-phosphorylated by Jaks and dimerized to translocate into the nucleus to activate target genes upon stimulations of cytokines and growth factors [2]. Studies have shown that the constitutive activation of STAT3 is presented in several cancer cell lines and associated with a number of human cancers including prostate, breast, lung, brain, and pancreatic cancers [2–7]. In recent publications, numerous groups have demonstrated that inhibition of STAT3 can suppress growth of cancer cells either by promoting apoptosis or by inhibiting cell proliferation [8].

The activity of STAT3 is regulated by both positive and negative factors in the cytoplasm and nucleus. Among the negative regulators, protein tyrosine phosphatases (PTPs) are apparently critical in down-regulating the STAT3 activity [9,10]. In particular, it has been documented that T cell protein tyrosine phosphatase TC-PTP negatively regulates the STAT3 activity either in the cytoplasm, through dephosphorylating protein tyrosine kinase JAK [11], or in the nucleus, through directly dephosphorylating STAT3 [10]. Other protein tyrosine phosphatases, including PTPTN9/Meg2 [12] and PTPN11/SHP2 [9], also regulate the STAT3 phosphorylation. However, it remains unclear how these phosphatases specifically regulate the STAT3 activity. Recently we demonstrated that an adaptor protein, GdX/Ubl4A, bridges the interaction of T cell protein tyrosine phosphatase TC45 with STAT3 and defines the specificity of TC45 to recognize phosphorylated STAT3 [13]. Since STAT3 functions under diverse physiological and pathological conditions, we speculated that more adaptor proteins are required for different phosphatases to interact with STAT3.

Author contributions: Fangli Ren: IP and dephosphorylation assays, draft manuscript writing; Yongtao Geng: IF experiments and discussion; Takayuki Minami: pull-down assay; Ying Qiu: western blot and experiment design; Yarui Feng: HEK293T cell culture and prepare plasmids; Chunxiao Liu: mapping IP experiments and statistical analysis; Juan Zhao: purify GST fusion proteins; Yinyin Wang: STAT3 mutant IP assay; Xuanzi Fan: B16 cell culture and statistical analysis; Yangmeng Wang: experiment design and discussion; Mengdi Li: luciferase experiments; Jun Li: experiment design, discussion and manuscript writing; Zhijie Chang: experiment design and direction, manuscript writing.

* Corresponding authors. Fax: +86 (10)62773624 (Z. Chang).
E-mail addresses: jun-li04@mails.tsinghua.edu.cn (J. Li), zhijiec@tsinghua.edu.cn (Z. Chang).

http://dx.doi.org/10.1016/j.febslet.2015.05.031

We previously reported that SIPAR (STAT3-Interacting Protein As a Repressor), also named Acpin1 [14], inhibits the phosphorylation of STAT3 [15,16]. Our previous observations showed that SIPAR interacted with STAT3 and suppressed the progression of melanoma by repressing the expression of STAT3 targeted genes [16]. However, no specific phosphatase catalytic domain was predicted for SIPAR. To reveal the mechanism by which SIPAR regulates the STAT3 activity, in the current study, we performed a co-immunoprecipitation experiment and identified that SIPAR interacted with TC45. Our data demonstrated that SIPAR inhibited the activity of STAT3 via TC45.

2. Materials and methods

2.1. Plasmids and antibodies

STAT3 related constructs were kept in this lab. pGL3/$(APRE)_4$-Luc reporter was provided by Dr. Sachiko Ezoe from Osaka University. TC45 and SIPAR plasmids were constructed in this lab [13,16]. Human interleukin-6, IL-6 soluble receptor and the TC-PTP antibody were purchased from R&D Biotechnology. Anti-STAT3 antibodies (C-20) and anti-Actin antibody (C-2) were purchased from Santa Cruz Biotechnology. Anti-p-STAT3 antibodies (9131L) were purchased from Cell Signaling Technology.

2.2. Cell culture and transfection

HEK293T, $MEF^{TC45+/+}$ and $MEF^{TC45-/-}$ cells were grown in DMEM containing 10% fetal bovine serum. B16 cells were grown in RPMI-1640 containing 10% fetal bovine serum. Lipofectamine 2000 (Invitrogen) was used for transient transfection of HEK293T, $MEF^{TC45+/+}$, $MEF^{TC45-/-}$ and B16 cells.

2.3. Luciferase assay

Luciferase assays were performed with the indicated plasmids using pGL3/$(APRE)_4$-luc reporter construct, a luciferase reporter driven by 4 repeats of the acute phase response elements (APRE). pRL-TK was used as an internal control. Data were normalized with the internal control. STAT3 luciferase activity was measured using a luciferase assay system (Promega) and results were presented as a relative mean with S.D. from triplicate experiments.

2.4. Immunoprecipitation and dephosphorylation assay

Immunoprecipitation and Western blot experiments were performed according to a previous protocol [17]. The dephosphorylation assay was performed under starvation conditions according to our previous report [13].

3. Results

3.1. SIPAR directly interacts with TC45

Our previous study showed that SIPAR interacted with STAT3 [16]. In an analysis of SIPAR interacting proteins, we speculated that SIPAR might associate with TC45, a phosphatase that regulates the activity of STAT3 in the nucleus [10]. Therefore, we examined the interaction of SIPAR and TC45 in mammalian cells. For this purpose, Myc-tagged SIPAR (Myc-SIPAR) and HA-tagged TC45 (HA-TC45) were co-expressed in HEK293T cells for an immunoprecipitation experiment with an anti-HA antibody. Western blot analyses demonstrated that HA-TC45 precipitated down Myc-SIPAR protein (Fig. 1A), suggesting that SIPAR interacts with TC45 in intact cells. Furthermore, we validated the interaction in a GST pull down experiment using GST-tagged TC45 protein (GST-TC45) purified from *Escherichia coli* and Myc-SIPAR protein expressed in HEK293T cells (Fig. 1B). To reveal a direct interaction of the proteins, we used purified His-SIPAR and GST-TC45 and performed an in vitro GST-pull down experiment. The results showed that GST-TC45 strongly pull down His-SIPAR (Fig. 1C), suggesting that TC45 and SIPAR interact directly in vitro.

To examine whether the endogenous SIPAR protein interacts with TC45, we used cell lysates from mouse embryos, where SIPAR is abundantly expressed. An immunoprecipitation experiment demonstrated that antibodies against mouse SIPAR precipitated down the endogenous TC45 protein but IgG failed (Fig. 1D), suggesting that SIPAR interacts with TC45 under physiological conditions.

To characterize the domains responsible for the interaction between SIPAR and TC45, we employed TC45 deletions of either PTP (protein tyrosine phosphatase) domain or CT (c-terminal) domain. An immunoprecipitation experiment indicated that only the region encoding the PTP domain is associated with SIPAR (Fig. 1E). These analyses indicated that SIPAR interacts with TC45 *via* the PTP domain of TC45.

To further examine whether TC45 and SIPAR co-localize in mammalian cells, we performed an immunostaining assay in MCF7 cells. The results demonstrated that GFP-SIPAR and TC45 co-localized in the nucleus (Fig. 1F). The co-distribution of SIPAR and TC45 in the nucleus provided a clue that the two proteins, possibly through their interaction, function in the nucleus.

3.2. SIPAR enhances the interaction of STAT3 and TC45

Since SIPAR interacts with both STAT3 and TC45, we questioned whether SIPAR affects the association of STAT3 with TC45 in mammalian cells. To examine the hypothesis, we co-expressed Flag-STAT3, Myc-SIPAR and HA-TC45 in HEK293T cells. An IP analysis revealed that HA-TC45 was able to form a complex with Flag-STAT3 under IL-6 treatment (Fig. 2A, middle lane). Interestingly, the interaction of HA-TC45 and Flag-STAT3 was greatly enhanced when Myc-SIPAR was co-expressed in cells treated with IL-6 (Fig. 2A, last lane). Simultaneously, we observed a strong interaction of HA-TC45 and Myc-SIPAR (Fig. 2A, second panel). A quantitative analysis showed that the interaction of Flag-STAT3 and HA-TC45 in the presence of Myc-SIPAR was enhanced more than 8-fold in comparison with that in the control (Fig. 2B). To examine whether the STAT3–SIPAR–TC45 complex forms in vitro, we used purified GST-TC45, Flag-STAT3 and Myc-SIPAR expressed in mammalian cells treated with IL-6 for a GST pull-down assay. The result indicated that the interaction between GST-TC45 and Flag-STAT3 was also enhanced in the presence of Myc-SIPAR (Fig. 2C and D).

To examine whether cytokines enhance the interaction of endogenous STAT3, SIPAR and TC45 proteins in mammalian cells, we performed an immunoprecipitation experiment using an antibody against TC45. The result showed that the antibody against TC45 precipitated down endogenous STAT3 and SIPAR in the presence of IL-6 (Fig. 2E). Furthermore, it appears that the interaction of SIPAR with TC45 was equally presented in the absence or presence of IL-6 (Fig. 2E, the second lane; Fig. 2F). To reveal whether SIPAR is required for the interaction of TC45 with STAT3 in the presence of IL-6, we depleted endogenous SIPAR using an siRNA against SIPAR in B16 cells. The results showed that depletion of SIPAR abrogated the interaction of TC45 with STAT3 (Fig. 2G). Taken together, these results suggest that SIPAR, STAT3 and TC45 form a complex and SIPAR enhances the interaction of STAT3 with TC45.