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Canine Lymphoma as a Comparative Model for Human Non-Hodgkin Lymphoma: Recent Progress and Applications

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Abstract

The term “lymphoma” describes a heterogeneous group of disorders involving monoclonal proliferation of malignant lymphocytes. As a group, lymphomas are among the most common tumors of dogs. Yet our enumeration and understanding of the many subtypes of lymphoma have been relatively slow, perhaps in part because for many years lymphoma was treated as a singular entity rather than a group of distinct diseases. The recognition that the full spectrum of lymphoid malignancies seen in humans also occurs in dogs, and that these tumors retain not only morphologic similarities and biological behavior but also synonymous driver molecular abnormalities, sets an ideal stage for dual-purpose research that can accelerate progress for these diseases in both species. Specifically, dogs represent exceptional models for defining causality, understanding progression, and developing new treatments for lymphoma in comparatively brief windows of time. Unique advantages of canine models include (1) spontaneous disease occurring without an isogenic background or genetic engineering; (2) chronology of disease adapted to lifespan, (3) shared environment and societal status that allows dogs to be treated as “patients,” while at the same time being able to ethically explore translational innovations that are not possible in human subjects; and (4) organization of dogs into breeds with relatively homogeneous genetic backgrounds and distinct predisposition for lymphomas. Here, we will review recent studies describing intrinsic and extrinsic factors that contribute to the pathogenesis of canine and human lymphomas, as well as newly developed tools that will enhance the fidelity of these models to improve diagnosis and develop new treatments.

2. Introduction

Non-Hodgkin lymphomas (NHL) are a heterogeneous group of relatively common human lymphoid malignancies that are increasing in incidence; the age-adjusted incidence of NHL has almost doubled in the past 30 years, with an estimated 70,130 new cases and 18,940 deaths in the UNITED STATES 2012 (Howlader et al., 2012). In Western countries, more than 80% of NHLs are mature B-cell tumors (Anon, 1997). The addition of Rituximab (chimeric anti-CD20) to multi-agent cytotoxic chemotherapy has significantly improved outcomes for patients with these B-cell malignancies (Traullé and Coiffier, 2005); however,

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causes for most subtypes of NHL remain unknown, therapeutic gains fail to extend life for all patients, and cures are elusive.

Lymphomas are among the most common types of tumors in dogs. There are remarkable similarities between the clinical features of canine lymphomas and human NHL, making the former attractive models for studying disease progression and therapy (reviewed in (Marconato et al., 2012)). Here, we will focus our attention on the molecular pathogenetic features of canine lymphomas, underscoring the concept that canine lymphomas also represent a heterogeneous group of diseases, and thus, each subtype is likely to best inform its unique disease homolog among human NHLs.

3. The Natural History of Canine Lymphomas

The data used to estimate the incidence of lymphomas in dogs in the United States date back to the 1960's. In the most widely cited study, Dorn reported that lymphomas accounted for ~6% of all malignancies in dogs in Alameda and Contra Costa Counties, CA (Dorn et al., 1968), and for ~90% of all hematopoietic cancers (Dorn et al., 1967). The incidence was estimated at 24 cases per 100,000 dog at risk (Dorn et al., 1967), which is slightly higher than the contemporary incidence rate reported for humans by the SEER program (19.6 per 100,000) (Howlader et al., 2012). More recent studies have estimated the incidence of canine lymphoma at 20 – 107 cases per 100,000, supporting the conclusion that the incidence of this group of diseases is higher in dogs than in humans (Dobson et al., 2002; Edwards et al., 2003; Merlo et al., 2008; Teske, 1994).

Dorn's work (Dorn et al., 1967), a large comprehensive survey of the Veterinary Medical Database in the United States and Canada by Priester and McKay in the late 1970's (Priester and McKay, 1980), and reports from hospital records and insurance databases in the United Kingdom and the Netherlands (Edwards et al., 2003; Teske, 1994) showed that certain dog breeds had statistically significant increased risk to develop lymphoma as compared to the average risk of all dogs. This suggests that heritable risk factors for the disease were introduced with the derivation of specific breeds. Other evidence supporting the existence of strongly embedded heritable factors for this disease in dogs could be inferred by familial clustering observed in Bullmastiff, Rottweiler, and Scottish Terrier lines (Onions, 1984; Teske et al., 1994). It is possible, however, that the demographics, incidence, and dynamics of the canine disease may have changed between then and now (Merlo et al., 2008; Ritt, 2010).

The introduction of multi-agent chemotherapy protocols as the standard of care for canine lymphoma in the 1980's and 1990's made this disease treatable, if not curable. Without treatment, dogs diagnosed with high-grade lymphoma generally survive less than 6 weeks, but survival increases progressively with chemotherapy: about 50% of dogs with high-grade lymphoma treated with multi-agent chemotherapy will stay in remission 7 – 10 months and will survive 10 – 14 months (Garrett et al., 2002; Keller et al., 1993; MacDonald et al., 2005). Breed type also might influence disease progression and response to therapy; for example, in one study, German Shepherd Dogs showed worse survival compared to other hospital populations (Garrett et al., 2002).

4. Pathological Classification of Canine Lymphoma According to the World Health Organization

The classification of human NHL into distinct subtypes has evolved over the past five decades to achieve a worldwide consensus in the latest World Health Organization (WHO) classification (Swerdlow et al., 2008). This classification utilizes morphology, topography, immunophenotype, and clinical progression to define approximately 30 distinct subtypes of human NHL; however, it is not static and continues to be refined. Work that attempted to develop a similar classification of canine lymphomas (Valli, 2002) was initially met with more resistance, but a modified WHO classification for canine lymphomas was recently published (Valli et al., 2011) and is slowly gaining acceptance.

A careful parallel assessment of the distribution of disease between both species is instructive to illustrate the strengths of the canine model (Figure 1). Specifically, diffuse large B-cell lymphoma (DLBCL) is the most commonly seen subtype in both species, but among the other five common subtypes that occur in dogs (Frantz et al., 2012; Ponce et al., 2010; Thomas et al., 2011; Valli et al., 2011), marginal zone lymphoma (MZL), peripheral T-cell lymphoma not otherwise specified (PTCL), nodal T-zone lymphoma (TZL), and lymphoblastic T-cell lymphoma (LBT) are quite rare in humans (Anon, 1997), providing resources that can help us to better understand these diseases at the cellular level.

It is worth noting that there appears to be a peculiar predilection for some dog breeds to develop certain subtypes of lymphoma. Overall, T-cell lymphomas account for about 35–40% of all lymphoma diagnoses in dogs (Modiano et al., 2005). However, this prevalence might be disproportionately influenced by heritable traits. Some modern Spitz (Northern) and Asian breeds develop T-cell lymphomas almost exclusively, whereas some modern European breeds develop B-cell lymphomas almost exclusively (Figure 2). Golden Retrievers and Boxers, two breeds with a high incidence of lymphoma (Dorn et al., 1967; Glickman et al., 1999; Priester and McKay, 1980), also show statistically different distributions of B-cell lymphomas and T-cell lymphomas when compared with the average for all dogs (Modiano et al., 2005). Somewhat predictably, the distribution of B-cell lymphomas and T-cell lymphomas in mixed breed dogs is comparable to that seen when all purebred dogs are considered as a single group (Modiano et al., 2005). This suggests that distinct factors that modulate lymphoma risk have been enriched during the process of breed derivation, providing unique opportunities to identify these traits through contemporary genome- wide studies (Tonomura et al., 2012). Furthermore, each breed is likely to reflect a small segment of outbred human (and mixed breed canine) populations; thus, the reduced genetic heterogeneity seen in dog breeds will help to lower the background noise of genome-wide approaches to identify the frequency of homologous risk factors (mutations) that are etiologically significant in both species.

5. Comparative Molecular Pathogenesis of Human NHL and Canine Lymphomas

5a. Shared Molecular Aberrations

Previous work has shown that shared pathognomonic molecular aberrations occur in some human and canine hematological malignancies. For example, Burkitt lymphoma (BL) in humans is characterized by a balanced translocation, whereby a region of human chromosome 8 (HSA 8q24) containing the MYC oncogene is juxtaposed on the region of chromosome 14 that contains the immunoglobulin heavy-chain (IgH) enhancer (HSA 14q32). This leads to constitutive MYC expression in B-cells, contributing to malignant transformation. In the sporadic disease, this translocation seems to occur stochastically. In the endemic form of BL, the translocation may be driven by instability associated with proliferation induced by Epstein-Barr virus (EBV) infection, and it potentiates EBV-associated lymphomagenesis (Ferry, 2006). In the dog, a corresponding MYC-IgH translocation was characterized where MYC from canine chromosome (CFA) 13 was juxtaposed with the IGH locus on CFA 8 (Figure 3) in sporadic BL (Breen and Modiano, 2008).

5b. Viral Etiologies in Lymphoma

Various types of endemic (transmissible) lymphoma have been described in humans. As noted above, endemic Burkitt lymphoma is associated with EBV infection, although a superimposed infection with the malarial parasite *Plasmodium falciparum* appears to be necessary for the complete manifestation of the malignant phenotype in this disease (Ferry, 2006). Along with EBV, the Human T-lymphotropic viruses (HTLV) HTLV-I and HTLV-II also are associated with endemic forms of T-cell lymphoma (Müller et al., 2004). There are few reports of canine lymphotropic retroviruses associated with leukemia and lymphoma (Safran et al., 1992; Tomley et al., 1983). Recently, a possible gamma herpes virus-like agent also was observed in five cases of canine B-cell lymphoma, (Huang et al., 2012). However, the significance of viral agents in the pathogenesis of canine lymphoma remains to be determined, as Koch's postulates have not been fulfilled through experimental transmission and there is no epidemiological evidence to suggest transmissibility in canine lymphomas.

5c. Comparative Cytogenetics – Heritable Traits and Gene Dosage

Human NHLs show relatively high levels of molecular diversity, consistent with their morphological diversity. Recurrent DNA copy number aberrations (CNA) exceed the frequency expected from normal copy number variability (Alvarez and Akey, 2012), but even within each WHO subtype, there is sufficient heterogeneity such that CNAs that are fundamentally associated with disease pathogenesis have remained largely elusive (Thomas et al., 2011).

CNAs are also relatively common in canine lymphomas, albeit the genomic imbalance in dogs is reduced compared to that seen in human NHL (Thomas et al., 2011). Breen et al have proposed that the confounding effects of genomic heterogeneity in domestic dogs are

limited as a consequence of the development of distinct and genetically isolated breeds, and thus detection of genetic factors that are intimately associated with tumor pathogenesis may be more penetrant, even if less frequent (Thomas et al., 2011). Several evolutionarily conserved CNAs were identified in canine lymphoma and human NHL using an innovative "genomic recoding" model, which organizes the genomes of different species according to syntenic blocks. The frequency of canine T-cell lymphomas may be especially useful in defining recurrent, pathogenetically significant CNAs. What is more, the observed breed bias may be especially informative in defining the possible role of heritable traits in genomic instability, as reflected by aneuploidy, and in tumor-specific alterations in gene dosage.

5d. Gene Expression Profiling

Gene expression profiling is another genome-wide method that has been applied to categorize NHLs based on their molecular features. This approach has been used most extensively in DLBCL, where a molecular classification is widely accepted (Alizadeh et al., 2000; Lossos et al., 2004; Rosenwald et al., 2002; Shipp et al., 2002). Recently, we reported that canine DLBCL, BL, MZL, LBT, PTCL, and TZL can be organized into three distinct molecular subgroups consisting of high-grade T-cell lymphomas (LBT, PTCL), low-grade T-cell lymphomas (TZL), and B-cell lymphomas (DLBCL, BL, and MZL) (Figure 4) (Frantz et al., 2012). Our data also suggest that DLBCL and nodal MZL may exist as a continuum of the same disease, with MZL representing a possibly distinct subgroup in both dogs and humans (Frantz et al., 2012). In the case of canine lymphomas, this molecular classification was prognostic and could be done using relatively simple quantitative real-time RT-PCR assays, which should make them translatable to more conventional diagnostic platforms (Frantz et al., 2012).

Our data did not support the organization of canine DLBCL into molecular groups that resemble human activated B-cell (ABC) DLBCL and germinal center-like B-cell (GCB) DLBCL. This could reflect the heterogeneity that is intrinsic to DLBCL as the largest single group of lymphoma in both species, or it could suggest that canine DLBCL represents only one subtype (a possibility that would be challenging to confirm in studies with small sample size and in the absence of the respective comparison group). As far as we know, there is no report that satisfies the criteria for subtyping ABC and GCB DLBCL in dogs. At present, we would caution investigators to avoid using canine lymphoma to model specifically ABC or GCB DLBCL subtypes.

6. Model Systems to Study the Biology of Canine Lymphoma

6a. In vitro culture of primary B-cell lymphomas

In general, hematologic malignancies have proven substantially less tractable for *in vitro* manipulation than their solid tumor counterparts. Unlike normal lymphoid cells that can survive for days to weeks in culture and can be manipulated to divide synchronously, primary lymphoma cells rarely survive >24–48 hours in culture, with or without exogenous mitogens. Canine B-cell lymphomas are particularly unstable *ex vivo*, and indeed only one canine B-cell lymphoma cell line exists that has consistently retained its *in vivo* phenotype with high fidelity after repeated passage (Rütgen et al., 2010; Rütgen et al., 2012).

Nevertheless, the utility of human lymphoma cell lines that are available to the scientific community is evident in the countless citations by investigators who have used them as resources to define signaling pathways in lymphocyte activation and lymphocyte development and, to some extent, in therapeutic development. Even so, these cell lines represent only a sliver of the diversity seen both within and among tumors. Therefore, the development of reliable methods to culture primary lymphomas *in vitro* and *in vivo* would provide a useful resource for preclinical studies. To this end, we adapted the "CD40 system," which was described as an effective method to maintain human B-cells *in vitro* (Andersen et al., 2000; Planken et al., 1996; Visser et al., 2000), to grow primary canine B-cell lymphoma cells in short-term and extended culture (Ito et al., 2012). We showed that CD40 signals can be delivered by feeder cells expressing CD40 ligand (CD40L, a member of the tumor necrosis factor family) (Mason et al., 2008) or in a cell-free culture system using soluble, trimeric CD40L. Canine B-cell lymphomas grown under these conditions retained their original phenotype, clonality, and known karyotypic abnormalities even after extended periods of expansion. The cell-free system was especially useful to assess targeted reagents against canine and human B-cell malignancies, highlighting its potential applications for preclinical development.

The observation that CD40 signaling supports growth of primary malignant canine B-cell lymphoma cells in culture is consistent with the notion that this pathway plays an important role in the oncogenic maintenance of B-cell malignancies. It is well established that the interaction of CD40L with CD40 promotes activation of nuclear factor kappa B (NFκB) transcription factors, which in turn promote survival of human and canine B-cell lymphoma cells (Challa et al., 2002; Davis et al., 2001; Gaurnier-Hausser et al., 2011; Pham et al., 2002), thus providing a series of rational targets for therapeutic development.

6b. Xenotransplantation models of primary B-cell lymphomas

In vivo experiments have been essential to understand the effects of potential therapies in complex systems that include the supporting tumor microenvironment. Xenotransplantation of human lymphoma cell lines into receptive, immunodeficient mice has been a powerful tool for preclinical development, and similar models are reported for two canine lymphoma cell lines (Kisseberth et al., 2007; Rütgen et al., 2012). However, the use of cell lines for *in vivo* xenotransplantation is subject to the same limitations regarding incomplete representation of tumor diversity, and most attempts to transplant primary B-cell lymphomas into mice have been unrewarding. In what is probably the most comprehensive study to address this, Mori and colleagues showed that only 10 of 50 human NHL tumors (and specifically 8 of 30 derived from B-cell NHL) injected into mice with severe combined immunodeficiency engrafted successfully based on the retention of phenotypic and genotypic properties of the primary tumors (Itoh et al., 1993). The other 13 engrafted tumors were found to be newly developed clones composed of EBV-transformed B-cells (Itoh et al., 1993). Two important limitations are apparent from this study; (1) only a limited number of human NHLs were able to engraft successfully in immunodeficient mice, and (2) the high prevalence of EBV infection in human populations will remain a persistent challenge to establish primary human lymphoma xenograft models.

As described above, EBV infection is unlikely to present a problem in development of xenotransplantation of canine lymphomas. So, we chose a different mouse model to enhance the rate of engraftment, using NOD/SCID/IL-2Rg^{null} (NSG) mice as recipients. These mice show improved engraftment of human hematopoietic stem cells over other mouse strain (Shultz et al., 2005), and in one study, they were used successfully to engraft a primary human follicular lymphoma and a DLBCL (Chao et al., 2010). We also added a conditioning step to improve engraftment. In our initial experiments, we noted that residual T-cells present in canine B-cell lymphomas caused severe graft-versus-host disease. But increasing the stringency of depletion was sufficient to overcome this problem and produced reliable, serially transplantable canine B-cell lymphoma xenografts (Figure 5) (Ito et al., 2011). In our experience, canine T-cell lymphomas also engraft with low frequency, but the conditioned NSG system may be sufficiently robust to overcome this challenge. Altogether, models using xenotransplantation of primary tumors will offer better models for assessing preclinical efficacy, especially when used as part of comparative approaches where primary tumor cells from humans and dogs are analyzed side by side.

7. Tumor-Initiating Cells in Lymphoma

The importance of cancer stem cells or tumor-initiating cells (TICs) in the pathogenesis of cancer is becoming increasingly well recognized (Nguyen et al., 2012). However, there are only a few reports supporting the existence of TICs in human lymphoma cell lines or in transgenic lymphoma mouse models (Lee et al., 2012; Vega et al., 2009; Wang et al., 2011). Our group identified a putative lymphoma-initiating cell (Ly-IC) population in primary canine B-cell lymphomas, which was characterized by co-expression of hematopoietic progenitor antigens CD34, c-Kit, and CD133, the lymphoid lineage marker CD22, and the common leukocyte antigen CD45 (Figure 6A) (Ito et al., 2011). When compared with the bulk of the tumor cells (BTCs), the putative Ly-IC population (enriched for CD34 expression) showed significantly lower expression of 44 genes across the genome that mapped to “Cell Cycle” and to “Membrane and Raft proteins” pathways using Ingenuity Pathway Analysis (Figure 6B). This suggests that Ly-ICs may exhibit the characteristic “slow proliferation” that is seen in normal bone marrow-derived hematopoietic stem cells. Importantly, the putative Ly-IC population persisted in the xenotransplantation setting (Figure 5), suggesting it is relevant to the biology of this disease *in vivo* (Ito et al., 2011).

The existence of TICs in other lymphoid malignancies is similarly controversial (Bernt and Armstrong, 2009). Several groups have reported putative TICs in acute lymphoblastic leukemia (ALL) based on co-expression of CD34, CD133, and CD19 (Castor et al., 2005; Cox et al., 2009; Cox et al., 2004; Hotfilder et al., 2002; le Viseur et al., 2008). We identified an Ly-IC-like population in human B-cell ALL that co-expressed KIT, CD19, and CD22 (Figure 6A), which may be equivalent to the canine B-cell Ly-ICs we described above. Additional work is needed to establish the significance of TICs in the pathogenesis of lymphoid malignancies of humans and dogs. However, if the hierarchical model applies to these tumors, it will be imperative to develop strategies that can target the TIC compartment in order to improve the outcome of patients with these tumors.

8. Canine Lymphoma in Pre-clinical Development

One of the major advantages of working with canine lymphomas is our capacity to utilize these tumors as spontaneous models of NHL for pre-clinical development. The capability of achieving rapid accrual and the potential benefit afforded by the shorter chronology of disease have been described in detail elsewhere (Khanna et al., 2006; Marconato et al., 2012; Paoloni and Khanna, 2008). As noted above, however, the capacity of these models to inform the process of pre-clinical development will require rigorous proof of anatomic, morphologic, phenotypic, and molecular homology to ensure that the canine disease is comparable to the subtype of NHL that is being modeled.

In their recent review of this topic ((Marconato et al., 2012), Comazzi and colleagues summarized recent drug trials that demonstrated safety and efficacy for treatment of canine lymphoma, including GS-9219 (a prodrug of the nucleotide analogue 9-(2-phosphonylmethoxyethyl) guanine), ABT526 (a modified thrombospondin-I peptide), and a NEMO-binding domain peptide that acts as an inhibitor of the NFκB pathway. Other contemporary trials include documentation of Btk target modulation by the small molecule inhibitor PCI-32765 (Honigberg et al., 2010) and tolerability of a pro-caspase-3 activating compound (Peterson et al., 2010).

Data from several multi-institutional efforts to develop new reagents and protocols also are reaching maturity. One double-blinded, placebo-controlled study examined safety and efficacy of neoadjuvant PSC-833 (Novartis, Inc.), a selective inhibitor of the ATP binding cassette B1 transporter (ABCB1, a.k.a., p-glycoprotein/multidrug resistance protein-1), in dogs with therapy-naïve DLBCL. This study was designed to test the hypothesis that modulation of ABCB1 activity in Ly-ICs by PSC-833 would sensitize these to doxorubicin-induced cytotoxicity and thus lengthen remission intervals. Interim data analyses showed a trend towards delayed progression in the PSC-833 group compared to placebo, and significantly delayed progression associated with stabilization or reduction in the percent of Ly-ICs in lymph nodes at the end of the neoadjuvant therapy period (Ito et al., 2013). Another study examined the therapeutic potential of KPT-335 (Karyopharm Therapeutics, Inc.), an orally available selective inhibitor of nuclear export (SINE) that binds exportin 1 (a.k.a., CRM1). SINE compounds inhibit export of tumor suppressors and other proteins, which in turn leads to selective death of tumor cells *in vitro*. This study identified a maximum tolerated dose for KPT-335, with disease stabilization and tumor reduction when used as a single agent, in dogs with relapsed DLBCL (Schacam et al., 2012).

9. Future directions

There has been remarkable progress in the development of new therapies that can improve or replace traditional chemotherapy protocols for NHL. Perhaps the best example is rituximab, which revolutionized treatment for most B-cell malignancies (Traullé and Coiffier, 2005); however, there is a wide array of additional promising reagents in the FDA pipeline, including Ibrutinib (PCI-32765) (Wiestner, 2013), Bortezomib (a proteasome/NFκB inhibitor) (Mato et al., 2012), Brentuximab vedotin (a CD30-specific antibody-drug conjugate) (Deng et al., 2013), and others. We are particularly enthusiastic about a new

multi-institutional effort using CD47 blockade (Chao et al., 2010) and a new anti-canine CD20 antibody to model development of this combinatorial approach for DLBCL and nodal MZL.

In summary, we advocate setting a high bar where spontaneous canine lymphomas are used judiciously to model homologous subtypes of human NHL. Careful attention to anatomic, morphologic, molecular, and clinical features of these diseases can accelerate discovery and improve the efficiency of translation, ultimately benefiting dogs and humans, and addressing unmet medical needs.

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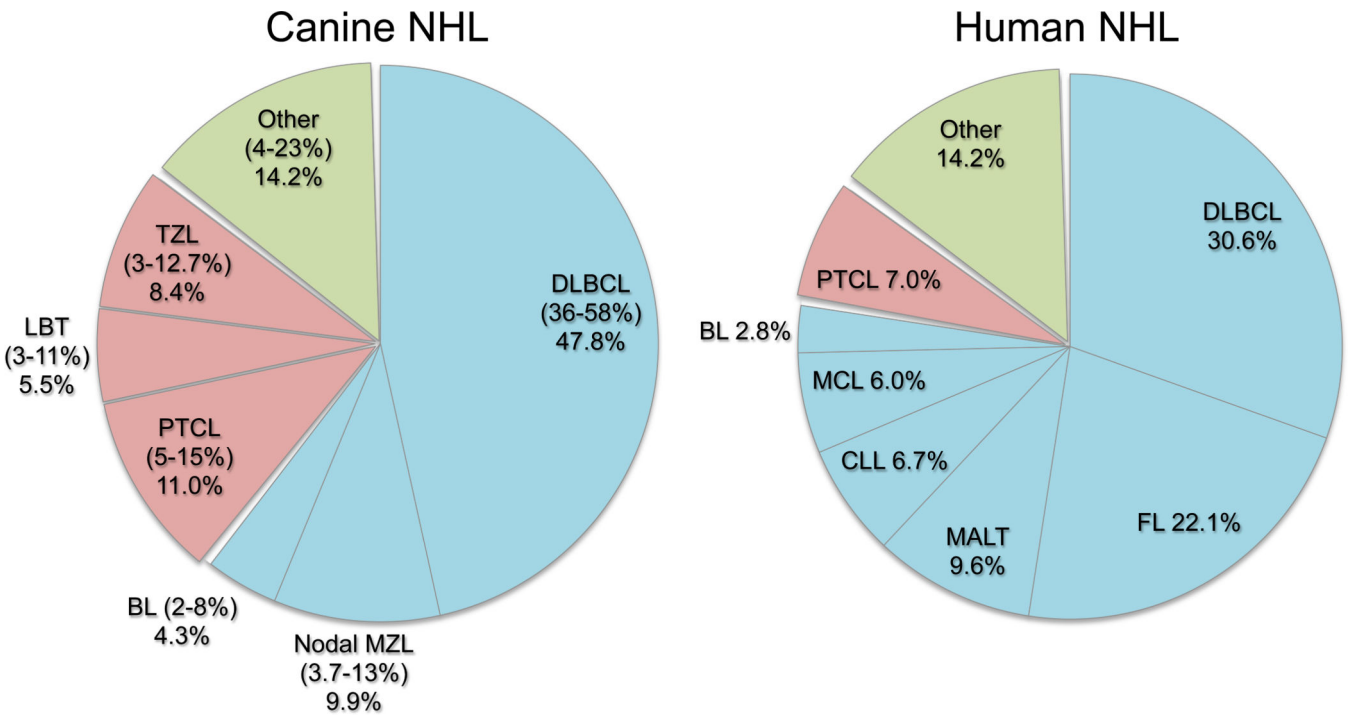

Figure 1.

Relative distribution of common subtypes of canine lymphoma (Frantz et al., 2012; Ponce et al., 2010; Thomas et al., 2011; Valli et al., 2011) and human NHL (Anon, 1997). DLBCL; diffuse large B-cell lymphoma, MZL; marginal zone lymphoma, BL; Burkitt lymphoma, PTCL; peripheral T cell lymphoma not otherwise specified, TZL; nodal T-zone lymphoma, LBT; lymphoblastic T-cell lymphoma, FL; follicular lymphoma, MALT; mucosa associated lymphoid tissue lymphoma (extranodal MZL), CLL; chronic lymphocytic leukemia, MCL; mantle cell lymphoma.

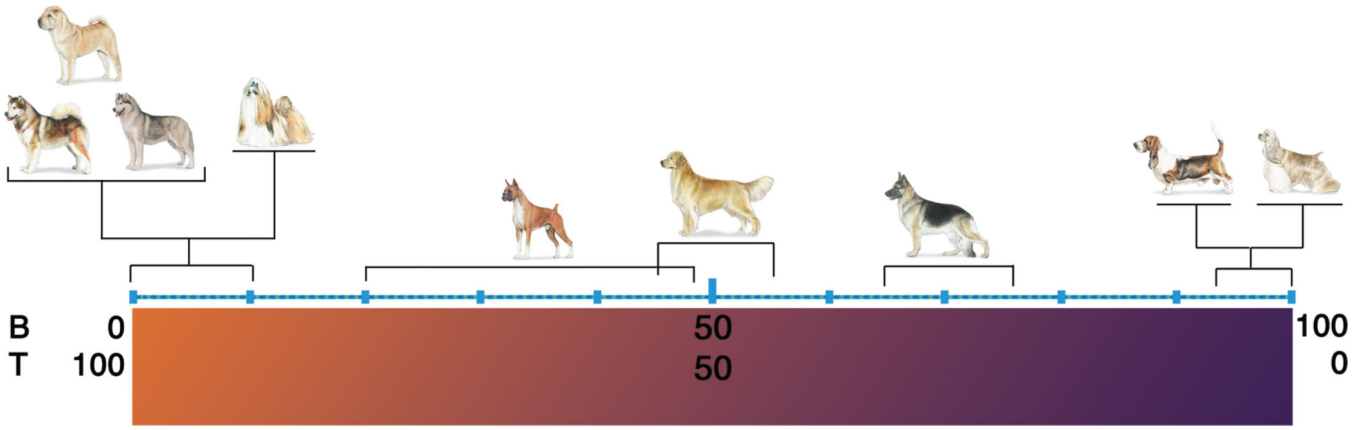

Figure 2.

Breed-specific distribution of B-cell and T-cell lymphomas in dogs. The prevalence of B-cell-derived and T-cell derived tumors in all dogs (considered as a single group) are ~65% and ~35%, respectively (Modiano et al., 2005). The incidence of B-cell tumors and T-cell tumors in most dogs and mixed breed dogs falls within these ranges (shown by German Shepherd Dog in this figure). In contrast, a prevalence of excess B-cell tumors and T-cell tumors has been shown in certain breeds. Reproduced with permission from (Modiano et al., 2006).

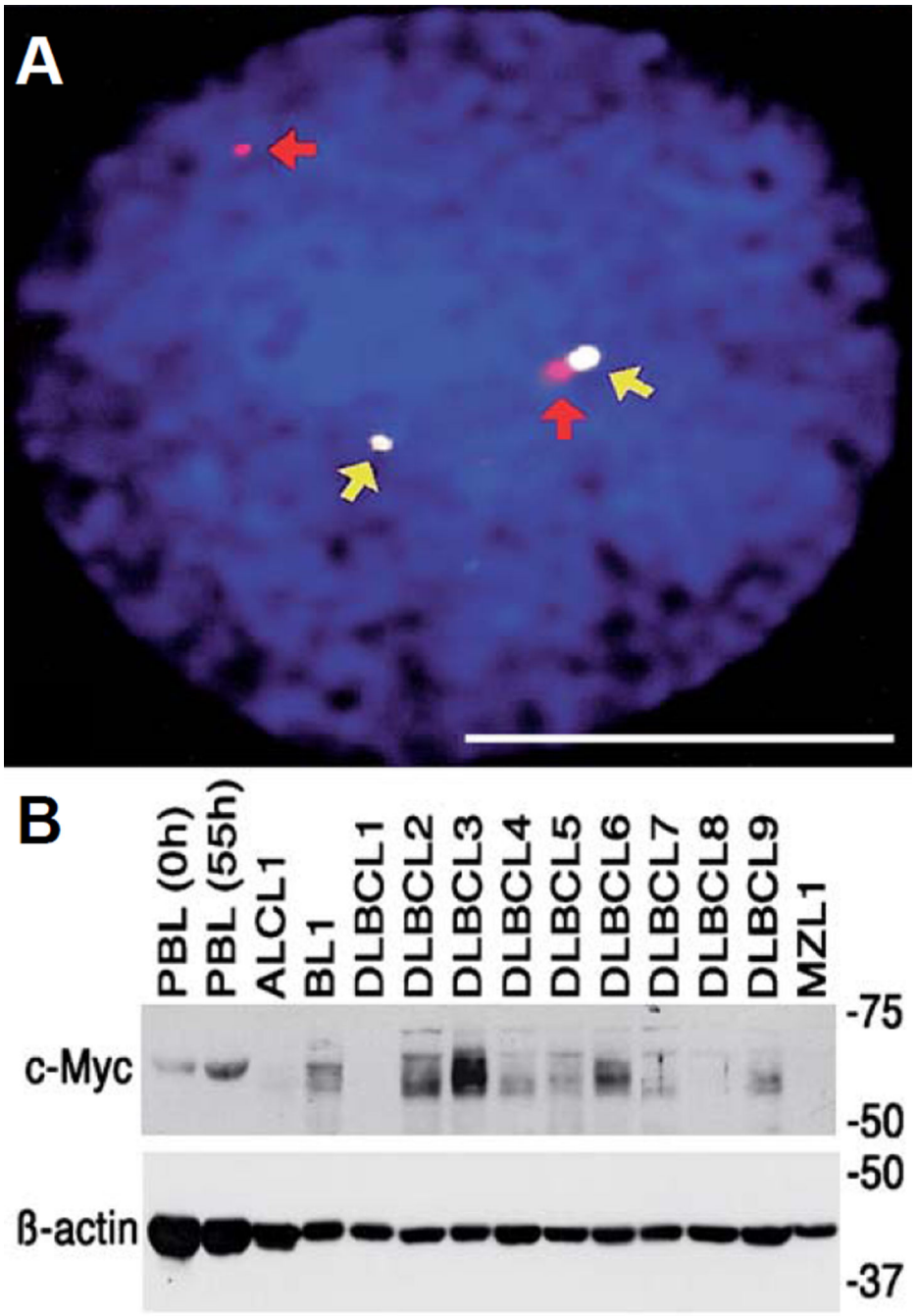

Figure 3.

Conserved cytogenetic rearrangement in canine Burkitt lymphoma. (A) Interphase tumor cell from a dog with BL showing heterozygous co-localization of a canine BAC clone containing the MYC gene (white spots indicated by yellow arrows) and a BAC clone that maps to the same cytogenetic band as the IgH locus (red spots, identified by red arrows). Scale bar = 5 μm. (B) Myc expression in non-stimulated normal canine peripheral blood lymphocytes (PBL) (0 h), mitogen-stimulated PBL (55 h), or lymph node cells from dogs with anaplastic large cell lymphoma (ALCL), BL, DLBCL (1–9) or nodal MZL. Beta-actin

was used as a loading control. Reproduced with permission from (Breen and Modiano, 2008).

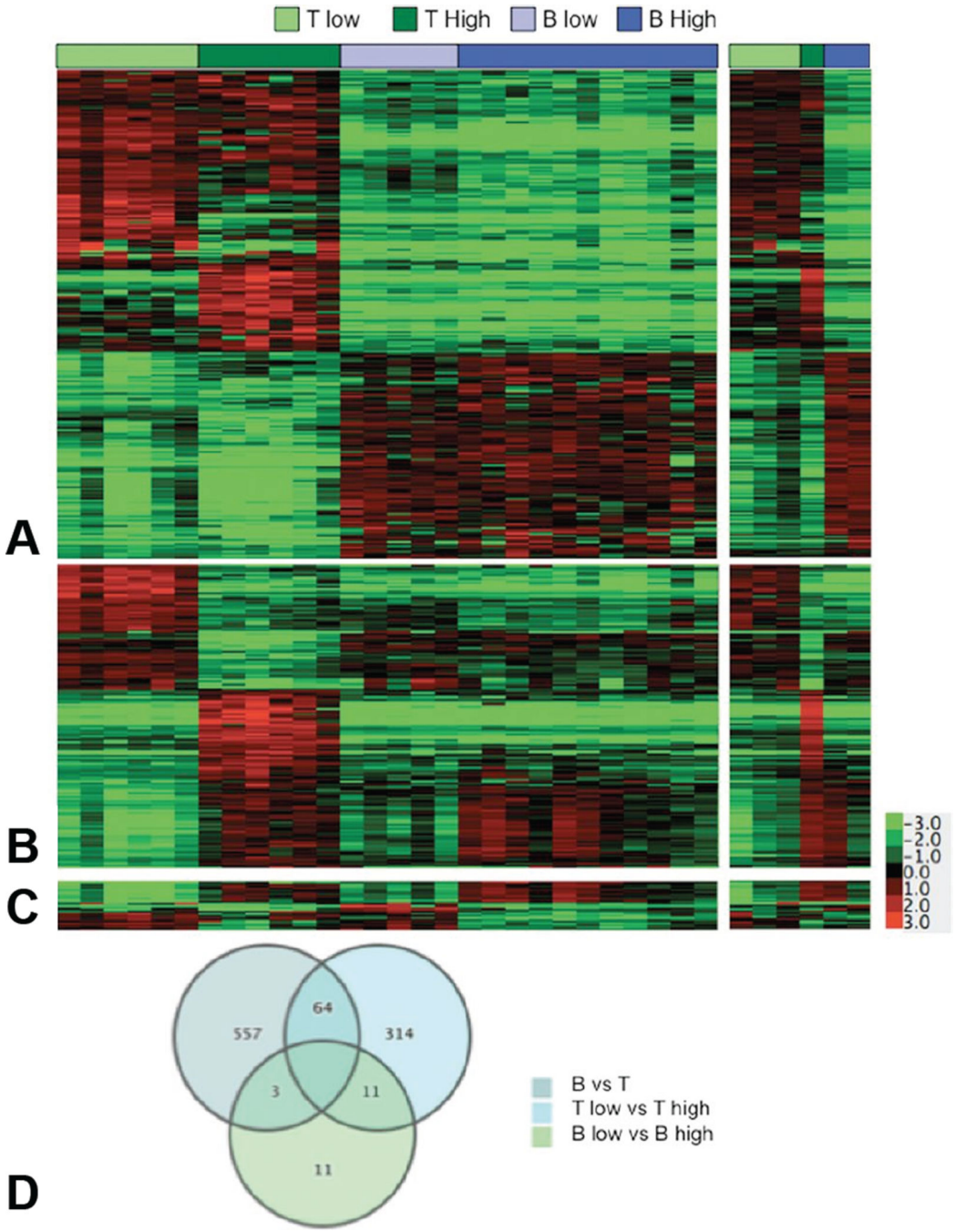

Figure 4.

Statistically significant genes define molecular subtypes of canine lymphoma. Genes differentially expressed with >3-fold average change and *P* values <0.001 were identified for the comparison of groups composed of (A) B-cell and T-cell lymphomas ($n = 624$), (B) high-grade and low-grade T-cell lymphomas ($n = 389$), and (C) high-grade and low-grade B-cell lymphomas ($n = 25$) using *t* test statistics. The second panel of (A–C) is an independent "validation" set (6 samples, right inset) of the results obtained in the initial set (29 samples,

left panel). (D) Venn diagram showing the number of unique and overlapping genes for each 2-group test. Reproduced with permission from (Frantz et al., 2012).

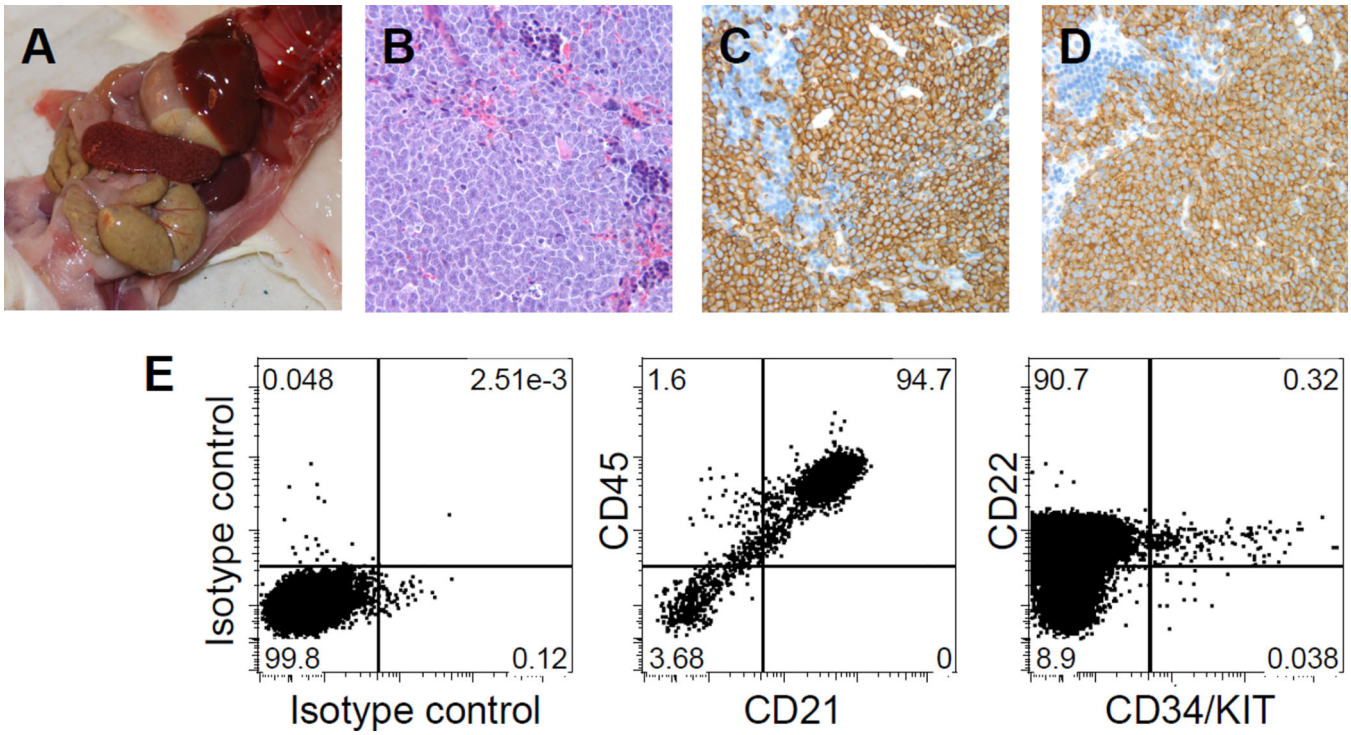

Figure 5.

Tumor engraftment of primary canine B-cell lymphoma in NSG mice. (A) Photomicrograph showing splenomegaly in an NSG recipient at autopsy. (B) Photomicrograph of diffuse infiltration of tumor cells in spleen. (C and D) Immunostaining of the donor (dog) cells for expression of canine CD20 (C) and CD79a (D). (E) Spleen cells of a secondary NSG recipient are virtually all canine B-cells, expressing CD21, CD22, and CD45, analyzed by multi-parameter flow cytometry. A small population of hematopoietic progenitor antigens CD34 and KIT positive cells is found within the $CD22^{+}$ B-cell tumor population. Reproduced with permission from (Ito et al., 2011).

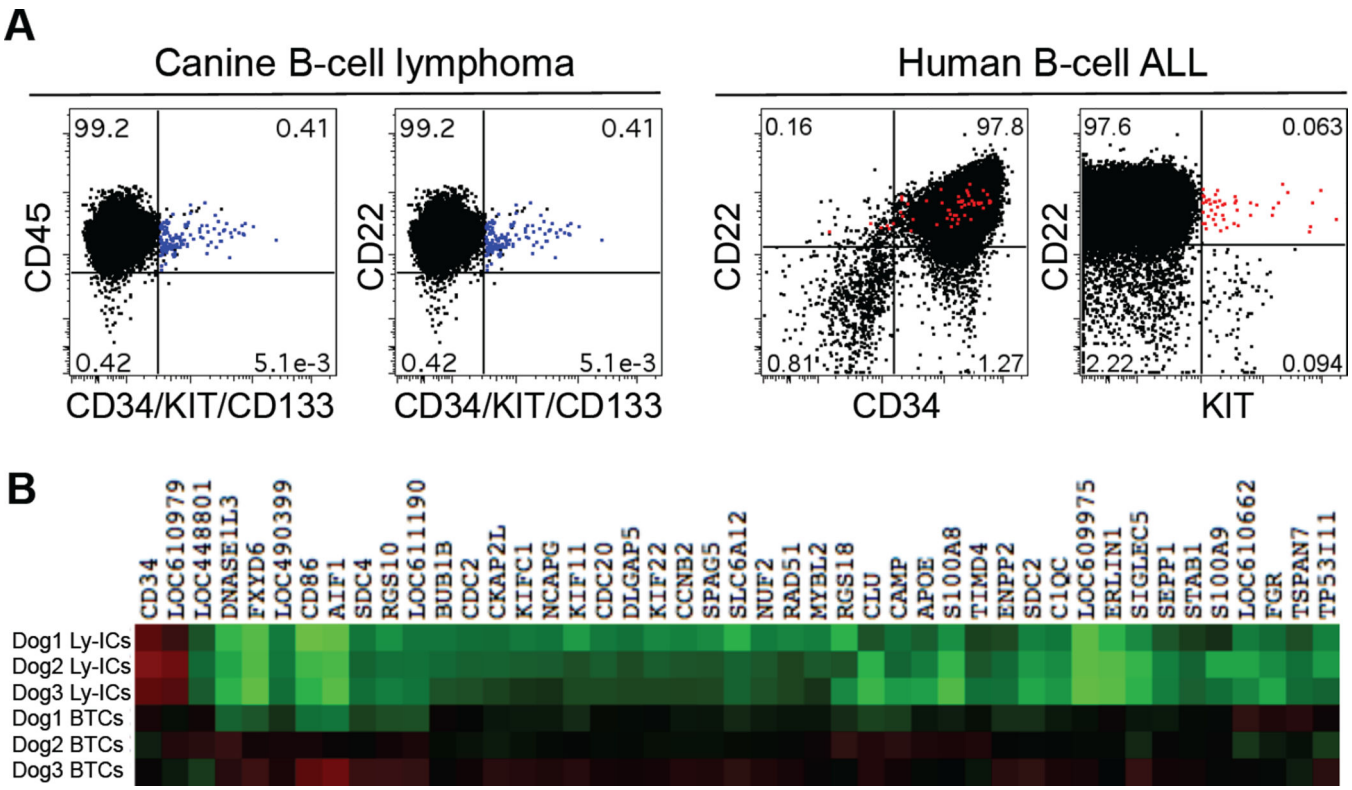

Figure 6.

Putative TICs in primary canine B-cell lymphomas and human B-cell ALLs. (A) Existence of a putative Ly-IC population ($CD22^{+}CD34/KIT/CD133^{+}$; blue dots) in primary canine B-cell lymphoma samples (n = 24) and a putative TIC population ($CD22^{+}KIT^{+}$; red dots) in primary human B-cell ALL samples (n = 2) were shown using multi-parameter flow cytometry. Reproduced with permission from (Ito et al., 2011). (B) Heat map showing 44 differentially expressed transcripts between enriched Ly-ICs and BTCs (greater than 2-fold change by a two group T-test, $p < 0.05$). Heat map colors represent median-centered fold change expression in log-space. Up-regulated genes are shown in red and down-regulated genes are shown in green.