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

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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.

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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.

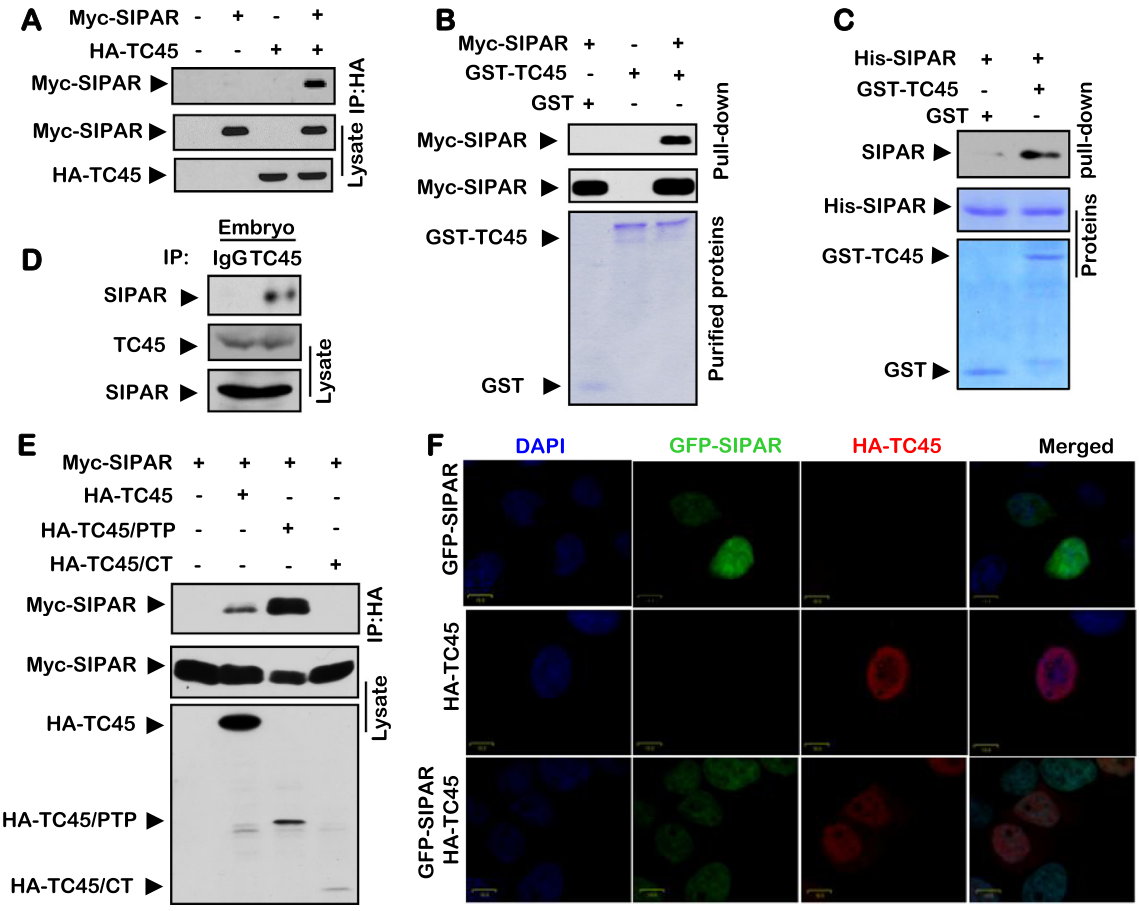


**Fig. 1.** SIPAR interacted with TC45. (A) SIPAR interacts with TC45 in mammalian cells. Myc-SIPAR was co-expressed with HA-tagged TC45 in 293T cells. IP was performed as indicated. (B) GST pull-down assay. GST and GST-fusion TC45 proteins were purified with GST beads. Myc-SIPAR was expressed in 293T cells. (C) SIPAR interacts with TC45 in vitro. A GST pull down experiment was performed using purified GST-45 and His-SIPAR. (D) Endogenous SIPAR interacts with TC45 in mouse embryo. The lysates from a mouse embryo (days post coitum 11.5) were used for an immunoprecipitation. (E) The PTP domain of TC45 responded to the interaction with SIPAR. An immunoprecipitation assay was performed with an HA antibody. (F) SIPAR colocalized with TC45 in nucleus.

### 3.3. SIPAR interacts with p-STAT3 and TC45 in the nucleus

As SIPAR interacts with STAT3 dependent on IL-6, we questioned whether SIPAR associates with p-STAT3, a tyronse phosphorylation form. For this purpose, we purified GST-STAT3 protein expressed from either mammalian cells under IL-6 stimulation or *E. coli* where no phosphorylation occurs. An IP result demonstrated that GST-STAT3 purified from mammalian cells strongly precipitated down Myc-SIPAR but GST-STAT3 purified from *E. coli* precipitated much less Myc-SIPAR in vitro (Fig. 3A). This result suggests that SIPAR preferably recognizes the phosphorylated STAT3.

To further confirm the phosphorylation of STAT3 is critical for the interaction with SIPAR, we used a mutant of STAT3, STAT3(CYF), which could not be phosphorylated at residue Y705, to perform an IP experiment. The result showed that Myc-SIPAR precipitated down wild type Flag-STAT3 but no Flag-STAT3(CYF) (Fig. 3B). Interestingly, we observed that a constitutively activated mutant, STAT3(CA), which was generated by a mutation of residues A661 and N663 into cysteine, remained of no interaction with SIPAR (Fig. 3B, the third lane). This is explainable as we did not stimulate the cells with IL-6 whereas the STAT3(CA) is a dimerized form to spontaneously translocate into the nucleus [13]. This result implies that the interaction of SIPAR with STAT3 dependent on the phosphorylation status but not on the nuclear localization. A reciprocal IP experiment using an anti-Flag antibody confirmed the result (Fig. 3C).

To clarify whether TC45 interacts with phosphorylated STAT3 with the assistance of SIPAR in the nucleus, we separated proteins from the cytoplasm and nucleus of cells treated with or without IL-6. An IP experiment using the fragments of the cells indicated that HA-TC45 interacts strongly with Flag-STAT3 and over-expression of SIPAR enhanced this interaction in the nucleus (Fig. 3D), implying that both TC45 and SIPAR prefer to interact with phosphorylated STAT3. As STAT3 contains SH2 domain which is important for interaction with TC45, we analyzed which domain of STAT3 is critical for the interaction with the SIPAR/TC45 complex. For this purpose, we expressed a variety of domains of STAT3 (Fig. 3E) and performed an IP experiment in the presence of HA-TC45 and Myc-SIPAR. The results showed that both Myc-SIPAR and HA-TC45 proteins are presented in the complex of Flag-STAT3(DB), Flag-STAT3(LD) or Flag-STAT3(SH2), indicating that SIPAR and TC45 interacts with DB, LD and SH2 domains of STAT3 (Fig. 3F). This result suggests that SIPAR and TC45 associate with STAT3 at the region of the DB, LD and SH2 domains.

### 3.4. SIPAR inhibits the activity of STAT3 through TC45

To reveal the biological function of SIPAR, we examined the activity of STAT3 at the transcriptional level and its phosphorylation status. A luciferase reporter linked to a STAT3 binding element (APRE) was transfected into $MEF^{TC45+/+}$ and $MEF^{TC45-/-}$ cells. The results showed that over-expression of SIPAR with an adenovirus

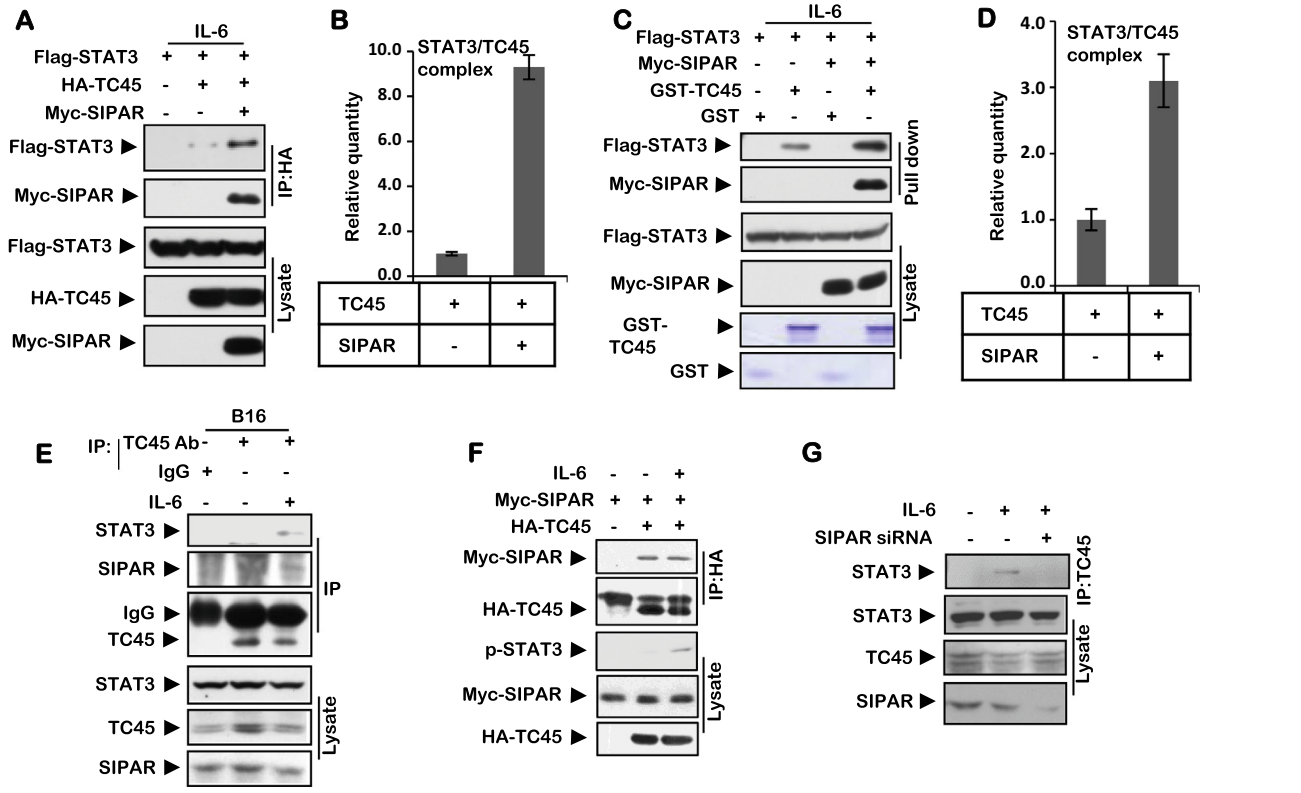


**Fig. 2.** SIPAR enhances the interaction of STAT3 and TC45. (A) SIPAR mediated the interaction of STAT3 and TC45. IP assay was performed with HEK293T cells. Flag-STAT3, HA-TC45 and Myc-SIPAR were over-expressed as indicated. (B) A quantitative analysis of the Flag-STAT3 and HA-TC45 complex. (C) SIPAR accelerated the interaction of STAT3 and TC45. GST pull down assay was performed with purified GST-TC45 and over-expressed STAT3 and SIPAR in mammal cells as indicated. (D) A quantitative analysis of the Flag-STAT3 and GST-TC45 complex is shown. (E) IL-6 induces the interaction of STAT3, TC45 and SIPAR in B16 cells. B16 cells were treated with/without IL-6 stimulation. (F) The interaction of SIPAR and TC45 is not regulated by IL-6. (G) Depletion of SIPAR impairs the interaction of TC45 with STAT3 in response to IL-6 stimulation.

(Ad/SIPAR) inhibited the transcriptional activity of STAT3 in $MEF^{TC45+/+}$ cells treated with IL-6 (Fig. 4A). However, over-expression of SIPAR had no significant effect on the inhibition of IL-6 stimulation in $MEF^{TC45-/-}$ cells (Fig. 4B). These results show that SIPAR inhibited the transcription activity of STAT3 mainly through TC45.

We next examined the level of phosphorylated STAT3 (p-STAT3) under over-expression of SIPAR. A Western blot analysis from HEK293T cells demonstrated that, while over-expression of TC45 alone led to decreased p-STAT3, co-expression of SIPAR with TC45 further decreased the level of p-STAT3 after IL-6 stimulation (Fig. 4C, last lane), suggesting SIPAR and TC45 reduced the phosphorylation of STAT3. A quantitative analysis of this result further demonstrated the effect of SIPAR and TC45 on the reduced phosphorylation of STAT3 (Fig. 4D). These results, together with that from our previous study [16], implied that SIPAR may promote the dephosphorylation of STAT3.

To examine whether SIPAR regulates the dephosphorylation of STAT3 through TC45, we performed a Western blot analysis in $MEF^{TC45+/+}$ and $MEF^{TC45-/-}$ cells, which were stimulated with IL-6 for 30 min and then subjected to cytokine withdrawal after different time periods. The results indicated that p-STAT3 remained at a high level for up to 120 min after cytokine withdrawal in the presence of Ad/GFP, but was dramatically decreased in the presence of Ad/SIPAR in 30 min after withdrawal of the cytokine in $MEF^{TC45+/+}$ cells (Fig. 4E). However, p-STAT3 remained at high levels in $MEF^{TC45-/-}$ cells at different times upon cytokine withdrawal in the presence of Ad/GFP or Ad/SIPAR (Fig. 4F), suggesting that the effect of SIPAR on the dephosphorylation of STAT3 was impaired when TC45 was depleted. Taken together, these results suggested that SIPAR promotes the dephosphorylation of STAT3 through TC45.

On the other hand, we further questioned whether SIPAR is reciprocally required for the dephosphorylation of p-STAT3 by TC45. A luciferase experiment indicated that depletion of SIPAR impaired, but not completely lost, the effect of over-expressed TC45 on the inhibition of STAT3 transcriptional activity (Fig. 4G, comparing the forth group of columns with the second group of columns). A Western blot analysis on the phosphorylation level of STAT3 indicated that depletion of SIPAR further facilitated the phosphorylation of STAT3 based on the depletion of TC45 (Fig. 4H). The results suggest that TC45 may have more adaptors in coordination of the dephosphorylation of p-STAT3.

In summary, we propose a model that SIPAR recruits TC45 to dephosphorylate STAT3 (Fig. 5). In the nucleus, SIPAR and TC45 forms a complex, which recognizes phosphorylated STAT3. The complex of SIPAR/TC45/p-STAT3 is de-associated after the dephophorylation of STAT3. In this way, the activity of STAT3 is negatively regulated by SIPAR.

## 4. Discussion

STAT3 plays a very important role in cell survival, differentiation and proliferation during the embryo development and difference diseases. Many tumors are attributed to the constitutive STAT3 phosphorylation, which can be a consequence of either increased expression and activation of positive regulators or decreased activities of negative regulators including phosphatases [18,19]. To date, several phosphatases have been identified to mediate the dephosphorylation of STAT3. We previously reported

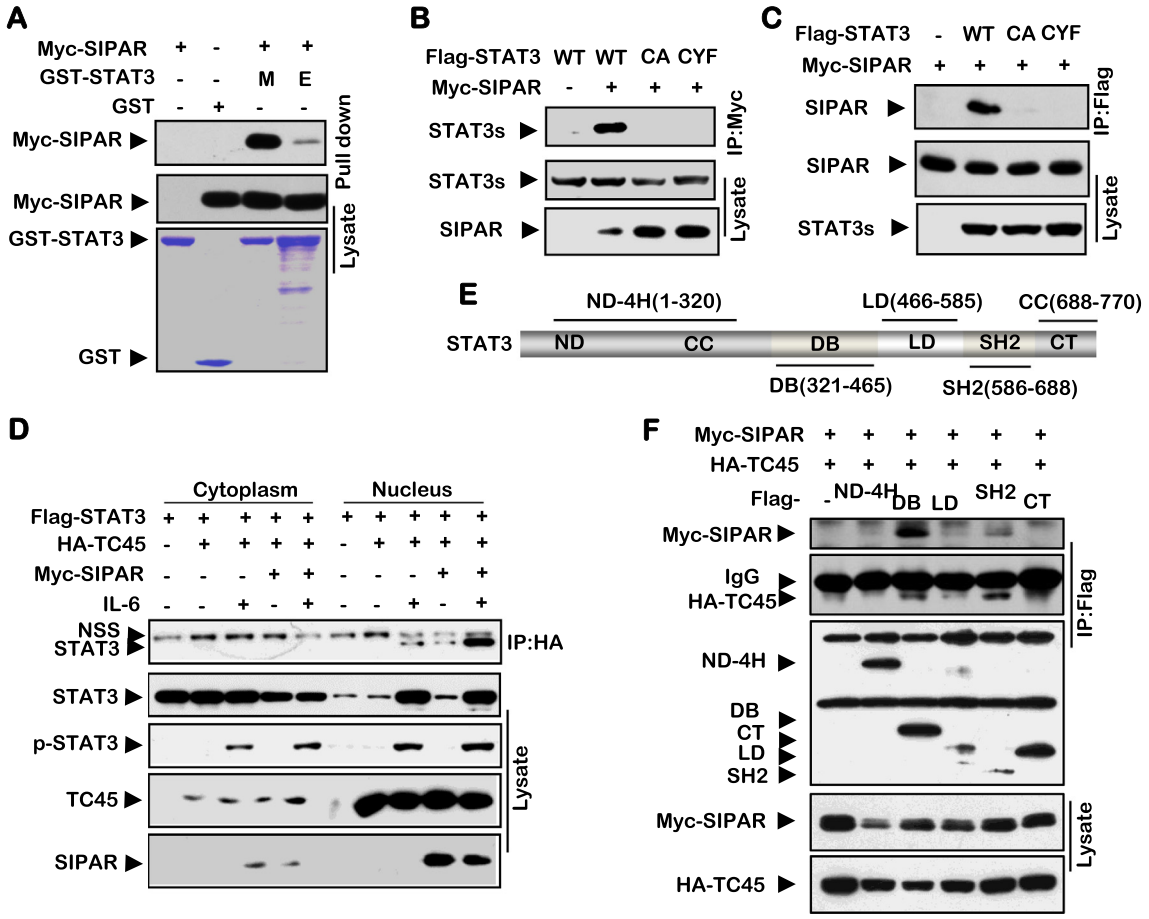


**Fig. 3.** SIPAR interacts with phosphorylated STAT3. (A) SIPAR interacts with STAT3 dependent on the phosphorylation of STAT3. Purified GST-STAT3 proteins which were expressed from either mammalian cells (M) or *E. coli* (E) were used to subject to an in vitro GST-pull down experiment using over-expressed Myc-SIPAR. (B–C) SIPAR failed to associate with STAT3 mutants. Wild type (WT), a constitutively active (CA) and a Y705 mutant (CYF) of STAT3 were used for performed IP experiments using either an anti-Myc antibody against SIPAR (B) or an anti-Flag antibody against STAT3 and its mutants (C). (D) SIPAR enhances the interaction of TC45 with STAT3 in the nucleus in response to IL-6 stimulation. Cells were fractioned as cytoplasm and nuclear sections for the immunoprecipitation experiment using an antibody against HA. A non-specific band was indicated as NSS. (E) A diagram to show the domains of STAT3. ND-4H: NH2-terminal and coiled-coil domains, DB: DNA binding domain, LD: linker domain, SH2: Src homology domain 2, CT: C-terminal. (F) SIPAR and TC45 associate with STAT3 at the DB, LD and SH2 domains.

that PTPN9/Meg2 directly interacted with STAT3 and mediated its dephosphorylation in breast cancer cells [12]. Interestingly we recently found that GdX, a ubiquitin-like domain containing protein, specifically interacted with STAT3 and TC45 to mediate specific dephosphorylation of STAT3 in colon cancers [13]. In this study, we revealed that SIPAR, a novel protein that interacts with STAT3 [5,15,16], also mediated the dephosphorylation of STAT3 through TC45. Our study provided another case that a non-phosphatase protein can bridge a phosphatase to associate with STAT3 (see Fig. 5).

It has been reported that other phosphatases also dephosphorylate STAT3. For example, SHP2 have been reported to dephosphorylate STAT3 in the nucleus [10,20]. Therefore it is speculated that whether SIPAR may associate with other phosphatases. Our data demonstrated that the inhibitory effect of SIPAR to mediate STAT3 dephosphorylation slightly decreased in $MEF^{TC45-/-}$ cells, suggesting that: (1) the primary role of SIPAR on STAT3 is dependent upon TC45, and (2) SIPAR may also function in cooperation with other phosphatases. This will be a direction for the future study.

It remains unclear how the interaction of SIPAR with STAT3 and TC45 is regulated as SIPAR has no pY-binding motif. In our study, we observed that IL-6 enhanced the interaction of SIPAR with TC45 and STAT3. Our results implied that SIPAR might recognize phosphorylated STAT3. This notion was supported by the analysis on the interaction of purified GST-STAT3 and Myc-SIPAR, as GST-STAT3 expressed from the mammalian cells (with strong phosphorylation) showed strong interaction with Myc-SIPAR (Fig. 3A). However, on the other hand, we observed that the interaction of SIPAR with TC45 remains constant in the presence or absence of IL-6 (Fig. 2F), suggesting that SIPAR might form a complex with TC45 before the recognition of p-STAT3. We speculate that the SIPAR–TC45 complex associates with phosphorylated STAT3 so that the catalytical domain of phosphatase could catch up the phosphorylated residue Y705 in STAT3. This process occurs after the phosphorylated STAT3 protein is translocated into the nucleus. How SIPAR recognizes the phosphorylated STAT3 proteins in the nucleus remains to be addressed. Also, it is interesting to question whether TC45 could be phosphorylated during the SIPAR, TC45 and p-STAT3 complex formation.

SIPAR was originally identified by our group from a yeast two hybrid screen using STAT3 as a bait [5,15]. In human, *SIPAR* is localized in chromosome 7p22.1, encoding 259 amino acid. SIPAR is conserved from mouse (with 260 amino acid) to human with a low homologous similarity in protein sequences [16]. In a homology search using NCBI blast algorithm we did not find any homology in other species evolutionarily lower than mouse (data not shown). To date, no reported was linked to the mutation

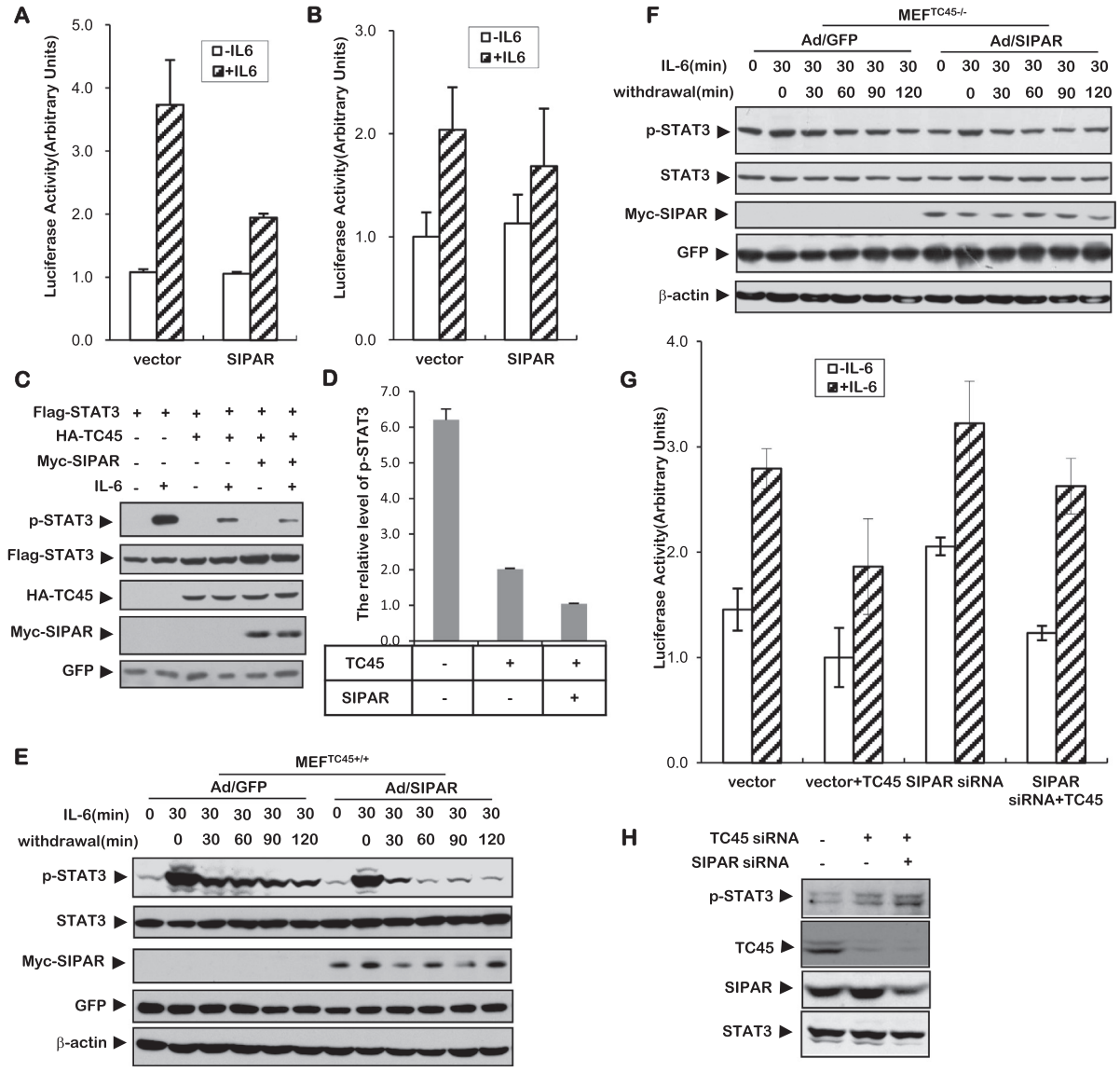


**Fig. 4.** SIPAR represses the transcriptional activity and phosphorylation of STAT3 by recruiting TC45. (A) SIPAR inhibits STAT3 transcriptional activity in MEF cells. Cells were infected with Ad/SIPAR or Ad/GFP (as a control) 24 h before starvation. After starvation of 12 h, cells were treated with/without IL-6 for 8 h. Results shown are mean ± S.D. of three independent experiments. (B) SIPAR regulated the transcriptional activity of STAT3 in MEF TC45-null cells. (C) SIPAR and TC45 synergistically dephosphorylate p-STAT3. 293T cells were stimulated by IL-6 for 30 min followed by IL-6 withdrawal for 30 min. (D) The p-STAT3 protein levels were analyzed by a quantitative method. (E) SIPAR accelerates STAT3 dephosphorylation. IL-6 was added to MEFs for 30 min and then withdrawn for the indicated times as above. (F) SIPAR failed to inhibit STAT3 phosphorylation activity in the absence of TC45. IL-6 was added to MEF TC45-null cells for 30 min and then withdrawn for indicated times. (G) Depletion of SIPAR impaired the function of TC45 in inhibition of STAT3 activity. (H) Depletion of SIPAR further enhances the phosphorylation of STAT3 based on the depletion of TC45.

or deletion of *SIPAR* in human diseases although the abnormalities of chromosome 7p22.1 (where *SIPAR* is located) were associated with autism [21–23]. Interestingly, inhibition of STAT3 was reported to oppose the occurrence of maternal immune activation induced autism [24]. This is very interesting as SIPAR is preferably expressed in brain tissues [16]. On the other hand, deletion and translocation in 7p22.1 were observed in myeloid leukemia [25,26], and STAT3 signaling is widely reported to be constitutively active and has been as a target for treatment of myeloid leukemia [27]. Those coincidences may imply a correlation of SIPAR and STAT3 in the regulation of these disease processes. It will be interesting to further study whether SIPAR is related to these diseases, in particular whether dysregulation of SIPAR together with STAT3 is related to the process of these diseases. A genetically SIPAR knock-out mouse model will be useful to reveal the functions of SIPAR in the physiology and pathological conditions.

Previously we observed that SIPAR was abundant in the heart, spleen, brain, lung and muscle in the adult mouse and is highly expressed in the whole mouse embryo [16]. During the embryo development, SIPAR is mainly expressed in the nerve system including brain, eye vesicles and vertebral column in the mouse [5]. Interestingly, SIPAR, also named as Acpin1, was reported to be highly expressed in the testis and appeared to function in spermatogenesis [14]. However, it was well known that the expression of STAT3 was ubiquitous in different adult tissues. Therefore the interaction of SIPAR with STAT3 and TC45 could be an event that occurs specifically in these tissues and during embryonic development. Indeed, in this study we observed the interaction of endogenous SIPAR with TC45 in the mouse embryo (Fig. 1D). However, the

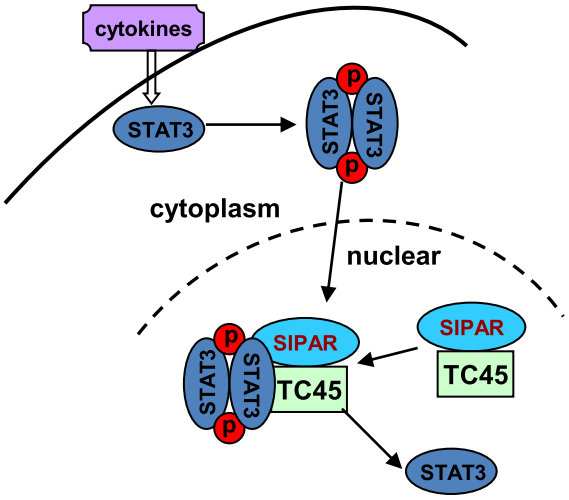


**Fig. 5.** A model of termination of STAT3 activity in the nucleus by SIPAR that interacts with TC45. STAT3 is phosphorylated in the cytoplasm after stimulation by cytokines. SIPAR interacts with TC45 to form a complex which then associates with phosphorylated STAT3. The SIPAR–TC45 complex mediates dephosphorylation of p-STAT3 and terminates its transcriptional activity in the nucleus.

specific biological function that enables SIPAR to mediate the dephosphorylation of STAT3 by interacting with TC45 during development and human diseases remains exclusive. Further experiments are needed to address these questions.

In conclusion, we demonstrated that SIPAR enhances the dephosphorylation of STAT3 and negatively regulates STAT3 activity through TC45. The next step should be to study whether SIPAR suppresses STAT3-mediated cell transformation, and the detailed mechanisms and functions of TC45 involvement in the regulation of SIPAR-mediated STAT3 dephosphorylation.

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