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Rapid communication

Development of the polymerase chain reaction assay based on the canine genome database for detection of monoclonality in B cell lymphoma

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Abstract

From the canine genome database and its bioinformatic analysis, we identified conserved sequences within the vast majority of 61 variable segments and 1 joining segment of the immunoglobulin heavy chain (IgH) gene, and designed optimal primers for polymerase chain reaction (PCR) amplification directed at these conserved sequences to evaluate the monoclonality of IgH in canine B cell lymphoma. Using the primers, a PCR-based assay was performed on fine-needle aspiration samples of normal, hyperplasia, and malignant lymph nodes and lymphoma cell lines. All fine-needle aspiration samples of five B cell lymphoma cases and the B cell lymphoma line GL-1 exhibited clonal amplification, whereas no amplification was observed in the samples from normal and hyperplasia lymph nodes, cases of T cell lymphoma, and the T cell lymphoma line CL-1. The primers we designed clearly distinguished malignant B lymphocytes from normal, reactive, and malignant T lymphocytes, indicating a potential utility of the primers for PCR-based routine clinical examination for canine B cell lymphoma.

Keywords: Immunoglobulin heavy chain; Rearrangement; PCR-based analysis; Dog; B cell lymphoma

Canine malignant lymphoma, which is defined as a monoclonal lymphoproliferative disease, is currently diagnosed primarily by morphological assessment of lymphoid tissue and cavity fluid, including ascites and pleural fluid. Although the diagnosis is easily performed by morphologic assessment in many cases, some cases of lymphoma, especially those of the well-differentiated type and those in the early stage of disease, are occasionally difficult to distinguish from lymphoid hyperplasia even with the use of immunochemistry.

Recent molecular techniques facilitate the diagnostic analysis of human clinical cases of B cell

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lymphoma by detection of the clonal gene rearrangement for the immunoglobulin heavy chain (IgH), using Southern blotting (Rezuke et al., 1997) or more recently, using polymerase chain reaction (PCR) (Diaz-Cano, 1996; Mitterbauer-Hohendanner et al., 2004; Xu et al., 2004; Bahloul et al., 2005). Although Southern blotting analysis is superior to PCR-based analysis for detecting unusual IgH gene configurations, it requires a complicated procedure and large amounts of DNA from freshly excised tissue. Therefore, PCR-based analysis is applicable for the diagnosis of lymphoma from limited biopsy samples, formalin-fixed/paraffin-embedded tissues (Tai and Peh, 2003; Wan et al., 1990), or even sections already mounted on slides and stained (Fend et al., 1999). Despite the technical advantage of PCR-based analysis of IgH rearrangement for the diagnosis of B cell lymphoma, only Burnett et al. (2003) have reported its use in dogs with B cell lymphoma. Limited information on the genomic sequence of immunoglobulin, especially variable (V), diversity (D), and joining (J) segments that become rearranged to generate IgH diversity, has made it difficult to develop a PCR-based assay system for canines. However, the current accumulation of genome resources from the progress of the Eukaryotic Genome Sequencing Projects has enabled us to obtain a huge amount of information regarding canine genomic sequences, including immunoglobulin genes. For the present study, we designed optimal PCR primers to analyze the monoclonality of canine IgH using a bioinformatic technique based on the canine genome database and established a PCR-based diagnostic assay for B cell lymphoma using these primers.

In most PCR methods for human IgH monoclonality analysis, PCR primers are directed at V and J regions to amplify rearranged V–D–J genes because three regions, which are called frameworks (FR) 1–3,

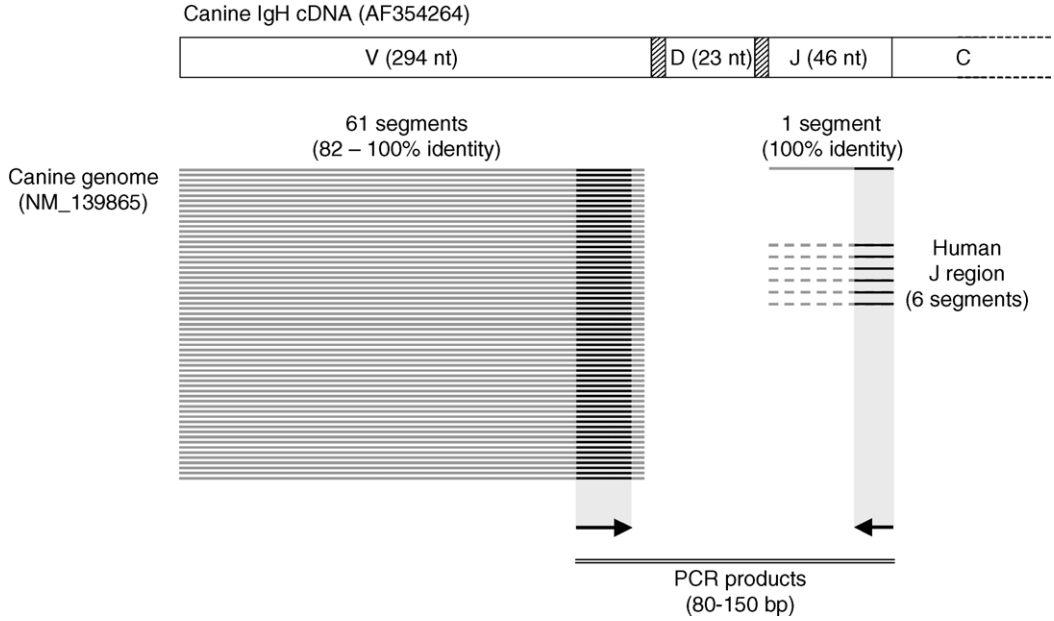

Fig. 1. Schematic representation of the rearranged V–D–J region of canine IgH and primer binding sites within conserved regions of genomic segments responsible for V and J regions. The variable (V), diversity (D), joining (J), and constant (C) regions in rearranged canine IgH cDNA (AF354264) are shown as boxes; striped boxes between V–D and D–J indicate N regions in which variable numbers of bases are inserted. The canine genomic database (NM_139865) derived 61 independent sequence segments, which showed 82–100% homology to 294 nt of the V region, and one segment that was identical in sequence with 46 nt of the J region; these are shown as gray bars under the boxed V and J regions. The conserved region among the 61 segments of the V region and a region within a segment of the J region, which corresponds to the consensus sequences of six segments of the human J region, are represented as black bars. The 5′ and 3′ primers were located on the conserved sequences of the V and J segments (arrows) to produce 80–150 bp of polymerase chain reaction amplification products.