Comparison between Immunohistochemistry and Genetic Clonality Analysis for Cellular Lineage Determination in Feline Lymphomas

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ABSTRACT. Clinical application of genetic clonality analysis by PCR has been available in feline lymphoma. However, it is in under dispute whether the analysis can be applied for cellular lineage determination. To evaluate utility of the analysis for lineage determination, the results between immunohistochemistry and the genetic analysis were compared. Based on the result with immunohistochemistry, sensitivity of the primer for *IgH* was 89% and specificity was 75%. Sensitivity of the primer for *TCRγ* was 25% and specificity was 100%. Cross-lineage *IgH* rearrangement was found in 25% of T-cell lymphomas. The present study suggests that the genetic analysis had better be applied as not a definite but an adjunctive tool for lineage determination in feline lymphomas.

KEY WORDS: cross-lineage rearrangement, feline, lymphoma, phenotype.

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Lymphoma is one of the most common neoplasm in cats and the etiology has been studied from several aspects. Immunologically, tumor cells are classified into B-cell, T-cell and non-B/non-T lymphomas. In human patients with non-Hodgkin's lymphoma, T-cell neoplasms have a poorer prognosis than B-cell neoplasms [5, 7]. Similarly, dogs with T-cell lymphoma have shorter survival times than those with B-cell lymphoma [12]. In cats, there have been present insufficient data on outcome between B and T lymphomas. In a study on feline alimentary lymphomas, there was no significant difference in survival time between cats with B-cell lymphoma and those with T-cell lymphoma [9]. However, the number of cases was so small in the study that further investigation should be required to evaluate the prognostic significance of immunophenotype in cats.

The lineage assignment of feline lymphomas has been performed mainly by immunohistochemistry [3, 4, 10]. Although immunohistochemistry requires tumor tissues, it is not always possible to perform tissue biopsy at the time of diagnosis in veterinary medicine. Recently, genetic clonality analyses of the antigen receptor rearrangement by PCR have been reported as an aid to the diagnosis of feline lymphoma [8, 14]. Since its utility is to require only small amount of samples, it can be performed by using fine needle aspiration samples even if tissue biopsy is impossible to be conducted. Although the potential application of this assay has been supposed to include determination of cellular lineages, genetic clonality results for lymphoma lineage assignment can be confounded because discordance between the gene rearrangement and the immunophenotype is sometimes observed in human medicine. In feline

lymphoid neoplasms, there has been no report on feasibility of applying the genetic analysis to cellular lineage determination. The aim of the present prospective study was to evaluate utility of genetic clonality analysis by PCR for lineage determination in feline lymphomas.

Twenty one cats referred to the Veterinary Medical Center of the University of Tokyo were diagnosed as lymphoma by histopathology. From the cases, lymphoma samples for immunohistochemistry and molecular analysis of genetic clonality were obtained prospectively.

Immunohistochemistry was performed as follows. Formalin-fixed paraffin embedded lymphoma tissues were sliced into 4 μ m sections and prepared on slides. The slides were dehydrated through graded alcohols and treated for antigen retrieval in 1% citrate buffer solution under the autoclave at 121°C for 15 min. Then, endogenous peroxidase activity was eliminated by 1% hydrogen peroxide in methanol, and blocking of nonspecific antigens was processed by 8% skim milk in Tris buffered saline. The sections were incubated with the panel of primary antibodies specific for CD3 (T cell marker, 1:400, A0452, Dako, Glostrup, Denmark), CD79a (B cell marker, 1:50, M7051, Dako) and BLA-36 (B cell marker, 1:50, sc-58694, Santa cruz biotechnology, Santa Cruz, CA) overnight at 4°C. Negative and positive control sections were also incubated at this stage. After primary incubation, they were treated by biotin-conjugated anti-mouse IgG antibody or anti-rabbit IgG antibody (Both from Cappel, Solon, OH) and by horse-raddish peroxidase labeled streptavidin (Dako). Finally, the immunolabeling was visualized by 0.05% 3,3' diaminobenzidine tetrahydrochloride with 0.01% hydrogen peroxide. Although the primary antibodies were developed for humans, they were validated to use for cats in previous feline lymphoma studies [2, 3, 10].

Molecular analysis of genetic clonality of antigen

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receptor rearrangement by PCR was performed as follows. DNA samples of 21 cases were extracted from fresh lymphoma cells obtained by fine needle aspiration or tissue biopsy. The primers and protocols for PCR analyses were previously described (*IgH*: [14]; *TCR γ* : [8]). All PCR products were assessed by heteroduplex analysis.

By Immunohistochemistry, 9 of the 21 cases with lymphoma were determined as the B-cell subtype and 12 cases were determined as the T-cell subtype (Table 1). In the B-cell subtype, all of 9 cases expressed BLA-36 whereas only 3 of 9 cases expressed CD79a.

By genetic clonality analysis by PCR, 11 of the 21 cases with lymphoma showed monoclonal rearrangement of *IgH* and 3 cases showed monoclonal rearrangement of *TCR γ* . The other 7 cases showed clonal gene rearrangement of neither *IgH* nor *TCR γ* .

Based on the result with immunohistochemistry, the sensitivity and the specificity of genetic clonality analysis were investigated (Table 2). The sensitivity of the primer for *IgH* was 89% (8/9) and the specificity of that was 75% (9/12). The sensitivity of the primer for *TCR γ* was 25% (3/12) and the specificity of that was 100% (9/9).

Although there were reports evaluating sensitivity of genetic clonality analysis for diagnostic application in feline lymphomas, there was no report investigating specificity of that for cellular lineage assignment. In this study, feasibility of applying the genetic analysis to lineage determination was evaluated by investigating specificity of the analysis for lineage determination in feline lymphomas. The specificity of primers for *IgH* and *TCR γ* was acceptably high as 75% and 100%, respectively. The sensitivity of the primer for *IgH* was 89% which was slightly higher than the previous report (68%) [14]. However, the sensitivity of the primer for *TCR γ* was 25% which was lower than the previous report (89%) [8]. Considering the case population, all cases were alimentary lymphoma in the previous study [8] and many cases were alimentary lymphoma (9/12) in the present study. Since the case population of this study was similar to the previous report, the reason for different sensitivity of the *TCR γ* analysis might be a discrepancy caused by small populations of both studies and the cases containing undetectable gene aberrations by the primers. Development of other primers and concurrent analyses using multiple primers can be required for the genetic clonality analysis in feline lymphoid neoplasms.

Of 12 cases with T-cell lymphoma determined by immunohistochemistry, 3 cases showed *IgH* monoclonal gene rearrangement. Such a repugnant phenomenon has been also reported in human lymphoid neoplasms, known as the cross-lineage rearrangement [11]. The occurrence of the cross-lineage *IgH* gene rearrangement in T-cell lymphoma can be explained in several mechanisms. *IgH* gene rearrangement in T-cell precursors may be an aberrant event directing cells into apoptotic pathway, unless they become immortalized by malignant transformation [6]. As an alternative explanation, cross-lineage *IgH* gene rearrangement in T-cell lymphoma can be postoncogenic

Table 1. Immunohistochemical findings of the study population

Immunophenotype	Number of cases	Number of cases expressing		
		CD3	CD79a	BLA-36
B-cell	9	0	3	9
T-cell	12	12	0	0

Table 2. Comparison between immunophenotype and clonal gene rearrangement

Clonal gene rearrangement	Immunophenotype	
	B-cell (n=9)	T-cell (n=12)
<i>IgH</i> (+)/ <i>TCRγ</i> (-)	8	3
<i>IgH</i> (-)/ <i>TCRγ</i> (+)	0	3
<i>IgH</i> (-)/ <i>TCRγ</i> (-)	1	6

events resulting from the ongoing activity of the common B- and T-cell V (D) J recombinase system on accessible gene loci [1, 6, 15]. By the same token, the latest study of feline lymphomas reported that the clonal rearrangement of *TCR γ* was present in 22% of B-cell lymphomas [13].

In B-cell lymphomas, all of 9 cases expressed BLA-36 whereas only 3 of 9 cases expressed CD79a. Previous studies also reported that there were cases determined as B-cell lymphoma without expression of CD79a [3, 4, 10]. Thus in feline lymphomas, identification of B-cell lymphoma should require another B-cell marker such as BLA-36 in addition to CD79a.

The present study suggested that genetic clonality analysis by PCR had better be applied as not a definite examination but an adjunctive tool for lineage determination and immunohistochemistry should be used mainly for lineage assignment in feline lymphomas. If the tissue biopsy is impossible to be performed, PCR clonality assessment using samples from the fine needle aspiration may be applicable for lineage determination since cell phenotype and gene rearrangement results can be concordant in many instances. However, the judgment of the gene rearrangement results should require extra caution. For lineage assignment, the result of genetic analysis can be different from that of immunohistochemistry in some cases. Furthermore, the result of the study investigating a prognostic significance of lineage determination with genetic analysis in feline lymphomas may be different from previous reports using immunohistochemistry for lineage assignment. Potential clinical utility of genetic clonality analysis can be investigated perspectively.

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