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Characterization and signaling pathway analysis of interferon-kappa in bovine

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ABSTRACT

A bovine interferon-kappa (BoIFN-κ) gene was amplified, which encodes a protein of 215 amino acids sharing 63% identity with human IFN-κ. BoIFN-κ was demonstrated to have antiviral and antiproliferative activities. Moreover, BoIFN-κ was shown to be highly sensitive to trypsin, however, it remained stable despite changes in pH and temperature. Result showed that BoIFN-κ can bind with bovine type I IFN receptors, and the antiviral activity can be blocked by antibodies against type I IFN receptors or BoIFN-κ. Additionally, BoIFN-κ can induce the transcription of Mx1, ISG15 and ISG56 gene, as well as the expression of Mx1 protein. The NF-κB, ISRE, and BoIFN-β promoter can all be activated by BoIFN-κ. This study revealed that BoIFN-κ exhibits the typical characteristics of type I IFNs and exerts antiviral activity via activation of the JAK-STAT signaling pathway. Overall, these findings will enrich the current knowledge about IFN-κ and facilitate further research on the role of type I IFN in antiviral defense responses in bovine.

1. Introduction

Interferons (IFNs) are a family of cytokines with antiviral, growth inhibitory, and immunomodulatory activities, which are produced naturally by white blood cells in response to a virus, bacteria, or other foreign intruder. All type I IFNs (α, β, ω, κ, ε, δ, and τ subtypes) interact with a single cell surface receptor (IFNAR), which is composed of IFNAR1 and IFNAR2 subunits (Doly et al., 1998). After IFN binds to their receptors on cells, signaling occurs via activation of the JAK-STAT signaling pathway, resulting in the activation of antiviral genes containing ISREs in their promoters (Darnell et al., 1994). During this process, IFN stimulates hundreds of downstream IFN-stimulated genes (ISGs) expression and induces the production of antiviral protein. ISGs take on a wide range of activities, many of them control viral, bacterial, and parasite infection by directly targeting pathways and functions required during pathogen life cycles, which leads to a remarkable antiviral state, effective against positive-, negative-, and double-stranded RNA viruses, DNA viruses, and intracellular bacteria and parasites (Schneider et al., 2014). NF-κB is an essential positive regulator for the activation of the IFN-β gene (Hiscott et al., 1989; Lenardo et al., 1989); this transcription factor leads to the expression of antiviral genes and plays a general role in gene regulation as a mediator of inducible signal transduction. Therefore, the activation of NF-κB is important in the inhibition of viral replication by stimulating innate and adaptive immune responses in the host (Lenardo et al., 1989).

IFN-κ represents a novel subclass of type I IFNs exhibiting structural homology and functional similarity to other type I IFN subclasses (LaFleur et al., 2001). Unlike IFN-α, the expression of human IFN-κ (HuIFN-κ) has been observed only in keratinocytes, monocytes, and monocyte-derived dendritic cells (LaFleur et al., 2001; Nardelli et al., 2002). The mRNA expression of HuIFN-κ can be upregulated by viral infection, as well as by other type I IFNs and IFN-γ (Nardelli et al., 2002). HuIFN-κ binds to the type I IFN receptor complex, and this interaction induces signaling events such as expression of the Mx1 protein (LaFleur et al., 2001). HuIFN-κ was demonstrated to protect human cells of fibroblastic origin from infection by two types of viruses, namely, vesicular stomatitis virus

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(VSV) and encephalomyocarditis virus (EMCV) (LaFleur et al., 2001). To date, studies on IFN-κ are largely restricted to humans and mouse, and bovine IFN-κ (BoIFN-κ) has not been explored in detail. BoIFN-κ, a novel member of the type I IFN family, herein was identified and characterized. BoIFN-κ was demonstrated to have antiviral and antiproliferative activities, transduces signals through binding with the type I IFN receptor complex, induction of ISGs expression, and activation of the NF-κB, ISRE and BoIFN-β promoter in the signaling pathway.

2. Materials and methods

2.1. Cells, virus, animals and antibodies

MDCK (Madin–Darby canine kidney) cells, MDBK (Madin–Darby bovine kidney) cells, PK-15 (porcine kidney) cells, BHK-21 (baby-hamster kidney) cells, and primary bovine testicular (BT) cells were preserved in our laboratory. Primary embryo bovine kidney (EBK) cells were kindly provided by Dr. Li Yu. Primary embryo bovine lung (BL) cells were kindly provided by Dr. Fei Xue. Bovine viral diarrhea virus (BVDV) cytopathic strain NADL and VSV were purchased from the China Institute of Veterinary Drug Control. Tissue samples were collected from three healthy Holstein bovines (male, 5–6 years old) at a dairy farm in Harbin, Heilongjiang, China. Two New Zealand White Rabbits (female, 4 weeks old) were purchased from the Laboratory Animal Center of Harbin Veterinary Research Institute. The animals used in this study were approved by The Laboratory Animal Ethical Committee of Northeast Agricultural University. The animal treatment was performed according to the Chinese Regulations of Laboratory Animals and the Guidelines for the Care of Laboratory Animals.

Rabbit polyclonal antibodies against Mx1 (GTX110256) and GAPDH (GTX100118) were purchased from GeneTex (CA, USA). HRP-conjugated goat anti-rabbit IgG was purchased from ZSGB (Beijing, China). Rabbit polyclonal antibody against BoIFN-αA was prepared and preserved in our laboratory. Antibodies against BoIFN-κ, BoIFNAR1, and BoIFNAR2 were prepared with the specific antigen in accordance with the standard procedure (Coligan et al., 2005).

2.2. Cloning and characterization

Pairs of specific primers for BoIFNK-F and BoIFNK-R (Table 1) were designed from the bovine genomic sequence containing the BoIFN-κ gene; bovine genomic DNA extracted from bovine liver was applied as template. PCR products were cloned into the pEASY Blunt-T Vector (TransGen, Beijing, China) and sequenced (BGI, Beijing, China). Characterizations were analyzed by bioinformatics software, including ORF Finder algorithm, NetGlycate 1.0 Server online, Lasergene 11 package, CLUSTALX, and MEGA 5.0.

Tissue expression was analyzed by RT-PCR; GAPDH was used as an internal reference. DNase-treated RNA was reverse-transcribed to cDNA with the following specific primers BoNK-A, BoNA-A, and BoGAPDH-A. PCR was separately conducted with the following primer pairs: BoNK-F and BoNK-R, BoNA-F and BoNA-R, BoGAPDH-F and BoGAPDH-R (Table 1) to amplify the BoIFN-κ, BoIFN-αA, and GAPDH genes, respectively. Additionally, the expression for BoIFN-κ gene was searched through the expressed sequence tag (EST) database according to the reported method (Radeva et al., 2008).

2.3. Protein expression and PAb preparation

pET32a(+) and pET30a(+) (Novagen, USA) were used as expression vectors. The forward primers BoNK-EIF and BoNK-NIF shared the same reverse primer BoNK-XIR (Table 1) were used to construct the recombinant plasmids pET32a-BoIFN-κ and pET30a-BoIFN-κ to yield recombinant proteins rHis-BoIFNκ and rBoIFNκ, respectively. Two specific primers BoIFNAR1-F and BoIFNAR1-R, BoIFNAR2-F and BoIFNAR2-R were used to amplify the extracellular regions of BoIFNAR1 and BoIFNAR2, then recombinant proteins rBoIFNR1-ER and rBoIFNR2-ER were expressed separately. rHis-BoIFNκ is the tagged fusion protein containing a His-Tag and Trx-tag, which can be purified on a nickel-chelated column (GenScript, Nanjing, China) to measure the biological activity. rBoIFNR1-ER and rBoIFNR2-ER are also the tagged fusion protein containing the His-Tag and can be purified on a nickel-chelated column. By contrast, rBoIFNκ contains no tag (rBoIFNκ was expressed in pET30a with *Nde* I and *Xho* I sites as a non-fusion protein) and was purified by SDS-PAGE gel extraction (Zhao et al., 2014). rBoIFNR1-ER, rBoIFNR2-ER and rBoIFNκ were used as the specific antigen to prepare the polyclonal antibody (PAb) against rBoIFNR1, rBoIFNR2 and rBoIFNκ respectively (Coligan et al., 2005).

Table 1
Nucleotide Sequence of the PCR primers used in this study.

Primer	Sequence (5′-3′)
BoIFNK-F	ACAACACTTACTGATGAGTTACTGT
BoIFNK-R	CATTTGGTCAACGTCTACGATT
BoNK-F	CAGAAGAACATCAAGGAGGC
BoNK-R	AATCTCCCAGGCGCAGTGAC
BoNA-F	TGGTCCTTCCTGCTATCCCT
BoNA-R	CTGACAACCTCCCAGGCACA
BoGAPDH-F	TTGGCATCGTGGAGGGACT
BoGAPDH-R	TTGGCATCGTGGAGGGACT
BoNK-NIF	ATCCATATG$_{Nde\ I}$CTGGACTGTAACTCGCTGAATG
BoNK-BIF	ATCGGATCC$_{BamH\ I}$CTGGACTGTAACTCGCTGAATG
BoNK-XIR	ATGCTCGAG$_{Xho\ I}$ TTATCTCCTCCTGAGTAATGCTG
BoIFNAR1-F	ATAGAATTC$_{EcoR\ I}$ AGATGGGTGCTGCCCGCCGC
BoIFNAR1-R	GCTGTCGAC$_{Sal\ I}$TTAGGTTTTGGAAGTATTTCCTG
BoIFNAR2-F	CGGCATATG$_{Nde\ I}$TCGTATGTTGCGCCTGTTCT
BoIFNAR2-R	CGGCTCGAG$_{Xho\ I}$TTATGTAGCAGATTCTGATGAC
BoMx1-F	TCAACCTCCACCGAACTG
BoMx1-R	TCTTCTTCTGCCTCCTTCTC
BoISG15-F	GCAGCCAACCAGTGTCTG
BoISG15-R	CCTAGCATCTTCACCGTCAG
BoISG56-F	TGGACTGTGAGGAAGGATGG
BoISG56-R	AGGCGATAGACAACGATTGC
BoGAPDH-qF	TTCAACGGCACAGTCAAGG
BoGAPDH-qR	ACATACTCAGCACCAGCATCAC

Note: The restriction enzyme sites that were introduced in primers are underlined.

2.4. Biological activity and physicochemical characteristics

The antiviral activity of BoIFN-κ against VSV was determined by inhibiting the cytopathic effect of the virus on cells as previously described (Cui, 2012; Guo et al., 2015) (Guo et al., 2015), which was conducted on different measurement systems separately, BoIFNαA was employed as control. The specificity of BoIFN-κ antiviral activity was analyzed using the antiviral-activity-blocking assay (Shao et al., 2015a,b).

The antiproliferative activity of MDBK cells was measured by MTT assay (Luo et al., 2015). The physicochemical characteristics of BoIFN-κ were analyzed using the MDBK/VSV system, and these characterizations included trypsin, pH, and temperature sensitivity.

2.5. Blocking assay with BoIFNAR or anti-BoIFNAR antibody

Briefly, plates were pre-coated with rBoIFNR1-ER, rBoIFNR2-ER or rBoIFNR1-ER plus rBoIFNR2-ER. After blocking, serial dilutions of rHis-BoIFNκ or BoIFN-αA were added before the anti-BoIFN-κ antibody or anti-BoIFN-αA antibody was added to each well. HRP-conjugated goat anti-rabbit IgG was introduced to each well and

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