Parasitology Research (2019) 118:551–557
https://doi.org/10.1007/s00436-018-6165-3

IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - ORIGINAL PAPER

Schistosoma japonicum soluble egg antigen inhibits TNF-α-induced IL-34 expression in hepatic stellate cells

Liuting Chen[1] · Yang Yu[1] · Ertao Liu[2] · Lian Duan[3] · Dandan Zhu[1] · Jinling Chen[1] · Jia Dai[1] · Yinong Duan[1]

Received: 30 August 2018 / Accepted: 22 November 2018 / Published online: 29 November 2018

Abstract

Hepatic fibrosis is characterized by the activation of the main collagen-producing cells of the liver, hepatic stellate cells, and is associated with inflammation. Although the involvement of numerous inflammatory cytokines has been reported, IL-34 in particular has recently been identified as a profibrotic factor in the development of hepatic fibrosis. Previous studies have found that schistosome eggs can lead to transcriptional downregulation of fibrosis-associated genes, and based on this evidence, we attempted to investigate whether or not IL-34 is regulated by soluble egg antigen (SEA). Our findings testified that SEA inhibited TNF-α-induced expression of IL-34 at both the mRNA and protein levels. Furthermore, results from reporter assays and qPCR experiments demonstrated that SEA impaired the activation of NF-κB triggered by TNF-α, as well as the transcription of downstream genes. More importantly, SEA decreased the phosphorylation and degradation of IκBα induced by TNF-α, two events that are hallmarks of canonical NF-κB activation. In conclusion, our results suggest that, in hepatic stellate cells, SEA impairs NF-κB activation and thereby inhibits TNF-α-induced IL-34 expression. These findings reveal a previously unidentified target and signaling pathway that support SEA's involvement in hepatic fibrosis and provide a new clue to guide ongoing research into the anti-fibrotic effects of SEA.

Keywords Hepatic fibrosis · Hepatic stellate cell · IL-34 · NF-κB

Introduction

Hepatic fibrosis has been identified as a major cause of morbidity and mortality worldwide. This disease results from a reversible wound-healing response characterized by the accumulation of connective tissue and extracellular matrix (ECM) that occurs alongside chronic viral hepatitis, non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, and schistosomiasis. During the process of hepatic fibrosis, the dominant pathogenic event is the activation of primary ECM-producing cells, hepatic stellate cells (HSCs) (Mederacke et al. 2013). In the past decade, numerous studies have identified inflammation as a key component and contributor to the development of hepatic fibrosis (Seki and Schwabe 2015), in which fibrosis is promoted by different mechanisms that involve multiple inflammatory cytokines, such as transforming growth factor-β1 (TGF-β1) (Dooley and ten Dijke 2012), interleukin (IL)-33 (McHedlidze et al. 2013), IL-20 (Chiu et al. 2014), and IL-17 (Meng et al. 2012; Tan et al. 2013).

Described for the first time in 2008 (Lin et al. 2008), IL-34 plays a vital role in several disparate systems, such as neuronal protection (Luo et al. 2013), autoimmune disease (Ciccia et al. 2013; Xie et al. 2018), and cancer (Baghdadi et al. 2018; Zhou et al. 2016). Recently, IL-34 has been identified as a profibrotic factor in hepatic fibrosis. In liver lesions, it causes the differentiation of monocytes into profibrotic type 2 macrophages, preventing the destruction of activated HSCs by NK cells, and thus increasing type I collagen (Preisser et al. 2014).

Liuting Chen, Yang Yu and Ertao Liu contributed equally to this work.

Section Editor: Xing-Quan ZHU

✉ Yinong Duan
yinongduan@aliyun.com

[1] Department of Pathogen Biology, School of Medicine, Nantong University, 19 Qixiu Road, Nantong 226001, Jiangsu, People's Republic of China

[2] Department of Orthopedics, Nantong Fourth People's Hospital, 37 Chenggang Road, Nantong 226005, Jiangsu, People's Republic of China

[3] Department of Medical Informatics, School of Medicine, Nantong University, 19 Qixiu Road, Nantong 226001, Jiangsu, People's Republic of China

Furthermore, a previous study found that IL-34 serves as an independent marker of hepatic fibrosis in NAFLD patients (Shoji et al. 2016), and another report found that, in patients with chronic hepatitis B (HBV) infection, IL-34 is an indicator of liver inflammation and fibrosis (Wang et al. 2018).

Previous research has demonstrated that both *Schistosoma mansoni* (*S. mansoni*) and *Schistosoma japonicum* (*S. japonicum*) eggs suppress the activation of HSCs, ultimately leading to the transcriptional downregulation of fibrosis-associated genes (Anthony et al. 2010, 2013). Consistently, our preceding studies have suggested that soluble egg antigen (SEA), a major complex mixture isolated from *S. japonicum* eggs, inhibits the TGF-β1-induced activation of human HSC line LX-2 cells, thus limiting the progression of hepatic fibrosis (Duan et al. 2014). However, whether or not IL-34 is regulated directly or indirectly by SEA has yet to be determined.

In the current study, we found that SEA inhibited the IL-34 expression induced by tumor necrosis factor α (TNF-α) in hepatic stellate cells. Mechanistically, SEA impaired the activation of NF-κB that was triggered by TNF-α, and hence impeded the induction of downstream genes. Our findings reveal a novel target and signaling pathway that involve SEA, as well as provide a previously unidentified avenue to research the anti-fibrotic effects of SEA.

Materials and methods

Reagents

We obtained the SEA of *S. japonicum* from the Jiangsu Institute of Parasitic Diseases (China) and sterile-filtered it before removing endotoxin using polymyxin B agarose beads (Sigma, USA). The removal of endotoxin was verified using Sensor chromogenic LAL endotoxin assay kit (GenScript, USA). Recombinant human TNF-α (PeproTech, USA), rabbit pAbs against GAPDH (Goodhere, China) or IκBα (Santa Cruz Biotechnology, USA), rabbit mAbs against p-IκBα (Cell Signaling Technology, USA), mouse mAbs against IL-34 (Abcam, UK), horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (Biosharp, China), and HRP-conjugated anti-mouse IgG (Santa Cruz Biotechnology, USA) were purchased from the indicated companies.

Constructs

The NF-κB luciferase reporter plasmid, mammalian expression plasmids for TNFR1, TRADD, RIP, and IKKβ were kindly provided by Hongbing Shu (Wuhan University).

Cell culture

The LX-2 cells were purchased from the Xiang Ya Central Experiment Laboratory (China) and the LO2 cells were purchased from the Shanghai Institutes for Biological Sciences (China). All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA), supplemented with 10% fetal bovine serum (Thermo, USA) at 37 °C in a humidified incubator with an atmosphere of 5% CO_2.

Western blot

Cells were lysed in RIPA buffer that included protease inhibitor (1 mM) and phosphatase inhibitors (1 mM). Equal amounts of protein extracts were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes. After blocking in 5% non-fat milk for 1 h, the membranes were incubated with the indicated primary antibodies at 4 °C overnight, and then incubated with the indicated secondary antibodies for 1 h at room temperature. After being extensively washed in TBS/Tween 20, the protein bands were visualized with ECL reagents (Millipore, USA).

Reverse transcription-quantitative real-time PCR

Total RNA was isolated from cells using TRIzol reagent (Invitrogen, USA) and then 4 μg was transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (Invitrogen, USA), according to the manufacturer's instructions. Aliquots of cDNA products were subjected to quantitative real-time PCR (qPCR) analysis to measure the level of mRNA expression of the tested genes using a SYBR Premix Ex Taq Kit (TAKARA, Japan) on a StepOnePlus Real-Time PCR System (Applied Biosystems, USA). Gene-specific primer sequences were as follows: *GAPDH*, 5′-GACAAGCTTCCCGTTCTCAG-3′ (forward) and 5′-GAGTCAACGGATTTGGTCGT-3′ (reverse); *IL-34*, 5′-GTGCTTAGGCCTCTGTGGAC-3′ (forward) and 5′-GCCAAGGAAGATCCCAAGATA-3′ (reverse); *IL-6*, 5′-TTCTCCACAAGCGCCTTCGGTC-3′ (forward) and 5′-TCTGTGTGGGGCGGCTACATCT-3′ (reverse); *CCL2*, 5′-TGTCCCAAAGAAGCTGTGATC-3′ (forward) and 5′-ATTCTTGGGTTGTGGAGTGAG-3′ (reverse).

Reporter assays

LX-2 cells were seeded in 24-well plates and transfected on the following day with Lipofectamine 2000 (Invitrogen, USA), according to the manufacturer's instructions. Empty

control plasmid was added to ensure that each transfection received an equal amount of total DNA. To normalize for transfection efficiency, the pRL-TK (Renilla luciferase) reporter plasmid (0.2 μg) was added to each transfection. Luciferase assays were performed using a dual-specific luciferase assay kit (Promega, USA). Firefly luciferase activities were normalized on the basis of Renilla luciferase activities.

Statistical analysis

All experiments were analyzed by the Student's *t* test. A p value < 0.05 was considered significant.

Results

TNF-α induces transcription of the *IL-34* gene in LX-2 cells

Previous reports have presented varying results on the source of IL-34 in hepatic fibrosis (Preisser et al. 2014; Shoji et al. 2016). We therefore used a human HSC line, LX-2 cells, and a human hepatocyte line, LO2 cells, to examine transcription of the *IL-34* gene in response to TNF-α, which is an identified cytokine that induces IL-34 expression (Chemel et al. 2012; Tian et al. 2013). Results from qPCR experiments indicated that TNF-α increased transcription of the *IL-34* gene in LX-2 cells but not in LO2 cells (Fig. 1). We therefore used LX-2 cells in the experiments going forward.

SEA inhibits TNF-α-induced IL-34 expression

In order to verify whether or not SEA had an effect on IL-34 expression, LX-2 cells were left untreated or treated with SEA and/or TNF-α, and then total RNA was extracted for further analysis by qPCR. The data revealed that SEA treatment resulted in a decrease in TNF-α-induced *IL-34* gene transcription in LX-2 cells, but had little effect on the transcription of the *IL-34* gene without TNF-α stimulation (Fig. 2a). Similar results were obtained at the protein level when examining IL-34 by western blot analysis (Fig. 2b). Together, these data suggested that SEA inhibits TNF-α-induced IL-34 expression.

SEA inhibits TNF-α-induced NF-κB activation

Because activation of NF-κB is required for TNF-α-induced IL-34 expression (Abdlla et al. 2015; Chemel et al. 2012; Yu et al. 2014), we therefore wondered whether or not SEA was involved in TNF-α-induced NF-κB activation. In our reporter assays, SEA inhibited TNF-α-triggered activation of NF-κB (Fig. 3a). Furthermore, SEA consistently inhibited TNF-α-induced transcription of other downstream genes, including *IL-6* and *CCL-2* (Fig. 3b). These findings suggest that SEA inhibits TNF-α-induced NF-κB activation. It has been previously reported that TNF-α-induced NF-κB activation leads to phosphorylation and degradation of IκBα (Hu et al. 2014; Zhang et al. 2014), and results of our western blot analysis showed that, in LX-2 cells, TNF-α treatment resulted in an increase in the phosphorylation and degradation of IκBα, when compared to untreated cells. Although SEA had little effect on the phosphorylation and degradation of IκBα, it did decrease the phosphorylation and degradation of IκBα induced by TNF-α (Fig. 3c). This result not only suggested that SEA modulates TNF-α-induced NF-κB activation, but also indicated that SEA acts on an upstream signaling step of IκBα or targeted IκBα directly. To further determine the molecular mechanisms of SEA's role in the regulation of TNF-α-induced NF-κB signaling, we examined the effects of SEA on the activation of NF-κB mediated by overexpression of several IκBα upstream molecules, including TNF receptor I (TNFR-I), TNFR-associated death domain protein (TRADD), receptor-interacting protein (RIP), and inhibitor κB kinase β (IKKβ). Unexpectedly, the findings showed that SEA failed to inhibit any of these molecules (Fig. 3d).

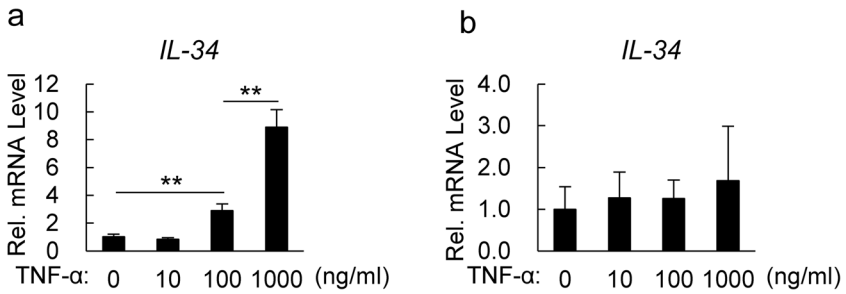

Fig. 1 TNF-α induces transcription of the *IL-34* gene in LX-2 cells. **a** and **b** LX-2 or LO2 cells (1×10^6) were left unstimulated or stimulated with increasing amounts of TNF-α for 36 h, and then total RNA was extracted for qPCR analysis. Graphs show the mean ± SD, $n = 3$. Double asterisks indicate $p < 0.01$

Discussion

Fibrosis is a wound-healing response to hepatic injury, occurring in virtually all types of diseases in which hepatocellular death occurs. Recent research on hepatic fibrosis has identified IL-34 as a profibrotic factor. It has been reported that IL-34 is highly expressed in the serum of patients infected with chronic HCV or HBV, and correlates with fibrosis (Seki and Schwabe 2015; Wang et al. 2018). Additionally, IL-34 is also highly expressed in the serum of NAFLD patients and was found to increase along with the progression of hepatic fibrosis (Shoji et al. 2016). However, the source of IL-34 in hepatic fibrosis has yet to be defined. In this study, TNF-α increased the transcription of the *IL-34* gene in a dose-dependent manner in LX-2 cells but not in LO2 cells. These findings are consistent with the results reported by Shoji et al. that, in NAFLD patients with liver fibrosis, IL-34 is primarily expressed in liver fibroblasts and that the level of IL-34 expression in liver fibroblasts and LX-2 cells increases in response to TNF-α (Shoji et al. 2016).

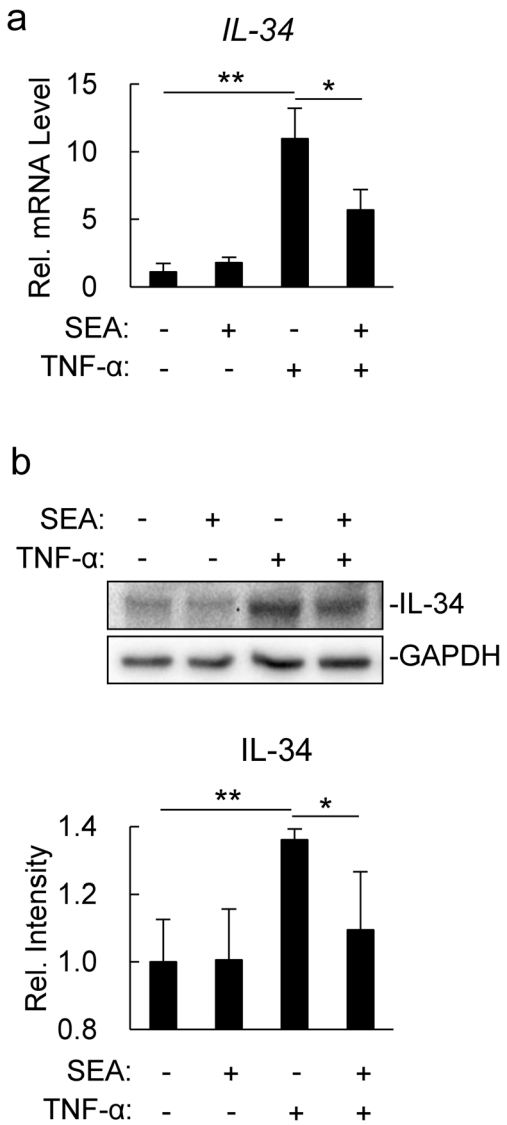

Fig. 2 SEA inhibits TNF-α-induced IL-34 expression. **a** LX-2 cells (1×10^6) were left unstimulated or stimulated with TNF-α (1 μg/ml) and/or SEA (20 μg/ml) for 36 h, and then total RNA was extracted for qPCR analysis. **b** LX-2 cells (1×10^6) were left unstimulated or stimulated with TNF-α (1 μg/ml) and/or SEA (20 μg/ml) for 36 h, and then immunoblot analysis was performed with the indicated antibodies. The histograms show the relative intensities of the bands, which were quantitated by densitometry using Image Lab, normalizing to GAPDH levels. Graphs show the mean ± SD, $n = 3$. Asterisk indicates $p < 0.05$, double asterisks indicate $p < 0.01$

Schistosome eggs and SEA are the primary pathogenic factors for the hepatic fibrosis that occurs in schistosomiasis, and have recently been heavily focused on for their potential anti-fibrotic effects on activated HSCs (Anthony et al. 2010, 2013). In our previous studies, multiple mechanisms on the anti-fibrotic effects of SEA have been elucidated. One study suggests that SEA inhibits activation of HSCs through increasing the expression of peroxisome proliferator-activated receptor γ (PPARγ), an important adipogenic transcriptional regulator involved in maintaining the quiescent HSC phenotype (Duan et al. 2014). Other studies suggest that SEA can promote the apoptosis and senescence of HSCs to remove activated HSCs, leading to attenuation of hepatic fibrosis (Duan et al. 2016; Wang et al. 2014). In this study, we found that SEA inhibited TNF-α-induced IL-34 expression in vitro. Because IL-34 is capable of generating profibrotic macrophages in vitro and then these macrophages induce collagen type I synthesis by HSCs (Preisser et al. 2014), it is therefore possible that SEA decreases profibrotic macrophages by inhibiting IL-34 expression, providing new insight into the possible regulatory mechanism for the anti-fibrotic effects of SEA.

Recent reports have also identified important roles for SEA in multiple signaling pathways, including Toll-like receptor (TLR) signaling (Chen et al. 2016; Correale and Farez 2009), TGF-β signaling (Duan et al. 2014), and Akt signaling (Wang et al. 2014). The results of the current study suggested that SEA is involved in TNF-α-triggered NF-κB signaling. Previous studies have indicated that activation of NF-κB is required for TNF-α-induced IL-34 expression. Furthermore, treatment with pharmacological inhibitors of NF-κB results in 80% inhibition of TNF-α-induced IL-34 (Zwicker et al. 2015). NEMO is one component of the canonical IκB kinase (IKK) complex that is the activator of NF-κB, and its knockout significantly inhibits the effects of TNF-α on *IL-34* gene expression (Chemel et al. 2012). Therefore, we reasoned that SEA inhibited TNF-α-induced IL-34 expression by impairing NF-κB activation. However, we failed to confirm with our current data the exact molecular mechanism by which SEA regulates the NF-κB activation triggered by TNF-α. According to the findings of our reporter assays, SEA inhibited TNF-α-triggered activation of NF-κB, but not the activation of NF-κB mediated by TNFR-I or its downstream

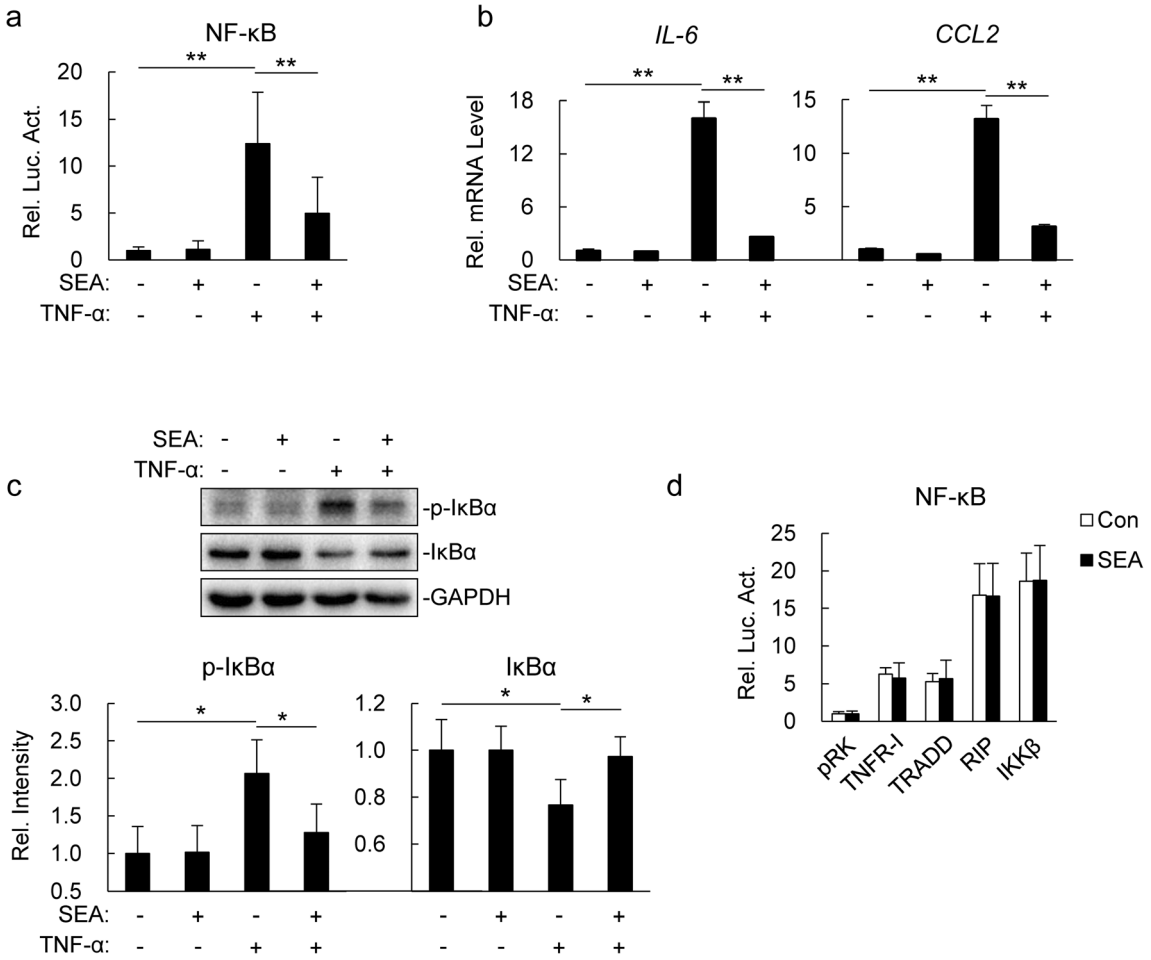

Fig. 3 SEA inhibits TNF-α-induced NF-κB activation. **a** LX-2 cells (1×10^5) were transfected with the NF-κB luciferase reporter plasmid (0.5 μg) and pRL-TK reporter plasmid (0.2 μg). After 24 h post-transfection, cells were left unstimulated or stimulated with TNF-α (1 μg/ml) and/or SEA (20 μg/ml) for another 36 h at which point luciferase assays were performed. **b** LX-2 cells (1×10^6) were left unstimulated or stimulated with TNF-α (1 μg/ml) and/or SEA (20 μg/ml) for 36 h, and then total RNA was extracted for qPCR analysis. **c** LX-2 cells (1×10^6) were left unstimulated or stimulated with TNF-α (1 μg/ml) and/or SEA (20 μg/ml) for 36 h before immunoblot analysis was performed with the indicated antibodies. **d** LX-2 cells (1×10^5) were transfected with the indicated expression plasmids (0.35 μg), NF-κB luciferase reporter plasmid (0.5 μg), and pRL-TK reporter plasmid (0.2 μg) for 24 h, after which cells were left unstimulated or stimulated with SEA (20 μg/ml) for 36 h before luciferase assays. The histograms show the relative intensities of the bands, which were quantitated by densitometry using Image Lab, normalizing to GAPDH levels. Graphs show the mean ± SD, $n = 3$. Asterisk indicates $p < 0.05$, double asterisks indicate $p < 0.01$

molecules. These results indicated that SEA might regulate TNF-α-induced NF-κB signaling upstream of TNFR-I. Since TNFR-I is the receptor of TNF-α, it is possible that SEA might disrupt the interaction of TNF-α and its receptor. Interestingly, we found that SEA inhibited TNF-α-induced IL-34 expression, but not constitutive expression of IL-34 in LX-2 cells. This observation may be due to low expression of IL-34. Others have also demonstrated that, under physiological conditions, IL-34 expression is weak or undetectable in the liver (Wang et al. 2012). In addition, we cannot exclude the possibility that SEA inhibits only ectopic expression of IL-34 in the inflammatory state, rather than its expression under physiological conditions.

In conclusion, we have shown that SEA inhibits TNF-α-induced IL-34 expression through impairing NF-κB activation in hepatic stellate cells. Our studies reveal a previously unidentified target and signaling pathway involving SEA and provide a new clue in the search to elucidate the anti-fibrotic effects of SEA.

Acknowledgments We thank Dr. Hongbing Shu (Wuhan University) for providing the indicated plasmids.

Funding This work was supported by the National Natural Science Foundation of China (81871677, 81471975), Jiangsu Planned Projects for Postdoctoral Research Funds (1701170B), and the Priority Academic Program Development of Jiangsu Higher Education Institution (PAPD).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdlla OA, Elboshy ME, Reisha EF, Gadlla HA, El-Khodery SA (2015) Tumor necrosis factor-alpha, interleukins-12(p40), 6, and 10 levels in cerebrospinal fluid and outcome prediction in Ossimi sheep with encephalitic listeriosis. Cytokine 73:283–287. https://doi.org/10.1016/j.cyto.2015.03.004

Anthony B, Mathieson W, de Castro-Borges W, Allen J (2010) Schistosoma mansoni: egg-induced downregulation of hepatic stellate cell activation and fibrogenesis. Exp Parasitol 124:409–420. https://doi.org/10.1016/j.exppara.2009.12.009

Anthony BJ, James KR, Gobert GN, Ramm GA, McManus DP (2013) Schistosomajaponicum eggs induce a proinflammatory, anti-fibrogenic phenotype in hepatic stellate cells. PLoS One 8:e68479. https://doi.org/10.1371/journal.pone.0068479

Baghdadi M, Endo H, Takano A, Ishikawa K, Kameda Y, Wada H, Miyagi Y, Yokose T, Ito H, Nakayama H, Daigo Y, Suzuki N, Seino KI (2018) High co-expression of IL-34 and M-CSF correlates with tumor progression and poor survival in lung cancers. Sci Rep 8:418. https://doi.org/10.1038/s41598-017-18796-8

Chemel M, Le Goff B, Brion R et al (2012) Interleukin 34 expression is associated with synovitis severity in rheumatoid arthritis patients. Ann Rheum Dis 71:150–154. https://doi.org/10.1136/annrheumdis-2011-200096

Chen J, Pan J, Wang J, Song K, Zhu D, Huang C, Duan Y (2016) Soluble egg antigens of Schistosoma japonicum induce senescence in activated hepatic stellate cells by activation of the STAT3/p53/p21 pathway. Sci Rep 6:30957. https://doi.org/10.1038/srep30957

Chiu YS, Wei CC, Lin YJ, Hsu YH, Chang MS (2014) IL-20 and IL-20R1 antibodies protect against liver fibrosis. Hepatology 60:1003–1014. https://doi.org/10.1002/hep.27189

Ciccia F, Alessandro R, Rodolico V, Guggino G, Raimondo S, Guarnotta C, Giardina A, Sireci G, Campisi G, de Leo G, Triolo G (2013) IL-34 is overexpressed in the inflamed salivary glands of patients with Sjogren's syndrome and is associated with the local expansion of pro-inflammatory CD14(bright)CD16+ monocytes. Rheumatology (Oxford) 52:1009–1017. https://doi.org/10.1093/rheumatology/kes435

Correale J, Farez M (2009) Helminth antigens modulate immune responses in cells from multiple sclerosis patients through TLR2-dependent mechanisms. J Immunol 183:5999–6012. https://doi.org/10.4049/jimmunol.0900897

Dooley S, ten Dijke P (2012) TGF-beta in progression of liver disease. Cell Tissue Res 347:245–256. https://doi.org/10.1007/s00441-011-1246-y

Duan Y, Gu X, Zhu D, Sun W, Chen J, Feng J, Song K, Xu F, He X, He X (2014) Schistosoma japonicum soluble egg antigens induce apoptosis and inhibit activation of hepatic stellate cells: a possible molecular mechanism. Int J Parasitol 44:217–224. https://doi.org/10.1016/j.ijpara.2013.11.003

Duan Y, Pan J, Chen J, Zhu D, Wang J, Sun X, Chen L, Wu L (2016) Soluble egg antigens of Schistosoma japonicum induce senescence of activated hepatic stellate cells by activation of the FoxO3a/SKP2/P27 pathway. PLoS Negl Trop Dis 10:e0005268. https://doi.org/10.1371/journal.pntd.0005268

Hu MM, Yang Q, Zhang J et al (2014) TRIM38 inhibits TNFalpha- and IL-1beta-triggered NF-kappaB activation by mediating lysosome-dependent degradation of TAB2/3. Proc Natl Acad Sci U S A 111:1509–1514. https://doi.org/10.1073/pnas.1318227111

Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D, Hollenbaugh D, Linnemann T, Qin M, Wong J, Chu K, Doberstein SK, Williams LT (2008) Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science 320:807–811. https://doi.org/10.1126/science.1154370

Luo J, Elwood F, Britschgi M, Villeda S, Zhang H, Ding Z, Zhu L, Alabsi H, Getachew R, Narasimhan R, Wabl R, Fainberg N, James ML, Wong G, Relton J, Gambhir SS, Pollard JW, Wyss-Coray T (2013) Colony-stimulating factor 1 receptor (CSF1R) signaling in injured neurons facilitates protection and survival. J Exp Med 210:157–172. https://doi.org/10.1084/jem.20120412

McHedlidze T, Waldner M, Zopf S et al (2013) Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. Immunity 39:357–371. https://doi.org/10.1016/j.immuni.2013.07.018

Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, Pradere JP, Schwabe RF (2013) Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. Nat Commun 4:2823. https://doi.org/10.1038/ncomms3823

Meng F, Wang K, Aoyama T, Grivennikov SI, Paik YH, Scholten D, Cong M, Iwaisako K, Liu X, Zhang M, Österreicher CH, Stickel F, Ley K, Brenner DA, Kisseleva T (2012) Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology 143:765–776.e763. https://doi.org/10.1053/j.gastro.2012.05.049

Preisser L, Miot C, Le Guillou-Guillemette H et al (2014) IL-34 and macrophage colony-stimulating factor are overexpressed in hepatitis C virus fibrosis and induce profibrotic macrophages that promote collagen synthesis by hepatic stellate cells. Hepatology 60:1879–1890. https://doi.org/10.1002/hep.27328

Seki E, Schwabe RF (2015) Hepatic inflammation and fibrosis: functional links and key pathways. Hepatology 61:1066–1079. https://doi.org/10.1002/hep.27332

Shoji H, Yoshio S, Mano Y, Kumagai E, Sugiyama M, Korenaga M, Arai T, Itokawa N, Atsukawa M, Aikata H, Hyogo H, Chayama K, Ohashi T, Ito K, Yoneda M, Nozaki Y, Kawaguchi T, Torimura T, Abe M, Hiasa Y, Fukai M, Kamiyama T, Taketomi A, Mizokami M, Kanto T (2016) Interleukin-34 as a fibroblast-derived marker of liver fibrosis in patients with non-alcoholic fatty liver disease. Sci Rep 6:28814. https://doi.org/10.1038/srep28814

Tan Z, Qian X, Jiang R, Liu Q, Wang Y, Chen C, Wang X, Ryffel B, Sun B (2013) IL-17A plays a critical role in the pathogenesis of liver fibrosis through hepatic stellate cell activation. J Immunol 191:1835–1844. https://doi.org/10.4049/jimmunol.1203013

Tian Y, Shen H, Xia L, Lu J (2013) Elevated serum and synovial fluid levels of interleukin-34 in rheumatoid arthritis: possible association with disease progression via interleukin-17 production. J Interf Cytokine Res 33:398–401. https://doi.org/10.1089/jir.2012.0122

Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, Barrow AD, Diamond MS, Colonna M (2012) IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat Immunol 13:753–760. https://doi.org/10.1038/ni.2360

Wang J, Xu F, Zhu D, Duan Y, Chen J, Sun X, He X, Li P, Sun W, Feng J (2014) Schistosoma japonicum soluble egg antigens facilitate hepatic stellate cell apoptosis by downregulating Akt expression and upregulating p53 and DR5 expression. PLoS Negl Trop Dis 8:e3106. https://doi.org/10.1371/journal.pntd.0003106

Wang YQ, Cao WJ, Gao YF, Ye J, Zou GZ (2018) Serum interleukin-34 level can be an indicator of liver fibrosis in patients with chronic hepatitis B virus infection. World J Gastroenterol 24:1312–1320. https://doi.org/10.3748/wjg.v24.i12.1312

Xie HH, Shen H, Zhang L, Cui MY, Xia LP, Lu J (2018) Elevated serum interleukin-34 level in patients with systemic lupus erythematosus is associated with disease activity. Sci Rep 8:3462. https://doi.org/10.1038/s41598-018-21859-z

Yu Y, Yang D, Qiu L, Okamura H, Guo J, Haneji T (2014) Tumor necrosis factor-alpha induces interleukin-34 expression through nuclear factorkappaB activation in MC3T3-E1 osteoblastic cells. Mol Med Rep 10:1371–1376. https://doi.org/10.3892/mmr.2014.2353

Zhang Y, Lei C-Q, Hu Y-H, Xia T, Li M, Zhong B, Shu H-B (2014) Krüppel-like factor 6 is a co-activator of NF-κB that mediates p65-dependent transcription of selected downstream genes. J Biol Chem 289:12876–12885. https://doi.org/10.1074/jbc.M113.535831

Zhou SL, Hu ZQ, Zhou ZJ, Dai Z, Wang Z, Cao Y, Fan J, Huang XW, Zhou J (2016) miR-28-5p-IL-34-macrophage feedback loop modulates hepatocellular carcinoma metastasis. Hepatology 63: 1560–1575. https://doi.org/10.1002/hep.28445

Zwicker S, Martinez GL, Bosma M, Gerling M, Clark R, Majster M, Söderman J, Almer S, Boström EA (2015) Interleukin 34: a new modulator of human and experimental inflammatory bowel disease. Clin Sci (Lond) 129:281–290. https://doi.org/10.1042/CS20150176