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ARTICLE

Comparison of the Nucleotide Sequence of Cloned Osteopontin from Hanwoo and Holstein

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Abstract

Osteopontin (OPN) is a secreted phosphorylated glycoprotein. It has an important role in multiple biological processes including cell survival, bone remodeling, inhibition of ectopic calcification, as well as, is thought to have potential immune modulation activities. In this work, we isolated and characterized a full-length open reading frame (ORF) of Korean native cow's OPN from Korean native cow's (Hanwoo) kidney, and successfully cloned firstly on Hanwoo's OPN. The sequencing results indicated that the isolated cDNA was 1190 bp in length containing a complete ORF of 837 bp. It encoded a precursor protein Hanwoo's OPN consisting of 278 amino acids with a signal peptide of 16 amino acids. Amino acid homology was found to be 99.3% as compared to the corresponding sequences of Holstein bone marrow OPN. Hanwoo's kidney OPN and Holstein bone marrow OPN are different only in two amino acid residues 42 and 56, amino acid residue 42 is Thr (T) ↔ Ile (I), and amino acid residue 56 is Ala (A) ↔ Thr (T) respectively. These results from the present work would be helpful to elucidate the biological function of Hanwoo's OPN and provided a foundation for further insight into role of Hanwoo's OPN.

Key words: Korean native cow (Hanwoo), osteopontin, cloning, amino acid

Introduction

Osteopontin (OPN) is an acidic, phosphorylated glycoprotein of M_r 60,000, secreted in body fluids (e.g., plasma, urine, and milk) and in mineralized tissues. Its expression is increased in many transformed cells and in normal cells exposed to various cytokines (Denhardt and Guo, 1993; Oldberg *et al.,* 1986). OPN is produced by activated T cells and is implicated in several aspects of immune cell functions, including stimulation of IgG production by B cells (Patarca *et al.,* 1993; Weber and Cantor, 1996). OPN contains a conserved Arg-Gly-Asp sequence, and binds to cells via integrin-mediated mechanisms such as the a_vb_3 as well as the a_vb_5 and a_vb_1 integrins (Liaw *et al.,* 1995). OPN was first isolated from bone and is also present in physiological fluids such as serum, urine (Kohri *et al.,* 1992; Kohri *et al.,* 1993; Shirage *et al.,* 1992) and breast milk (Senger *et al.,* 1989; Sorensen *et al.,* 1993). OPN acts as an opsonin that enhances bacterial phagocytosis (Schack *et al.,* 2009) and plays a pivotal role in the development and maintenance of immune responses (Wang and Denhardt, 2008). OPN induces the NFκB-mediated pro-MMP-2 activation through IKK-regulated phosphorylation of IκBα and curcumin inhibits OPN-induced cell migration, tumor growth, and NFκB-mediated MMP-2 activation by inhibiting signal leading to IKK activity (Philip and Kundu, 2003).

In human milk, OPN were highly expressed at both mRNA and protein levels during lactation (Nagatomo *et al.,* 2004), and studies proposed that it might be useful in preventing rotavirus infections during lactating period (Naficy *et al.,* 1999). OPN is also present in bovine milk with a concentration of approximately 18 mg/L, which is considerably lower than the corresponding OPN concentrations in human breast milk (approximately 138 mg/L) (Bayless *et al.,* 1997; Nagatomo *et al.,* 2004). When the bovine OPN was used as a substrate for transglutaminase, it revealed the presence of two reactive acceptor glutamins (Gln-34 and Gln-316) (Sorensen *et al.,* 1994). Also Sorensen *et al.* (2003) carried out purification and characterization of osteopontin from human milk.

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In this work, we isolated and characterized a full length open reading frame (ORF) of OPN cDNA from kidney of Korean native cow's (Hanwoo). These results would be helpful to elucidate the biological function of Korean native cow's (Hanwoo) OPN and will also provide a basis for genetic engineering studies of Korean cow's (Hanwoo) OPN.

Materials and Methods

Molecular cloning and sequencing of the OPN cDNA from Hanwoo's kidney

Hanwoo's kidney was gained from slaughterhouse and total RNA was separated by guanidium/acidic phenol extract method (Chomczynski and Sacchi, 1987). Protein and DNA fractions were extracted from the homogenate by acidic phenol (pH 4.0) extraction. The upper aqueous phase was transferred into a new reaction tube to precipitate RNA by the addition of 2.5 vol. absolute ethanol. The purity of the RNA was determined in a denaturing MOPS-buffered 1.5% agarose gel. RNA concentration was measured using spectrophotometer. cDNA was synthesized using oligo-dT primers (Promega, USA) and Superscript II+ reverse transcriptase (Life Technologies Inc., USA) from purified RNA. The cDNA encoding the whole open reading frame (ORF) contained signal sequence for the Korean native cow's OPN.

cDNA was amplified by PCR using the following primer. A sense primer (OPN-up) is 5'-GGATCCATGAGAATTGCAGTGATTTG-3', and an antisense primer (OPN-down) is 5'-AAGCTTTCATAGTGACATCAAATTTT-3' resulted in an approximately 1190 bp fragment. Sequence and primer information of the bovine OPN genome were retrieved from GenBank (no. M66236). PCR was performed with *Pfu* DNA polymerase (Stratagene) for 35 cycles as follows: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 1 min 30 s. The PCR products were separated by 1.5% agarose gel electrophoresis, excised from the gel, purified using the Gel Extraction kit (Qiagen, Switzerland). The amplified fragment was inserted into the Blunt II site of pCR-Blunt II-TOPO cloning vector (Invitrogen, USA) according to the manufacturer's instructions. All primers were purchased from Genotech Ltd. (Korea). Unambiguous sequences of 1190 nucleotides of the OPN cDNA were determined using the ABI PRISM cycle sequencing method (Perkin Elmer, USA).

Sequence analysis of OPN

We performed the alignment and amino acid translation of nucleotide sequences of OPN using the web site software (http://web.expasy.org).

Nucleotide sequence Accession Numbers

The sequence of the Korean cow's (Hanwoo) OPN was submitted to GenBank under accession number AF492837.

Results and Discussion

Cloning of OPN

As shown in Fig. 1, the OPN cDNA were obtained by PCR using a Korean native cow's (Hanwoo) kidney DNA as a template. Expected PCR product in lane 1 having size of 1190 bp corresponded to mature form of OPN (Fig. 1A). These PCR products were inserted into the Blunt II site of pCR-Blunt II-TOPO vector and transformed into the *E. coli* strain (DH5a) on LB/Kana agar plate. The procedure of cloning the OPN cDNA into this vector schematically showed in Fig. 1B.

Properties of Hanwoo's OPN

As shown in Fig. 2, Korean native cow's kidney and Holstein bone marrow OPN (GenBank: M66236) amino acid sequence were compared to DNA sequences from NCBI database. Signal peptides (1-16) of Korean native cow's kidney OPN and Holstein bone marrow OPN marked on bold letters [Met (M)-Arg (R)-Ile (I)-Ala (A)-Val (V)-Ile (I)-Cys (C)-Phe (F)-Cys (C)-Leu (L)-Leu (L)-Gly (G)-Ile (I)-Ala (A)-Ser (S)-Ala (A)]. Korean native cow's (Hanwoo) kidney OPN and Holstein bone marrow

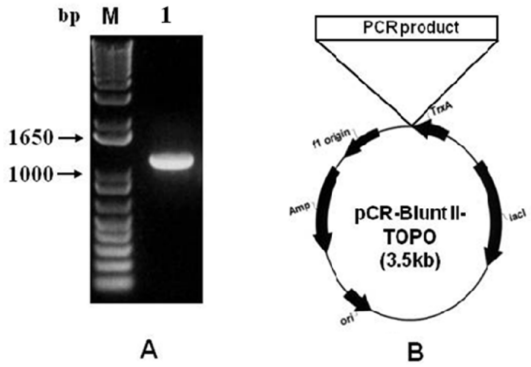

Fig. 1. Agarose gel electrophoresis of Korean-native cow's (Hanwoo) OPN. (A) cDNA encoding the mature form of OPN was amplified by PCR. PCR product were electrophoresed on 1.2% agarose gel. M, DNA marker; lane 1, PCR product of Korean native cow's (Hanwoo) kidney OPN (B) Schematic representation of the OPN cDNA cloning vector. cDNA encoding the mature form of OPN was amplified by RT-PCR and cloned into the pCR-Blunt II-TOPO vector.

DNA Sequence

ATGAGAATTGCAGTGATTTGCTTCTGCCTCTTGGGCATTGCCTCCGCCCTTCCAGTTAAACCGA
CCAGTTCTGGCAGCTCTGAGGAAAAGCAGCTTAACAACAAATACCCAGATGCTGTAGCC**ACA**TGG
CTAAAGCCTGACCCATCTCAGAAGCAGACTTTCCTA**GCA**CCACAGAATTCTGTGTCCTCTGAGGAA
ACTGATGACAACAAACAAAATACCCTCCCAAGTAAGTCCAATGAAAGCCCTGAGCAAACAGACGA
TCTAGATGACGATGATGATAACAGCCAGGACGTCAACTCTAATGACTCCGACGACGCTGAAACCAC
TGATGACCCTGACCATTCCGACGAGTCTCACCATTCTGATGAATCTGATGAAGTTGATTTTCCCACT
GATATTCCAACAATCGCAGTTTTCACTCCGTTTATCCCTACGGAAAGCGCAAATGATGGCCGAGGTG
ATAGTGTGGCTTACGGACTGAAGTCAAGATCTAAGAAGTTCCGCCGATCTAACGTTCAGAGTCCAG
ATGCCACAGAGGAGGACTTCACATCACACATAGAGAGTGAGGAGATGCATGACGCACCTAAGAAG
ACGAGTCAGCTGACTGACCACAGCAAGGAAACCAACAGTAGCGAGCTTTCCAAAGAACTCACGCC
AAAGGCCAAGGATAAGAACAAGCATTCCAATCTGATTGAGAGTCAGGAAAATTCCAAACTCAGCC
AAGAATTCCATAGCCTTGAAGACAAGCTAGACCTAGATCATAAGAGTGAAGAAGACAAACACCTG
AAAATTCGTATTTCTCATGAATTAGATAGTGCCTCTTCTGAGGTCAATTGA

Amono Acid Sequence Alignment

K 1 **MRIAVICFCLLGIASA**LPVKPTSSGSSEEKQLNNKYPDAVA**T**WLKPDPSQKQTFL**A**PQNS
H **MRIAVICFCLLGIASA**LPVKPTSSGSSEEKQLNNKYPDAVA**I**WLKPDPSQKQTFL**T**PQNS
61 VSSEETDDNKQNTLPSKSNESPEQTDDLDDDDDNSQDVNSNDSDDAETTDDPDHSDESHH
VSSEETDDNKQNTLPSKSNESPEQTDDLDDDDDNSQDVNSNDSDDAETTDDPDHSDESHH
121 SDESDEVDFPTDIPTIAVFTPFIPTESANDGRGDSVAYGLKSRSKKFRRSNVQSPDATEE
SDESDEVDFPTDIPTIAVFTPFIPTESANDGRGDSVAYGLKSRSKKFRRSNVQSPDATEE
181 DFTSHIESEEMHDAPKKTSQLTDHSKETNSSELSKELTPKAKDKNKHSNLIESQENSKLS
DFTSHIESEEMHDAPKKTSQLTDHSKETNSSELSKELTPKAKDKNKHSNLIESQENSKLS
241 QEFHSLEDKLDLDHKSEEDKHLKIRISHELDSASSEVN
QEFHSLEDKLDLDHKSEEDKHLKIRISHELDSASSEVN

Fig. 2. Comparison of Korean native cow's (Hanwoo) kidney and Holstein bone marrow OPN amino acids. The amino acid sequence corresponding to the mature processed form of Korea native cow's (Hanwoo) kidney OPN. Signal peptides marked on bold letters (1~16). Amino acid sequences differences from the Holstein DNA are bold letter. K : Korean native cow's (Hanwoo) kidney OPN, H : Holstein bone marrow OPN

OPN were different only in two amino acid residues 42 and 56, amino acid residue 42 is Thr (T) ↔ Ile (I), and amino acid residue 56 is Ala (A) ↔ Thr (T) respectively (bold letter). Overall, the sequence identity was 99.3% in comparison to the Holstein bone marrow OPN.

These findings suggest that cloning of Korean native cow's (Hanwoo) kidney OPN can make the expression from eukaryotic cell such as insect cell or mammalian cell. In addition, these results would be helpful to elucidate the biological function of Korean native cow's (Hanwoo) OPN and may be utilized in foodstuffs due to its active biological function.

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