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Short Communication

Comprehensive analysis of gene expression profiles reveals novel candidates of chemotherapy resistant factors in canine lymphoma

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ABSTRACT

The molecular mechanisms of acquisition of chemotherapy resistance in canine lymphoma have not been elucidated. The aim of the present study was to identify novel molecular mechanisms of chemotherapy resistance by a comprehensive analysis of changes in gene expression profiles (GEPs). Tumor samples were obtained from 10 dogs with lymphoma at chemotherapy sensitive and chemotherapy resistant phases. During chemotherapy resistance, the expression of genes associated with immune responses and inflammatory reactions was decreased compared to chemotherapy sensitive phases. In addition, 11 genes, with significant changes in expression ($P < 0.05$), were extracted from seven dogs with chemotherapy resistant lymphoma. Further studies are needed to investigate the associations of these changes in GEPs with the acquisition of chemotherapy resistance in canine lymphoma.

Chemotherapy resistance is one of the leading causes of treatment failure in humans and dogs with malignancies including lymphoma. A variety of mechanisms associated with the development of chemotherapy resistance have been reported in veterinary medicine (Tomiyasu and Tsujimoto, 2015). However, the molecular mechanisms of chemotherapy resistance in canine tumor cells have not been elucidated as yet. The purpose of the present study was to investigate molecular mechanisms of chemotherapy resistance and to identify candidates of specific genes associated with chemotherapy resistance in dogs with lymphoma.

Ten dogs with multicentric high-grade B-cell lymphoma were enrolled in the study. The signalment of these dogs is presented in Appendix: Supplementary Table 1. All dogs were treated with chemotherapy, and tumor samples were collected at both chemotherapy sensitive and chemotherapy resistant phases. Details of dogs, treatments, and tumor samples are described in Appendix: Supplementary Materials and methods. Written informed consent was obtained from all dog owners prior to study enrollment. The Institutional Animal Care and Use Committee (IACUC) approval (No. 18315; Approved from 18 August, 2014 to 18 August, 2017) was obtained for sample collections that occurred at the University of California, Davis, and the sampling procedures were approved by the Animal Care Committee of the Veterinary Medical Center of the University of Tokyo.

Microarray analysis was performed as previously described (Tomiyasu et al., 2013). Total RNA was extracted from fine needle aspirates of peripheral lymph nodes in dogs diagnosed with multicentric lymphoma. Data have been annotated and deposited according to Minimum Information About a Microarray Gene Experiment guidelines with Gene Expression Omnibus (GEO) accession number GSE54744 and GSE83274.

First, hierarchical clustering using 20 samples was conducted based on the fluorescence intensities of all probes on the array plate. This analysis yielded the smallest clusters composed of chemotherapy sensitive and chemotherapy resistant samples from each dog (Appendix: Supplementary Fig. 1). Next, gene expression profiles (GEPs) were compared between the chemotherapy sensitive and chemotherapy resistant phases using 20 samples from 10 dogs, and differentially expressed genes (DEGs) between the two phases were extracted (Appendix: Supplementary Table 2). The hierarchical clustering using these DEGs divided the 20 samples into three clusters (A–C, Appendix: Supplementary Fig. 2). The

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progression-free survival time and overall survival time of Dogs 3, 4, 9 and 10, which were included in cluster B, were compared to other dogs over a longer period (Appendix: Supplementary Table 1). However, further studies with more dogs are needed to investigate possible associations between the expression profiles of these DEGs and the prognosis in dogs with lymphoma.

Based on these results, GEPs were compared between chemotherapy sensitive and chemotherapy resistant samples from seven dogs with similar changes in GEPs. The differentially expressed genes of the two phases in these seven dogs were extracted (Appendix: Supplementary Table 3). These DEGs included 33 genes that were also found in DEGs extracted when GEPs of two phases were compared between all 10 dogs. Hierarchical clustering of these DEGs divided the 14 samples from the seven dogs into two clusters that were composed of only chemotherapy sensitive samples or chemotherapy resistant samples (Fig. 1). These results suggest that changes in GEPs varied among dogs and that the comparison of GEPs between the two-phase samples from all 10 dogs mainly reflected those of the seven dogs.

Pathway analysis using Ingenuity Pathway Analysis (IPA) was conducted using the 57 DEGs. A total of six signaling pathways (Table 1) and 66 biological functions (representative functions are shown in Table 2) were extracted at significance levels ($P < 0.05$ and Z score ≥ 2.0 or ≤ -2.0). Of the 64 biological functions that were inactivated in chemotherapy resistant samples, 32 functions (50%) were associated with activation, recruitment, and degranulation of leukocytes and nine functions (14%) were associated with activation, movement, and metastasis of tumor cells. These findings suggest that immune responses and inflammatory reactions are suppressed at chemotherapy resistant phases in dogs with multicentric high-grade B-cell lymphoma. Our results are comparable to a previous study in humans with diffuse large B-cell lymphoma (DLBCL) that demonstrated an enhanced immunological reaction in tissues obtained from patients that achieved complete remission (Linderoth et al., 2008). There were no obvious changes in the complete blood count (CBC) and serum biochemistry in dogs with chemotherapy resistant lymphoma. However, C-reactive protein, a major positive acute phase protein in dogs, was decreased in three dogs (Appendix: Supplementary

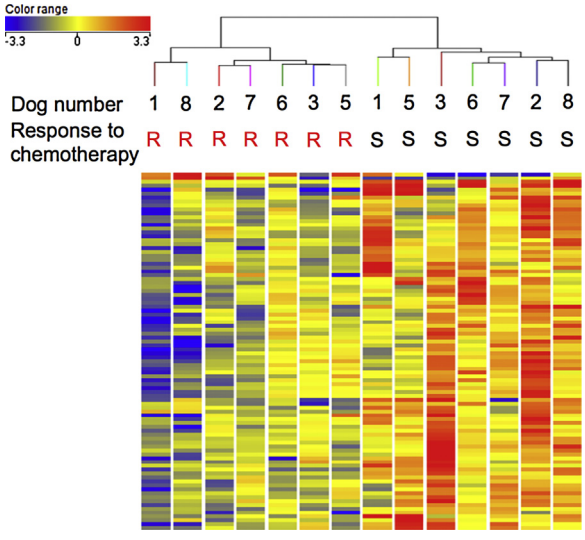

Fig. 1. Hierarchical clustering of chemotherapy sensitive and chemotherapy resistant samples from seven dogs with lymphoma (dogs 1–3 and 5–8). The comparison between the sensitive and resistant phases revealed 57 genes (92 probes) that were extracted. Gene analyses resulted in two major clusters composed of chemotherapy sensitive and chemotherapy resistant samples. 'S': chemotherapy sensitive samples; 'R': chemotherapy resistant samples.

Table 1
Activated (Z score ≥ 2.0) and inactivated (Z score ≤ -2.0) signaling pathways at chemotherapy resistant phases in seven dogs with multicentric B-cell lymphoma.

Signaling pathways	*P*	Z score
PPAR signaling	0.0002	2.000
IL-6 signaling	<0.0001	−2.236
HMGB1 signaling	<0.0001	−2.000
Endothelin-1 signaling	0.0002	−2.236
p38 MAPK signaling	0.0005	−2.000
Acute phase response signaling	0.0018	−2.000

Table 5). Further studies are needed to investigate a potential role of the tumor microenvironment in inducing immunosuppression in dogs with chemotherapy resistant lymphoma.

Among the differentially expressed genes identified, 16 DEGs were selected as candidates for specific genes associated with chemotherapy resistance (Table 3). Significant differences ($P < 0.05$) between the two phases were confirmed in 11 of the 16 DEGs by evaluation with RT-qPCR; *ASNS*,*CXCL8*, *CALCA*, *SASH1*, *IL1R2*, *FCER1A*, *SELE*, *ENDRB*, *PDK4*, *SERPINA1* and *PLAUR* (Fig. 2A–K).

Table 2
Activated (Z score ≥ 2.0) and inactivated (Z score ≤ -2.0) biological functions at chemotherapy resistant phases in seven dogs with lymphoma.

Biological functions	*P*	Z score
Organismal death	<0.0001	4.104
Morbidity or mortality	<0.0001	3.869
Inflammatory response	<0.0001	−2.849
Cell movement	<0.0001	−2.614
Cell movement of tumor cell line cells	<0.0001	−2.795
Cell movement of mononuclear leukocytes	<0.0001	−2.539
Cell movement of neutrophils	<0.0001	−2.177
Stimulation of cells	<0.0001	−2.253
Activation of cells	<0.0001	−3.257
Activation of phagocytes	<0.0001	−3.218
Activation of neutrophils	<0.0001	−2.147
Activation of antigen presenting cells	0.0002	−2.400
Chemotaxis of cells	<0.0001	−2.537
Chemotaxis of phagocytes	<0.0001	−2.047
Chemotaxis of monocytes	<0.0001	−2.415
Degranulation of cells	<0.0001	−2.168
Degranulation of phagocytes	<0.0001	−2.566
Degranulation of mast cells	<0.0001	−2.163
Migration of cells	<0.0001	−2.242
Leukocyte migration	<0.0001	−2.574
Migration of tumor cell line cells	<0.0001	−2.069
Accumulation of phagocytes	0.0001	−2.187
Recruitment of neutrophils	<0.0001	−2.403
Binding of cells	<0.0001	−2.406
Binding of blood cells	0.0001	−2.177
Binding of tumor cell line cells	0.0004	−2.149
Adhesion of mononuclear leukocytes	0.0001	−2.193
Cellular infiltration by mononuclear leukocytes	0.0004	−2.219
Proliferation of cells	<0.0001	−2.699
Proliferation of tumor cells	<0.0001	−3.067
Proliferation of connective tissue cells	<0.0001	−2.269
Invasion of tumor cells	<0.0001	−2.211
Differentiation of leukocytes	<0.0001	−2.054
Secretion of lipid	<0.0001	−2.031
Transport of molecules	<0.0001	−2.246
Cellular homeostasis	<0.0001	−2.679
Ion homeostasis of cells	<0.0001	−2.395
Mobilization of Ca^{2+}	<0.0001	−2.426
Flux of Ca^{2+}	<0.0001	−2.189
Angiogenesis	<0.0001	−2.601
Vasculogenesis	<0.0001	−2.597
Vascularization	<0.0001	−2.387
Microtubule dynamics	<0.0001	−3.499
Formation of cytoskeleton	<0.0001	−2.093
Organization of cytoskeleton	<0.0001	−3.596
Formation of connective tissue cells	<0.0001	−2.202
Quantity of cells	<0.0001	−2.316
Hypersensitive reaction	<0.0001	−2.190
Release of eicosanoid	0.0001	−2.207