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Establishment of a Patient-Derived Xenograft of Canine Enteropathy-Associated T-Cell Lymphoma, Large Cell Type

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Summary

The pathogenesis of canine T-cell lymphoma remains incompletely understood, partly because there are no well-established in-vivo models to study these malignancies. For this study, we generated a patient-derived tumour xenograft (PDTX) from a 10-year-old neutered male golden retriever dog with enteropathy-associated intestinal T-cell lymphoma, large cell type. One of two female, 15-week-old beige/nude/XID mice developed a visible tumour 7 weeks after sections of tumour material from the spleen were surgically implanted. The histological appearance, immunophenotype and clonal antigen receptor rearrangements of the tumour from the recipient mouse showed that it was derived from the primary canine tumour. Our results indicate that immunodeficient mice are receptive hosts to develop in-vivo PDTX models to study the pathogenesis and management of canine T-cell lymphomas.

Keywords

dog; enteropathy-associated T-cell lymphoma; immunodeficient mouse model; patient-derived tumour xenograft

T-cell lymphomas comprise about 30% of all canine lymphomas, including both indolent and aggressive tumour types (Modiano *et al.*, 2005; Ito *et al.*, 2014). The biology of these tumours remains incompletely understood, partly because there are no well-established

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laboratory models to reproduce the disease. Here, we report on the establishment of a patient-derived tumour xenograft (PDTX) from a dog with enteropathy-associated T-cell lymphoma, large cell type.

A 10-year-old, male neutered golden retriever was presented to the Internal Medicine Service of the Veterinary Medical Center, University of Minnesota, with signs of increasing inappetence and lethargy over a period of 1–2 months, and severe, abrupt onset vomiting for several days. The dog did not exhibit any remarkable signs on physical examination. Ultrasound imaging showed an infiltrative condition of the small intestine, spleen and liver. Abdominal exploratory surgery was performed and multiple thickened areas of the small intestine and hepatic nodules (~1 cm diameter) were biopsied and tissues were fixed in 10% neutral buffered formalin. Multiple, well-circumscribed, variably sized (0.5–3.5 cm diameter) masses were noted on the ventral surface of the spleen, so a complete splenectomy was done. The dog had severe post-surgical gastrointestinal haemorrhage that did not respond to medical therapy, and it was humanely destroyed 3 days later.

Formalin fixed tissues were submitted to Marshfield Laboratories and to the Comparative Pathology Shared Resource of the Masonic Cancer Center, University of Minnesota, for histopathological analysis. Review of haematoxylin and eosin (HE)-stained sections of small intestine revealed extensive erosion, blunting and fusion of the duodenal and ileal villi with near full-thickness infiltration, expansion and effacement of the epithelium, lamina propria and muscularis propria by a neoplastic lymphoid population (Figs. 1A, 1B). The neoplastic lymphoid cells were intermediate to large sized with modest amounts of blue–grey cytoplasm. Nuclei were oval to irregular in shape with rare sharp, shallow indentations. Chromatin was granular, unevenly distributed, and 1–3, variably-sized nucleoli were identified. The mitotic index was 5. The same population of neoplastic lymphocytes was observed in the hepatic nodule and, in addition to foci of nodular hyperplasia, multifocally within the spleen (Fig. 2).

Immunohistochemistry (IHC) was performed on the spleen samples in order to determine the lymphocyte immunophenotype. Canine tonsil was used as a positive control; for negative controls, primary antibodies were substituted with irrelevant antibodies of the equivalent isotype. The malignant lymphocytes showed strong immunolabelling with anti-CD3 antibody (clone CD3-12; MCA1477T, AbD Serotec, Hercules, California, USA; Fig. 2); in contrast anti-CD79a (clone HM47/A9; CM057C, Biocare medical, Concord, California, USA) and anti-CD20 (rabbit polyclonal; RB-9013-P; Thermo Fisher Scientific, Fremont, California, USA) antibodies only labelled scattered lymphocytes in the tumour, and these were interpreted as being residual normal B cells (supplementary Figs. 1A, 1B). Based on these observations, the diagnosis was enteropathy-associated T-cell type 1 (large cell) lymphoma with hepatic and splenic metastasis.

To generate patient-derived tumour xenograft (PDTX) models, a ~ 0.5 cm diameter fragment from a splenic nodule was transferred aseptically into sterile F-12K medium (30-2004, ATCC, Manassas, Virginia, USA) supplemented with 10% fetal bovine serum (FBS) and maintained at 4°C. The freshly preserved nodule was then minced into 0.5 mm³ fragments that were washed five times in sterile phosphate buffered saline solution and

placed on ice for xenotransplantation. Two healthy, female, 15-week-old beige/nude/XID mice ($Lyst^{bg-J}Foxn1^{nu}Btk^{xid}$) weighing 23.3 and 24.2 g, respectively, were used as recipients. Xenotransplantation experiments were done with approval of the Institutional Animal Care and Use Committee of the University of Minnesota (protocol number 130630712A). Ketamine (100 mg/kg) and xylazine (10 mg/kg) were used to achieve a surgical plane of anaesthesia, a small subcutaneous incision was made on the dorsal thoracic region, and a small pocket was made at the point of incision by blunt dissection. A piece of viable splenic tumour was implanted into the subcutaneous pocket of each mouse and the incisions were closed with surgical staples. The mice were observed daily for general health and tumour growth. A mass was grossly visible in one of the two mice within 7 weeks of implantation. The mouse showed no systemic ill health effects from the tumour and a tumour endpoint (2 cm³ volume) was reached on the 54th day after implantation, at which time the mouse was killed. At necropsy examination, two distinct, round to oval, well-demarcated, movable subcutaneous masses were present at the implantation site. Splenomegaly was the only other gross abnormality.

The subcutaneous masses were white and glistening on cut surface. The tumours and spleen were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Microscopical examination of HE-stained sections showed that the xenografted tumours had histological architecture comparable with the primary T-cell lymphoma from the dog (Fig. 3). The tumours consisted of intermediate to large round cells with pale cytoplasm, round to oval nuclei with finely stippled chromatin, prominent nucleoli and a mitotic index of 11. Immunohistochemically, the cells also showed strong reactivity with anti-CD3 antibody (Fig. 3), and again, only small lymphocytes scattered throughout the tumour matrix were consistent with residual, long lived B-cells, labelled with antibodies against CD79a and CD20 (supplementary Figs. 2A, 2B). A similar population of CD3⁺ tumour cells was seen in the parenchyma and invading the capsule of the spleen (supplementary Fig. 3).

To confirm the canine origin of the malignant T cells, we investigated if related, clonally expanded lymphocyte populations could be detected by polymerase chain reaction (PCR) for clonal antigen receptor rearrangements (PARR) in the primary tumour and the xenograft (Comparative Immunology Laboratory, Colorado State University, Fort Collins, Colorado, USA). Briefly, DNA was extracted from snap-frozen samples of the dog spleen and the mouse subcutaneous masses (QIAamp DNA mini kit, Qiagen, Valencia, California, USA). The PARR assay was carried out in two separate reactions: the first, containing the immunoglobulin gene primers detects complete V_H-J_H rearrangements, and incomplete D_H-J_H rearrangements. The second, containing the T-cell receptor (TCR) gene primers results in amplification of three V_γ gene families, which are distinguishable by the size of the PCR product and the dye attached to each primer. The results from the canine sample showed a clonal rearrangement using the first V_γ cassette (approximately 60 base pairs [bp]; supplementary Fig. 4A). However, selection in the recipient mouse allowed for enrichment of the malignant clone, with a reduction of background from normal T cells, which highlighted rearrangements in the other V_γ cassettes detectable at approximately 415 and 469 bp (supplementary Fig. 4B).

Considering the genetic background of the recipient mouse, which results in deficiency of B cells, T cells and natural killer (NK) cells, and the detection of a clonal population of lymphocytes with rearrangements of the canine TCR- γ gene, we confirmed that the disseminated T-cell lymphoma originated from the transplanted canine tissue and expanded in the microenvironment provided by the immunodeficient mouse.

Several examples of PDTX from human T-cell tumours have been reported, including acute T cell leukaemias (Guimaraes-Correa *et al.*, 2011; Agnusdei *et al.*, 2014) and one example of an anaplastic large T-cell lymphoma (Abate *et al.*, 2015). A cell line (OSW) derived from the pleural effusion of a dog with malignant peripheral T-cell lymphoma that forms tumour xenografts in SCID/NOD mice was reported (Kisseberth *et al.*, 2007). However, the OSW xenografts did not retain the immunophenotype of the original tumour. A second instance of a successful T-cell lymphoma xenograft used disaggregated cells from the popliteal lymph node of a dog with disseminated, stage IV T-cell lymphoma (Nadella *et al.*, 2008). In this case, the xenograft tumours had similar characteristics to the parent tumour, showing systemic dissemination in the mice after intraperitoneal injection and retaining the CD3⁺ immunophenotype. To our knowledge, however, this represents the first report of a canine T-cell lymphoma PDTX derived by implantation of intact tumour tissues into immunocompromised mice. The observation that the tumour became established in the spleen indicates that the biological behaviour and capacity to achieve systemic dissemination also were preserved in this PDTX model.

PDTX models in immunocompromised mice are valuable in the study of cancer (Jin *et al.*, 2010; Hidalgo *et al.*, 2014). These models have several unique features that complement the use of conventional cell lines, including providing pathological tissues for histological and molecular characterization, as well as acting as tumour-bearing laboratory animals in which novel treatment strategies can be tested under controlled conditions (Dong *et al.*, 2010; Jin *et al.*, 2010). PDTXs also could help to overcome the historical challenges and inefficiencies that characterize the establishment of immortalized cell lines from solid T-cell lymphomas (Kisseberth *et al.*, 2007), avoid the genetic drift that alters the biological properties of cell lines, and maintain the inherent heterogeneity of tumours that is lost over time in cultured cells, which represent a single clone from the tumour (Hidalgo *et al.*, 2014). Although, as is shown by our data, these tumours are also subject to selection that might narrow the clonal pool. Intriguingly, the environment allowed for long-term survival of residual canine B cells in the tumour. The precise subtypes and the functional significance of these B cells to support tumour growth merit further investigation.

In summary, we report the successful establishment of a PDTX from a canine enteropathy-associated T-cell lymphoma, large cell type, providing the basis for a platform that can be used to improve our understanding the biology of these tumours, as well as for drug discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Enteropathy-associated canine T-cell lymphoma, large cell type in small intestine. (A) Transmural effacement and expansion by neoplastic lymphocytes. HE. Bar, 345 μ m. (B) Neoplastic lymphocytes are intermediate to large with irregular nuclei, granular chromatin, multiple nucleoli and frequent mitotic figures. HE. Bar, 34.5 μ m.

Fig. 2. Enteropathy-associated canine T-cell lymphoma, large cell type in spleen. Neoplastic lymphocytes are morphologically similar to those noted in the small intestine. HE. Bar, 34.5 mm. Inset: cell surface expression of CD3 by neoplastic lymphocytes. IHC. Bar, 34.5 μ m.

Fig. 3. Subcutaneous mass of patient-derived tumour xenograft retains characteristics of canine T-cell lymphoma. Morphological characteristics of neoplastic lymphocytes resemble those from the implanted enteropathy-associated canine T-cell lymphoma, large cell type shown in Figs. 1 and 2. The cells are intermediate to large-sized with round to oval nuclei, fine chromatin and numerous nucleoli. HE. Bar, 34.5 μ m. Inset: cell surface expression of CD3 by patient-derived neoplastic lymphocytes. IHC. Bar, 34.5 μ m.