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Use of a recombinant protein containing major epitopes of hnRNP G to detect anti-hnRNP G antibodies in dogs with systemic lupus erythematosus

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Abstract

The objective of this study was to express major epitopes of heterogeneous nuclear ribonucleoprotein G (hnRNP G) for detecting anti-hnRNP G antibodies in dogs with systemic lupus erythematosus (SLE). HnRNP G cDNA clone was isolated from HEp-2 cells, and a DNA fragment encoding immunodominant region (residues 189–272) of hnRNP G (hnRNP Gi) was subcloned into pET32 vector to construct a prokaryotic expression plasmid named pEThnRNPGi. After induction, *Escherichia coli* carrying pEThnRNPGi expressed a recombinant protein of 28 kDa, comprising recombinant hnRNP Gi and fusion tag. Purified recombinant hnRNP Gi protein was further analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and its identity was confirmed. Western blot analysis showed that recombinant hnRNP Gi was specifically recognized by anti-hnRNP G positive sera of SLE dogs, and not by negative control sera. In conclusion, recombinant hnRNP Gi protein expressed in this study may serve as a useful reagent to assist in the immunological diagnosis of canine SLE.

Keywords: Canine systemic lupus erythematosus; Heterogeneous nuclear ribonucleoprotein G; Immunodominant region of hnRNP G; Anti-hnRNP G antibody; Antinuclear antibodies

1. Introduction

Canine systemic lupus erythematosus (SLE) is a spontaneously occurring autoimmune disease that shares many similar symptoms with human SLE (Lewis, 1972). The manifestations of SLE in dogs include polyarthritis, symmetrical or focal dermatitis, glomerulonephritis, leukopoenia, haemolytic anaemia, thrombocytopenia, myositis, and neuropathy (Ettinger and Feldman, 2005; Birchard and Sherding, 2006). Dogs with SLE may present with different signs of the disease, depending on the severity and the type of tissues or organs involved (Ettinger and Feldman, 2005; Birchard and Sherding, 2006). Many of the clinical signs are considered to be the result of the depositions of immune complexes on basement membranes or in blood vessels (Gershwin, 2005). Although the clinical manifestations of canine SLE are highly diverse, the disease is consistently accompanied by the presence of antinuclear antibodies (ANAs) directed against a variety of nuclear proteins. A positive ANA is expected in 97–100% of cases of canine SLE (Chabanne et al., 1999a). Detection of ANA is therefore listed as a

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common diagnostic test for canine SLE (Gershwin, 2005). However, ANA is not exclusive to dogs with SLE. Positive ANA can also be observed in 20% of dogs with various infectious diseases (Chabanne et al., 1999a).

Of ANAs that have been reported in SLE dogs, anti-heterogeneous nuclear ribonucleoprotein G (hnRNP G) antibodies were found in 20% of SLE dogs (Chabanne et al., 1999a). Although anti-hnRNP G antibody is not a sensitive marker for canine SLE, it is considered highly specific for canine SLE (Monier et al., 1992) and therefore listed as one of the important markers of canine SLE (Chabanne et al., 1999b). HnRNP G is a glycosylated protein modified with O-linked *N*-acetylglucosamine (Soulard et al., 1991). As a component of hnRNPs complexes, hnRNP G may participate in post-transcriptional processing of pre-mRNA (Soulard et al., 1993; Weighardt et al., 1996). Epitope mapping of hnRNP G shows that major epitopes or immunodominant regions recognized by anti-hnRNP G antibody of SLE dogs reside within amino acid residues 189–272 (Soulard et al., 2002). Therefore, the protein domain encompassing such an immunodominant region may be employed for examining anti-hnRNP G antibody.

The specific aim of this study was to express the immunodominant region of hnRNP G (hnRNP Gi) for detecting anti-hnRNP G antibodies in SLE dogs or, possibly, in ANA positive dogs that might develop SLE later. We cloned cDNA of hnRNP G from HEp-2 cells and constructed a pET32-based expression plasmid encoding amino acid residues 189–272 of hnRNP G. The resultant plasmid, designated as pEThnRNPGi, was introduced into *Escherichia coli* to express recombinant hnRNP Gi (rhnRNP Gi) protein. After purifying the recombinant protein by metal chelation chromatography, the identity of hnRNP Gi was verified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Finally, Western blot analysis was performed to evaluate the specificity of rhnRNP Gi protein for detecting anti-hnRNP G antibodies in SLE dogs.

2. Materials and methods

2.1. Dog sera and ANA detection

Sera of SLE dogs (E23, E58, B223, and B899) were collected and characterized in our previous study (Chiou et al., 2004). Control sera were collected from healthy dogs whose owners had volunteered to bring their dogs in for a check up. Presence or absence of serum ANA was determined by indirect immunofluorescence assay (IFA) as described (Chiou et al., 2004).

2.2. Molecular cloning of human hnRNP G cDNA

RNA was extracted from HEp-2 cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). By employing reverse transcription-polymerase chain reaction (RT-PCR), cDNA of hnRNP G was cloned and the sequence was deposited on GenBank (Accession No. AY464692).

2.3. Construction of a prokaryotic expression plasmid of hnRNP Gi

Using the above hnRNP G cDNA as template, PCR was performed for 35 cycles as follows: denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR primers included 5′-GACTCAGAATTCAGTTATGGAGGTCCACCTCG-3′ (sense primer) and 5′-TCGTACAAGCTTACTAATAGTCACGATCACGACCATA-3′ (antisense primer). Sequences of *Eco*RI and *Hin*dIII restriction sites are underlined. DNA fragment encoding hnRNP Gi was subcloned into a prokaryotic expression vector pET32a(+) (Novagen, Madison, WI, USA). The resultant pEThnRNPGi plasmid was introduced into *E. coli* strain BL21(DE3)pLysS (Novagen) for protein expression.

2.4. Induction and purification of the recombinant hnRNP Gi protein

Escherichia coli strain BL21(DE3)pLysS carrying pET-hnRNPGi was grown at 37 °C until OD_{595} (optical density) of the bacterial broth reached 0.8. After isopropyl-β-D-thiogalactopyranoside (IPTG, 0.5 mM) induction of the recombinant protein at optimal time course, the bacterial pellets were resuspended in binding buffer (0.5 M NaCl, 20 mM Tris at pH 7.9), lysed under 2500 pounds per square inches (psi) with a French pressure cell press (Thermo IEC, Needham Height, MA, USA) and the bacterial lysate containing recombinant protein was passed through a Ni^{2+} resin column, washed with lower levels of imidazole (0, 20, 40 mM) in binding buffer, and the recombinant protein was finally eluted with 125 mM of imidazole. Protein concentration was determined by a BCA protein assay reagent kit (Pierce, Rockford, IL, USA).

2.5. Protein identification by MALDI-TOF MS

Purified recombinant protein was resolved by 12.5% SDS–PAGE to perform in gel trypsin digestion as described previously (Havlis et al., 2003). Trypsin-digested peptides were analyzed by a Bruker *autoflex* time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany). The resultant peptide masses were searched against the NCBI protein sequence data base using the Mascot program (Perkins et al., 1999).

2.6. Western blot analysis

Purified recombinant hnRNP Gi protein (250 ng per lane) was resolved by 12.5% SDS–PAGE, and transferred to nitrocellulose membrane for Western blot analysis. Briefly, the membrane was incubated with buffer A (phos-