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ECHS1 interacts with STAT3 and negatively regulates STAT3 signaling

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ABSTRACT

Signal transducer and activator of transcription 3 (STAT3) is a critical transcriptional factor in a variety of cellular processes, and is frequently over-activated in a range of human tumors. However, the processes that regulate STAT3 activation need to be further clarified. With a yeast two-hybrid screening, we identified enoyl-CoA hydratase short chain 1 (ECHS1) as a novel STAT3 binding protein. We further confirmed the interaction between STAT3 and ECHS1 by GST-pull down and co-immnunoprecipitation. Importantly, we found that ECHS1 specifically represses STAT3 activity and negatively regulates the expression of several target genes of STAT3 through inhibiting STAT3 phosphorylation. Therefore, our findings will provide new insights into the mechanism of STAT3 signaling regulation.

Structured summary of protein interactions:
STAT3 physically interacts with **ECHS1** by pull down (View interaction)
STAT3 physically interacts with **ECHS1** by two hybrid (View Interaction: 1, 2)
ECHS1 physically interacts with **STAT3** by anti tag co immunoprecipitation (View Interaction: 1, 2)
STAT3 physically interacts with **ECHS1** by anti bait co immunoprecipitation (View interaction)

1. Introduction

STAT3 is a member of the STAT transcriptional factor family, which comprises multiple members, termed STAT1–6. STAT3 was first reported to be activated by interleukin-6 (IL-6) and epidermal growth factor (EGF), and interacts with an enhancer element in the promoters of acute phase genes [1,2]. Later studies demonstrated that STAT3 could be activated by several other cytokines and growth factors, such as interferon (IFN) and leptin. Binding of ligand triggers JAK1-mediated phosphorylation of specific tyrosine residues in the cytoplasmic portion of the receptor, then STAT3 is recruited to the receptor complex and phosphorylated at tyrosine 705 residue by activated JAK1, phosphorylated STAT3 dimerizes and subsequent translocates into the nucleus and transactivates target genes [3]. Activation of STAT3 is critical for a variety of cellular processes such as cell proliferation, differentiation, inflammation and immune responses. It has been reported that STAT3 is constitutively activated in oncogenic transformed cells and various primary tumors [4]. Constitutive activation of STAT3 induces persistent expression of many target genes, such as BCL2 (an important anti-apoptotic factor), BCL2L1 (BCL-2-like protein 1), Cyclin D1 (a key protein involved in G1-S progression), MMP-2 (matrix metalloproteinase 2) and so on. Up-regulation of these genes contributes to tumor development or progression through promoting cell proliferation, tumor angiogenesis and inducing tumor cell invasion or suppressing anti-tumor immune responses [5–9]. Therefore, STAT3 is considered to be a key player in the pathogenesis of diverse human cancers and an ideal target for cancer therapy.

Enoyl-CoA hydratase short chain 1 (ECHS1) is a key enzyme that catalyzes the second step in the physiologically important β-oxidation pathway of fatty acid metabolism. Except for its pivotal roles in regulation of fatty acid metabolism, accumulating literatures have demonstrated that down-regulation of ECHS1 might be in-

Abbreviations: ECHS1, enoyl-CoA hydratase short chain 1; STAT3, signal transducer and activator of transcription 3; APRE, acute phase response elements

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volved in tumor development. Lake et al. found that ECHS1 was frequently deleted in metastatic conjunctival melanomas [10]. It has been reported that ECHS1 expression is also decreased in renal cell carcinomas and in hepatocellular carcinomas [11,12]. These reports indicated that ECHS1 might play an important role in controlling tumor metastasis or progression. However, it remains largely unknown about the detailed mechanism that ECHS1 is involved in these processes.

Considering the critical role of STAT3 in tumor development and progression, regulation of STAT3 signaling has been concerned more and more. In this study, to further identify potential novel regulators of STAT3, we performed a yeast two-hybrid screening for STAT3-interacting proteins from a human mammary cDNA library, and identified ECHS1 as a new STAT3 interacting protein. This interaction was further confirmed by GST pull-down and co-immnunoprecipitation assays. Importantly, we found that ECHS1 specifically inhibits STAT3 transcriptional activity induced by IL-6 and EGF. Furthermore, we showed that ECHS1 negatively regulates IL-6 and EGF induced STAT3 phosphorylation at tyrosine 705 residue, which is the essential molecular event that leads to STAT3 transcriptional activation. Additionally, ECHS1 controlled the expression of several pro-oncogenic target genes of STAT3, like MMP-2, BCL2 and Cyclin D1. Taken together, our findings identified a novel STAT3 modulator and revealed a new biological function of ECHS1.

2. Materials and methods

2.1. Cell culture, transfection and reporter gene analysis

HEK293T and HeLa cells were cultured in DMEM containing 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2. HepG2 cells were maintained in MEM containing 10% fetal bovine serum plus Sodium Pyruvate (100×) (Gibco) and Non-essential Amino Acid (100×) (Gibco). Transfection of plasmids was performed by using Lipofectamine 2000 (Invitrogen) and siRNAs were transfected by using Lipofectamine RNAiMAX (Invitrogen) following the manufacture's instruction. Luciferase activities were determined using a Dual-Luciferase Reporter Assay System (Promega).

2.2. Immunoprecipitation, immunoblotting and antibodies

For co-immunoprecipitation experiments, HEK293T cells were lysed in E1A buffer (50 mM Hepes, pH = 7.5, 250 mM NaCl, 0.1% Nonidet P-40, 5 mM EDTA) containing certain protease inhibitors. The whole cell homogenates were incubated with indicated antibodies and rocked at 4 °C for 6 h after the pre-incubating with protein A/G-Sepharose (Santa Cruz Biotechnology). Immunoprecipitates were washed three times and resuspended in 40 μl of 1× SDS sample buffer, then resolved by SDS–PAGE. All samples for immunoblotting assays were prepared in M2 buffer (20 mM Tris–HCl pH = 7.5, 250 mM NaCl, 0.5% NP-40, 3 mM EDTA, 3 mM EGTA) with a mixture of protein inhibitors. For endogenous immunoprecipitation experiments, HepG2 cells were untreated or treated with IL-6 (50 ng/ml) for 30 min followed by serum starvation for 16 h, cells were collected in E1A buffer, then the cell lysates were immunoprecipitated by rabbit anti-STAT3 antibody or normal rabbit IgG. Rabbit anti-ECHS1 (sc-133534) antibody, anti-STAT3 (sc-7179) antibody and mouse anti-c-Myc (sc-40) antibody were purchased from Santa Cruz Biotechnology. Rabbit anti-JAK1 antibody (3332) and anti-pJAK1 antibody (Tyr1022/1023) (3331) were purchased from cell signaling technology. Mouse anti-Flag (M2) (F3165) and α-Tubulin (T5168) monoclonal antibodies were from Sigma. Rabbit anti-pSTAT3 (Tyr-705) (2236-1) was purchased from Epitomics Company.

2.3. RNA extraction and real-time RT-PCR analysis

HepG2 cells in 6 wells were transfected with ECHS1 siRNA (40 nM) for 48 h or Myc-ECHS1 (1.5 μg/well) for 24 h, and starved using MEM with 0.5% serum for 16–18 h, then stimulated with IL-6 (50 ng/ml) for indicated times. Cell pellets were collected and total RNA were extracted by Trizol (Invitrogen). The RNA isolation and data analyses were performed as described in previous study [13]. The real-time PCR were performed specifically with primers as following:

MMP-2 (5′-AGGGCACATCCTATGACAGC-3′/5′-ATTTGTTGCCCAG GAAAGTG-3′),
BCL2L1 (5′-TTCAGTGACCTGACATCCCA-3′/5′-CTGCTGCATTGTT CCCATAG-3′),
BCL2 (5′-CTGAGTACCTGAACCGGCA-3′/5′-GAGAAATCAAACAGA GGCCG-3′),
Cyclin D1 (5′-GACCTTCGTTGCCCTCTGT-3′/5′-TGAGGCGGTAG-TAGG ACAGG-3′),
GAPDH (5′-CCAGGTGGTCTCCTCTGACTTC-3′/5′-TGGTCGTTGAG GGCAATG-3′).

2.4. Statistical analysis

Comparisons between groups were analyzed by Student's *t* test. A *P* value of less than 0.05 was considered significant.

3. Results

3.1. ECHS1 interacts with STAT3 in yeast

To further explore the regulation and function of STAT3, we performed a yeast two-hybrid screening in attempt to identify new STAT3 interacting proteins. Since full-length STAT3 leads to the constitutive activation of the reporter genes, we used two truncation mutants of STAT3 as baits to screen human mammary cDNA library. 1–330 aa is the N-terminal of STAT3 containing the Coil–Coil domain, which was reported to be important for protein–protein interaction, 575–710 aa contains SH2 domain, which was also critical to mediate protein association. Finally, 95 positive clones from 1.6×10^6 independent transformants were obtained by using the bait 1–330, and two was found to encode the same protein ECHS1 with DNA sequencing. The interaction between STAT3 and ECHS1 was first confirmed in yeast system. As shown in Fig. 1A and B, ECHS1 interacted with full-length STAT3 and the N-terminus (1–330 aa), but not with the SH2 domain (575–710 aa) of STAT3 or the negative control pGBKT7-LAM.

3.2. ECHS1 interacts with STAT3 in vitro and in vivo

To further confirm the physical interaction between STAT3 and ECHS1, we performed GST pull-down experiment, and the result showed that substantial amounts of ECHS1 were pulled down by GST-STAT3 but not by GST alone (Fig. 1C). To determine whether STAT3 interacts with ECHS1 in mammalian cells, we carried out a co-immunoprecipitation experiment in HEK293T cells. Myc-ECHS1 and Flag-STAT3 were co-expressed in HEK293T cells, and then the cell lysates were immunoprecipitated with an anti-Myc antibody followed by immunoblotting with an anti-Flag antibody. The result showed that Flag-STAT3 was precipitated by Myc-ECHS1, but not Myc-vector (Fig. 1D). Consistently, when the cell lysates were subjected to immunoprecipitation with an anti-Flag antibody, Myc-ECHS1 was detected in the precipitant (Fig. 1E). To study the possible physiological significance of ECHS1, we investigated the interaction between endogenous STAT3 and ECHS1 in HepG2 cells.

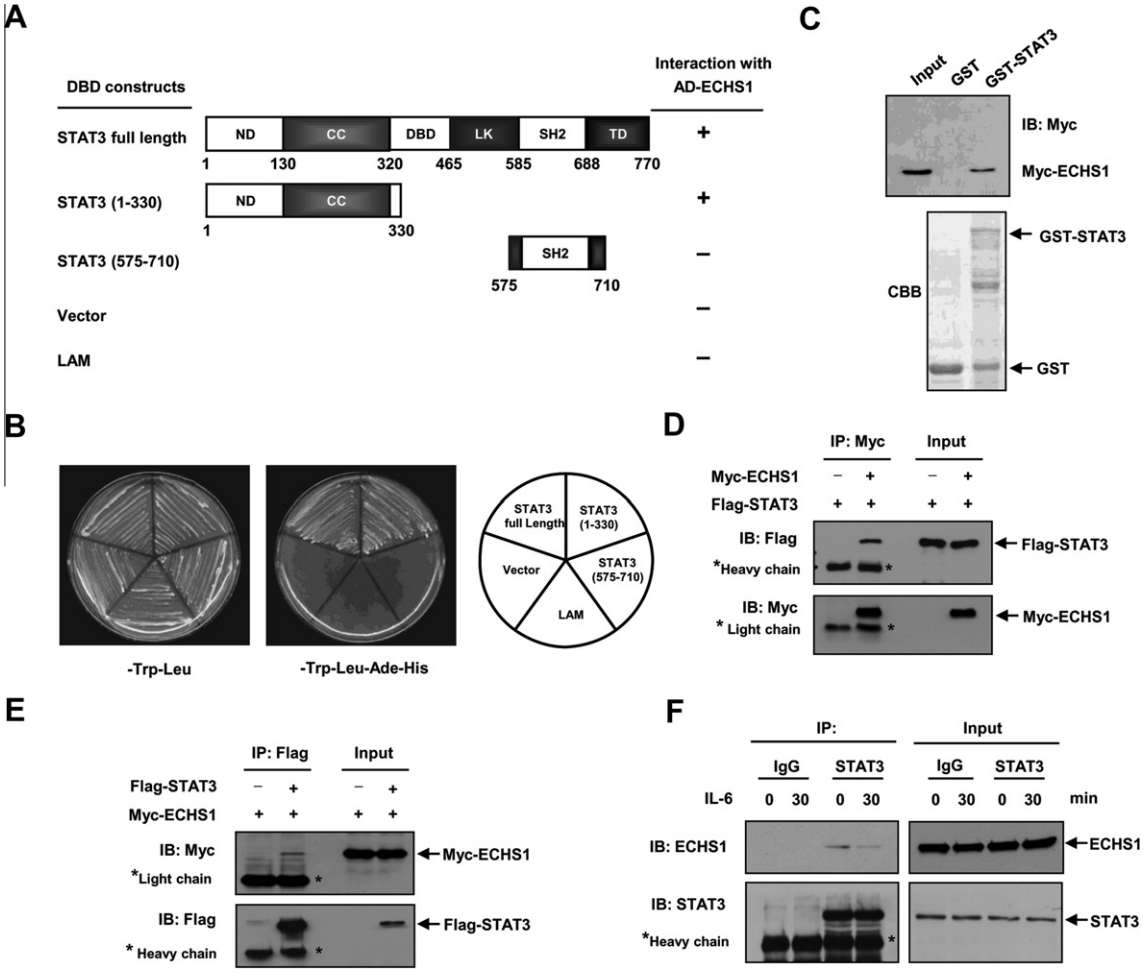

Fig. 1. ECHS1 interacts with STAT3 in vitro and in vivo. (A) Schematic illustration of full-length human STAT3 protein. The DNA fragment encoding the N-terminal (1–330 aa) and C-terminal (575–710aa) were fused in-frame with the yeast GAL4 DNA binding domain and used as baits to screen for STAT3 interacting protein in yeast two-hybrid assay. The numbers refer to the amino acids of STAT3. Interaction is scored as "+" and no interaction as "−". (B) Growth of AH109 co-transformed with different GAL4-DBD-STAT3 and GAL4-AD-ECHS1 constructs on SD medium. Co-transformants were isolated on the SD medium lacking tryptophan and leucine (-Trp-Leu, left plate) or on the SD medium lacking tryptophan, leucine, adenine, and histidine (-Trp-Leu-Ade-His, right plate). (C) Cell lysates of HEK293T cells transfected with Myc-ECHS1 were incubated with GST or GST-STAT3, and the bound proteins were detected with anti-Myc antibody. CBB, Coomassie Brilliant Blue staining. (D and E) HEK293T cells were cotransfected with Flag-STAT3 and Myc-ECHS1 or control vector, and 24 h after transfection, cell lysates were immunoprecipitated with anti-Myc or anti-Flag antibody, then immunoblot analysis was performed with anti-Flag and anti-Myc antibodies. (F) HepG2 cells were serum starved for 16 h, and then treated or untreated with IL-6 (50 ng/ml) for 30 min, cell lysates were immunoprecipitated with rabbit normal IgG or rabbit anti-STAT3 antibodies, then western blot was analyzed using anti-ECHS1 and anti-STAT3 antibodies.

As shown in Fig. 1F, ECHS1 was co-immunoprecipitated by STAT3 but not rabbit IgG in unstimulated cells, and interestingly, the amount of immunoprecipitated ECHS1 was significantly lower when the cells were treated with IL-6 for 30 min, suggesting that interaction between STAT3 and ECHS1 is regulated by the upstream signaling. Collectively, these results indicate that ECHS1 associates with STAT3 specifically in vitro and in vivo.

3.3. ECHS1 inhibits the transcriptional activity of STAT3 induced by IL-6 and EGF

To investigate the potential biological function of ECHS1 interacting with STAT3, we used reporter luciferase assay to test whether ECHS1 affects STAT3 transcriptional activity. As shown in Fig. 2A, HepG2 cells were transfected with pACT-Luc, a luciferase reporter plasmid containing two STAT3 binding sites, and increasing dose of Myc-ECHS1 constructs. Results showed that ECHS1 inhibits STAT3 transcriptional activity induced by IL-6 in a dose-dependent manner. Similar results were also obtained in exogenous STAT3 transcriptional activity (Fig. 2B). To further explore the physiological function of ECHS1 on STAT3 transcriptional activity, endogenous ECHS1 was knocked down by using two different siRNAs in HepG2 cells and STAT3 transcriptional activity was detected. As expected, ECHS1 depletion augmented the STAT3 transcriptional activity induced by IL-6 (Fig. 2C). To investigate whether ECHS1 is also involved in STAT3 activation mediated by other JAK1/STAT3 activators, we examined the effect of ECHS1 on EGF-mediated STAT3 activation using APRE-Luc, another STAT3-responsive luciferase reporter. The results showed that ECHS1 overexpression could also suppress EGF-induced STAT3 activation in HeLa cells (Fig. 2D), and knocking down ECHS1 up-regulated STAT3 transcriptional activity induced by EGF (Fig. 2E). Considering the comparable effects and the similar knockdown efficiency of these two different siRNAs (Fig. 2C and E), ECHS1 siRNA #2 was used in most of the ECHS1 RNAi experiments unless indicated otherwise.

To test the specificity of ECHS1 on STAT3 activation, we also detected luciferase activity of NF-κB-responsive reporter NF-κB-Luc and p53-responsive reporter pp53-TA-Luc when ECHS1 or control plasmids were transfected. As shown in Fig. 2F and G, ECHS1 had no effect on the transcriptional activity of either NF-κB or p53, while SOX4 could obviously promote p53 activity as reported

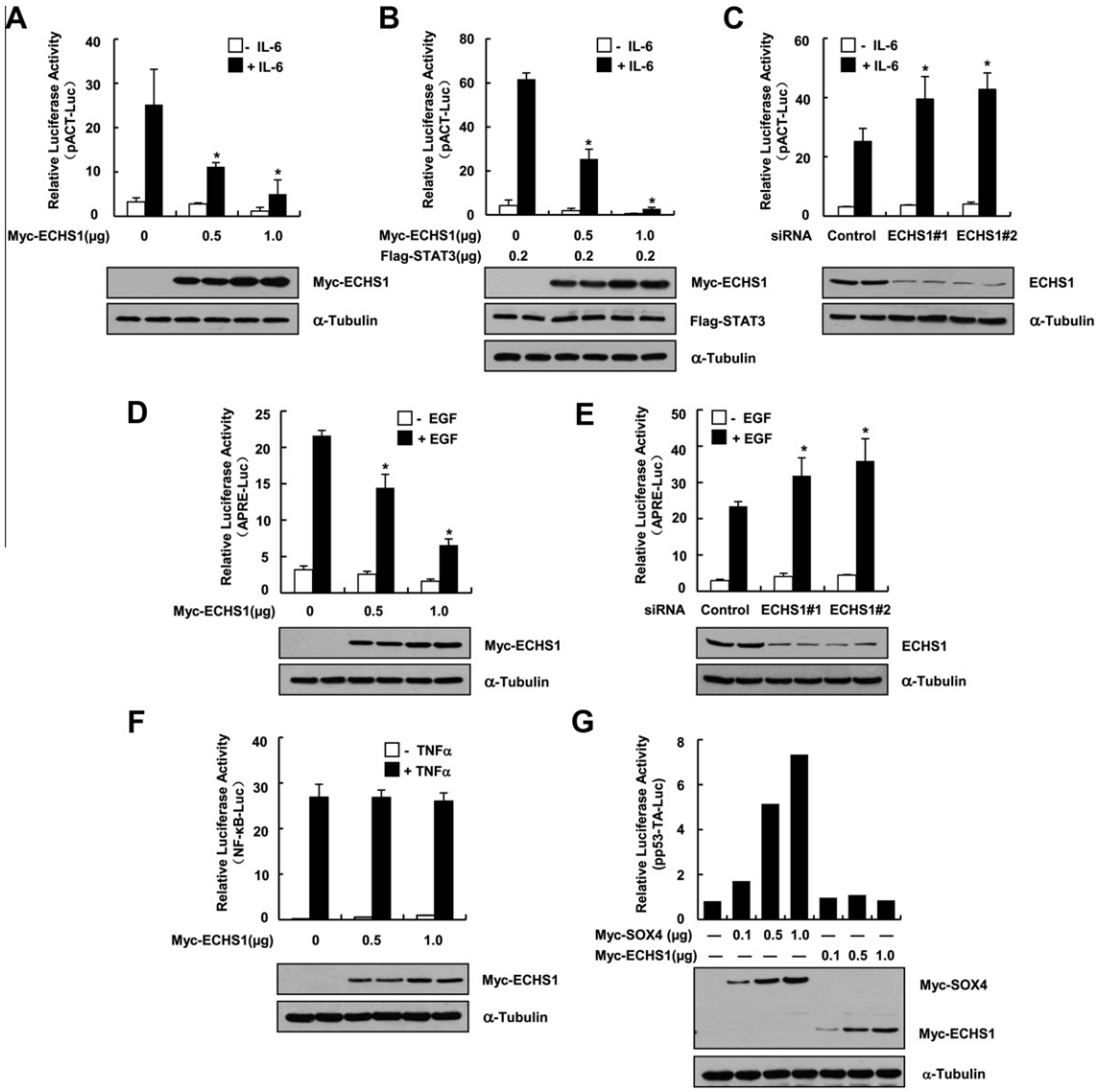

Fig. 2. ECHS1 inhibits STAT3 transcriptional activity induced by IL-6 and EGF. (A) HepG2 cells in a 12-well plate were transiently transfected with pACT-Luc (200 ng/well) and increasing amounts of Myc-ECHS1 plasmids. 24 h after transfection, cells were starved for 16–18 h in MEM with 0.5% serum and then stimulated with or without IL-6 (50 ng/ml) for additional 6 h, luciferase activity was measured. Renilla reporter pRL-TK vector were used as an internal control for transfection efficiency. The cell lysates were immunoblotted using anti-Myc and α-Tubulin antibodies. (B) HepG2 cells were transfected and treated the same as in A, except for the addition of Flag-STAT3 (200 ng/well). (C) HepG2 cells in 12-well plate were transfected with control siRNA or the two different ECHS1 siRNAs (40 nM) (#1 and #2). 48 h after transfection, pACT-Luc (200 ng/well) and pRL-TK-Luc (20 ng/well) were cotransfected. Another 24 h later, cells were treated with or without IL-6 (50 ng/ml) for 6 h. Luciferase activity was detected and western blot was performed using anti-ECHS1 and α-Tubulin antibodies. (D and E) HeLa cells were transfected and treated the same as in A and C, except for the APRE-Luc and EGF treatment. (F) HEK293T cells were cotransfected with NF-κB-Luc (200 ng/well) and indicated dosage of Myc-ECHS1 plasmids. 24 h later, cells were stimulated by TNFα (10 ng/ml) for 6 h, then luciferase activity and western blot were performed. (G) H460 cells were cotransfected with pp53-TA-Luc (500 ng/well), Myc-vector or increasing dosage of Myc-SOX4 or Myc-ECHS1 plasmids. 24 h after transfection, luciferase activity was measured and immunoblotting was performed. All the results above are mean ± S.D. of three independent experiments except G. Statistic analyses were done using Student's *t* test between control group and ECHS1 overexpression/knockdown group after EGF/IL-6 stimulation (*$P < 0.05$ indicate statistically significant differences).

[14]. Taken together, our findings suggest that ECHS1 is a negative regulator of STAT3 transcriptional activity.

3.4. ECHS1 inhibits STAT3 phosphorylation

Phosphorylation of STAT3 at specific residues, particularly Tyr-705, is very important for its activation. To further investigate the molecular mechanism that ECHS1 regulates STAT3 transcriptional activity, we next tested whether ECHS1 inhibits STAT3 activity through regulating its phosphorylation at Tyr-705. To do so, we examined the kinetics of STAT3 Tyr-705 phosphorylation induced by IL-6 in HepG2 cells transfected with either Myc-vector or Myc-ECHS1. As shown in Fig. 3A, the amounts of phosphorylated STAT3 obviously decreased in Myc-ECHS1 overexpressed cells compared with control cells, while the levels of total STAT3 protein were similar. To test whether ECHS1 regulate STAT3 phosphorylation through its kinase JAK1, the phosphorylation level of JAK1 was also detected. The result showed that JAK1 phosphorylation level remained unchanged. Conversely, knockdown of ECHS1 in HepG2 cells led to an elevated level of phosphorylated STAT3 (Fig. 3B). Then we examined the impact of ECHS1 on EGF-induced STAT3 phosphorylation. We found that EGF-induced phosphorylation of STAT3 at Tyr-705 also decreased in ECHS1-overexpressing cells compared with control cells (Fig. 3C), and the STAT3 phosphoryla-

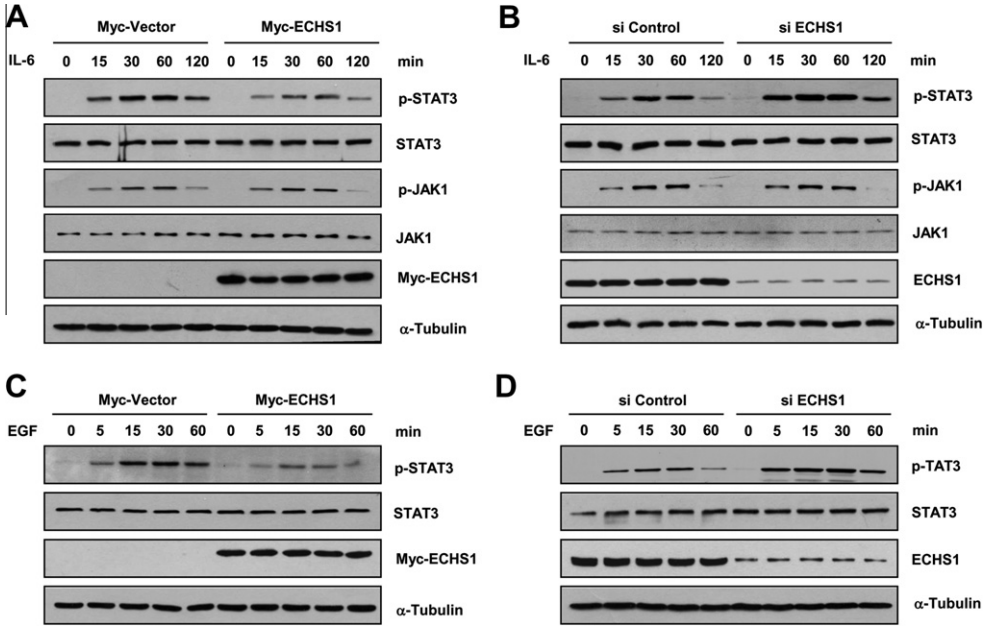

Fig. 3. ECHS1 decreases STAT3 tyrosine phosphorylation. (A) HepG2 cells in 6-well plates were transfected with Myc-ECHS1 or Myc-vector (1.5 μg /well). 24 h after transfection, cells were starved for 16 h in MEM with 0.5% serum and stimulated by IL-6 (50 ng/ml) for 0–120 min, then the total cell lysates were immunoblotted using anti-pSTAT3 (Tyr-705), anti-STAT3, anti-pJAK1, anti-JAK1, anti-Myc and α-Tubulin antibodies. (B) HepG2 cells were transfected with ECHS1 siRNA (#2) or control siRNA (40nM), 48 h after transfection, cells were starved for 16 h and stimulated by IL-6. Immunoblotting were analyzed with the antibodies as indicated. (C and D) HeLa cells were transfected and treated the same as in A and B, except that cells were treated with EGF at indicated times.

tion was up-regulated in ECHS1 knocking down cells (Fig. 3D), suggesting that ECHS1-mediated suppression of STAT3 phosphorylation is not restricted to a particular stimulation. Taken together, these results suggest that ECHS1 inhibits STAT3 transcriptional activation through attenuating its phosphorylation.

3.5. ECHS1 negatively regulates expression of STAT3 target genes

Constitutive activation of STAT3 contributes to tumor development or progression mainly through regulating target genes that involving in promoting cell proliferation, inhibiting apoptosis or inducing cancer cell metastasis. To clarify the roles of ECHS1 in the process of STAT3 promoting tumorigenesis, we examined the effect of ECHS1 on the expression of several STAT3 target genes. We measured the expression of MMP-2, BCL2L1, BCL2 and Cyclin D1 induced by IL-6 by real time RT-PCR in ECHS1-overexpressing or knockdown HepG2 cells. As shown in Fig. 4A, overexpression of ECHS1 led to reduced MMP-2, BCL2L1, BCL2 and Cyclin D1 mRNA expression, and depletion of ECHS1 caused up-regulated expression of these genes (Fig. 4B). Therefore, these results indicate that ECHS1 might play a key role in regulating the expression of STAT3 target genes.

4. Discussion

As one of the most important transcriptional factors involved in tumorigenesis or tumor progression, STAT3 has been studied for decades. Despite rapid progress in elucidating the molecular mechanisms of activation of JAK1/STAT3 pathway, the processes that regulate JAK1/STAT3 deactivation are still largely unknown. Several lines of evidences have shown that JAK1/STAT3 signaling pathway could be modulated at multiple steps through different mechanisms. Protein tyrosine phosphatases (PTP), such as SHP1, SHP2, CD45, PTP-1b, negatively regulate JAK1/STAT3 pathway by dephosphorylating cytokine receptors, JAKs, or some other essential pathway component [15,16]. SOCS proteins are another group of critical inhibitors of JAK1/STAT3 signaling. Distinct SOCS family members regulate cytokine signaling through several different mechanisms, including competitively binding to STATs members, inhibiting JAKs catalytic activity [17,18] and so on. Our recent study has shown that CUE domain containing 2 (CUEDC2) specifically interacts with SOCS3 and acts as a novel SOCS3 co-operator required for inhibition of JAK1/STAT3 signalling [19]. Except for the PTP and SOCS family inhibitors, STAT3 is reported to be regulated by some suppressors directly binding to STAT3, like PIAS3 [20], GRIM-19, Daxx and LMW-DSP2 [21–23]. Inhibition of STAT3 by these suppressors is committed either through blocking of STATs-DNA binding, prevention of their nuclear import, or through undefined mechanisms. In this study, we found that ECHS1 inhibits the transcriptional activation of STAT3 through direct interaction. We demonstrate that ECHS1 suppresses STAT3 phosphorylation without altering JAK1 phosphorylation, suggesting that inhibition of ECHS1 on STAT3 activity might not be achieved by regulating JAK1 activity. It is likely that ECHS1 suppresses JAK1-mediated STAT3 phosphorylation through allosteric interaction. This hypothesis will be investigated by further experiments.

ECHS1 belongs to the low homology hydratase/isomerase enzyme superfamily and is responsible for catalyzing the second step in the physiologically important β-oxidation pathway of fatty acid metabolism. Evidence has demonstrated that ECHS1 catalyzes the hydration of α,β-unsaturated enoyl-CoA thioesters to the corresponding β-hydroxybutyryl-CoA thioesters [24,25]. Down regulation of ECHS1 was detected both in rat models and in patients with simple steatosis. Knocking down of ECHS1 by small interfering RNA (siRNA) significantly exacerbated lipid accumulation in hepatocytes induced by free fatty acid (FFA) [26,27]. Recently, ECHS1 is reported to be involved in other biological processes or diseases. For example, expression of ECHS1 was found to be altered during corpus luteum (CL) maintenance and most likely involved in mechanisms allowing the CL to produce progesterone during early pregnancy [28]. ECHS1 also participates in VPA biotransformation [29]. These evidences suggest that ECHS1 is a multifunc-

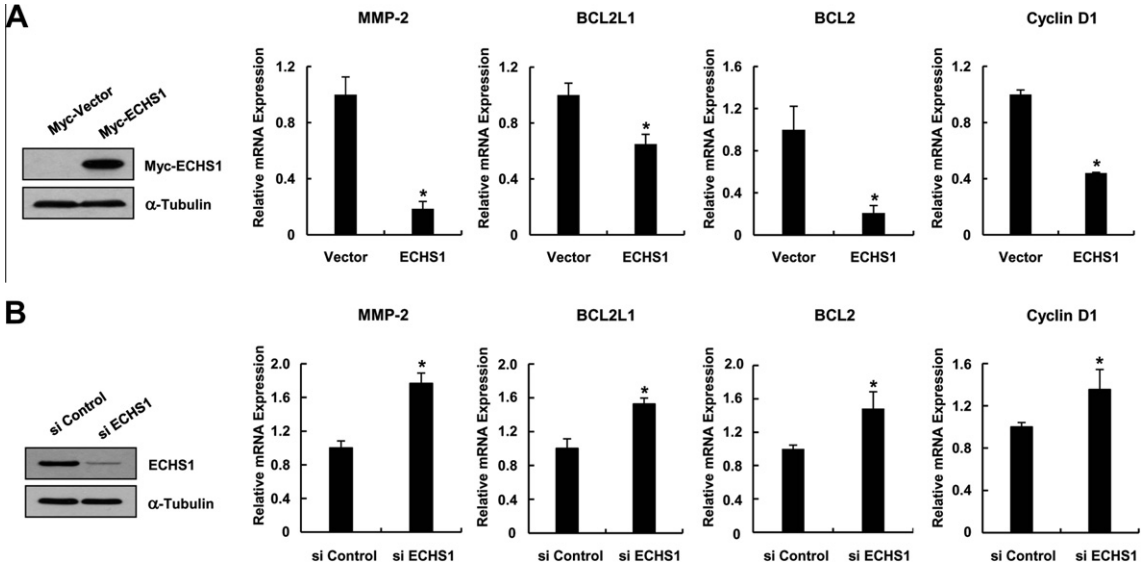

Fig. 4. ECHS1 affects STAT3 target genes expression. (A) HepG2 cells in 6-well plate were transfected with Myc-Vector or Myc-ECHS1(1.5 μg/well), 24 h after transfection, cells were starved for 16–18 h in MEM with 0.5% serum, and stimulated by IL-6 (50 ng/ml) for different times. One half of cells were lysed in M2 buffer and immunoblotted using anti-Myc and α-Tubulin antibodies, and another half of cells were determined the relative mRNA expression levels of MMP-2, BCL2L1, BCL2 and Cyclin D1 by real-time RT-PCR. Samples were respectively harvested at 6 h (MMP-2) or 24 h (BCL2L1, BCL2 and Cyclin D1) after IL-6 stimulation as described in Materials and methods. (B) HepG2 cells were transfected with ECHS1 siRNA (#2) or control siRNA(40 nM), 48 h after transfection, cells were starved and treated with IL-6 as in A. All the results above are mean ± S.D. of three independent experiments Statistic analyses were done using Student's *t* test between control group and ECHS1 overexpression/knockdown group after IL-6 stimulation. (*$P < 0.05$ indicate statistically significant differences).

tional protein, but the exact functions of ECHS1 remain to be uncovered.

Recently, a large body of literatures has demonstrated that ECHS1 was down-regulated in a variety of cancers. Liu et al. reported that ECHS1 was not only down-regulated in MCF-7 cells but also in other carcinoma cells, such as MDA-MB-231 (breast adenocarcinoma cell line), T47D (breast ductal carcinoma cell line), DU145 (prostate carcinoma cell line), HCT-8 (colon adenocarcinomacell line) and SKOV-3 (ovarian adenocarcinoma cell line) [30]. In metastatic conjunctival melanomas, ECHS1 was frequently deleted [10]. It has also been reported that ECHS1 expression decreased in renal cell carcinomas and in hepatocellular carcinomas [11,12]. These findings suggested that down regulation of ECHS1 might contribute to tumorigenesis or tumor metastasis. However, little is known about the function of ECHS1 in these processes. In this study, we found that ECHS1 binds to STAT3, negatively regulates STAT3 activity and inhibits expression of its critical target genes which play important roles in tumor metastasis and cell growth. Thus, down-regulation of ECHS1 might lead to constitutive STAT3 activation and result in tumor development or progression. Our findings provide one possibility to explain the significance of ECHS1 down regulation in multiple tumor types.

In conclusion, we identified ECHS1 as a novel STAT3 binding protein. Further study demonstrated that ECHS1 inhibits STAT3 phosphorylation, transcriptional activity and the subsequent target genes expression. Therefore, our work not only identified a novel STAT3 inhibitor but also provided important insight into the critical roles of ECHS1 in the intricate STAT3 signal transduction network, although a more detailed mechanism that ECHS1 regulates STAT3 need to be further studied.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.02.005.

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