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Prognostic efficacy of the human B-cell lymphoma prognostic genes in predicting disease-free survival (DFS) in the canine counterpart

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Abstract

Background: Canine B-cell lymphoma is deemed an ideal model of human non-Hodgkin's lymphoma where the lymphomas of both species share similar clinical features and biological behaviors. However there are some differences between tumor features in both species. In the current study, we sought to evaluate the prognostic efficacy of human B-cell lymphoma prognostic gene signatures in canine B-cell lymphoma.

Methods: The corresponding probe sets of 36 human B-cell lymphoma prognostic genes were retrieved from 2 canine B-cell lymphoma microarray datasets (GSE43664 and GSE39365) (76 samples), and prognostic probe sets were thereafter detected using the univariate and multivariate Cox proportional-hazard model and the Kaplan–Meier analysis. The two datasets were employed both as training sets and as external validation sets for each other. Results were confirmed using quantitative real-time PCR (qRT-PCR) analysis.

Results: In the univariate analysis, CCND1, CCND2, PAX5, CR2, LMO2, HLA-DQA1, P53, CD38, MYC-N, MYBL1, and BIRCS5 were associated with longer disease-free survival (DFS), while CD44, PLAU, and FN1 were allied to shorter DFS. However, the multivariate Cox proportional-hazard analysis confirmed CCND1 and BIRCS5 as prognostic genes for canine B-cell lymphoma. qRT-PCR used for verification of results indicated that expression level of CCND1 was significantly higher in B-cell lymphoma patients with the long DFS than ones with the short DFS, while expression level of BIRCS5 wasn't significantly different between two groups.

Conclusion: Our results confirmed CCND1 as important gene that can be used as a potential predictor in this tumor type.

Keywords: Canine B-cell lymphoma, Prognosis, Cox proportional-hazard analysis, Survival

Background

Lymphoma is one of the most common malignancies in dogs and occurs in different forms, including multicentric, mediastinal (thymic), alimentary, cutaneous, and solitary types [1, 2]. Investigators have proposed canine B-cell lymphoma as a suitable model of human non-Hodgkin lymphoma (NHL) because the tumors of both species have common clinical manifestations and biological properties. However there are some differences between tumor features in both species [3, 4].

Some clinical and histological features have been proposed as prognostic factors in canine lymphoma [2, 5, 6]. For example, there are conflicting data on the use of the Kiel and Working Formulation classifications insofar as studies have revealed that both classifications are unreliable prognosticators [2, 5]. Nonetheless, in a study by Teske et al. (1994), the Working Formulation classification and Kiel classification were suggested as prognostic factors for the overall survival and time-to-relapse in treated dogs with malignant lymphoma, respectively [7]. Moreover, investigations have reported that such clinical parameters

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as age, sex, animal weight, and clinical stage have no robust efficiency for predicting overall survival and disease-free survival (DFS) times [5]. Some cellular proliferation markers such as Ki-67, PCNA, and AgNOR have been evaluated as suitable prognosis predictor. Indeed, Ki-67 and AgNOR have been reported as appropriate prognostic markers in human and canine malignant lymphoma [5, 8, 9], where AgNOR can be utilized for the grading of the canine and human NHL [10, 11].

Molecular phenotyping is a robust method for the definition of tumor subtypes and the detection of prognostic gene genes [12–15]. For instance, the gene expression profile analysis divided human diffuse large-B-cell lymphoma (DLBCL) into 3 distinct subtypes: activated germinal center-like B-cell lymphoma, B-cell lymphoma, and peripheral mediastinal B-cell lymphoma [12]. A similar investigation classified canine malignant lymphoma based on molecular profiling [6]. In different studies, 36 genes have been suggested as prognostic markers for human B-cell lymphoma (majorly DLBCL) (Table 1). To the best of our knowledge, there is limited information on the prognostic efficacy of these important gene markers in canine B-cell lymphoma as an ideal model of human NHL. In the present study, the robustness of these genes for the prediction of DFS in 2 canine B-cell lymphoma microarray datasets was investigated using the univariate/multivariate Cox proportional-hazard model and the Kaplan–Meier analysis. The prognostic efficacy of selected gene(s) in each dataset was validated via the other dataset.

Table 1 List of human B-cell lymphoma prognostic genes used in our study

BCL2 [1–4]	Ki-67 [5]
BCL6 [4, 6, 7]	LMO2 [4, 8]
BCL7A [4]	LRMP [4]
BIRC5 [9]	MYBL1 [4]
CCND1 [10]	MYCN [6]
CCND2 [8, 11]	NPM3 [6]
CD10 [4]	NR4A3 [12]
CD38 [4]	P53 [13]
CD44 [14]	PAX5 [15]
CFLAR [4]	PDE4B [12]
CR2 [4]	PIK3CG [4]
EEF1A1L4 [6]	PLAU [6]
FN1 [6]	PMS1 [4, 11]
HGAL [4, 6]	PRDM1 [11]
HLA-DQA1 [6]	SCYA3 [8, 11]
HLA-DRA [6]	SLA [4]
ICAM1 (CD54) [16]	SLAM [4]
IRF4 [4]	WASPIP [4]

References were provided in Additional file 1

Methods

Microarray expression datasets

Two canine B-cell lymphoma microarray datasets, namely GSE43664 [16] and GSE39365 [6] (platform: GPL3738), were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). Expression data were downloaded in the CEL file format. The GSE43664 and GSE39365 datasets comprised 58 and 36 samples, respectively, where the GSE43664 samples were solely canine B-cell lymphoma (mainly diffuse large B-cell lymphoma [DLBCL]) and the GSE39365 samples contained both B-cell ($n = 18$) and T-cell lymphoma ($n = 18$). In the GSE39365 dataset, only B-cell lymphoma samples were included in the study. B-cell lymphoma samples in GSE43664 included DLBCL (mainly), MZL, and unknown. Additionally, B-cell lymphoma samples in GSE39365 included DLBCL (mainly), MZL, and BL. The clinical features of the studied cases are summarized in Additional file 1: Table S1 and S2. The data were first converted into expression values and then transformed logarithmically using the Affy package [17] in R environment, version 3.0.2 (http://www.r-project.org/). Survival time, compared using Student's t-test between two datasets (GSE43664: 9.6 ± 8.7 months and GSE39365:11.7 ± 12.1 months), wasn't statistically different ($P = 0.42$).

Extraction of prognostic gene expression values

Thirty-six human-specific genes, presumed as prognostic genes, were tested in the current study (Table 1). The literatures were mined to retrieve papers exploring prognostic genes or gene signatures in human B-cell lymphoma. Public databases (especially PubMed) were screened for papers describing genes predicting survival in human B-cell lymphoma. Finally, 36 genes were extracted from papers, where some of these genes weren't evaluated as a single prognostic gene and proposed as a prognostic gene signature with the other genes. So, to perform a comprehensive assessment, we included all genes in our analysis. The corresponding probe sets of these genes and the related expression value for each probe set were retrieved from both datasets using MATLAB 7.8.0 (R2009a) (MathWorks, Natick, MA).

Survival analysis and external validation

Survival analysis was performed using *Survival* (http://cran.r-project.org/package=survival) and *Survcomp* [18] packages in R environment. The Cox proportional-hazards analysis was used for constructing a model for the prediction of survival. In this analysis, the association between a group of covariates (genes) and the response variable (DFS) was evaluated. Two datasets were employed as training and validation (test) groups, where important prognostic gene(s) was identified in a group (training group) and then validated in the other dataset

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(validation group). We used an external validation instead of internal validation, as the former is generally more robust to the overfitting problem [19].

First, the univariate Cox analysis was performed and genes with a z score greater than 1.5 or less than -1.5 [13, 20] were selected for the multivariate Cox analysis, where a negative score and a positive score associated with longer and shorter survival respecivley. In the multivariate Cox analysis, statistically significant genes were entered into the analysis and significant covariate(s) was detected at a *P*-value lower than 0.05. Survival curves were depicted by Kaplan–Meier method and compared using the log-rank test. Furthermore, some clinical prognosis parameters such as animal age, sex, and tumor grade (high or low) (Additional file 1: Table S2) were assessed in the Cox analysis to determine their roles in the prediction model.

Next, the external validation of the resulted prognostic genes was determined. The prognostic gene(s) in each group was tested in the other group via the Kaplan-Meier method and the log-rank test. In addition, the expression of the prognostic genes were compared in human ABC-like (activated B-cell like) and human GCB-like (germinal center B like) groups, because GCB-like and ABC-like cases are associated with better and poorer prognoses, correspondingly [21]. For this analysis, the patients were categorized as GCB-like and ABC-like groups based on 1,180 canine-specific differentially expressed probe sets proposed by Richards et al. (2013) [16]. Grouping was carried out using the hierarchal clustering analysis provided in geWorkbench 2.5.1 package [22]. Subsequently, the expressions of the prognostic genes were compared between the two groups using the Student's t-test analysis provided in geWorkbench 2.5.1 package.

Verification of the results by quantitative real-time PCR (qRT-PCR)

qRT-PCR procedure was performed as previously described [23, 24] on lymph node biopsy samples obtained from 60 dogs with B-cell lymphoma. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Biopsy samples were processed using hematoxylin and eosin (H&E) staining method for the routine histopathology evaluation. Samples were diagnosed and subtyped based on the World Health Organization classification of hematopoietic and lymphoid tissues [25]. CD79a and CD3 antibodies (Dako, Denmark) were used for the confirmation of B-cell phenotype. CD79a-positive and CD3-negative samples were selected for subsequent analysis. Because mean survival time of GEO datasets samples that had lower expression (short survival) and higher expression (long survival) values than *CCND1* or *BIRCS5* median value

were 6.9 months and 12.1 months respectively (see results), the selected cases for qRT-PCR included 30 dogs with DFS <7 months and 30 dogs with DFS >12 months. Mean age of the dogs with DFS >12 months and dogs with DFS <7 months were 8.3 years (range: 3-12 years) and 7 years (range: 2-10 years) respectively.

In brief, total RNA was extracted using Tripure isolation reagent (Roche, Germany) according to the manufacturer's protocol. cDNA was synthesized using Maxime RT PreMix Kit (Intron biotechnology, Korea) according to the manufacturer's instructions. The cDNA synthesis reaction was run at 45 °C for 60 min, followed by 95 °C for 5 min. Synthesized cDNA was used for final PCR assay. SYBR green-based quantitative real-time PCR (qRT-PCR) was performed using the Applied Biosystems 7500 Real- Time PCR system. Cycle conditions were 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 s, 52 °C for 45 s, and 72 °C for 1 min. Data were analyzed by SDS 2.0 software (Applied Biosystems). Specific primers used for *CCND1* and *BIRCS5* were presented in Additional file 1: Table S3. HPRT was used as the reference gene for normalization of target gene expression. Comparative ΔCT-method was used for calculation of relative expression of the target gene [23]. Data are presented as fold change in gene expression level of the target gene. Fold changes in gene expression was compared between two groups (DFS <7 months vs. DFS >12 months) by Student's t-test. A *P* value lower than 0.05 was considered significant.

Results

Probe sets corresponding to the prognostic genes were obtained from both datasets and subjected to subsequent survival analysis. Ninety one probe sets corresponding to 36 genes were retrieved from the each datasets. In the univariate analysis, the genes with a z score higher than 1.5 or lower than -1.5 were selected for the multivariate analysis. The results of the univariate analysis are summarized in Table 2. In the 58-sample dataset, *CCND1, CCND2, PAX5, CR2, BCL2L14, LMO2, HLA-DQA1, P53, MYC-N*, and *BIRCS5* had z scores lower than -1.5, which is associated with longer DFS. Conversely, *CD44, PLAU*, and *FN1* had positive z scores (higher than 1.5), which is correlated with shorter DFS. Moreover, in the 18-sample dataset, *CCND1, BIRCS5, MYC-N, LMO2, MYBL1*, and *CD38* had significant negative z scores (lower than -1.5). No genes with a z score higher than 1.5 was detected in the univariate analysis of the GSE39365 dataset (Table 2). Our subsequent multivariate analysis indicated that *CCND1* was a robust predictor in both datasets. Furthermore, *BIRCS5* in the GSE39365 dataset reached a statistically significant level (Table 3).

Appropriate external validation was confirmed by validating the prognostic gene(s) in each group in the other

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Table 2 Univariate Cox proportional-hazard analysis of B-cell lymphoma prognostic gene signatures in GSE43664 and GSE39365 datasets

	Coef	Exp (coef)	SE (coef)	z score	P
GSE43664 dataset					
Cfa.21188.1.S1_s_at: (CCND2)	-0.672	0.511	0.207	-3.24	0.0012
Cfa.19972.1.S1_at: (BCL2L14)	-3.44	0.0321	1.26	-2.72	0.0065
CfaAffx.18137.1.S1_at: (CR2)	-0.902	0.406	0.371	-2.43	0.015
CfaAffx.4397.1.S1_x_at: (PAX5)	-1.03	0.357	0.446	-2.31	0.021
Cfa.37.1.S1_at: (BIRC5)	-1.44	0.236	0.627	-2.3	0.021
Cfa.16248.1.S1_at: (CCND1)	-0.505	0.604	0.257	-2.1	0.049
Cfa.16217.1.S1_s_at: (CR2)	-0.527	0.59	0.272	-1.94	0.052
Cfa.182.1.S2_at: (HLA-DQA1)	-0.467	0.627	0.241	-1.93	0.053
Cfa.10937.1.S1_at: (LMO2)	-0.676	0.509	0.354	-1.91	0.056
CfaAffx.6511.1.S1_at: (MYCN)	-1.19	0.304	0.63	-1.89	0.059
Cfa.15639.1.A1_at: (TP53)	-0.373	0.688	0.202	-1.85	0.064
Cfa.5536.1.A1_at: (MYCN)	-0.781	0.458	0.443	-1.76	0.078
CfaAffx.18218.1.S1_at: (CR2)	-0.391	0.677	0.225	-1.74	0.083
CfaAffx.18149.1.S1_s_at: (CR2)	-0.429	0.651	0.248	-1.73	0.083
CfaAffx.18202.1.S1_s_at: (CR2)	-0.454	0.635	0.272	-1.67	0.095
Cfa.19191.1.S1_at: (PDE4B)	-0.857	0.425	0.518	-1.65	0.098
CfaAffx.4400.1.S1_at: (PAX5)	-1.65	0.192	1.05	-1.58	0.11
CfaAffx.11868.1.S1_at: (MYBL1)	-1.21	0.297	0.773	-1.57	0.12
Cfa.3707.1.A1_s_at: (FN1)	0.622	1.86	0.415	1.5	0.13
Cfa.3800.2.S1_at: (CD44)	0.226	1.25	0.14	1.61	0.11
CfaAffx.11235.1.S1_s_at: (CD44)	0.259	1.3	0.142	1.82	0.068
Cfa.127.1.S1_s_at: (PLAU)	0.884	2.42	0.463	1.91	0.057
Cfa.3707.2.S1_at: (FN1)	0.277	1.32	0.129	2.15	0.031
CfaAffx.22155.1.S1_s_at: (FN1)	0.347	1.41	0.154	2.26	0.024
Cfa.3707.3.S1_s_at: (FN1)	0.827	2.29	0.336	2.46	0.014
GSE39365 dataset					
Cfa.15826.1.S1_s_at: (BIRC5)	-0.832	0.435	0.477	-1.74	0.081
Cfa.16248.1.S1_at: (CCND1)	-0.923	0.397	0.54	-1.71	0.088
Cfa.5536.1.A1_at: (MYCN)	-2.44	0.0875	1.44	-1.69	0.091
Cfa.10937.1.S1_at: (LMO2)	-0.825	0.438	0.516	-1.6	0.11
Cfa.3619.1.S1_at: (CD38)	-0.804	0.448	0.512	-1.57	0.12
Cfa.3619.1.S1_s_at: (CD38)	-0.605	0.546	0.39	-1.55	0.12
CfaAffx.11868.1.S1_at: (MYBL1)	-1.76	0.171	1.16	-1.52	0.12
Clinical features (GSE39365 dataset)					
Age at diagnosis (years)	0.0774	1.08	0.0893	0.866	0.39
Sex	0.165	1.18	0.576	0.287	0.77
Grade	-1.04	0.354	0.68	-1.53	0.13

Genes with z score higher than 1.5 or lower than -1.5 were listed. Exp (coef) indicates hazard ratio. Positive and negative z score denotes shorter and longer survival time respectively

group. The correlation between *CCND1* and *BIRCS5* expression and DFS time was tested using the Kaplan-Meier estimator and log-rank test. The patients were divided into high-risk and low-risk groups based on the median of the *CCND1* and *BIRCS5* expression values, and their survival durations were compared using the log-rank test. High-risk and low-risk groups had expression values lower than and higher than the median value

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Table 3 Multivariate Cox proportional-hazard analysis of B-cell lymphoma prognostic gene signatures in GSE43664 and GSE39365 datasets

	Coef	Exp (coef)	SE (coef)	z score	P
GSE43664 dataset					
CCND1	-0.72	0.487	0.353	-2.041	0.041
GSE39365 dataset					
BRICS5	-2.322	0.098	0.834	-2.785	0.0054
CCND1	-3.017	0.0489	1.427	-2.114	0.035

Exp (coef) indicates hazard ratio

respectively. The DFS time in the GSE43664 dataset was statistically different in the survival curves constructed based on *CCND1* ($P = 0.007$) and *BIRCS5* ($P = 0.0042$) expressions (Fig. 1 b and c). However, the DFS time in high-risk and low-risk groups of the GSE39365 dataset tended to be significant ($P = 0.058$) (Fig. 2 b). Additionally, the expression levels of *CCND1* and *BIRCS5* were tested in the GCB-like and ABC-like groups. To that end, the samples were first classified into two groups based on 1,180 canine-specific probe sets. Then, the expression level of *CCND1* was compared between the two groups. For the GSE43664 dataset, a clear clustering pattern was reconstructed (Additional file 1: Figure S1),
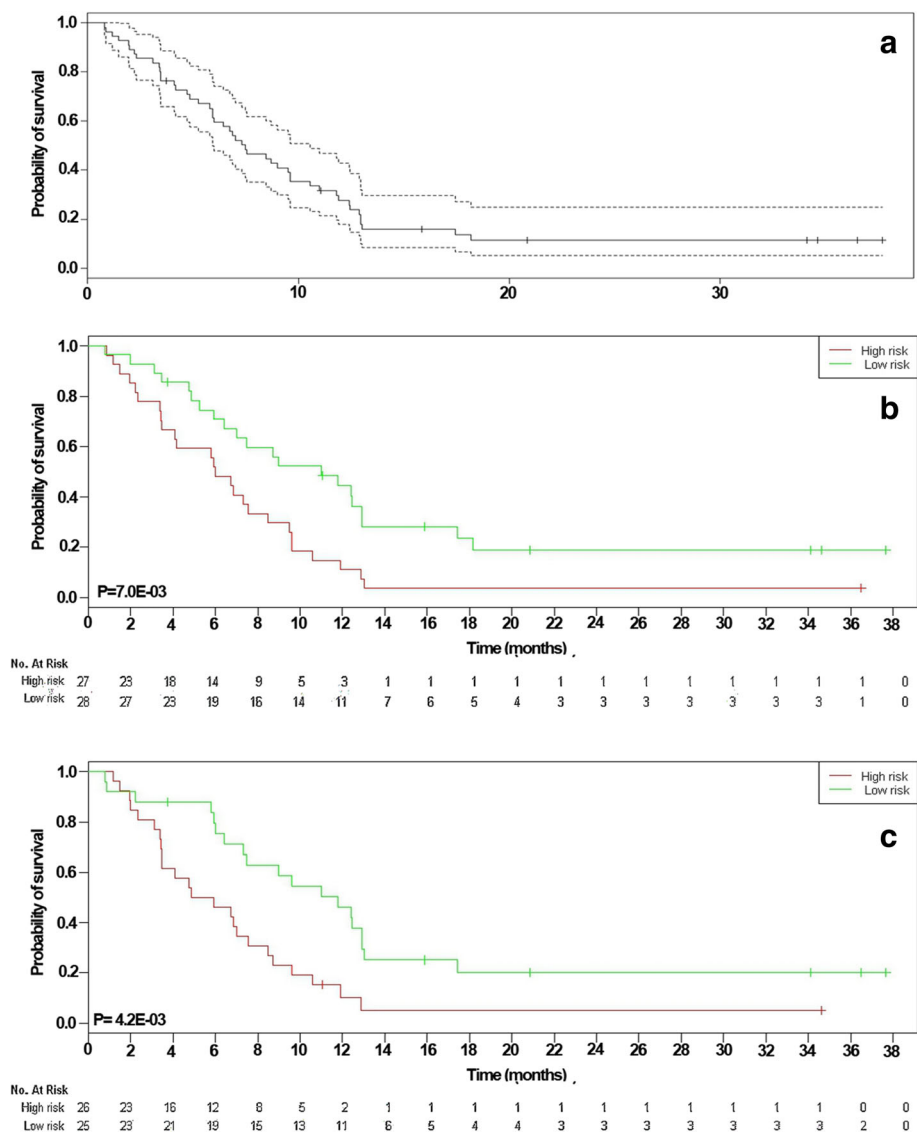

Fig. 1 Survival analysis for evaluation of the correlation between GSE39365 prognostic genes and DFS time in GSE43664 dataset. Panel **a** indicated Kaplan-Meier estimate with 95% confidence bound in GSE43664 dataset. There was significant correlation between DFS with *CCND1* (**b**) ($P = 0.007$) and *BIRCS5* (**c**) ($P = 0.042$). *Green* and *red lines* indicated samples had higher and lower expression value than median value respectively
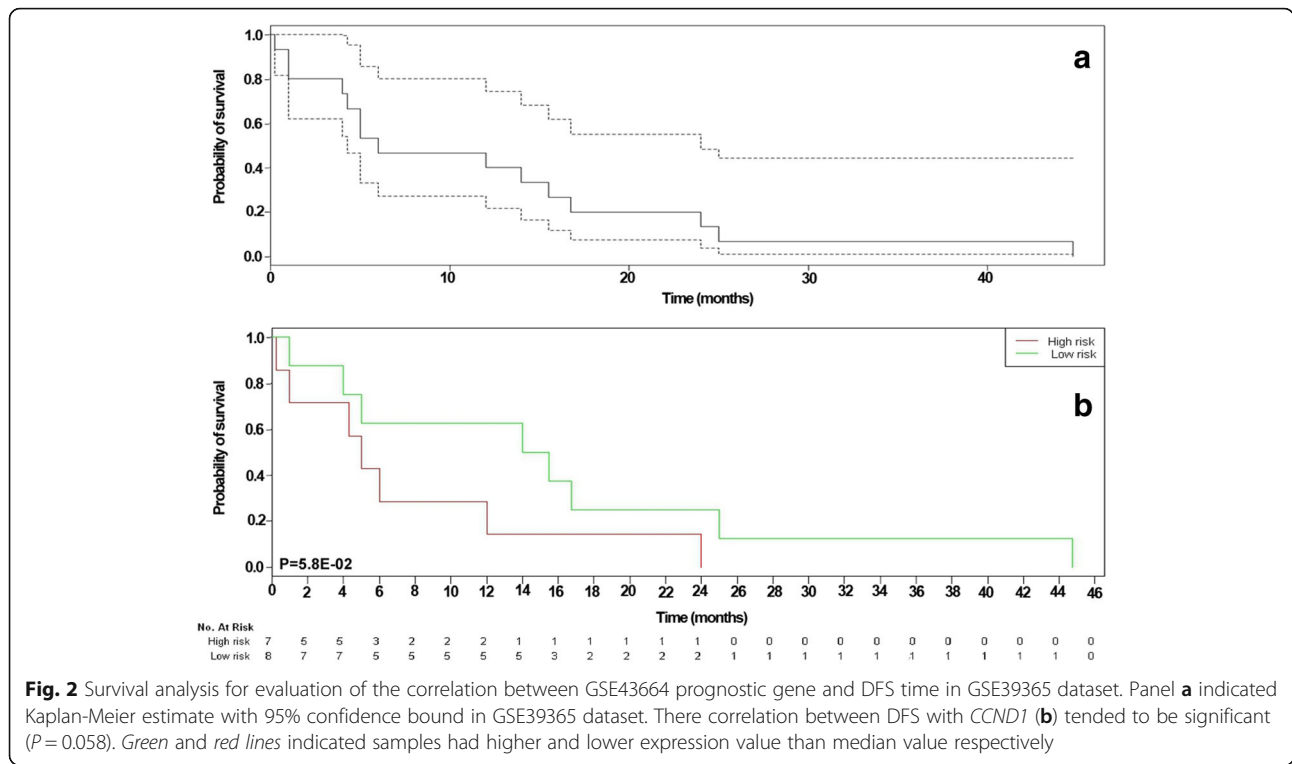
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Fig. 2 Survival analysis for evaluation of the correlation between GSE43664 prognostic gene and DFS time in GSE39365 dataset. Panel **a** indicated Kaplan-Meier estimate with 95% confidence bound in GSE39365 dataset. There correlation between DFS with *CCND1* (**b**) tended to be significant (*P* = 0.058). *Green* and *red lines* indicated samples had higher and lower expression value than median value respectively

while the GCB-like and ABC-like groups were not clearly created for the GSE39365 dataset maybe because of its small sample size (Additional file 1: Figure S2). Hence, the T-test analysis was performed only on the GSE43664 dataset and reveled that the differences between *CCND1* expression in the GCB-like (mean ± SD = 8.03 ± 0.86) and ABC-like (mean ± SD = 7.7 ± 0.54) groups tended to be significant (*P* = 0.052) while *BIRCS5* expression in the GCB-like (mean ± SD = 5.32 ± 0.21) and ABC-like (mean ± SD = 5.26 ± 0.23) groups wasn't significant (*P* = 0.36).

qRT-PCR analysis confirmed *CCND1* as final prognostic gene because *CCND1* expression was significantly higher in the dogs with DFS >12 months than the dogs with DFS <7 months while expression level of the *BIRCS5* wasn't significantly different between two groups (Fig. 3).

As was expected in light of our literature review [2, 6], clinical characteristics such as age, sex, and tumor grade were not significant predictor components in canine B-cell lymphoma. More statistical details about the patients' clinical characteristics are summarized in Table 2.

Discussion

Although prognostic gene genes for human NHL and especially DLBCL have been meticulously investigated by various researchers, there is little information on molecular prognostic genes for canine B-cell lymphoma. For example, Rosenwald et al. (2002) [14] proposed germinal-center B-cell, MHC class II, lymph-node, and cell proliferation signatures as a molecular profiling for

predicting progression-free survival after chemotherapy in patients with DLBCL. HLA-DPα, HLA-DQα, HLA-DRα, and HLA-DRβ as members of the MHC class II module; BCL-6 as a member of the germinal-center B-cell module; fibronectin, α-Actinin, connective-tissue growth factor, urokinase plasminogen activator, collagen type IIIα1, and KIAA0233 as members of the lymph-node module; and E21G3, c-myc, and NPM3 as members of the proliferation module constituted the more
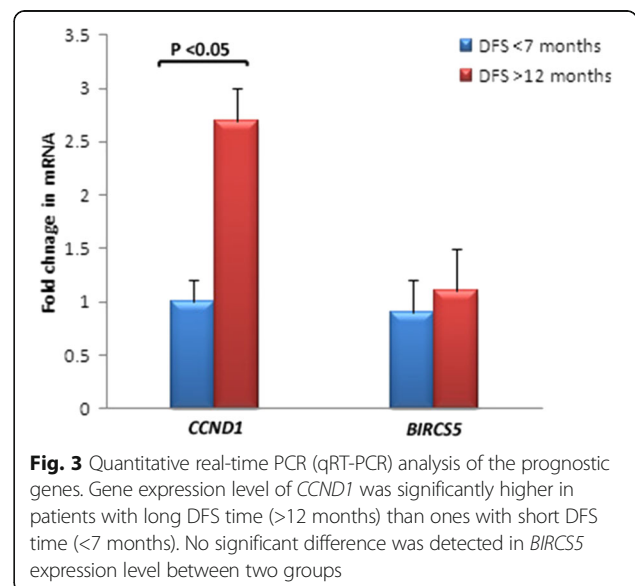

Fig. 3 Quantitative real-time PCR (qRT-PCR) analysis of the prognostic genes. Gene expression level of *CCND1* was significantly higher in patients with long DFS time (>12 months) than ones with short DFS time (<7 months). No significant difference was detected in *BIRCS5* expression level between two groups

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prominent elements of prognostic signatures [14]. Furthermore, Lossos et al. (2004) [13] proposed a complex of LMO2, FN1, BCL6, SCYA3, CCND2, and BCL2 as a suitable predictor in patients with DLBCL, independent of the International Prognostic Index (IPI). In addition, the authors reported HGAL and BCL6 as predictors of overall survival, independent of the IPI [26, 27]. A comprehensive list of the prognostic genes in human B-cell lymphoma and related studies is presented in the Materials/Methods (Table 1).

The current study utilized human B-cell lymphoma prognostic genes so as to detect valuable genes that can serve as prognostic predictors in canine B-cell lymphoma. There is currently no counterpart for the IPI as regards canine lymphoma inasmuch as the prognostic efficacy of the IPI is evaluated alongside molecular genes. Among genes analyzed in our investigation, *CCND1* was found to be the most appropriate prognostic factor. *CCND1* was confirmed in both datasets while BIRCS5 was solely verified in one dataset. Additionally qRT-PCR verified *CCND1* prognostic efficacy.

CCND1, encoding cyclin D1 protein, plays a critical role in the cell cycle machinery; i.e. in G1-S transition. The overexpression of *CCND1* has been indicated in various human B-cell lymphoma subtypes, including mantle cell lymphoma (MCL) [28, 29], DLBCL [30–32], and plasma cell myeloma [33]. Nevertheless, the overexpression of cyclin D1 is regarded as an unusual characteristic in human DLBCL [34, 35]. In general, cyclin D1 has been proposed as the most critical prognostic gene majorly in MCL [36, 37] and seldom for other human B-cell lymphoma subtypes. In one study, cyclin D1 was verified as an independent prognostic factor from the IPI and 5-year overall survival was significantly higher in cyclin D1-negative MCL than cyclin D1-positive MCL (86% vs. 30%) [37]. Furthermore, the m-RNA level of *CCND1* in blood and bone marrow has been proposed as an appropriate prognostic factor in patients with MCL [38]. Cyclin D1 overexpression showed a correlation with longer survival in breast carcinoma [39] and colorectal cancer [40]. The results of our study demonstrate that *CCND1* is a favorable potential prognostic predictor for canine B-cell lymphoma. The results of the present study confirm that *CCND1* is an important potential prognostic gene in canine B-cell lymphoma (especially DLBCL subtype) and should, accordingly, be considered for further investigation in future studies. There is no significant comparable data about prognostic efficacy of the *CCND1* in human DLBCL while studied samples in our study were majorly DLBCL. In an study by lossos et al [13], although univariate Cox proportional-hazard analysis revealed that *CCND1* was a genes with negative z score (longer survival), *CCND1* didn't reach a significant level for entering final multivariate analysis.

Additionally previous investigation revealed that patients with cyclin-D1+ CD5+ DLBCL tended to be associated with inferior survival, but the correlation was not statistically significant [32]. Discrepancy between prognostic efficacy of the *CCND1* in human (especially MCL) and canine B-cell lymphoma can be described in some ways. This discrepancy may stem from the use of different methods for the analysis of *CCND1* expression (e.g. imunohistochemistry, Western blotting, or gene expression analysis) [39, 40] or may related to the species-dependent characteristics. Relationship between *CCND1* expression and survival time in human MCL was evaluated using immunophenotyping methods [37], while we used a gene expression profiling approach in our study. Additionally, some obvious reverse findings have been found between canine B-cell lymphoma and human counterpart. For example, previous investigation indicated that in contrast to human DLBCL, BCL6 and MUM1/IRF4 rarely expressed in canine B-cell lymphoma [16]. Moreover, an inverse expression pattern for p65 and p52 were found in canine and human DLBCL [41]. Furthermore, some potential confounders such as microsatellite instability (MSI), the CpG island methylator phenotype (CIMP), and BRAF mutation have been suggested as another source of the inconsistent findings regarding association between *CCND1* expression and clinical outcome [40]. These genetic aberrations haven't been examined in canine B-cell lymphoma.

Another gene regarded as a prognostic factor in our study was *BIRCS5* (*survivin*), but it wasn't confirmed in final qRT-PCR assay. *BIRCS5* is one of the most important inhibitors of apoptosis proteins (IAP) involved in the inhibition of induced cell death in vitro and in vivo [42]. Previous investigations have revealed that overall survival is significantly shorter in patients with high *survivin* expression in patients with MCL [43] and DLBCL [44]. Analysis has confirmed that *survivin* can play a role in the prediction of survival independent of the IPI in DLBCL cases [44, 45]. Be that as it may, some authors have indicated that there is no correlation between *survivin* expression and survival or response to treatment in patients with DLBCL [46]. The overexpression of *survivin* in other cancers such as colorectal cancer and neuroblastoma is associated with higher proliferation activity and higher relapse rate [47, 48]. In our study, although *BIRCS5* was found to be a predictor of DFS in canine B-cell lymphoma in multivariate Cox proportional-hazard analysis, this gene wasn't considered as suitable prognostic factor because its expression level wasn't significantly different between patients with long or short survival time.

Conclusions

To the best of our knowledge, this has been one of the few studies to explore prognostic genes in canine lymphoma using gene expression data analysis. Although microarray

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data from human cancers are very extensive and informative, microarray data related to animal cancers are rare and incomplete. When mining microarray databases such as GEO and ArrayExpress, there are limited studies exploring canine cancers using gene expression profiling. Similarly, there is same problem with the canine lymphoma. Our mining in the databases provided three datasets (GSE43664, GSE39365, and GSE30881) with ideal sample size on canine B-cell lymphoma, where clinical metadata (including survival time) haven't been provided for GSE30881 dataset. Therefore, we excluded this dataset and used other two datasets. However, to gain more robust and reliable results, both datasets were used as training and validation groups. Although our results may affect by small sample size of a dataset, we hope that with extending larger canine datasets, future studies by our group or other veterinary oncology researchers will provide more remarkable findings. In conclusion, although the results of the present study reveal *CCND1* as a potential prognostic factor in canine B-cell lymphoma, further studies on more extensive gene expression databases are required to clarify other prognostic genes which can be used as robust survival predictors.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Additional file

Additional file 1: Additional figures and tables. (DOCX 910 kb)

Abbreviations

ABC-like: Activated B-cell like; DFS: Disease-free survival; DLBCL: Diffuse large B-cell lymphoma; GCB-like: Germinal center B like; qRT-PCR: Quantitative real-time PCR

Availability of data and materials

The datasets in the manuscript were deposited in GEO database (http://www.ncbi.nlm.nih.gov/geo/) with the accession number GSE43664 and GSE39365. Other supporting data are included as Additional file 1.

Authors' contributions

MZA, SA, and KI participated in the study design, performing the experiments, and analysis of the data. MZA and SA wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interest.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Informed consent was obtained from pet owners participants included in the study. This study was not based on an animal experiment and used naturally occurring cases. A local ethics committee ruled that no formal ethics approval was required in this particular case.

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