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Construction and expression of human scFv-Fc against interleukin-33

Yingchun Ye, Siji Nian, Wenfeng Xu, Tong Wu, Xu Wang, Yan Gao, Qing Yuan *

Department of Basic Medicine, Luzhou Medical College, Luzhou, Sichuan Province 646000, China

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ABSTRACT

Interleukin-33 (IL-33) is a member of the IL-1 family and the ligand of orphan ST2 molecules. IL-33 is widely expressed in multiple tissues and cells, and mainly involved in regulating Th2 immune and inflammatory responses. Inhibiting IL-33 signaling pathways relieves the symptoms of allergic inflammation, indicating that IL-33 is a potential target for the treatment of allergic diseases. In this study, the recombinant vectors SP-scFv-Fc/pcDNA3.1 and SP-scFv-Fc/PMH3^{EN} were constructed to express a human scFv-Fcs against IL-33. The size of the inserted SP-scFv-Fc was approximately 1540 bp. The RT-PCR results showed that SP-scFv-Fcs were successfully transfected into CHO K1 cells. Western blot analysis indicated specific binding of the expressed scFv-Fcs fusion protein (approximately 60 kDa under reduced condition) with a goat anti-human IgG1 Fc antibody. The expression level of the scFv-Fcs from SP-scFv-Fc/PMH3^{EN} was higher than that from SP-scFv-Fc/pcDNA3.1. A single high-expressing cell line was selected after three rounds of screening and the fusion protein was expressed in a suspension culture in serum-free medium. The level of expression products reached 20 mg/L and the expressed and purified scFvs was further characterized and analyzed for bioactivity and functionality. The recombinant vectors for eukaryotic expression of scFv-Fcs against IL-33 were successfully constructed and the expressed scFv-Fcs was shown to be a suitable candidate for the development of a new therapy for allergic and autoimmune diseases.

Introduction

Interleukin-33 (IL-33) is a member of the IL-1 cytokine family. Based on the cDNA sequence, this protein contains 270 amino acids with a predicted molecular mass of 30 kDa. It is expressed by many cells and tissues, including the spleen, stomach, brain, heart, bronchial epithelial cells, fibroblasts, smooth muscle cells, macrophages, keratinocytes, and dendritic cells (DCs) [1–3]. The IL-33 receptor, ST2, was first identified as an IL-1 receptor-like molecule (the gene symbol was designated *IL1R1*) by Tominaga in 1989. Two forms of the receptor exist; a membrane-bound form expressed on hematopoietic tissues and lung and a soluble form induced by stimulation of fibroblasts [4].

IL-33 is considered to be crucial for the induction of T-helper type Th2 cell-dominant immune responses, such as host defense against allergic diseases [5,6]. IL-33 is released by epithelial cells during injury or necrosis caused by exogenous triggers such as mechanical trauma, viruses, smoke, airborne allergens, or endogenous stimuli. These triggers also lead to activation of pattern recognition receptors such as TLRs, and as a consequence, the release of IL-33 from epithelial cells. The released IL-33 binds to the ST2L and stimulates the intracellular signaling pathway, leading to the activation of NF-κB and mitogen-activated protein kinases. IL-33 signaling via ST2L plays important roles in Th2 cell-mediated immunological responses including the production of Th2 cytokines [7,8]. IL-33 is believed to be involved in Th2-mediated inflammatory responses in allergic diseases such as asthma, anaphylaxis, and atopic dermatitis; thus, inhibiting IL-33 signaling pathways could relieve the symptoms of allergic inflammation [9–12].

Based on the important roles of IL-33 in allergic diseases, anti-IL33 antibodies are implicated as potential therapeutic molecules. In previous studies, we have successfully selected a human single-chain Fv fragment (scFvs) against human IL-33 from a non-immune human scFv library by phage display [13]; however, this scFvs is not stable and its half-life time in vivo is short. In this study, we aimed to develop a stable antibody fragment (scFv-Fc) by fusing the scFv with a human IgG1 Fc to improve the stability and half-life of the molecule. Here, two recombinant vectors

Abbreviations: IL-33, interleukin-33; DCs, dendritic cells; scFv, single-chain Fv fragment; PBMC, peripheral blood mononuclear cells; SP, signal peptide; ECL, chemiluminescence.

* Corresponding author at: Room 218, Hanguang Building, No. 319, Zhongshan Road, Luzhou Medical College, Luzhou City, Sichuan Province 646000, China.

E-mail addresses: 351584908@qq.com (Y. Ye), sijinian@hotmail.com (S. Nian), x.wenfeng@yahoo.com (W. Xu), 7631046@qq.com (T. Wu), wangxu322@163.com (X. Wang), 747094571@qq.com (Y. Gao), yuanqing_ok@yahoo.com (Q. Yuan).

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(SP-scFv-Fc/pcDNA3.1, SP-scFv-Fc/PMH3^{EN}) were constructed for expression of scFv-Fcs, and the expression levels were compared. Finally, high-expressing cell lines were selected and the scFv-Fcs were expressed and indentified.

Materials and methods

Materials

Peripheral blood was obtained from healthy volunteers for isolation of peripheral blood mononuclear cells (PBMC) using procedures approved by the Ethics Committee of Luzhou Medical College (No. 5105025012142). The volunteers were adults provided informed consent to participation in this study.

Construction of recombinant vector SP-scFv-Fc/pcDNA3.1

PBMCs were isolated from healthy volunteers using TRIzol Reagent (Invitrogen, USA) and the total RNA was extracted with an RNA extract kit (Invitrogen). The cDNA of IgG1 Fc (hinge + CH2 + CH3) was synthesized and amplified by RT-PCR with the following primer pair: Fc-F-*BamH*I/Fc-R-*Not*I (Table 1). The plasmid DNA of pcDNA 3.1 (+) (bought from Invitrogen) and IgG1 Fc were digested by *BamH*I and *Not*I, then ligated with T4 DNA ligase to generate the Fc/pcDNA3.1 (+) recombinant vector and then transformed into *Escherichia coli* TOP10. The positive clones with the correct Fc insert were identified by PCR and sequencing.

The signal peptide (SP; MDWTWRILFLVAAATGTHA) was synthesized by GenScript (USA). The Fc/pcDNA3.1 (+) plasmid and the SP were digested with *Hind* III and *Kpn* I, ligated by T4 ligase to generate the SP-Fc/pcDNA3.1 (+) recombinant vector, which was then transformed into *E. coli* TOP10. Positive clones with the correct SP were identified by PCR and sequencing.

The anti-human IL-33 scFv88 was selected and identified in our previous study. The SP-Fc/pcDNA3.1 (+) plasmid and scFv88 (PCR amplified by scFv88F-*Kpn* I/scFv88R-*BamH* I, Table 1) were digested by *Kpn* I and *BamH* I and ligated by T4 ligase to generate the SP-scFv-Fc/pcDNA3.1 (+) recombinant vector, which was then transformed into *E. coli* TOP10. Positive clones with the correct scFv88 were identified by PCR and sequencing (Fig. 1).

Construction of recombinant vector SP-scFv-Fc/PMH3^{EN}

The SP-scFv-Fc/pcDNA3.1 (+) plasmid was extracted and the SP-scFv-Fc fragment was excised from the recombinant vector. The PMH3^{EN} plasmid (bought from Amprotein. Co, China, and the number of the international patent was WO/2008/091276) and SP-scFv-Fc were digested with *Hind* III and *Not* I, ligated with T4 DNA ligase and transformed into *E. coli* TOP10. The positive clones with the correct SP-scFv-Fc inserts were identified by PCR and sequencing.

Table 1
The primers used in this study.

Primers	Sequences
Fc-F-*BamH* I	5′CGGGATCCGAGTCCAAATCTTGTGAC3′
Fc-R-*Not* I	5′ATAAGAATGCGGCCGCTCATTTACCCGGAGACAGGGA3′
P1	5′CTGTGGCATCCACGAAACTA3′
P2	5′ACATCTGCTGGAAGGTGGAC3′
ScFv88F-*Kpn* I	5′GGGGTACCCGGCCCAGCCGGCCCAGGT3′
scFv88R-*BamH* I	5′CGGGATCCACGTTTGATATCCACTTTGGTCCC3′

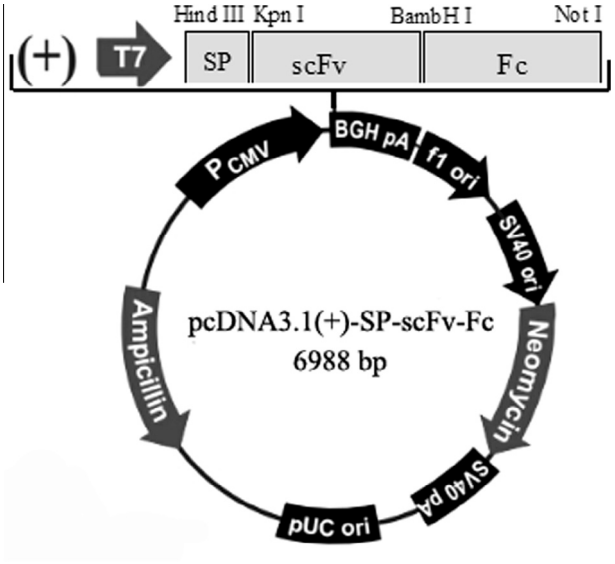

Fig. 1. The plasmid map of pcDNA3.1-SP-scFv-Fc.

Transfection of CHO K1 cells

CHO K1 cells were cultured to 85% confluence in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C under 5% CO_2. The recombinant plasmids SP-scFv-Fc/pcDNA3.1 (+) and SP-scFv-Fc/PMH3^{EN} were then transfected into CHO K1 cells using the Lipofectamine 2000 transfection reagent (Invitrogen). After 24 h, the cells were screened with G418 (600 μg/mL, life technologies, USA) and the medium was changed every 5 days. Non-transfected CHO K1 cells were used as a negative control. When the control cells were all dead, the transfected cells were cultured in medium containing 300 μg/mL G418 for a further 6 weeks; positively transfected cells were then identified by RT-PCR and Western blot analyses.

Identification of transfected cells

When the proportion of transfected cells in the culture reached to 85%, the medium was replaced with serum-free medium (sigma). The total RNA of cells transfected with the SP-scFv-Fc/pcDNA3.1 (+) and SP-scFv-Fc/PMH3^{EN} plasmids was extracted. The Fc fragments were amplified by RT-PCR with the following primer pair: Fc-F-*BamH* I/Fc-R-*Not* I (Table 1) to detect the transcriptional level of scFv-Fcs; non-transfected cells were used as a control and β-actin was used as an internal reference (amplified with primer pair P1/P2; Table 1).

The transfected cells were lysed and the total proteins were extracted. The extracted proteins and the cell culture supernatant were separated by SDS–PAGE and electrotransferred onto a nitrocellulose membrane. scFv-Fcs were detected using an HRP-conjugated goat anti-human IgG Fc antibody (Abcam, UK) and an enhanced chemiluminescence (ECL) reagent (Millipore). β-Actin was used as an internal reference.

Screening of high-expressing stable cell lines

The positively transfected cells were cultured for 21 d, and then counted. Single cells were picked and cultured in 96-well plates at 37 °C under 5% CO_2. When the proportion of transfected cells reached 85%, the medium were replaced with serum-free medium, and the cells were cultured for a further 5 d at 37 °C under 5% CO_2. The scFv-Fcs secreted into the serum-free medium was detected by