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Short Communication

Cloning and Expression of Equine β-Nerve Growth Factor

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A R T I C L E I N F O

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ABSTRACT

Nerve growth factor (NGF) is a neurotrophic factor that is essential for the maintenance of peripheral and central neurons. While importance of such physiological activities as well as the potential of it as a stress marker in *Equus caballus* has been implicated, the sequence of equine NGF remained unknown. In this study, we identified the sequence of equine NGF from the mRNA expressed in the peripheral blood in seven Thoroughbreds and three warmblood horses. There were no polymorphisms among samples analyzed, and the homology is more than 90% in comparison to human, mouse, rat, dog, and cow. When the sequence corresponding to the biologically active β -nerve growth factor was expressed in CHO-K1 cells, they were stained with an anti-NGF antibody that recognizes human NGF. Thus, it provides a rationale for using antibodies that react to other species' NGF to measure the equine NGF for further studies.

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1. Introduction

Nerve growth factor (NGF) is a polypeptide necessary for the growth and support of neuron cells, which is produced from various kinds of cells such as hematopoietic cells, keratinocytes, and fibroblasts [1–4]. Besides nerve cell maintenance, it is involved in a wide variety of physiological activities such as pain sensation and wound healing [2–7]. In *Equus caballus*, NGF has been implicated as a novel stress marker because serum NGF levels elevated after running exercise or truck transportation [8,9]. In those studies, we estimated the elevation of NGF in an in vitro assay using rat PC12 pheochromocytoma cell line based on the premise that equine NGF sequence is highly conserved and crossreacts among species, leaving the sequence of equine NGF remained undetermined. Recently, Wade et al [10] provided a predicted sequence of equine NGF from a shotgun sequence analysis of a Thoroughbred genome. It indicates that equine NGF shares high homology with other species, though more data are required to conclude the equine NGF sequences taking polymorphisms into account.

In this study, we identified a sequence of equine NGF using blood samples from 10 horses and analyzed whether a commercially available antibody against human NGF can be applicable for the detection of the protein.





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Α	B
1 atgtecatgt tgttetacae tetgattaca getettttga teggeacaea ggeagaacee M S M L F Y T L I T A L L I G T Q A E P 61 cacacagaga geaatgteee ageaggacae gecateceee aageecaetg gaetaaaett H T E S N V P A G H A I P Q A H W T K L	1 60 Horse MSMLFYTLITALLIGTQAEPHTESNVPAGHAIPQAHWTKLQHSLDTALRRARSAPARAIA Human MSMLFYTLITAFLIGUQAEPHTSSNVPAGHTIPQAHWTKLQHSLDTALRRARSAPAAAIA Mouse MSMLFYTLITAFLIGUQAEPTTSSNVPEGDSVPEAHWTKLQHSLDTALRRARSAPATPAIA NGMLFYTLITAFLIGUQAEPTTSSNVPEGDSVPEAHWTKLQHSLDTALRRARSAPAGAIA Cow MSMLFYTLITALLIGUAAPHTESNVPAGHAIPQAHWTKLQHSLDTALRRARSAPAGAIA Cot MSMLFYTLITALLIGUAAPHTESNVPAGHAIPQAHWTKLQHSLDTVLRRARSAPAGAIA
121 cagcattege ttgacacage ecteegeaga geeegtageg ecceageegg ggegataget Q H S L D T A L R R A R S A P A R A I A	61 120
181 gcaagggtgg cagggcagac ccgcaacatc actgtggacc ccaaactttt taaaaagcgg A R V A G Q T R N I T V D P K L F K K R	Horse ARVAGQTRNITVDPKLFKKRRLRSPRVLFSTQPPPVAADTQDLDFEAGGAASFNRTHRSK Human ARVAGQTRNITVDPRLFKKRRLRSPRVLFSTQPPPEAADTQDLDFEVGGAAFFNRTHRSK Mouse ARVTGQTRNITVDPRLFKKRRLRSPRVLFSTQPPPTSSDTLDLDFQAHGTIPFNRTHRSK Rat ARVTGQTRNITVDFLFKKRRLRSPRVLFSTQPPPTSSDTLDLDFQAHGTISFNRTHRSK
241 egactgegit eaccegegi getgittage acceageee eactgiege igeagataet R L R S P R V L F S T Q P P P V A A D T	Dog ARVTGQTRNITVDPKLFKKRRLRSPRVLFSTHPPPVAADAQDLDLEAGSTASVNRTHRSK Cow ARVAGQTHNITVDPKLFKKRRLRSPRVLFSTQPPPVAADTQDLDFEAGGASSFNRTHRSK Cat ARVAGQTHNITVDPKLFKKRRLRSPRVLFSTUPPPVAADTQDLDEAGGASSFNRTHRSK
301 caggatetgg acttigagge egggggigee geeteettea acaggaetea caggageaag Q D L D F E A G G A A S F N R T H R S K	121 180 Horse R5SSHPVFHRGEFSVCDSVSVWVGDKTTATDIKGKEVMVLGEVNINNSVFK0YFFErKCR
361 cggtcttcct cccaccccgt cttccacagg ggggagttct ccgtgtgcga cagcgtcagc R [S S S H P V F H R G E F S V C D S V S	Human RSSSHPIFHRGEFSVCDSVSVWVGDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCR Mouse RSSTHPVFHMGEFSVCDSVSVWVGDKTTATDIKGKEVTVLAEVNINNSVFRQYFFETKCR Rat RSSTHPVFHMGEFSVCDSVSVWVGDKTTATDIKGKEVTVLGEVNINNSVFKQYFFETKCR
421 gtctgggtgg gggataagac caccgccacg gacatcaagg gcaaggaggt gatggtgtg ∨ ₩ ∨ G D K T T A T D I K G K E V M ∨ L	Dog RSSSHPVFHRGEFSVCDSVSVWVGDKTATDIKGKEVMVLGEVNINNSVFRQYFFETKCR Cow RSSSHPVFHRGEFSVCDSISVWVGDKTATDIKGKEVMVLGEVNINNSVFRQYFFETKCR Cat RSSSHPVFHRGEFSVCDSVSVWVGDKTATDIKGKEVMVLGEVNINNSVFRQYFFETKCR
481 ggagaggtga acattaacaa cagtgtattc aagcagtact tttttgagac caagtgccgg G E V N I N N S V F K Q Y F F E T K C R	181 240 Horse DPNPVDSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKTGRK
541 gaccccaatc ccgtggacag cgggtgccgg ggcattgact cgaagcactg gaactcctat D P N P V D S G C R G I D S K H W N S Y	Human DPNPVDSGCRGIDSKHWNSYCTTTHTFVKALTMDCKQAAWRFIRIDTACVCVLSRKAVRR Mouse ASNPVESGCRGIDSKHWNSYCTTHTFVKALTDEKQAAWRFIRIDTACVCVLSRKARR Rat APNPVESGCRGIDSKHWNSYCTTHTFVKALTDEKQAAWRFIRIDTACVCVLSRKARR
601 tgcaccacaa cccacacctt cgtcaaggeg ctgaccatgg acggcaagea ggctgcctgg C T T T H T F V K A L T M D G K Q A A W	Dog DPTPVDSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAGRR Cow DPNPVDSGCRGIDAKHWNSYCTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAGRR Cat DPTPVDSGCRGIDSKHWNSYCTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAGRR
661 cggttcatcc ggatcgacac ggcctgtgtg tgcgtgctca gcaggaaaaac cgggaqaaaa R F I R I D T A C V C V L S R K T G R]K 721 gcctga A stop	241 Horse A Human A Mouse G Rat G Dog A Cow A
	Cat A

Fig. 1. (A) Sequence of cloned NGF sequence. Square brackets indicate the beginning and the end of the predicted β -NGF sequence based on the amino acid comparison between species. (B) Comparison of amino acids of prepro-NGF in species. Framed sequences are corresponded to the β -NGF in each species. β -NGF, β -nerve growth factor; NGF, nerve growth factor.

2. Materials and Methods

2.1. Animals

All experiments with horses had ethical permission along with the standards specified in the guidelines of the University Animal Care and Use Committee of the Tokyo University of Agriculture and Technology, as well as with the guidelines for the use of laboratory animals provided by the Science Council of Japan. A total of 10 healthy male adults horses (seven Thoroughbreds and three warmblood horses) were included. Approximately 30 mL of blood were obtained from one Vena jugularis externa of each horse into conical tubes containing EDTA (Maxim Biotech Inc, San Francisco, CA). After hemolysis with ACK lysis buffer (0.15 M NH₄Cl, 1.0 mM KHCO₃, 0.1 mM Na₂EDTA, pH 7.2), total RNA from white blood cells was extracted with ISO-GEN (Nippongene, Tokyo, Japan) and reverse transcribed into cDNA by using random oligo(dT) primers and Prime-Script (Takara Bio, Shiga, Japan) according to the manufacturer's instructions.

2.2. Cloning of Equine NGF

Polymerase chain reactions (PCRs) with PrimeStar Max DNA polymerase (Takara Bio) were carried out using a pair of primers 5'-ATGTCCATGTTGTTCTACACT-3' (sense primer) and 5'-TCAGGCTTTTCTCCCCGGTTTT-3' (antisense primer) and the equine blood cDNA as templates. Polymerase chain reaction products were cloned into a pGEM-T TA cloning vector (Promega, Madison, WI), followed by PCR reactions using BigDye Terminator v3.1 Cycle Sequencing kits (Life Technologies, Gaithersburg, MD) with M13-forward and M13-reverse primers. The sequence was analyzed by ABI3100 sequencer (Life Technologies) according to the manufacturer's instruction.

2.3. Cell Culture

CHO-K1 cells were obtained from Riken Cell Bank (Tsukuba, Japan). Cells were cultured in RPMI 1640 medium (Life Technologies) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin, and 100 μ g/mL streptomycin in a humid atmosphere of 5% CO₂ in air at 37°C.

2.4. Expression of Equine NGF and Immunocytochemistry

The β -nerve growth factor (β -NGF) sequence was cloned into pIRES2-AcGFP vector (Clontech, Palo Alto, CA), which is designated pIRES2- β -NGF-AcGFP and transfected into CHO-K1 cells by using a Cell Line Nucleofector kit T (Lonza, Walkersville, MD) with a Nucleofector I electroporation device (Lonza) according to the manufacturer's instructions. Cells were cultured for 48 hours in the culture medium and then fixed with 4% paraformaldehyde (Wako, Download English Version:

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