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A novel canine lymphoma cell line: A translational and comparative model for lymphoma research

William C. Kisseberth [a,c,*], Murali Vara Prasad Nadella [b], Matthew Breen [d,e], Rachael Thomas [d], Shannon E. Duke [d], Sridhar Murahari [a], Carrie E. Kosarek [a], William Vernau [f], Anne C. Avery [g], Mary Jo Burkhard [b], Thomas J. Rosol [b,c]

[a] *Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA*
[b] *Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA*
[c] *The Ohio State University Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA*
[d] *Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA*
[e] *Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC, USA*
[f] *Department of Pathology, School of Veterinary Medicine, University of California, Davis, CA, USA*
[g] *Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA*

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Abstract

A novel canine lymphoma cell line, OSW, was established from the malignant pleural effusion of a dog with peripheral T-cell lymphoma. The immunoprofile as determined by flow cytometry was as follows: positive for CD45, CD49d, CD18, CD11a; weakly positive for CD11b, CD11c, CD11d; and negative for CD45RA, CD1a, CD1c, CD3, TCRαβ, TCRγδ, CD4, CD5, CD8a, CD8b, CD90(Thy1), CD21, MHCII, CD14(TUK4), CD34, and MPO. Immunocytochemistry of cytospin preparations was negative for cytoplasmic CD3, CD79a, and MPO, but was positive for CD20. The cell line had an oligoclonal T-cell receptor gamma (TCRγ) gene rearrangement. Array comparative genomic hybridization (aCGH) and single locus probe (SLP) analysis showed that there were copy number increases of loci on dog chromosome 13 (CFA 13), and copy number decreases were evident for regions of CFA 11, 22, 26, 30 and 32, which include several of the more common chromosomal aberrations reported previously in canine lymphoma. The OSW cell line grows rapidly in vitro and is tumorigenic as a xenograft in SCID/NOD mice. OSW represents one of only a few reported canine lymphoma cell lines and is the most thoroughly characterized. This cell line and xenograft represent significant in vitro and in vivo models, respectively, for comparative and translational lymphoma research.

Keywords: Canine lymphoma; Cell line; Array comparative genomic hybridization; Bioluminescent imaging; Xenograft

1. Introduction

Spontaneously occurring lymphoma in the dog has many of the same histopathological, molecular, and clinical features as non-Hodgkin's lymphoma (NHL) in people [1–3]. Most of the lymphoma subtypes recognized in humans have histopathologically identical counterparts in the dog [4] and recent investigations show similar molecular characteristics in the two species [1,5] (Kisseberth et al., submitted; Fosmire et al., submitted). Likewise, spontaneous lymphoma in the dog has a similar clinical presentation, response to chemotherapy, and clinical progression compared to NHL in people [1–3]. Until recently, utilization of spontaneous cancers in dogs as a model for human cancers was limited by the unavailability of reagents and techniques that could be used in the dog. However, recent advances in canine genomics,

* Corresponding author at: Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Veterinary Teaching Hospital, 601 Tharp Street, The Ohio State University, Columbus, OH 43210, USA.
Tel.: +1 614 247 7201; fax: +1 614 292 6464.
E-mail address: kisseberth.2@osu.edu (W.C. Kisseberth).

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including sequencing of the dog genome, availability of gene microarrays, development of advanced cytogenetic and comparative genomic hybridization techniques and development of species-specific flow cytometry, FISH, and other antibody techniques now makes possible most of the experimental techniques that are routinely available for in vitro and in vivo use in humans and mice. Given these similarities and the advantage of larger subject size and presence of spontaneous disease (in contrast to small subject size and experimentally induced disease in xenograft and genetically modified mouse models of lymphoma); spontaneously occurring lymphoma in the dog represents an excellent large animal model for the study of lymphoma in people, including investigation of new therapeutic agents [1–3].

Laboratory study of spontaneous canine lymphoma has been severely limited by the lack of validated, well characterized, and widely disseminated cell lines. Those previously reported were either only partially characterized, were not widely distributed, or were established from leukemias, rather than lymphomas [6–10]. These canine hematopoietic cell lines include: 17–71, established from the lymph node of a dog with multicentric lymphoma, GL-1 established from a dog with B-cell leukemia, CL-1 established from the pleural fluid of a dog with thymic lymphoma, DLC-01 established from the lymph node of a dog with cutaneous lymphoma, and DLC-02 established from peripheral lymphocytes of a dog with large granular lymphocyte (LGL) leukemia [6–10]. As in humans, establishment of new lymphoma cell lines is difficult. To further accelerate the use of spontaneous cancers in dogs as a model system for new anticancer drug development and cancer cell biology, we established a novel T-cell lymphoma cell line. The cell line OSW was established from the malignant pleural effusion of a dog with peripheral T-cell lymphoma. The comparative molecular and biological properties of OSW, grown as a cell line and xenograft, are the subject of this study. The OSW cell line grows rapidly in vitro and is tumorigenic as a xenograft in SCID/NOD mice. The cell line has an oligoclonal TCRγ gene rearrangement, but does not express T-cell differentiation antigens. OSW contains several of the more common chromosomal aberrations reported previously in canine lymphoma [5,11,12] resulting in decreased copy number of p16/INK4a (also known as CDKN2A), RB1, and PTEN and increased copy number of MYC. As canine lymphoma has many similarities, both clinically and molecularly, to NHL in people, this cell line and xenograft will be useful for translational studies of novel therapeutics using spontaneous lymphoma in the dog as a model for human disease.

2. Materials and methods

2.1. Case report

A 5.5-year-old male Airedale Terrier was referred to The Ohio State University Veterinary Teaching Hospital in February 2004 for evaluation of lethargy and mandibular lymphadenopathy. Thoracic radiographs showed severe pleural effusion with hilar lymphadenopathy. Thoracocentesis was performed and 1000 mL of serosanguinous fluid was removed. An abdominal ultrasound showed multicentric lymphadenopathy, small hypoechoic nodules throughout the spleen, and a diffusely hyperechoic and enlarged liver. A biopsy of an enlarged popliteal lymph node was performed. Histopathological examination showed that the normal architecture of the lymph node was obliterated by sheets of large round cells with a high nuclear to cytoplasmic ratio, mild anisocytosis and anisokaryosis and a mitotic index of two per hpf (400×). The majority of these cells stained positive for CD3 and CD18, and negative for CD79a, CD20, and BLA36. Based on World Health Organization classification criteria, the tumor was classified as a peripheral T-cell lymphoma, unspecified [4]. The dog was treated with the combination chemotherapy protocol, COAP (cyclophosphamide, vincristine, cytosine arabinoside, prednisone) [13]. Three weeks later, thoracocentesis was repeated and approximately 900 mL of serosanguinous fluid was removed, an aliquot of which was used to establish the OSW cell line. The dog had minimal response to further chemotherapy and was euthanized 8 weeks after initial diagnosis due to progressive disease.

2.2. Establishment of the T-cell lymphoma line "OSW"

Cells from the pleural effusion were centrifuged, washed in RPMI 1640 medium, and cultured in T25 flasks in RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS), penicillin, streptomycin, and L-glutamine. The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. For the first 3 weeks, both adherent and non-adherent cells were passaged together weekly. After 3 weeks in culture, the non-adherent cell population was passaged and grown as a suspension culture consisting primarily of single cells and small clumps. The morphologic appearance of the cells was assessed on cytospin preparations stained with Wright's-Giemsa stain. The OSW cell line was subsequently maintained in continuous culture for over 1 year. For storage, cells are kept at a concentration of $1–5 \times 10^6$ cells/mL in 90% RPMI 1640 (supplemented with 20% FBS) and 10% DMSO and stored in liquid nitrogen.

2.3. Cell surface makers analysis and immunocytochemical staining

Immunophenotyping of the OSW cell line was determined on exponentially growing cells by two different laboratories (MJB, WV). Experiments were performed at least twice by each laboratory. Flow cytometry was carried out using the antibodies listed in Table 1. The sources of these reagents and the methods used have been described in detail elsewhere [14].