International Immunopharmacology 18 (2014) 365–372

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Equine adipose-derived stem cell (ASC) expresses BAFF and its receptors, which may be associated with the differentiation process of ASC towards adipocyte

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ARTICLE INFO

Article history:
Received 28 March 2013
Received in revised form 17 December 2013
Accepted 18 December 2013
Available online 3 January 2014

Keywords:
Equine
Horse
BAFF
Adipose-derived stem cell
Adipogenic differentiation

ABSTRACT

B cell activating factor (BAFF) and its receptors were regarded as elements of the immune system, regulating the fate of B cell. In recent years, these molecules were identified in a number of normal and pathological tissues, expanding their potential functions beyond the immune system. In this study, on the basis of molecular clone and prokaryotic expression of equine BAFF, we reported that equine adipose-derived stem cell (ASC) expressed BAFF and its receptors, which exhibited the increased expression during ASC adipogenic differentiation *in vitro*. Moreover, with the addition of recombinant protein His_6-sBAFF, an increased differentiation of equine ASC towards adipocyte was detected. These results suggested that BAFF and its receptors might be associated with the differentiation process of ASC towards adipocyte in horse.

1. Introduction

The TNF superfamily members and their receptors modulate diverse biological functions including cell proliferation, differentiation, survival, and apoptosis. B cell activating factor belonging to the TNF family (BAFF, or BLyS, THANK, TALL-1, TNFSF13B) is a key B cell survival factor [1]. It specifically binds to BAFF receptor (BAFF-R), and shares two receptors, transmembrane activator and CAML interactor (TACI), B cell maturation antigen (BCMA), with A proliferation-inducing ligand (APRIL, TNFSF13) [2]. BAFF is mainly produced by innate immune cells such as neutrophil, macrophage, monocyte and dendritic cell [3]. It is also produced by malignant B cell and acts as an essential autocrine survival factor for malignant B cell [4]. A recent development is the identification of non-hematopoietic cells that express BAFF, such as cytotrophoblast [5], epithelial cell [6], astrocyte [7], adipogenic-differentiated bone marrow mesenchymal stem cell [8], adipocyte, and adipose-derived stem cell [9,10], indicating that the role of BAFF may extend beyond that of B cell biology.

Adipose tissue is now recognized as an endocrine and immune organ, secreting hormone, cytokine, chemokine, and growth factor that influence a wide variety of processes [11]. Moreover, the adipose tissue of adult animal contains a population of mesenchymal stem cell, termed adipose-derived stem cell (ASC), which is capable of differentiating into different mesenchymal tissue types, including adipocyte [12], osteoblast [13], chondrocyte [14], myocyte [15], and cardiomyocyte [16]. As adipose cell is mesodermal in origin, the differentiations of ASC into neural tissue of ectodermal origin [17], pancreatic-like cell [18] and hepatocyte-like cell [19] of endodermal origin are thought to be very surprising. Furthermore, ASC may also provide angiogenic [20] and hematopoietic [21] support. Consequently, ASC has a wide range of potential clinical uses in regenerative therapy.

Horses (*Equus caballus*) have substantial value in sports and recreation fields, and hold enormous potential as a model for a range of medical conditions commonly found in humans, such as injury to muscle, tendon, ligament, and joint. Stem cell is of particular interest both for basic research and for the therapeutic approach to these damages. Recently, equine induced pluripotent stem line was established successfully [22]. The therapeutic approach using autologous mesenchymal stem cell grafting was also developed in horse [23,24]. In this study, we explored the relationship between equine BAFF and its ASC, which may provide the basis for investigation into the role of BAFF in regulating ASC adipogenesis in horse.

2. Materials and methods

2.1. Cloning of equine BAFF cDNA

The human BAFF mRNA (GenBank accession no. AF116456) was used to search for the equine *BAFF* gene from NCBI horse (*E. caballus*)

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http://dx.doi.org/10.1016/j.intimp.2013.12.018

genomic database (http://blast.ncbi.nlm.nih.gov/). Total RNA was isolated from blood of a Mongolian female horse (Nanjing Hongshan Forest Zoo) using TRIzol (GIBCO/BRL, USA). First-strand cDNA was synthesized from 1 μg of RNA using M-MLV Reverse Transcriptase (Promega, USA). Nest PCR primers, B1 and B2, B3 and B4, were used for BAFF cDNA amplification (Table 1). The PCR product of expected size was cloned with *pEASY*-T1 Vector (TransGen, China) and sequenced with the ABI Prism automated sequencing method (YingJun, China). At least three independent sequencing experiments were conducted to rule out error introduced by PCR. Finally, the equine BAFF genomic DNA, cDNA and putative protein sequences were submitted to NCBI GenBank under the accession numbers of GU989642, GU982934, and ADZ54788.

2.2. Bioinformatics analysis

In order to determine the exact location of the exon–intron boundary, the mRNA-to-genomic alignment program (www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/index.html) was used. Multiple sequence alignment was performed with the ClustalW Multiple Alignment program (http://www.ebi.ac.uk/clustalw/). The protein was analyzed with the Expert Protein Analysis System (http://www.expasy.org/). The three dimensional (3D) structure of protein was determined by homology modeling program on ESyPred3D Web Server 1.0 (http://www.fundp.ac.be/sciences/biologie/urbm/bioinfo/esypred/) and visualized by PyMOL Molecular Viewer (http://www.pymol.org/).

2.3. Protein prokaryotic expression and purification

The DNA fragment that encodes equine soluble BAFF (sBAFF, aa139–290) was generated by PCR with the primers B5 and B6 (Table 1). The PCR product was ligated into the *Nde* I/*Hind* III sites within pET28a vector (Novagen, USA), forming a sequence that encodes a fusion protein of equine sBAFF and a NH_2-terminal His_6-tag.

The recombinant plasmid was transformed into *Escherichia coli* BL21 (DE3) (Novagen, USA) to express His_6-tagged equine sBAFF. The bacteria were cultured in LB medium with vigorous shaking (220 rpm) at 37 °C to a density of 0.6 (OD600). Then, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the final 0.5-mM concentration, and bacteria were grown at 20 °C with continuous shaking for another 20 h. The induced culture was centrifuged at 6000 *g* for 15 min, and resuspended in binding buffer (20 mM Tris–HCl, 500 mM NaCl, and 50 mM imidazole, pH 7.9). After sonication and 10,000-*g* centrifugation at 4 °C for 15 min, the supernatant was applied to Ni^{2+}-IDA affinity resin that was pre-equilibrated with binding buffer. The column was washed with increasing concentration of imidazole, and eluted with elution buffer (20 mM Tris–HCl, 500 mM NaCl, 250 mM imidazole, pH 7.9). Finally, with an Amicon Ultra-15 3K centrifugal filter device (Millipore, USA), purified sample was concentrated and desalted with Phosphate Buffered Saline (PBS, 2 mM KH_2PO4, 10 mM Na_2HPO4, 2.7 mM KCl, 137 mM NaCl).

The expression and purification of His_6-sBAFF was analyzed by using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot with rabbit anti-His_6 antibody and HRP-conjugated goat anti-rabbit IgG (Boster, China).

2.4. Immunofluorescence staining

Equine adipose-derived stem cell (ScienCell, USA) was cultured in normal medium consisting of high glucose DMEM (GIBCO/BRL, USA) supplemented with 10% fetal bovine serum (GIBCO/BRL, USA) and 100 U/mL penicillin/streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

For immunofluorescence staining, equine ASC (passage 3, 80% confluent) was fixed and permeabilized in PBS with 4% paraformaldehyde and 0.2% Triton X-100 at 37 °C for 30 min. After washing with PBS, the cells were incubated with 200 ng/mL His_6-sBAFF or BAFF-R-Fc (based on the cloning of horse BAFF-R cDNA (GenBank accession no. JF502272), its extracellular domain fused with Fc fragment was expressed in *E. coli* BL21 (DE3) and purified with protein A affinity chromatography, data not shown) at 37 °C overnight. Afterwards, the cells were washed, and blocked with 3% bovine serum albumin at 37 °C for 1 h. For the detection of BAFF-R-Fc binding to ASC, the cells were incubated with 1:100 dilution of FITC-conjugated goat anti-human IgG (Invitrogen, USA) at 37 °C for 2 h. For the detection of His_6-sBAFF binding to ASC, the cells were incubated with 1:2000 dilution of mouse anti-His antibody (TIANGEN, China) at 4 °C overnight, and then 1:100 dilution of R-PE-conjugated goat anti-mouse IgG (Invitrogen, USA) at 37 °C for 2 h. The cells were then washed and counterstained with 0.5 μg/mL 4′ 6-diamidino-2-phenylindole (DAPI) at 37 °C for 20 min. After a further wash, the culture slide was mounted in antifade mounting medium (Beyotime, China). Analysis

Table 1
Primers used in this study.

Amplification	Primer	Sequence (5′–3′)	Nucleotide position
Cloning of equine BAFF	B1 (Forward)	CGACAGCCAAGCTGGGCGATGTAGTC	−122 to −97 (gene)
	B2 (Reverse)	CGCCCTACAGATGTGGGCACCGG	31,201 to 31,223 (gene)
	B3 (Forward)	GGTCACTTATTCTAAAGGCCCGAACC	−46 to −21 (gene)
	B4 (Reverse)	GACACCCTTTGGCTGTGGTTGTCGG	31,007 to 31,031 (gene)
Prokaryotic expression of equine sBAFF	B5 (Forward)	AAGAATTCCATATGGCCGCTCAGGACTCAG	415 to 430 (cDNA)
	B6 (Reverse)	GCGAAGCTTTCACAGAAGTTTCAGTGC	856 to 873 (cDNA)
qRT-PCR of equine BAFF	Forward	ACCCAAGACTGCTTGCAACT	442 to 461 (cDNA)
	Reverse	GGCAAAGGTGTTATCGGTGT	617 to 636 (cDNA)
qRT-PCR of equine BAFF-R	Forward	AGGACGAGGCTCTGGACAAT	365 to 384 (cDNA)
	Reverse	TCTTGGTGGTCACCAGCTC	511 to 529 (cDNA)
qRT-PCR of equine TACI	Forward	TGTTGAGGGACTGTGTCAGC	200 to 219 (cDNA)
	Reverse	GCTCTGGTCCCTGGTACCTT	357 to 376 (cDNA)
qRT-PCR of equine BCMA	Forward	CTTGGGCCTGAGCTTGATAG	480 to 499 (cDNA)
	Reverse	AGGCCTCTCGGAAGAAGAAC	640 to 659 (cDNA)
qRT-PCR of equine PPARγ	Forward	CCAGAAAGCGATTCTTTTGC	106 to 125 (cDNA)
	Reverse	CAACCATGGGATCAGCTCTT	324 to 343 (cDNA)
qRT-PCR of equine LPL	Forward	GAAGCATTGGGATCCAGAAA	635 to 654 (cDNA)
	Reverse	AGCCCTTTCTCAAAGGCTTC	862 to 881 (cDNA)
qRT-PCR of equine GUSB	Forward	GTCCAGGGCAGTGAACATTT	559 to 578 (cDNA)
	Reverse	CCACAGGGAGAGCGTAGAAG	765 to 784 (cDNA)