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Evaluation of the drug sensitivity and expression of 16 drug resistance-related genes in canine histiocytic sarcoma cell lines

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ABSTRACT. Canine histiocytic sarcoma (HS) is an aggressive tumor type originating from histiocytic cell lineages. This disease is characterized by poor response to chemotherapy and short survival time. Therefore, it is of critical importance to identify and develop effective antitumor drugs against HS. The objectives of this study were to examine the drug sensitivities of 10 antitumor drugs. Using a real-time RT-PCR system, the mRNA expression levels of 16 genes related to drug resistance in 4 canine HS cell lines established from dogs with disseminated HS were determined and compared to 2 canine lymphoma cell lines (B-cell and T-cell). These 4 canine HS cell lines showed sensitivities toward microtubule inhibitors (vincristine, vinblastine and paclitaxel), comparable to those in the canine B-cell lymphoma cell line. Moreover, it was shown that P-gp in the HS cell lines used in this study did not have enough function to efflux its substrate. Sensitivities to melphalan, nimustine, methotrexate, cytarabine, doxorubicin and etoposide were lower in the 4 HS cell lines than in the 2 canine lymphoma cell lines. The data obtained in this study using cultured cell lines could prove helpful in the developing of advanced and effective chemotherapies for treating dogs that are suffering from HS.

KEY WORDS: canine, drug resistance, histiocytic sarcoma, microtubule inhibitors, *TP53* gene

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Canine histiocytic sarcoma (HS) is a rare tumor type originating from histiocytic cell lineages, including dendritic cells (DCs) and macrophages [2, 26], and are characterized by aggressive biological behavior and poor prognosis. Canine HS is subdivided into two categories: solitary HS and disseminated HS. In addition, hemophagocytic HS has recently been described as a different subtype of canine HS that originates from macrophages. Monotherapy with lomustine (CCNU) is often the treatment of choice for HS [34], and there have been several reports describing its response to chemotherapy with doxorubicin [39], liposomal entrapped doxorubicin [39] and paclitaxel [28]. Nevertheless, HS often acquires multidrug resistance to these antineoplastic agents within a short time, leading to a median survival time of less than 100 days [30, 34, 35]. Therefore, there is a pressing need to develop effective antitumor drugs against HS and identify the factors that lead to the chemoresistance. Although the drug sensitivities of HS cells against some of the chemotherapeutic agents have been examined *in vitro* [14, 17, 36, 42], so far there have been no comprehensive studies on the drug sensitivity / resistance against a series of antineoplastic agents of various categories in canine HS cell lines.

To date, many studies have been carried out in order to elucidate drug resistance in various human tumors, and a number of genes related to drug resistance have been uncovered. Over-expression of ATP-binding cassette transporters (ABC transporters) including *ABCB1* [27], *ABCC1* [27] and *ABCG2* [6] is known to be the major mechanism underlying reduced drug accumulation in tumor cells. Mutant *TP53* and over-expression of *Bcl-2* lead to decreased apoptosis [5, 21]. GSTA4 and GSTP1, members of the Glutathione S-transferase (GST) family, are known to induce detoxification of cytotoxic drugs [7, 18]. Moreover, DNA repair pathways are also known to be involved in the development and acquisition of drug resistance. Previous reports suggested that O^6-alkylguanine DNA alkyltransferase, which is encoded by the O^6-methylguanine DNA methyltransferase *(MGMT)* gene, is associated with resistance to alkylating agents [4, 13, 19]. Loss of DNA mismatch repair (MMR) genes is also known to lead to drug resistance [1, 10, 11].

In veterinary medicine, the expression of P-gp is enhanced in tumor cells that were obtained from dogs with relapsed lymphoma and chemotherapy-resistant lymphoma [22, 24]. Our recent study revealed that the epigenetic regulation of *ABCB1* was associated with sensitivity to vincristine in canine lymphoma cell lines [37]. Inhibition of survivin increased sensitivities to CCNU and doxorubicin, and also influences the biological behavior of canine HS cell lines [42]. However, no studies have yet been able to adequately account for the underlying cause of the short survival time even after chemotherapy treatment in dogs afflicted with HS.

The objectives of this study were to evaluate the sensitivities of HS cell lines toward a series of antineoplastic agents and investigate the associations between this sensitivity and the expression levels of drug resistance-related genes in

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canine HS cell lines.

MATERIALS AND METHODS

Cell lines and cell culture: Four canine HS cell lines (CHS-1, CHS-2, CHS-3 and DH82) and 2 lymphoma cell lines (Ema and CLBL-1) were used in this study. CHS-1, CHS-2 and CHS-3 [3] were established from the tumor tissues of dogs with HS. None of the 3 dogs from which these HS cell lines were established had received any chemotherapy. DH82 [41] was established from a dog with malignant histiocytosis. Ema [15] was established from a dog with abdominal T-cell lymphoma showing drug resistance, whereas CLBL-1 [32] was established from a dog with diffused large B-cell lymphoma (DLBCL) that had not been subjected to chemotherapy. CLBL-1 was used as a representative canine B-cell lymphoma cell line sensitive to vincristine, whereas Ema was used as a representative canine T-cell lymphoma cell line that is resistant to vincristine [37]. Our previous study revealed that due to epigenetic regulation, the expression level of *ABCB1* was much higher in Ema than in the CLBL-1 cell line [37]. HS cell lines were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (Biowest, Nuaille, France) in a humidified atmosphere containing 5% CO_2. The 2 lymphoma cell lines were cultured at 37°C in RPMI-1640 culture medium containing 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2.

Preparation and storage of antitumor drugs: Ten antitumor drugs were used in this study, melphalan (Sigma-Aldrich, St. Louis, MO, U.S.A.), nimustine hydrochloride (Tokyo Chemical Industry, Tokyo, Japan), methotrexate (Tokyo Chemical Industry), cytarabine (Wako, Tokyo, Japan), vincristine sulfate (Wako), vinblastine sulfate (Wako), paclitaxel (Wako), doxorubicin hydrochloride (Wako), etoposide (Wako) and Paccal Vet®, water-soluble micellar paclitaxel (WSMP). WSMP was kindly provided by Oasmia Pharmaceutical AB and the Nippon Zenyaku Kogyo Co., Ltd. (ZENOAQ). The stock solutions of all drugs were prepared according to the manufacturer's instructions and stored at −20°C. Working concentrations of the chemotherapeutic agents were prepared by diluting stock solutions with saline.

Drug sensitivity assay: The 50% inhibitory concentration (IC_{50}) values for each drug were determined for the 6 cell lines. After pre-incubation for 24 hr, cells (5×10^4 cells/m*l*) were co-cultured in a 96-well plate with various concentrations of each drug or saline for 48 hr. After cultivation, cell viability was measured using Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's instruction. The absorbance was measured using a Model 680 Microplate Reader (Bio-Rad Laboratories, Hercules, CA, U.S.A.), and IC_{50} values for the 6 cell lines were determined. All samples were examined in three independent experiments.

Quantitative analysis of mRNA by real-time RT-PCR: Expression levels of mRNAs for *ABCB1*, *ABCC1*, *ABCG2*, *LRP*, *TP53*, *p21^waf1^*, *Bcl-2*, *survivin*, *GSTA4*, *GSTP1*, *MGMT*, *MSH2*, *MSH3*, *MSH6*, *MLH1* and *PMS2* were evaluated using a real-time RT-PCR system. *MSH2*, *MSH3*, *MSH6*, *MLH1* and *PMS2* are genes involved in DNA mismatch repair [23]. Primer sequences for these genes related to drug resistance were designed using the Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/) and are listed in "Supplementary file 1". Primers for *surviving* and *p21^waf1^* were prepared as described previously [38, 42]. For normalization purposes, *HMBS* and *TBP* were selected as appropriate internal controls. Detailed information of the real-time RT-PCR procedure is shown in "Supplementary file 2".

Examination of TP53 mutations: To examine mutations in the coding regions of the *TP53* gene in the 6 cell lines, genomic DNA sample was extracted from each cell line using the QIAamp® DNA Blood Mini Kit (QIAGEN, Limburg, Netherlands). The sequence of the *TP53* genomic DNA was divided into seven fragments, and seven pairs of primers were selected based on previous report [9]. Primer sequences are listed in "Supplementary file 3". The DNA samples were amplified by PCR using AmpliTag Gold® 360 (Applied Biosystems, Foster City, CA, U.S.A.), and primer pairs were constructed according to the manufacturer's instructions for each fragment as well as *MMP3*, which is located on the same chromosome as *TP53*. Amplification of products was confirmed by electrophoresis. The PCR products were inserted into a T/A cloning vector (pGEM-T Easy) (Promega Corporation, Leiden, The Netherlands) and subjected to sequence analysis. The sequence of each fragment was analyzed using the BigDye terminator v3.1/1.1 Cycle Sequencing Kit (Applied Biosystems) and the Applied Biosystems 3130XL genetic analyzer (Applied Biosystems). At least five clones from each sample were sequenced.

Nucleotide sequencing of ABCB1 cDNA: The sequence of entire cDNA of *ABCB1* gene was divided into eight fragments (fragments 1 to 8), and eight primer pairs were designed (Supplementary file 4). Sequences of the primers were based on the GenBank database (AF269224). Nucleotide sequences of the amplified fragments were analyzed directly from the PCR products using the BigDye terminator v3.1/1.1 Cycle Sequencing Kit (Applied Biosystems) and the Applied Biosystems 3130XL genetic analyzer (Applied Biosystems). The results were confirmed by two independent experiments.

Western blot analysis for P-gp: Ten μg of protein extracted from each cell line was separated by SDS-PAGE and blotted onto a PVDF membrane. The membranes were blocked in 5% skimmed milk and incubated with primary antibodies against P-gp (murine monoclonal: C219, Merck Millipore, Darmstadt, Germany) diluted at 1:100 for 12 hr at 4°C, or β-actin (murine monoclonal: AC-15, Sigma-Aldrich) diluted at 1:5,000 for 1 hr at room temperature. Then, the membranes were incubated with HRP-labeled goat anti-mouse IgG antibodies (1:2,000; Santa Cruz Biotechnology, Dallas, TX, U.S.A.) for 1 hr at room temperature. After incubation, positive immunoreactivity was detected using Luminata Forte Western HRP Substrate (Merck Millipore) and visualized using a ChemiDoc XRS Plus (Bio-Rad Laboratories).

Rhodamine 123 efflux test: Function of P-gp was evaluated by testing its efflux function for its substrate dye as reported

Table 1. The IC_{50} values for the 6 cell lines

Drug examined	IC_{50} values					
	CHS-1	CHS-2	CHS-3	DH82	CLBL-1	Ema
Melphalan	36 μg/m*l*	49 μg/m*l*	29 μg/m*l*	65 μg/m*l*	158 *n*g/m*l*	689 *n*g/m*l*
Nimustine hydrochloride	398 μg/m*l*	467 μg/m*l*	190 μg/m*l*	587 μg/m*l*	1.42 μg/m*l*	876 *n*g/m*l*
Methotrexate	>100 μg/m*l*	8.56 μg/m*l*	495 *n*g/m*l*	>100 μg/m*l*	7.10 *n*g/m*l*	17.3 *n*g/m*l*
Cytarabin	14.4 μg/m*l*	40.8 μg/m*l*	9.29 μg/m*l*	142 μg/m*l*	91.7 *n*g/m*l*	736 *n*g/m*l*
Vincristine sulfate	2.53 *n*g/m*l*	2.69 *n*g/m*l*	1.77 *n*g/m*l*	2.66 *n*g/m*l*	1.86 *n*g/m*l*	52.4 *n*g/m*l*
Vimblastine sulfate	2.47 *n*g/m*l*	2.78 *n*g/m*l*	1.75 *n*g/m*l*	2.42 *n*g/m*l*	12.0 *n*g/m*l*	39.7 *n*g/m*l*
Paclitaxel	53.2 *n*g/m*l*	47.1 *n*g/m*l*	23.8 *n*g/m*l*	58.4 *n*g/m*l*	133 *n*g/m*l*	263 *n*g/m*l*
WSMP	46 *n*g/m*l*	92.8 *n*g/m*l*	15.6 *n*g/m*l*	19.2 *n*g/m*l*	12.3 *n*g/m*l*	85.5 *n*g/m*l*
Doxorubicin hydrochloride	187 *n*g/m*l*	343 *n*g/m*l*	95.0 *n*g/m*l*	113 *n*g/m*l*	24.8 *n*g/m*l*	69.5 *n*g/m*l*
Etoposide	3.71 μg/m*l*	8.18 μg/m*l*	755 *n*g/m*l*	2.19 μg/m*l*	67.5 *n*g/m*l*	230 *n*g/m*l*

previously. One million cells of each cell line were incubated with 200 *n*g/m*l* of Rhodamine 123 (Sigma-Aldrich) in medium at 37°C for 15 min. After washing in medium, the cells were incubated in Rhodamine 123-free medium at 37°C for 1 hr, either with or without 2 μM of cyclosporine (Sigma-Aldrich). After incubation, the cells were washed in medium and subjected to flow cytometric analysis using the FACSCalibur (BD Biosciences, Franklin Lakes, NJ, U.S.A.). Cells that had not been exposed to Rhodamine 123 were used as negative controls. The results were confirmed by two independent experiments.

Statistical analysis: Data were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post-hoc test was performed for multiple comparisons of relative mRNA quantities using the STATMATE (ATMS, Tokyo, Japan) software. *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Drug sensitivity assay: The IC_{50} values for the 6 cell lines are shown in Table 1 and Fig. 1. For alkylating agents (i.e., melphalan and nimustine hydrochloride), the 4 HS cell lines showed higher IC_{50} values than the 2 lymphoma cell lines. Similarly, the HS cell lines showed higher IC_{50} values for antimetabolites (methotrexate and cytarabine), doxorubicin and etoposide compared to the lymphoma cell lines. Meanwhile, the HS cell lines demonstrated similar or lower IC_{50} values for microtubule inhibitors (vincristine sulfate, vinblastine sulfate, paclitaxel and WSMP) compared to the 2 lymphoma cell lines. The IC_{50} values for vincristine sulfate in the HS cell lines were similar to that in CLBL-1, a canine B-cell lymphoma cell line reported to be sensitive to vincristine [37]. For vinblastine and paclitaxel, HS cell lines showed lower IC_{50} values than CLBL-1.

Quantitative analysis of mRNA by real-time RT-PCR: The expression levels of 16 drug resistance genes are shown in "Supplementary file 5". In addition, comparisons of 5 genes showing the relative quantities that were significantly different between HS and lymphoma cell lines are illustrated in the graphs (Fig. 2). Among the drug efflux transporters (*ABCB1*, *ABCC1*, *ABCG2* and *LRP*), the expression levels of *ABCB1* in 3 HS cell lines (CHS-1, CHS-2 and DH82) were significantly higher than those in CLBL-1. Furthermore, the expression level of *ABCB1* was significantly higher in Ema. The expression levels of *ABCG2* in the 4 HS cell lines were significantly higher, compared to those in the 2 lymphoma cell lines. However, there were no significant differences observed in the expression levels of *ABCC1* and *LRP* between HS cell lines and lymphoma cell lines.

The 4 HS cell lines exhibited significantly lower expression levels of *TP53* than the 2 lymphoma cell lines. The expression levels of $p21^{waf1}$ were significantly higher in the 4 HS cell lines than in the 2 lymphoma cell lines. No significant differences were observed in the levels of *survivin* and *Bcl-2* between the HS and lymphoma cell lines. With regard to the DNA repair genes (*MSH2*, *MSH3*, *MLH1*, *PMS2* and *MGMT*), the expression levels of *MSH6* in the 4 HS cell lines were significantly lower than in the 2 lymphoma cell lines. However, there were no significant differences between the HS and lymphoma cell lines with respect to the other 4 genes.

Mutation of the *TP53 gene*: Of the 6 cell lines, mutations in the *TP53* gene were identified in 3 HS cell lines and 1 lymphoma cell line (Fig. 3). CHS-1 harbored a point mutation in exon 10, resulting in the introduction of a stop codon at codon 326. CHS-2 harbored 3 nucleotide deletions in exon 6 at codon 206 (Val). In these cell lines, no clone representing the wild-type *TP53* sequence was detected in the five clones examined. No gene amplification could be observed by PCR using 7 primer pairs complementary to the canine *TP53* gene in the DH82 cell line, although *MMP3* was amplified normally. CLBL-1 had heterozygous point mutations at exon5 (Ala^{125} to Val), but no mutations were observed in the CHS-3 and Ema cell lines.

Nucleotide sequencing of the ABCB1 gene: In the sequence analysis, no mutation was found in the cDNAs of *ABCB1* gene of 6 cell lines. The results were compared to the coding sequence of canine *ABCB1* gene (GenBank association number AF269224).

Western blot for P-gp: In Western blotting, a distinct band of approximately 170 kD corresponding to P-gp was detected in Ema (Fig.5). In CHS-1 and DH82, a faint corresponding band was found. Expression of P-gp was not detected in CHS-3 and CLBL-1.

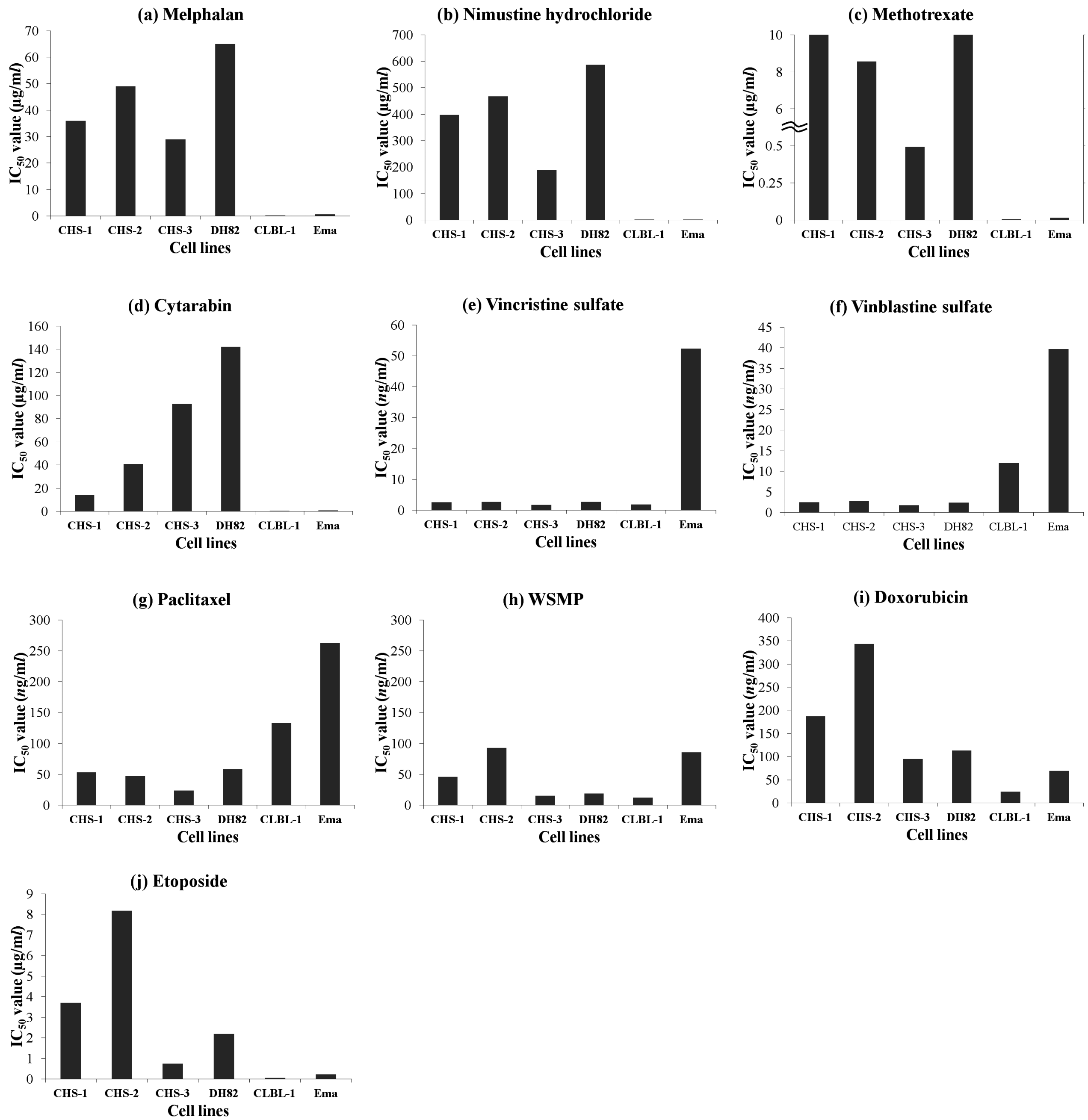

Fig. 1. Graphs illustrating the IC_{50} values for (a) melphalan, (b) nimustine hydrochloride, (c) methotrexate, (d) cytarabine, (e) vincristine sulfate, (f) vinblastine sulfate, (g) paclitaxel, (h) WSMP, (i) doxorubicin and (j) etoposide. CHS-1, CHS-2, CHS-3 and DH82: canine HS cell lines. CLBL-1 and Ema: canine lymphoma cell lines.

Rhodamine 123 efflux test: In the Rhodamine 123 efflux test, efflux of the dye was observed in Ema, whereas the dye was retained in other cell lines (Fig. 4).

DISCUSSION

In the 4 HS cell lines examined in this study, the IC_{50} values of vincristine were 1.77 to 2.69 *n*g/m*l*, which were similar to or less than that in a vincristine-sensitive canine B-cell lymphoma cell line (CLBL-1) (1.86 *n*g/m*l*), but much lower than that in a vincristine-resistant canine T-cell lymphoma cell line (Ema) (52.4 *n*g/m*l*). Similar results were obtained for the IC_{50} values of vinblastine, paclitaxel and WSMP, indicating that the 4 HS cell lines demonstrated sensitivities toward the tested microtubule inhibitors, comparable to the chemosensitive canine B-cell lymphoma cell line. Cur-

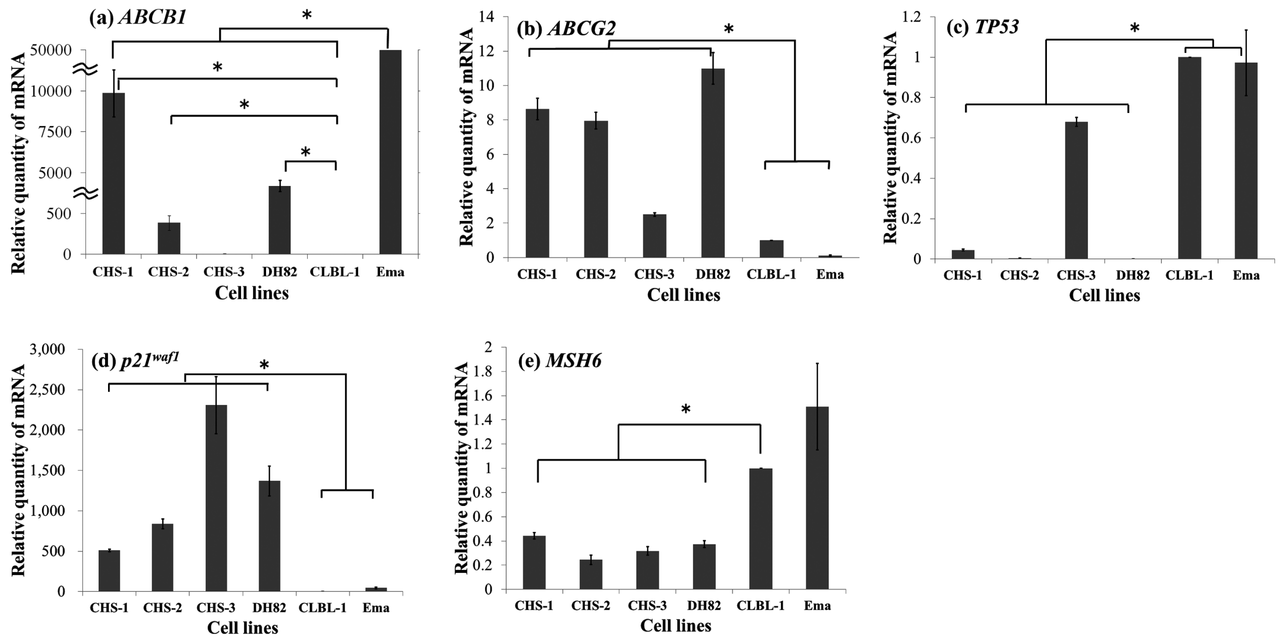

Fig. 2. Graphs illustrating the mRNA expression levels of (a)*ABCB1*, (b)*ABCG2*, (c)*TP53*, (d)*p21^{waf1}* and (e)*MSH6*. All data represent the mean ± SD of three independent experiments. *P<0.05 (one-way ANOVA followed by Tukey's post-hoc test).

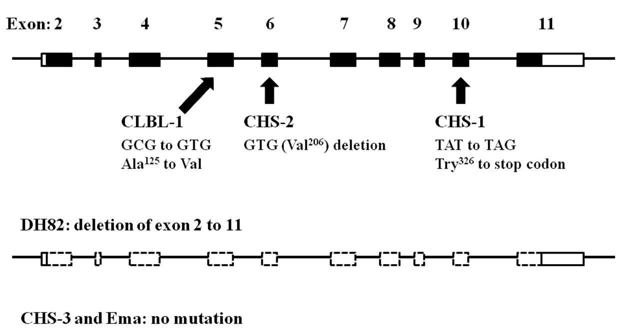

Fig. 3. Schematic diagram of the locations of the mutations of *TP53* gene found in this study. Dark boxes represent the coding exons of the *TP53* gene. Open boxes represent the non-coding regions, and open boxes with dotted line represent the deletion of exons. The mutations identified in this study are represented by arrows under the *TP53* gene map.

rently, there are only a few published studies available that examined the pharmacokinetics of antitumor drugs in dogs; however, the C_{2min} value of vincristine was reportedly 39.8 ± 9.18 *n*g/m*l* in 5 beagle dogs when intravenously injected at a dose of 0.07 mg/kg [44]. Moreover, the C_{max} value of WSMP was recently reported as 14 ± 6.6 μg/m*l* in dogs when it is intravenously administered at a dose of 150 mg/m^2 [40], and a complete or partial response was observed in 59% of dogs with mastocytoma when injected intravenously at a median dose of 145 (range, 135–150) mg/m^2 [31]. The suggested dose of vincristine is 0.7 mg/m^2 in representative chemotherapeutic protocol for canine lymphoma [12, 25], and the recommended dose of paclitaxel is 132 mg/m^2 [29]. Therefore, these results suggest that the IC_{50} values of the microtubule inhibitors that were used in this study can be achieved at a clinical dosage level, and that these drugs may be good candidates for the effective treatment of canine HS. However, no reports have thus far described the efficacy of monotherapy with microtubule inhibitors for the treatment of dogs with HS. Consequently, further studies would be required to evaluate the efficacies of these candidate drugs in treatment applications for canine HS.

The 4 HS cell lines showed relatively high IC_{50} values for alkylating agents (melphalan and nimustine hydrochloride), antimetabolites (methotrexate and cytarabine), antitumor antibiotic (doxorubicin hydrochloride) and topoisomerase inhibitor (etoposide) in comparison to CLBL-1. Although there have been several reports on the treatment of canine HS with CCNU and nimustine hydrochloride (ACNU) [35], from their response rates (46% and 50%, respectively) and medium survival times (59 and 48 days, respectively), efficacy of these agents does not seem to be satisfactory. Moreover, the number of dog patients evaluated for the efficacy of ACNU was small (6 dogs), and 2 of the 3 responders did not achieve complete remission. Canine HS cell lines examined in this study were generally resistant to ACNU; therefore, further effort is necessary to examine the clinical efficacy of other chemotherapeutic agents in dog patients suffered from HS.

Here, it was shown that the expression levels of *ABCB1* in 3 HS cell lines (CHS-1, CHS-2 and DH82) were significantly higher than that in CLBL-1, though significantly lower than that in Ema. It was previously reported that the product of *ABCB1*, P-gp, was involved in the resistance to vincristine, vinblastine and paclitaxel [8, 16]. However, the

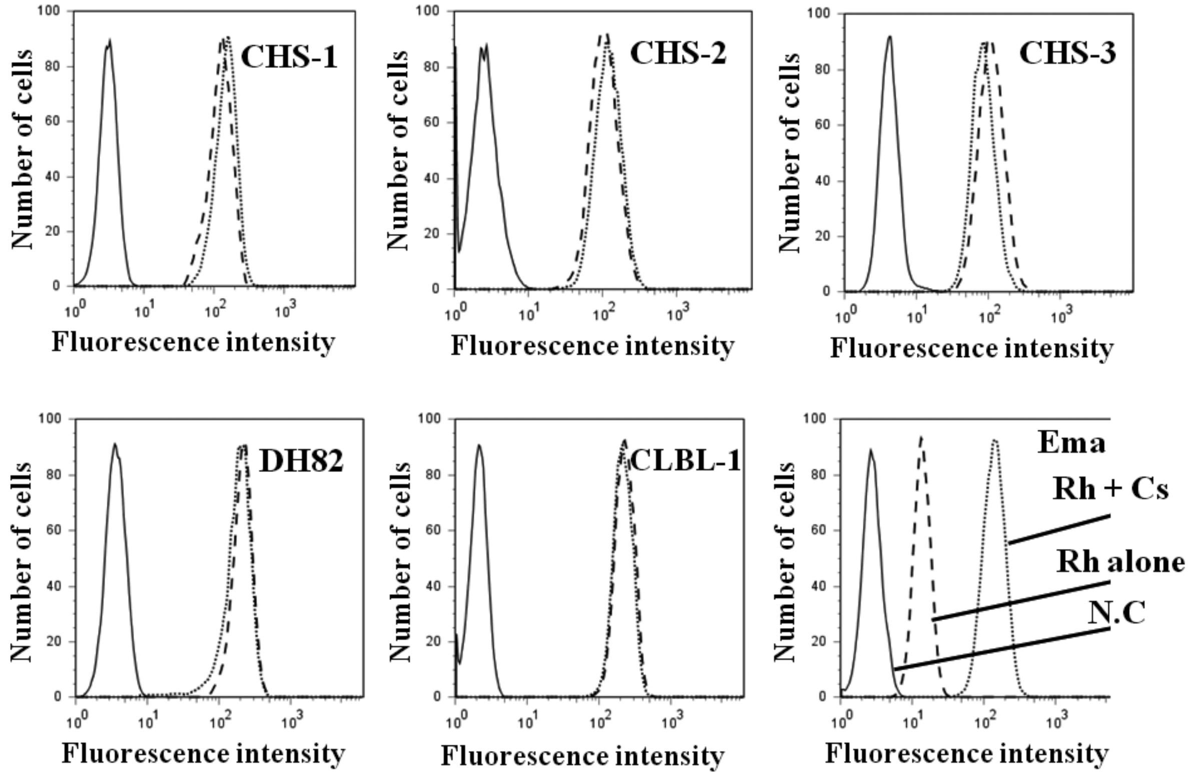

Fig. 4. Efflux of the Rhodamine 123 dye was obvious in the Ema, whereas its efflux was not observed in other cell lines (CHS-1, CHS-2, CHS-3, DH82 and CLBL-1). The solid line, dashed line and dotted line represent the histograms of negative control (N.C), Rhodamine 123 only and Rhodamine 123 with Cyclosporine (Cs), respectively.

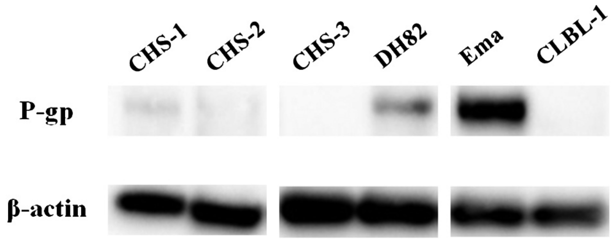

Fig. 5. In Western blot analysis, the expression of P-gp was detected in CHS-1, DH82 and Ema, whereas no signal was detected in CHS-2, CHS-3 and CLBL-1.

IC_{50} values of these 3 drugs were similar or lower in HS cell lines, compared to CLBL-1. In Western blot, the band corresponding to P-gp was not found in CHS-2 and CHS-3 and very faint in CHS-1 and DH82. Moreover, no efflux of the substrate dye was observed in the 4 HS cell lines in the Rhodamine 123 efflux test, and no mutation of the *ABCB1* gene was identified in the 4 HS cell lines. These results indicated that the 4 canine HS cell lines did not express P-gp with sufficient function, although 3 of them had measurable amount of *ABCB1* mRNA. It was conceivable that translation of the mRNA to P-gp was impaired for some reasons.

The expression levels of *TP53* in the 4 HS cell lines were very low in comparison to the 2 lymphoma cell lines. Especially, in the DH82 cell line, nearly no *TP53* mRNA was detected by the quantitative PCR analysis. Therefore, down regulation of *TP53* might be associated with resistance to a wide range of antitumor drugs possibly via a mechanism that circumvents apoptosis. Moreover, 2 out of the 4 HS cell lines harbored mutations in the *TP53* gene, and it was shown that in these mutations, there were no wild-type alleles present. Result obtained from sequencing of *TP53* also indicated that the coding region of *TP53* was in fact lost in the DH82 cell line. These results implied that aberrations in the *TP53* gene might be a common genetic alteration in canine HS. A further study is needed to know the frequency of the aberration of *TP53* gene in the primary tumor tissues obtained from dogs that developed HS. Moreover, direct experiment of transferring wild-type *TP53* gene into *TP53*-deficient canine HS cell line will reveal its influence on the drug resistance in the neoplastic cells of canine HS. Since the expression of $p21^{WAF1}$ is induced by P53, its expression level is usually reduced in cells with inactivated p53. However, the expression levels of $p21^{waf1}$ were significantly higher in the HS cell lines than in the lymphoma cell lines (Supplementary file. 5). Although wild-type p53 promotes the expression of $p21^{WAF1}$ [20], p53-independent induction of *p21* expression has been observed in several cell types including human breast cancer cells and the canine MDCK cell line [33, 43]. Therefore, it is suggested that the expression of $p21^{waf1}$ in the HS cell lines examined in this study may have been induced by p53-independent mechanisms.

In this study, we screened sensitivities demonstrated by canine HS cell lines to conventional antitumor drugs in comparison to canine lymphoma cell lines. The 4 canine HS cell lines examined have shown high IC_{50} values of alkylating agents, antimetabolites, doxorubicin and etoposide, whereas they exhibited relatively low IC_{50} values of microtubule inhibitors compared to lymphoma cell lines. Microtubule inhibitors may be considered potential drug candidates for the treatment of canine HS. On the other hand, it was suggested that alkylating agents, antimetabolites, antitumor antibiotics and topoisomerase inhibitors might not be effective as treatment of HS. Moreover, the expression levels of 16 genes related to drug resistance were examined to elucidate the resistance mechanism toward the antitumor agents used in this study. Finally, the low expression level of *TP53* in HS cell lines might play a role in the resistance against a diverse range of chemotherapeutic agents. Although the expression level and mutation of various genes related to drug resistance were examined in cultured canine HS cell lines in this study, similar analyses of selected genes will be needed using primary tumor cell samples obtained from dog patients suffered from HS. The present study provided insights into the mechanisms of chemosensitivity / chemoresistance of canine HS cell lines which can be further investigated to gain further information regarding the pharmacokinetics, safety and efficacy of candidate drugs in treating dogs with HS.

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