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Characterization of C-, J- and V-region-genes of the feline T-cell receptor γ

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Abstract

Lymphomas and leukemias are important neoplasias of domestic cats and human beings. In some cases it can be difficult to differentiate these tumors from reactive lymphatic hyperplasia. To overcome this problem, the diagnosis of lymphomas and leukemias in man is often supported by molecular techniques.

To be able to establish such a technique in the cat we had to sequence the genes coding for the antigen receptors. As primary target in this study we choose the T-cell receptor γ. Using 5′-and 3′-RACE techniques we were able to clone and sequence four different V-region genes, which can be clustered into two subgroups as well as six variants of the C-region gene. Additionally, we found eight J-region genes which can be classified into three subgroups. One of the V-region genes, six of the J-region genes and all C-region genes had not been described previously. All together we analysed 112 clones containing V- and J-region genes and 31 clones containing C-region genes. Sixty-six of these clones were full length containing the L-region as well as the 5′-UTR of the feline T-cell receptor γ.

The sequences of the V-region- and J-region-genes show sufficiently homologous areas that can be used to establish a small number of consensus-primers to be applied in molecular diagnosis of feline lymphomas and leukemias.

Keywords: T-cell receptor γ; Cat; Lymphoma; V-region; J-region; C-region

1. Introduction

Lymphomas and leukemias are among the most important neoplasias of domestic cats. The diagnosis of lymphomas relies mostly on the histopathological examination of hematoxylin–eosin-stained tissue sections. In some cases this can be challenging because the neoplastic cells can look like activated lymphocytes or normal small lymphocytes (Avery and Avery, 2004). And sometimes benign lymphadenopathies can show features characteristic of malignant neoplasias like infiltration of the lymphnode capsule or high mitotic index (Mooney et al., 1987).

To overcome these problems in man, molecular techniques have been developed to assess the clonality of lymphocytic infiltrates (Avery and Avery, 2004; Spagnolo et al., 2004). Though clonality is not synonymous with malignancy these techniques can nevertheless give additional information about the

Abbreviations: V, variable; D, diversity; J, joining; RAG, recombination activating gene; FR, framework region; CDR, complementarity determining region; TRD, T-cell receptor δ; TRG, T-cell receptor γ; TRA, T-cell receptor α; TRB, T-cell receptor β; fTRG, feline T-cell receptor γ.

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lymphocytic infiltrate. One of these techniques applicable on virtually all lymphatic neoplasias is the analysis of clonally recombined antigen receptor genes.

During lymphocyte development the V-domains of immunoglobulin genes and T-cell receptor genes are somatically rearranged using two or three different regions in a process called the V(D)J recombination (Nossal, 2003). This has been extensively studied in human cell lines and in mice. In this process, the joining of a D- to a J-region is followed by the joining of a V-region to the DJ-region. The connection to the C-region follows during RNA maturation by splicing (Tonegawa, 1983; Jung et al., 2006). In the case of immunoglobulin light chains, TcRγ and TcRα, there is no D-region and the V-region is joined directly to the J-region. The diversity is further enhanced by imprecise joining during V(D)J-recombination (Tonegawa, 1983).

The V(D)J-recombination is initiated by the product of the recombination activating genes 1 and 2 (RAG1 and RAG2) that bind to the recombination signal sequence (RSS) and induce a DNA double-strand break (van Gent et al., 1995; McBlane et al., 1995). The signal ends and coding ends are processed by the ubiquitous process of non-homologous end joining (NHEJ) (Jung and Alt, 2004). In this process some bases are lost at the signal ends and others are added by the enzyme terminal transferase without template. In consequence the resulting so-called N-region has no definite length and the resulting V-domains also vary in the number of base pairs. Because of the triplet structure of the genetic code statistically only one-third of the recombined chains have a productive open reading frame (Jung et al., 2006).

The fact that the N-region varies in length, is used in the molecular diagnosis of lymphomas. By using primers directed against the FR3 and FR4 of the V-domain the area of varying length can be amplified and the result after electrophoresis is a smear for a polyclonal population and a definite band for a clonal population (Avery and Avery, 2004).

In the TRG-locus of humans the different V-region genes are followed by two sets of J-region genes and C-region genes in tandem arrangement (Lefranc et al., 1989). In some species like mouse, sheep, and cattle, however, the TRG-locus is arranged in clusters. The term cluster refers to genes that are grouped in proximity within an antigen receptor locus. Each cluster contains one or more variants for the V-, J- and C-region and recombination takes place mostly between variants included in one cluster (Vernooij et al., 1993; Vaccarelli et al., 2005; Herzig et al., 2006). Variants are distinguishable genes coding for V-, J- or C-regions of antigen receptors.

The TcRγ structurally resembles the other antigen receptors (Allison et al., 2001). Like immunoglobulins, the TcRγ comprises an approximately 110-aa long V-domain with three CDRs and four FRs. CDR1 and CDR2 are germline encoded, CDR3 is created during V(D)J-recombination and includes the N-region and parts of the V- and J-regions. D-regions are not present in the TRG-locus. Ahead of the V-domain, an 18–20-aa long signal peptide is positioned (Saito et al., 1984; Lefranc et al., 1986a).

Typical protein sequence motifs for the TcRγ are ''IHWY'' at the beginning of FR2 and ''YYCA'' at the end of FR3 (Hayday, 2000). In most antigen receptors, there is a ''WYRQ'' two amino acids after the beginning of FR2 (Rast and Littman, 1994). To be able to compare V-domains of different species, Lefranc (1999) and Lefranc et al. (2003) developed a unique numbering system for the functional regions of these chains. They are defined by the conserved amino acids cystein 23, tryptophan 42, leucin 89, and cystein 104. In the case of FR4 the conserved residue is a tryptophan or phenylalanin that is placed at position 118. An essentially identical system has also been developed for C-domains of antigen receptors (Lefranc et al., 2005a). In T-cell receptors the C-domain is made up of the first exon of the C-region.

Moore et al. (2005) already described a system for the molecular diagnosis of feline intestinal T-cell lymphomas. They found fourteen V-region variants belonging to three subgroups and six different J-region variants belonging to one subgroup. They used 80% nucleotide homology as cut off in creating these subgroups. Our goal in this study was to characterize the variants of regions in the feline TRG more comprehensively to be able to extend the diagnostic tools for other types of feline lymphomas. Our results describe one further V-region variant and three additional J-region variants. And for the first time the C-regions of the TRG-locus of the cat could be characterized.

2. Material and methods

2.1. Animals and RNA extraction

Total RNA was extracted out of spleen tissue samples of three domestic shorthair cats of different ages and genders using the Purescript® RNA Isolation Kit (Biozym, Oldendorf, Germany) as recommended by the manufacturer. From each sample RNA was isolated and RACE-experiments were conducted separately.