TO THE EDITOR:

Shared and distinct genetic features in human and canine B-cell lymphomas

Krysta Mila Coyle,1,* Tiana Hillman,1,* Matthew Cheung,1 Bruno M. Grande,1 Kevin R. Bushell,1 Sarah E. Arthur,1 Miguel Alcaide,1 Nicole Thomas,1 Kostiantyn Dreval,1 Stephanie Wong,1 Krishanna Campbell,1 and Ryan D. Morin1-3

1Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; and 2Genome Sciences Centre, and 3Lymphoid Cancer Research, BC Cancer, Vancouver, BC, Canada

Animal models of human cancers are an important tool for the development and preclinical evaluation of therapeutics. Canine B-cell lymphoma (cBCL) is an appealing alternative to murine preclinical models because of its frequent, spontaneous incidence and its clinical and histological similarities to some human mature B-cell neoplasms.1,2 Dogs are particularly relevant for comparative oncology, as they show a higher sequence similarity in cancer genes to humans, relative to mice, and telomerase is largely inactive in adult dog tissues, as in humans.3,4 Current veterinary care for cBCL includes many of the same chemotherapeutic agents used for human B-cell lymphomas (hBCLs), and the accelerated lifespan of dogs and relative acceleration in cancer progression may allow for more rapid observations of experimental treatments.1,5,6 Of note, trials of the Bruton tyrosine kinase inhibitors ibrutinib6 and acalabrutinib7 in canines provided evidence of clinical efficacy before the first in-human studies. Subsequent approval of these inhibitors for treatment of hBCLs speaks to the relevance of canine models.

The most common form of cBCL resembles human diffuse large B-cell lymphoma (hDLBCL),3 with other subtypes, including Burkitt-like cBCL, less frequently diagnosed.8,9 Genomic characterization of hDLBCL continues to reveal novel subtypes with different clinical features and responses to therapy.10 Given the mutation patterns that underlie molecular heterogeneity in hDLBCL, we hypothesized that the molecular heterogeneity of cBCL and its relationship to hDLBCL remains incomplete and is not adequately captured by current diagnostic methods.11 Moreover, the utility of cBCL as a veterinary model of human disease would be bolstered by an enhanced understanding of the genetic alterations that collectively underlie cBCL.

We obtained fresh frozen and matched plasma/serum from 86 dogs from the Canine Comparative Oncology Genomic Consortium (CCOGC), with 61 confirmed as having BCL by immunophenotyping (supplemental Table 1). Immunophenotype was not available for the remaining samples. We extracted total RNA and DNA from 29 tumor samples and performed RNA sequencing as previously described (supplemental Table 2).12 Genomic DNA was extracted from the remaining tumors by using either the AllPrep DNA/RNA Universal Kit or the DNeasy Blood and Tissue Kit (Qiagen). DNA was extracted from plasma or serum with the MagMAX Cell-free DNA Isolation Kit (Thermo Fisher, Waltham, MA).

We used STAR to align RNA-seq reads to the canFam3 reference13 and identified single nucleotide variants and indels, as described previously.12 After identifying genes with evidence for recurrent mutations, we performed targeted sequencing of candidate mutations using custom polymerase chain reaction (PCR) primers. We produced custom capture baits by PCR amplification of each exon of interest using genomic DNA from a healthy dog as a template.14 Tumor DNA libraries were prepared by using the QIAseq FX DNA Library Kit (Qiagen). Plasma and serum DNA libraries were prepared with the NebNext Ultra II DNA Library Prep Kit (New England BioLabs), followed by enrichment using our baits. Sequencing data are available through the National Center for Biotechnology Information (NCBI) PRJNA797476. We aligned reads to canFam3.1 and visually confirmed mutations using Gencode. Variants were annotated with Variant Effect Predictor and human-dog pairwise alignments were extracted from Ensembl to identify human positions for all canine variants.

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*K.M.C. and T.H. contributed equally to this study.

Other data are available by email request to the corresponding author (rdmorin@sfu.ca).
Figure 1.
resistant to FBXW7-mediated degradation.21,22 In human MYC, these are known to stabilize the protein by rendering it conserved Cdc4 phosphodegron (CPD) sequence (Figure 1C). In (Figure 1B). Twelve mutations (80%) affect MYC box I, located in a focal pattern that is not consistent with the pattern in hDLBCL tumor DNA contaminating some of the normal samples.12 can be attributed, in part, to our prior observation of high levels of mutations in cBCL (Figure 1A), including the previously noted high frequency of mutations in TRAF3 (45%) and FBXW7 (20%; Figure 1A).12,15,16 DDX3X (20%) and MYC (13%) were mutated at a higher frequency than has been previously described in cBCL.15 These higher rates can be attributed, in part, to our prior observation of high levels of tumor DNA contaminating some of the normal samples.12

We compared the pattern and incidence of mutations between cBCL, hDLBCL, and human Burkitt lymphoma (hBL, from a variety of in-house and published sources (supplemental Figure 2).17-19 MYC is commonly deregulated by translocation in hDLBCL and hBL, and these events are commonly associated with point mutations related to aberrant somatic hypermutation.20 We observed a low frequency of MYC mutations in our cBCL cohort with a more focal pattern that is not consistent with the pattern in hDLBCL (Figure 1B). Twelve mutations (80%) affect MYC box I, located in a conserved Cdc4 phosphodegron (CPD) sequence (Figure 1C). In human MYC, these are known to stabilize the protein by rendering it resistant to FBXW7-mediated degradation.21,22

FBXW7 mutations are of particular interest, as we never observe both MYC and FBXW7 mutations in cBCL (Figure 1D). The most recurrent FBXW7 mutation affected R470, corresponding to the human R465 codon, which is also a hot spot in hDLBCL (Figure 1E). These mutations are predicted to yield a dominant negative form of FBXW7 that does not effectively degrade target substrates including MYC and NOTCH1.23 We hypothesize that mutations in FBXW7 and the MYC CPD represent alternative approaches to stabilizing MYC protein, possibly resulting in overexpression.

DDX3X was one of the most frequently mutated genes in our cohort (20%) and is among the most frequently mutated genes in hBL (46%), but with a strikingly different pattern (supplemental Figure 2). Interestingly, only missense mutations were observed in cBCL, whereas hBLs included a high proportion of truncating mutations (Figure 2A).24 We explored various clinical features of patients with hBL to identify possible explanations for this difference. Separating DDX3X mutations from male and female cases of hBL resolved a similar pattern only in female cases of hBL (Figure 2B), whereas stratification on Epstein-Barr viral status showed no clear pattern (supplemental Figure 3). In males, mutations were found across the entire length of the DDX3X coding region with a large proportion of truncating mutations, including both nonsense and frameshift, whereas the pattern in females was predominantly missense mutations affecting the DEAD box and helicase domains. In contrast, although all DDX3X mutations in cBCL were missense, there was no sex bias observed in frequency or location (Figure 2C).

DDX3Y, a paralog of DDX3X, is encoded on the Y chromosome. Based on high sequence similarity (Figure 2D) and functional evidence, DDX3X and DDX3Y proteins may have partially redundant functions in humans.24,25 We considered the possibility that DDX3Y plays a compensatory role in males with DDX3X mutations. We found a significantly higher expression of DDX3Y in males with hBLs with DDX3X mutations when compared with males without these mutations (Figure 2E). A similar comparison was not possible for cBCL because of the small sample size; however, our findings support the premise that these 2 proteins may have functional redundancy in the context of human lymphomagenesis, but may not in canine lymphomagenesis. This represents an important difference between cBCLs and hBLs.

cBCL has value as an intermediate between rodent models and clinical trials; however, our data identified 2 key factors, FBXW7 and DDX3X, that may promote the use of cBCL as a preclinical model for mature hBCLs. In human cancers, FBXW7 is most commonly mutated in cholangiocarcinoma and T-cell acute lymphoblastic leukemia,29 but is rarely observed in the mature B-cell malignancies used in this study. We hypothesize that FBXW7 mutations in cBCL have a redundant function to the mutations affecting the MYC phosphodegron, which may be the cause of the apparent mutual exclusivity observed in this study. This redundancy should be considered in future
**Figure 2.**

**A**
Protein percent identity matrix

|            | DDX3X human | DDX3X canine | DDX3Y canine | DDX3Y human |
|------------|-------------|--------------|--------------|-------------|
| DDX3Y human| 92.56       | 89.31        | 92.72        | 100         |
| DDX3Y canine| 96.52      | 93.45        | 100          |             |
| DDX3X canine| 96.81      | 100          |              |             |
| DDX3X human| 100         |              |              |             |

**B**

**C**

**D**

**E**

**Unmutated**

**Mutated**

0.037
studies of potential MYC-targeted therapies for canine lymphomas. Although differences in hBL DDX3X mutation frequency have been observed between sexes, we are the first to describe a sex-specific pattern of mutations affecting DDX3X in hBL; this pattern is not recapitu-
lated in cBCL. The discrepancy in mutation patterns between can-
ines and human represents an important distinction that may indicate
differences in the biology of these cancers.

This study revealed key differences in the mutational profiles of
cBCLs and hBCLs and provides an impetus for enhanced genomic
characterization of canine lymphomas, particularly in their continued
use as a preclinical model for human disease. The relevance of
canine models will vary, depending on the intervention being pur-
sued, and these findings may diminish the utility of canine models
for future clinical studies of targeted agents in DLBCL.

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K.R.B., B.M.G., and R.D.M. conceived and designed experiments;
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ORCID profiles: K.M.C., 0000-0002-1309-4873; B.M.G., 0000-
0002-4621-1589; S.E.A., 0000-0002-5341-7868; R.D.M., 0000-
0003-2932-7800.

Correspondence: Ryan D. Morin, Simon Fraser University, 8888
University Dr, Burnaby, BC V5A 1S6, Canada; e-mail: rdmorin@sfu.
ca; and Krysta M. Coyle, Simon Fraser University, 8888 University
Dr, Burnaby, BC V5A 1S6, Canada; e-mail: kcoyle@sfu.ca.

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