RNA-Seq analysis of gene expression in 25 cases of canine lymphoma undergoing CHOP chemotherapy

Miles W. Mee1, Sydney Faulkner1, Geoffrey A. Wood2, J. Paul Woods3, Dorothee Bienzle2 and Brenda L. Coomber1*

Abstract

Objectives: Canine lymphoma, the most common hematological cancer in dogs, shares many molecular and clinical characteristics with human Non-Hodgkin lymphoma (NHL). The standard treatment for canine lymphoma is “CHOP” multiagent chemotherapy protocol consisting of Cyclophosphamide, Doxorubicin (Hydroxydaunorubicin), Vincristine (Oncovin™), and Prednisone. Approximately 70–85% of patients treated with CHOP achieve clinical remission. However, duration of remission varies and the majority of dogs eventually relapse. To identify possible biomarkers for patients failing to achieve remission, we performed RNA-Seq analysis on 25 cases of canine lymphoma obtained prior the start of their CHOP therapy regime and assessed gene expression associated with patient progression free survival (PFS).

Data description: The data consists of (1) raw RNA-Seq reads in 75 bp fastq format from fine needle aspirate samples of enlarged lymph nodes from canine patients with naturally occurring lymphoma; (2) Fragments Per Kilobase Million (FPKM) values for each sample; (3) raw transcript counts for each sample; (4) anonymized patient details including PFS; (5) heat map of gene expression and (6) Cox proportional hazard analysis showing significantly expressed genes. These data may be useful for comparative analysis of gene expression in human NHL and analysis of gene expression associated with disease outcome in canine lymphoma.

Keywords: Cancer, Lymphoma, Dog, Gene expression, Comparative oncology, Chemotherapy, RNA sequencing

Objective

Canine lymphoma is the most common haematopoietic neoplasm in dogs [1] and is similar to human non-Hodgkin lymphoma (NHL). Both canine and human NHL have similar clinical presentation, molecular biology, therapy, and treatment response [2, 3]. Lymphoma is also common in dogs, and is treated with similar multiagent chemotherapy regimens as human disease yet has a much faster disease progression (time to relapse of 6–8 months is typical [4]) than that seen in NHL. This makes canine lymphoma an attractive comparative oncology model for the most aggressive human NHL cases.

The standard treatment is standard in both canine lymphoma and NHL, consisting of a multi-agent chemotherapy protocol of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), often with the addition of rituximab in humans [5]. There is a high initial response rate for canine lymphoma to CHOP of about 70% to 85%. However, the duration of remission varies and the majority of patients will eventually relapse [4, 6]. The failure of many canine lymphoma patients to achieve long term remission has raised interest in developing methods to predict their response to CHOP therapy.
While several molecular biomarkers have been proposed [7–9] in general they lack a clear therapeutic target, thus additional research is required. The data provided here may be useful for analysis of gene expression changes related to disease outcome in canine lymphoma, and for comparative analysis of gene expression in human NHL.

Data description

Case enrolment

Dogs with naturally occurring lymphoma, diagnosed by cytology or histology at the Mona Campbell Centre for Animal Cancer, University of Guelph, who had received no prior treatment other than a single injection of prednisone were eligible for this study. No breed, sex or age restrictions were in place, but dogs with other concurrent neoplasms or prior neoplasms including lymphoma were excluded. Lymphomas were immunophenotyped by flow cytometry [10]. Dogs were enrolled with the intention to treat with standard CHOP therapy and were monitored by physical examinations ± diagnostic imaging for a minimum of 6 months to categorize response as complete remission or not by WHO criteria [11]. Dogs lost to follow-up, or not progressed at last check were censored for PFS.

RNA-Seq data generation and processing

Tumor samples were collected by fine needle aspirate and expressed into sterile collection vials containing 1.0 ml of RNAProtect Cell Reagent (Qiagen). Poly A-RNA was isolated from the tissue samples using QIAGEN RNeasy isolation kit and quantified using a Nanodrop spectrophotometer. The RNA integrity number (RIN) was calculated for each sample using Bioanalyzer analysis. Twenty-five samples with RIN above 9 were selected for sequencing, including 16 immunophenotyped as B-cell lymphoma, three immunophenotyped as T-cell lymphoma, and six patients with missing immunophenotype. The selected samples were sequenced using the Illumina NextSeq platform by the London Regional Genomics Centre, London, ON, Canada. The sequence data were returned in single read 75 bp fastq format. Data Set 1 contains the RNA sequencing results for this publication, which have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE130874 [12]. The RNA-Seq reads were assessed for quality using FastQC [13]. The reference genome (CanFam3.1) and gene model (CanFam3.1.88.gtf) were downloaded from Ensembl [14]. The raw fastq reads were aligned to the CanFam3.1 reference sequence and CanFam3.1.88.gtf annotation file using Hisat2 [15]. StringTie [16] was used to annotate genes and quantify their expression. Fragments Per Kilobase Million (FPKM) values were calculated using the R package Ballgown [17] and are available in Data file 1 [12]. Raw transcript counts for expressed genes are available in Data file 2 [12]. Key patient details, including PFS are available in Data file 3 [18]. When we hierarchically clustered and visualized the RNA-Seq data from all sequenced samples, we observed two patient clusters corresponding to samples isolated by separate technicians, suggesting some batch effects existed between the patient clusters (Data file 4 [18]). While there were 1052 genes with unadjusted Cox Proportional Hazard model p-values < 0.05 (Data file 5 [18]), we were unable to confirm their significance in a validation set of cases. Thus, no research publication from this study was feasible. However, we feel the available data may be of use to others who are researching lymphoma in dogs and in humans (see Table 1).

Limitations

This study is limited by the small sample size, and technical issues resulting in batch effects further reducing the statistical power. The lack of complete immunophenotype information for all cases, and the lack of technical replicates (repeat sequencing) are also limitations. The

| Table 1 Overview of data files/data sets |
|------------------------------------------|
| **Label** | **Name of data file/data set** | **File types (file extension)** | **Data repository and identifier (DOI or accession number)** |
| Data set 1 | Raw sequence reads for 25 canine lymphoma cases | FASTQ files (fastq.gz) | NCBI GEO; https://identifiers.org/geo:GSE130874 [12] |
| Data file 1 | FPKM values for expressed genes | TSV file (tsv.gz) | NCBI GEO; https://identifiers.org/geo:GSE130874 [12] |
| Data file 2 | Raw transcript counts for expressed genes | TSV file (tsv.gz) | NCBI GEO; https://identifiers.org/geo:GSE130874 [12] |
| Data file 3 | Clinical outcome for 25 canine lymphoma cases | TAB file (tab) | Harvard Dataverse; https://doi.org/10.7910/DVN/PGL3BO [18] |
| Data file 4 | Heatmap of gene expression | Adobe Acrobat portable document format file (.pdf) | Harvard Dataverse; https://doi.org/10.7910/DVN/PGL3BO [18] |
| Data file 5 | Results of Cox proportional hazard analysis of gene expression | TAB file (tab) | Harvard Dataverse; https://doi.org/10.7910/DVN/PGL3BO [18] |
selection of cases to sequence was initially influenced by client decision to treat their dogs with CHOP, and client consent to participate in this study. Recovery of sufficient total RNA of suitable quality from available cases and finances limited the RNA-Seq analysis to 25 samples. All or any of the above may have had unintended and unknown influence on the data obtained.

Acknowledgements
The authors wish to thank Allison Majeed, Karolina Skowronski and Victoria Sabine at the Mona Campbell Centre for Animal Cancer for help with sample collection and clinical monitoring. We appreciate the technical advice and assistance with sample preparation from Jodi Morrison, Department of Biomedical Sciences, and from RNA Diagnostics Inc.

Authors’ contributions
MWM isolated RNA from patient samples, performed bioinformatics analysis, and was a major contributor to the writing of the manuscript; SF isolated RNA from patient samples; GAW assisted in study design; JPW supervised case recruitment and assisted in study design; DB supervised case immunophenotyping and assisted in study design; BLC assisted in study design and was a major contributor to the writing of the manuscript; all authors read and approved the final manuscript.

Funding
This study was funded by a VIP II award (#24467) to BLC, DB and JPW from the Ontario Centre of Excellence in conjunction with funds from RNA Diagnostics Inc. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials
The raw RNA sequencing results for this publication are available from the NCBI Gene Expression Omnibus and are accessible through GEO Series accession number GSE130874 https://identifiers.org/geo:GSE130874. The FPKM values and raw transcript counts are also available through GEO accession number GSE130874. The data on patient outcome, information on batch effects, and the results of a Cox Proportional Hazard evaluation are available at https://doi.org/10.7910/DVN/PGL3BO. Please see Table 1 and references [12, 18] for details and links to the data.

Declarations
Ethics approval and consent to participate
This study was conducted in accordance with the Canadian Council on Animal Care (CCAC) Guidelines, as approved and supervised by the University of Guelph Animal Care Committee. Informed consent for sample collection for this study was obtained from all clients prior to enrollment of their dogs.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada. 2Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada. 3Department of Clinical Studies and Mona Campbell Centre for Animal Cancer, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada.

Received: 14 October 2021 Accepted: 14 March 2022

References
1. Marconato L, Gelain ME, Comazzi S. The dog as a possible animal model for human non-Hodgkin lymphoma: a review. Hematol Oncol. 2013;31(1):1–9.
2. Sverdlov SH, Campo E, Pilieri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
3. Ito D, Frantz AM, Modiano JF. Canine lymphoma as a comparative model for human non-Hodgkin lymphoma: recent progress and applications. Vet Immunol Immunopathol. 2014;159(3–4):192–201.
4. Soerenko K, Overley B, Knick E, Ferrara T, LaBlanc A, Shoffer F. Outcome and toxicity associated with a dose-intensified, maintenance-free CHOP-based chemotherapy protocol in canine lymphoma: 130 cases. Vet Comp Oncol. 2010;8(3):156–208.
5. Coiffer B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346(6):325–42.
6. Flory AB, Rassnuck KM, Erb HN, Garrett LD, Northrup NC, Seiling KA, et al. Evaluation of factors associated with second remission in dogs with lymphoma undergoing retreatment with a cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy protocol: 95 cases (2000–2007). J Vet Med Assoc. 2011;12(8):501–6.
7. Richards KL, Motzingier-Reif AA, Chen HW, Fedoriyi Y, Fan C, Nielsen DM, et al. Gene profiling of canine B-cell lymphoma reveals germinal center and postgerminal center subtypes with different survival times, modeling human DLBCL. Cancer Res. 2013;73(16):5029–39.
8. Reibhun RB, Lana SE, Ehhrart EJ, Charles JB, Thamm DH. Comparative analysis of survivin expression in untreated and relapsed canine lymphoma. J Vet Intern Med. 2008;22(4):989–95.
9. Sierra Matiz OR, Santilli J, Anai LA, Da Silva MCL, Sueiro FA, Sequeira JL, et al. Prognostic significance of Ki67 and its correlation with mitotic index in dogs with diffuse large B-cell lymphoma treated with 19-week CHOP-based protocol. J Vet Diagn Invest. 2018;30(2):263–7. https://doi.org/10.1177/1040637717743280.
10. Deravi N, Berke O, Woods JP, Bienez D. Specific immunotypes of canine T cell lymphoma are associated with different outcomes. Vet Immunol Immunopathol. 2017;191:15–13.
11. Vezzali E, Parol AD, Marcato PS, Bettini G. Histopathologic classification of 171 cases of canine and feline non-Hodgkin lymphoma according to the WHO. Vet Comp Oncol. 2010;8(1):38–49.
12. Mee WM, Faulker S, Wood GA, Woods JP, Bienez D, Coomber BL. Replication Data for RNA-Seq analysis of gene expression in 25 cases of canine lymphoma undergoing CHOP chemotherapy. NCBI Gene Expression Omnibus (GEO). 2021. https://identifiers.org/geo:GSE130874.
13. Andrews S. FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Accessed 16 Mar 2022.
14. Yates A, Akamri W, Amode MR, Barrett DC, Billis K, Carvalho-Silva D, et al. Ensembl 2016. Nucleic Acids Res. 2016;44(D1):D710–6.
15. Kim D, Langmead B, Salzberg SL. HISAT: fast spliced aligner with low memory requirements. Nat Methods. 2015;12(4):357–60.
16. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol. 2015;33(3):296–5.
17. Frazee AC, Pertea G, Laffie AE, Langmead B, Salzberg SL, Leek JT. Ballgown bridges the gap between transcriptome assembly and expression analysis. Nat Biotechnol. 2015;33(3):243–6.
18. Mee WM, Faulker S, Wood GA, Woods JP, Bienez D, Coomber BL. Replication Data for RNA-Seq analysis of gene expression in 25 cases of canine lymphoma undergoing CHOP chemotherapy. Harvard Dataverse. https://doi.org/10.7910/DVN/PGL3BO. Accessed 16 Mar 2022.
Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.