Regulations of *ABCB1* and *ABCG2* Expression Through MAPK Pathways in Acute Lymphoblastic Leukemia Cell Lines

HIROTAKA TOMIYASU[1], MANABU WATANABE[2], KANJI SUGITA[3], YUKO GOTO-KOSHINO[1], YASUHITO FUJINO[1], KOICHI OHNO[1], SUMIO SUGANO[2] and HAJIME TSUJIMOTO[1]

[1]*Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan;*
[2]*Laboratory of Functional Genomics, Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Minato-ku, Tokyo, Japan;*
[3]*Department of Pediatrics, School of Medicine, University of Yamanashi, Chuo, Japan*

Abstract. *Background: One of the major causes of failure in chemotherapy for patients with acute lymphoblastic leukemia (ALL) is the acquisition of multidrug resistance (MDR). Predominant mechanisms for MDR acquisition include the overexpression of efflux pumps. In the present study, the regulation of the expression of two genes that encode efflux pumps, ATP-binding cassette, sub-family B, member 1 (ABCB1) and ABCG2, through mitogen-activated protein kinase (MAPK) pathways was examined. Materials and Methods: ABCB1 and ABCG2 mRNAs were quantified in a T-ALL cell line, CCRF-HSB-2 and a B-ALL cell line, YAMN90 using real-time RT-PCR. Changes in the mRNA amounts of these genes were examined after activation or inhibition of MAPK/extracellular signal-regulated kinase (ERK) pathway and c-Jun NH2-terminal kinase (JNK) pathway. Results: Activation of MAPK/ERK pathway up-regulated ABCB1 expression but down-regulated ABCG2 expression. Activation of JNK pathway up-regulated ABCG2 gene expression. Conclusion: The expressions of ABCB1 and ABCG2 genes were regulated through MAPK/ERK and JNK pathways in the human ALL cell lines.*

Acute lymphoblastic leukemia (ALL) is a biologically and clinically heterogeneous group of diseases that are considered to be the result of clonal proliferation of malignant lymphoid progenitors. ALL commonly originates from the B-cell lineage, which accounts for 67.6% of overall incidence of ALL, and T-cell lineage ALL is an uncommon disorder that accounts only for 15.2% of overall incidence of ALL (1). Approximately 25% of children with ALL experience relapse and cannot be cured with current chemotherapy (2). One of the major causes of chemotherapy failure for patients with ALL is intrinsic or acquired multidrug resistance (MDR) (2).

Although various molecules have been reported to contribute to the development of MDR (3, 4), acquisition of MDR is not well-understood. One of the most important mechanisms associated with MDR is the reduction of intracellular concentrations of chemotherapeutic agents due to overexpression of efflux pumps, such as the ATP-binding cassette (ABC) transporters (5). The efflux pumps encoded by *ABCB1*, *ABCC1* and *ABCG2* genes are representative ABC transporters associated with the acquisition of MDR, and overexpression of these genes has been found in various types of tumor cells with MDR phenotypes (6). In hematopoietic tumors, expression of P-glycoprotein (encoded by the *ABCB1* gene) and ABCG2 is frequently observed after chemotherapy in patients with acute myeloid leukemia (AML) (7, 8) and multiple myeloma (9). In ALL patients, high expression of *ABCB1* gene at diagnosis has been reported to be correlated with a poor prognosis (10), and a possible correlation between ABCG2 expression and shorter disease-free survival was also reported (11).

The mechanisms that induce overexpression of these transporters are still unclear. Various intracellular signaling systems have been reported to regulate the expressions of ABC transporters. Mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway (12) and c-JUN NH_2-terminal kinase (JNK) pathway (13) were reported to be involved in the regulation of *ABCB1* gene expression. These two MAPK pathways were also reported to regulate the expression of *ABCG2* gene (14, 15). In addition, we recently reported that the

Correspondence to: Hajime Tsujimoto, Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan Tel: +81 358415402, Fax: +81 358415640, e-mail: atsuji@mail.ecc.u-tokyo.ac.jp

Key Words: ABCG2, ALL, BCRP, MAPK, multidrug resistance.

Table I. *Primer pairs used for real-time reverse transcription-polymerase chain reaction measurement of the relative quantity of each gene.*

Gene	Sense primer	Reverse primer	Amplicon size (bp)	Genebank accession number
ABCB1	5'-AGGCCAACATACATGCCTTC-3' (3945-3964)	5'-CCACCAGAGAGCTGAGTTCC-3' (4010-4029)	85	NM_000927
ABCC1	5'-CCTGTTCAACGTCATTGGTG-3' (3466-3485)	5'-AGCCACGTAGAACCTCTGGA-3' (3561-3580)	115	NM_004996
ABCG2	5'-TTCGGCTTGCAACAACTATG-3' (785-804)	5'-TCCAGACACACCACGGATAA-3' (894-912)	128	NM_001257386
LRP	5'-TGAGGAGGTTCTGGATTTGG-3' (779-798)	5'-TGCACTGTTACCAGCCACTC-3' (894-913)	135	NM_005115
ABL1	5'-TGGAGATAACACTCTAAGCATAACTA AAGGT-3' (372-401)	5'-GATGTAGTTGCTTGGGACCCA-3'(475-495)	124	NM_005157

ABCB1: ATP-binding cassette, sub-family B, member 1; *LRP*: lung resistance-related protein; *ABL1*: c-abl oncogene 1. The numbers in the parenthesis indicate the nucleotide numbers registered in GenBank.

MAPK/ERK pathway regulates the expression of *lung resistance-related protein* (*LRP*) and *ABCB1* genes in lymphoma cell lines derived from dogs (16). However, the association of these pathways with regulation of transporter expression has not been studied in ALL cells.

The purpose of the present study was to examine the regulation of the expression of four transporter genes, *ABCB1*, *ABCC1*, *ABCG2* and *LRP*, through MAPK pathways.

Materials and Methods

Cell culture. CCRF-HSB-2 human T-ALL cell line (17), which was provided by RIKEN BRC (Tsukuba, Ibaraki, Japan) through the National Bio-Resource Project of the MEXT, JAPAN, and the human pre-B-ALL cell line YAMN90, which was established at Yamanashi University (18), were used in this study. These cell lines were grown in RPMI-1640 supplemented with 10% fetal calf serum and cultured at 37˚C in a humidified atmosphere of 5% CO_2.

Relative quantification of efflux pump mRNAs. The expression levels of *ABCB1, ABCC1, ABCG2* and *LRP* genes were relatively quantified by a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) system as reported in our previous study (19) with the primer pairs listed in Table I. For normalization of the amount of cDNA sample, *c-abl oncogene 1 gene* (*ABL1*, gene accession number; NM_005157) was used as an internal reference, based on a previous study (20). For relative quantification, assay-specific standard curves were prepared, and the relative quantity of each gene was calculated by plotting the quantification cycle in the prepared standard curves. To normalize the amount of cDNA sample, the rations of the relative quantities of cDNA for *ABCB1, ABCC1, ABCG2* and *LRP* genes to that of *ABL1* gene were adopted. Real-time RT-PCR assays were performed in triplicate.

Changes in the expression levels of transporter genes due to activation of MAPK/ERK and JNK pathways. For activation or inhibition of MAPK/ERK and JNK pathways, phorbol 12-myristate 13-acetate (PMA) (protein kinase C activator, Wako, Osaka, Japan), U0126 (MAPK/ERK kinase inhibitor; Promega, Leiden, the Netherlands), and SP600125 (JNK inhibitor; Promega), were used as previously described (15, 21, 22). Briefly, CCRF-HSB-2 and YAMN90 cell lines untreated or pre-incubated with 10 μM U0126 for 1 h or 50 μM SP600125 for 40 min were cultured in the presence of 200 nM PMA for 8 h. The relative quantity of *ABCB1, ABCC1, ABCG2* and *LRP* mRNA in these cells was then compared to those in non-treated cells using real-time RT-PCR as described above. The fold-change in the relative quantity of each mRNA in treated cells was calculated by comparing it to that in non-treated cells.

Western blotting for MAPK/ERK and JNK pathway proteins. For confirmation of activation or inhibition of MAPK/ERK and JNK pathways by PMA, U0126 and SP600125, western blotting analysis was performed as described in our previous study (16). In brief, CCRF-HSB-2 and YAMN90 cells untreated or pre-incubated with U0126 or SP600125 were cultured in the presence of 200 nM PMA for 30 min, and whole-cell lysates were extracted. Equal amounts of the extracted proteins were then separated by sodium dodecyl sulfate-polyaclylamidegel electropheresis (SDS-PAGE) and blotted onto a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked in 5%-skimmed milk/tris-buffered saline with Tween20, and then incubated with primary antibodies against phospho-ERK1/2 (1:2,000), ERK1/2 (1:1,000), phospho-JNK (1:1,000), JNK (1:1,000), phospho-c-JUN (1:1,000), c-JUN (1:1,000), and β-actin (1:1,000) (Cell Signaling Technology, Danvers, MA, USA) overnight at 4˚C. After incubation with a secondary antibody of horseradish peroxidase-labeled anti-rabbit IgG (1:3,000; Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at room temperature, immunoreactivity was detected using Luminata Forte Western HRP Substrate (Millipore, Billerica, MA, USA) and visualized using ChemiDoc XRS Plus (Bio-Rad Laboratories).

Rhodamine-123 efflux test. To examine the changes in the function of transporters following the activation or inhibition of MAPK/ERK and JNK pathways, rhodamine-123 efflux test was performed as described in our previous study (23). One million cells of each cell line with or without treatment of PMA alone or combination of PMA and U0126, as described above, were incubated with 200 ng/ml rhodamine-123 (Sigma-Aldrich, St. Louis, MO, USA) in RPMI-1640 medium at 37˚C for 20 min. After washing twice with phosphate-buffered saline (PBS), the cells were incubated in rhodamine-123-free medium at 37˚C for 60 min, either with or without 2 μM cyclosporin. After incubation, the cells were washed with ice-cold PBS three times, placed on ice in the dark, and

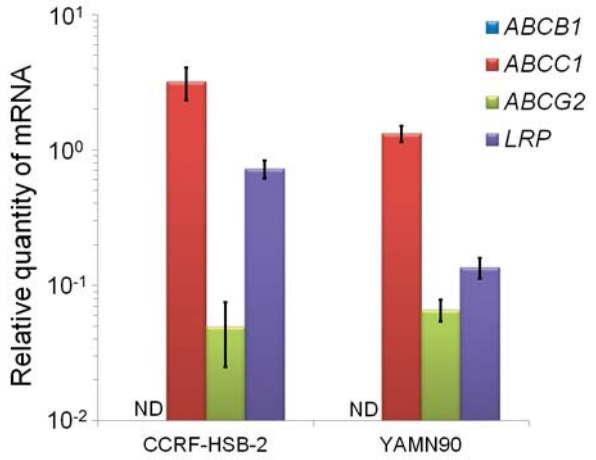

Figure 1. *The relative quantities of ATP-binding cassette, sub-family B, member1 (ABCB1), ABCC1, ABCG2 and lung resistance-related protein (LRP) gene mRNA in CCRF-HSB-2 and YAMN90 cells. Expression of ABCC1, ABCG2 and LRP genes was detected in both cell lines, although the expression of ABCB1 gene was not observed in either cell line. ND: Not detected.*

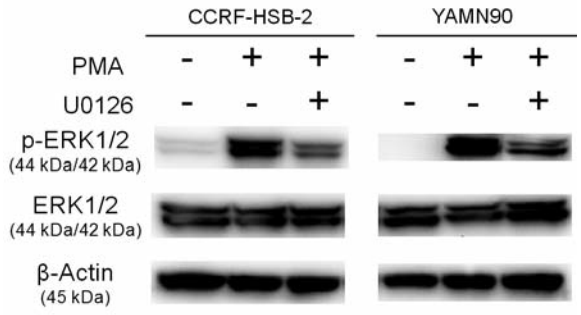

Figure 2. The results of western blotting for phospho-extracellular signal-regulated kinase (ERK), ERK, and β-actin in CCRF-HSB-2 and YAMN 90 cells after treatment with phorbol 12-myristate 13-acetate (PMA) or the combination of PMA and U0126. The quantity of phospho-ERK clearly increased following treatment with PMA, and this increase was inhibited by treatment with PMA and U0126 in both of the cell lines.

subjected to flow cytometric analysis. Cells that had not been exposed to rhodamine-123 were used as negative controls. A canine lymphoma cell line, Ema, which exhibits MDR and expresses ABCB1 (23), was used as a positive control. Twenty thousand events were counted using a FACSCalibur™ instrument (Becton Dickinson, San Jose, CA, USA) and analyzed with FlowJo software (Tomy Digital Biology, Tokyo, Japan). Rhodamine-123 efflux index (REI) was calculated as [the mean fluorescence intensity (MFI) of the sample incubated with rhodamine-123 and cyclosporin – the MFI of the negative control]/(the MFI of the sample incubated with rhodamine-123 – the MFI of the negative control). The rhodamine-123 efflux test was performed in triplicate.

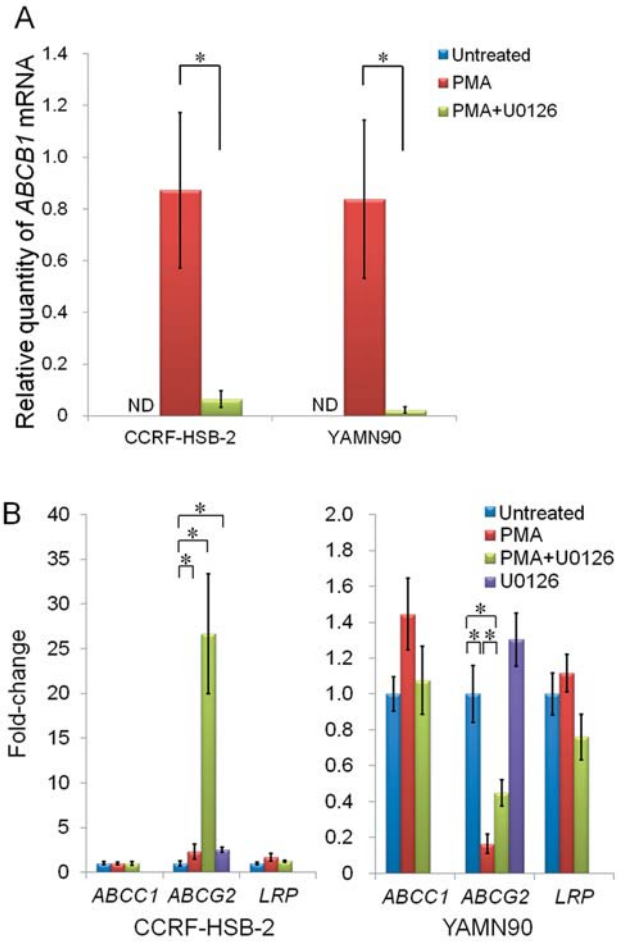

Figure 3. *The change of the expression of ATP-binding cassette, sub-family B, member 1 (ABCB1) (A), ABCC1, ABCG2 and lung resistance-related protein (LRP) (B) genes after treatment with phorbol 12-myristate 13-acetate (PMA) or PMA plus U0126. Although no expression of ABCB1 gene was detected in CCRF-HSB-2 and YAMN90 before treatment, its mRNA expression was observed after treatment with PMA, and the expression of this gene significantly decreased following treatment with PMA and U0126. The expression of ABCG2 gene significantly increased with PMA treatment in CCRF-HSB-2 cells compared to that of untreated cells. Although treatment with U0126 also increased the expression of ABCG2 mRNA in this cell line, the relative quantity of ABCG2 mRNA was further increased by the combined treatment with PMA and U0126. However, in YAMN90 cells, treatment with PMA reduced the expression of ABCG2 mRNA compared to that of untreated cells. When treated with the combination of PMA and U0126, the relative quantity of ABCG2 gene was significantly greater than that of cells treated with PMA, although it was significantly lower compared to that of untreated cells. The expression of ABCC1 and LRP genes did not change significantly in either of the two cell lines when treated with PMA or combination of PMA and U0126. *p<0.01. ND: Not detected.*

Statistical analysis. The non-parametric Mann-Whitney *U*-test was used for comparison of relative quantities of genes. All statistical tests were two-sided and were performed using JMP version 5.0.1 (SAS Institute, Cary, NC, USA). For all tests, $p<0.05$ was used as the level of significance.

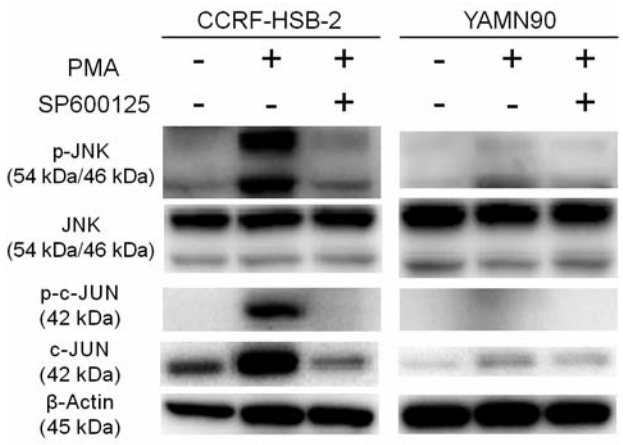

Figure 4. *The results of western blotting for phospho-c-JUN NH2 terminal kinase (JNK), JNK, phospho-c-JUN, c-JUN and β-actin after treatment with phorbol 12-myristate 13-acetate (PMA) or the combination of PMA and SP600125 in CCRF-HSB-2 and YAMN90 cells. The quantities of phospho-JNK and phospho-c-JUN apparently increased by treatment with PMA, and this increase was inhibited by treatment with PMA and SP600125 in CCRF-HSB-2 cells. However, changes in quantities of phopho-JNK and phospho-c-JUN were not observed after treatment with PMA or the combination of PMA and SP600125 in YAMN90 cells.*

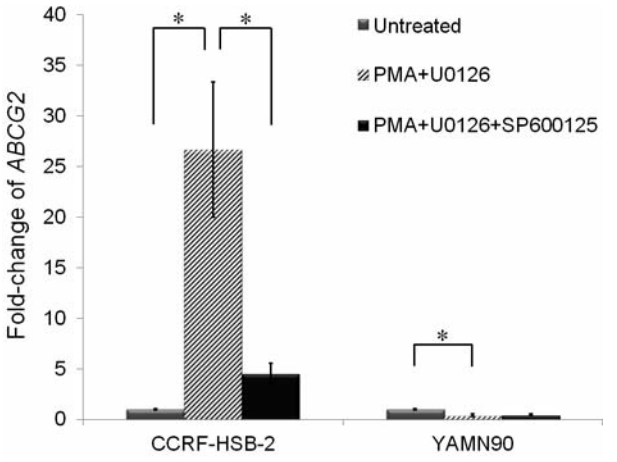

Figure 5. *Fold-change of relative quantities of ATP-binding cassette, sub-family G, member 2 (ABCG2) gene in CCRF and YAMN90 cells after treatment with phorbol 12-myristate 13-acetate (PMA) plus U0126, or the combination of PMA, U0126 and SP60012. When CCRF-HSB-2 cells were treated with PMA, U0126 and SP600125, the relative quantity of ABCG2 mRNA significantly decreased compared with when they were treated with PMA and U0126. However, in YAMN90 cells, the expression of ABCG2 gene in cells treated with PMA, U0126 and SP600125 was not different from those of cells treated with PMA and U0126. *p<0.01.*

Results

Relative quantification of efflux pump mRNAs. In real-time RT-PCR, the relative expression levels of *ABCB1, ABCC1, ABCG2* and *LRP* genes were measured in CCRF-HSB-2 and YAMN90 cells (Figure 1). Among these four genes, the expression of *ABCC1, ABCG2* and *LRP* mRNAs was detected in both cell lines, whereas the expression of *ABCB1* mRNA was not observed in either of the cell lines.

MAPK/ERK pathway regulated ABCB1 and ABCG2 gene expression. In both CCRF-HSB-2 and YAMN90 cells, the quantity of phospho-ERK1/2 markedly increased by treatment with PMA, and this increase was partially inhibited by treatment with U0126 (Figure 2). After treatment with PMA, the amount of *ABCB1* mRNA increased; it decreased after treatment with U0126 in both cell lines (Figure 3A). The expression of *ABCG2* gene significantly increased by treatment with PMA in CCRF-HSB-2 cells (2.3-fold; Figure 3B) compared with that in untreated cells. In addition, the quantity of *ABCG2* mRNA further increased (28-fold) by combined treatment with PMA and U0126, although treatment with U0126-alone also increased the expression levels of this gene in this cell line (2.5-fold). However, in YAMN90 cells, treatment with PMA reduced the expression of *ABCG2* mRNA (0.16-fold) compared to that in untreated cells. When this cell line was treated with the combination of PMA and U0126, the relative quantity of the *ABCG2* gene was significantly larger than that in cells treated with PMA, although it was significantly lower (0.45-fold) compared with to in untreated cells. The expression of *ABCC1* and *LRP* genes did not change in either of the two cell lines when treated with PMA or the combination of PMA and U0126.

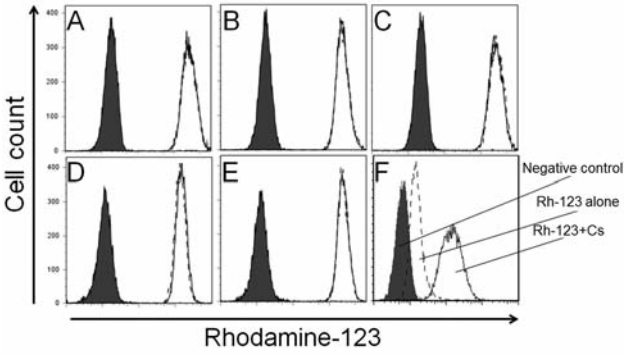

Figure 6. *The results of the rhodamine-123 (Rh123) efflux test. Efflux of Rh123 was not observed in CCRF-HSB-2 and YAMN90 cells (A and D), although obvious efflux was observed in Ema cells when Rh123-alone was administered compared to when Rh123 and cyclosporin (Cs), an inhibitior of ABC transporters, were administered (F). After treatment of CCRF-HSB-2 cells with phorbol 12-myristate 13-acetate (PMA) or the combination of PMA and U0126, no efflux of Rh 123 was observed (B and C). In addition, no efflux of Rh 123 was observed in YAMN90 cells after treatment with PMA (E).*

JNK pathway up-regulated ABCG2 gene expression. Because the changes of the expression of *ABCG2* gene when cell lines were treated with PMA and U0126 were different between CCRF-HSB-2 and YAMN90 cells, it was hypothesized that pathways other than the MAPK/ERK pathway might be associated with regulation of *ABCG2* gene expression. Therefore, the changes in the relative quantity of *ABCG2* mRNA were examined in cells treated with PMA, U0126 and SP600125. When CCRF-HSB-2 cells were treated with PMA, the quantities of phospho-JNK and phospho-c-JUN clearly increased, and these increases were inhibited by treatment with SP600125 (Figure 4). However, quantities of phospho-JNK and phospho-c-JUN did not change after treatment with PMA in YAMN90 cells. When CCRF-HSB-2 cells were treated with PMA, U0126 and SP600125, the relative quantity of *ABCG2* mRNA (4.6-fold increase compared with untreated cells) significantly decreased compared to cells treated with PMA and U0126 (28-fold increase compared to untreated cells; Figure 5). However, in YAMN90 cells, the relative quantity of *ABCG2* gene mRNA in cells treated with PMA, U0126 and SP600125 (0.45-fold increase compared to untreated cells) was not different from that in the cells treated with PMA and U0126 (0.50-fold increase compared to untreated cells).

Rhodamine-123 efflux test. Finally, changes of the function of transporters by treatment with PMA and U0126 were examined by the rhodamine-123 efflux test. Efflux of rhodamine-123 was not observed in either CCRF-HSB-2 or YAMN90 cells (Figure 6A and D), and the REI values were 1.01±0.0139 and 1.05±0.0318, respectively, although efflux was obvious in Ema cells (Figure 6F), with an REI value of 17.0±2.15. After treatment of CCRF-HSB-2 cells with PMA or the combination of PMA and U0126, efflux of rhodamine-123 was not observed (Figure 6B and C) (REI values of 1.01±0.0403 and 0.983±0.0783, respectively). In addition, efflux of rhodamine-123 was not also observed in YAMN90 cells after treatment with PMA (Figure 6E) (REI value=0.989±0.0144).

Discussion

The present study showed that in human ALL cell lines, expression of *ABCB1* gene was regulated through MAPK/ERK pathway and that of *ABCG2* gene was regulated through MAPK/ERK and JNK pathways.

In both CCRF-HSB-2 and YAMN90 cells, mRNA expression of *ABCB1* gene was detected after treatment with PMA, a protein kinase C activator, although the expression of *ABCB1* gene was not observed before treatment. In addition, this increase in *ABCB1* gene expression was inhibited by treatment with U0126, a MAPK/ERK kinase inhibitor. These findings indicated that the expression of *ABCB1* gene was up-regulated through the MAPK/ERK pathway in these two ALL cell lines. This observation agreed with findings of previous studies using human B-cell lymphoma cells or canine lymphoma cells (12, 16).

The expression of *ABCG2* gene increased after treatment with both PMA, and U0126, and expression was further increased by treatment with the combination of PMA and U0126 in CCRF-HSB-2 cells. In contrast, the expression of this gene apparently decreased after treatment with PMA, and this decrease was inhibited by treatment with U0126 in YAMN90 cells. These results indicate that *ABCG2* gene expression was down-regulated through the MAPK/ERK pathway, and that some other pathways up-regulated the expression of this gene. Based on this possibility, the role of the JNK pathway in the regulation of the expression of *ABCG2* gene was examined. In CCRF-HSB-2 cells, the JNK pathway was activated by treatment with PMA and was inhibited by treatment with the combination of PMA with SP600125. The large increase in *ABCG2* gene expression that was observed when CCRF-HSB-2 cells were treated with PMA and U0126 was abolished when cells were treated with PMA, U0126 and SP600125. However, in YAMN90 cells, activation of the JNK pathway was not observed, despite treatment with PMA, and the expression of *ABCG2* gene was not significantly different between the cells treated with PMA and U0126 and those treated with PMA, U0126 and SP600125. These results indicate that the JNK pathway up-regulated the expression of *ABCG2* gene in CCRF-HSB-2 cells. In previous studies, it was shown that the transcription of *ABCG2* gene was down-regulated through MAPK/ERK pathway in a human breast cancer cell line (14) and the expression of this gene was up-regulated through JNK pathway in human colon cancer (15). The findings in the present study were consistent with the results of these previous studies. However, there has been no study revealing the simultaneous inverse regulation of *ABCG2* gene expression through MAPK/ERK and JNK pathways as shown in the present study.

The expression of *ABCC1* and *LRP* genes did not change after treatment with PMA and U0126 in the present study. The MAPK/ERK pathway was previously shown to be essential for collagen-induced *ABCC1* gene expression in human T-ALL cell lines (24). Combined with the results in the present study, it is reasonable to consider that the MAPK/ERK pathway might be associated with the regulation of *ABCC1* gene expression in cooperation with other mechanisms in human T-ALL cell lines, although activation of the MAPK/ERK pathway alone did not increase the *ABCC1* gene transcription. Our previous study showed that the MAPK/ERK pathway up-regulated *LRP* gene expression in canine lymphoma cell lines (16). The reason for the differences between the results of our present study and those obtained in a previous study (16) is not clear;

however, it is possible that the state of activation of factors downstream of ERK differed between human ALL cell lines and canine lymphoma cell lines after treatment with PMA.

In the present study, the relative quantity of mRNA of *ABCB1* and *ABCG2* genes in CCRF-HSB-2 cells increased after treatment with PMA or the combination of PMA and U0126, and *ABCB1* gene expression increased after treatment with PMA in YAMN90 cells. However, in the rhodamine-123 efflux test, it was shown that the function of the efflux pumps was not enhanced despite the increase of the amount of ABC transporter gene mRNAs after treatment with PMA or the combination of PMA and U0126. Previous studies showed that inhibition of the MAPK/ERK pathway, which up-regulated *ABCB1* gene, or inhibition of the JNK pathway, which up-regulated *ABCG2* gene, could reduce the function of transporters encoded by these genes (12, 15), indicating that the inhibition of these pathways could re-establish sensitivity to chemotherapeutic agents in tumor cells with an MDR phenotype. However, the results of the present study raise the possibility that the activation of MAPK/ERK or JNK pathways alone cannot up-regulate the function of ABCB1 and ABCG2 sufficiently to confer MDR phenotype on the ALL cells.

In conclusion, the present study revealed the regulation of *ABCB1* and *ABCG2* genes through MAPK/ERK and JNK pathways in human ALL cell lines. However, it is suggested that other mechanisms might be important for enhancement of transporter function during MDR acquisition in tumor cells lacking ability for drug efflux. Further study is needed to elucidate the mechanisms of MDR acquisition *via* overexpression of efflux pumps in addition to the mechanism revealed in the present study.

Acknowledgements

This study was supported by the Japan Society for the Promotion of Science, KAKENHI 23380182, 24658265 and 11J03651. We are indebted to Professor Takuya Mizuno (Yamaguchi University, Japan) for providing the Ema cell line.

References

1 Dores GM, Devesa SS, Curtis RE, Linet MS and Morton LM: Acute leukemia incidence and patient survival among children and adults in the United States, 2001-2007. Blood *119*: 34-43, 2012.

2 Swerts K, De Moerloose B, Dhooge C, Laureys G, Benoit Y and Philippe J: Prognostic significance of multidrug resistance-related proteins in childhood acute lymphoblastic leukaemia. Eur J Cancer *42*: 295-309, 2006.

3 Gimenez-Bonafe P, Tortosa A and Perez-Tomas R: Overcoming drug resistance by enhancing apoptosis of tumor cells. Curr Cancer Drug Targets *9*: 320-340, 2009.

4 Harris AL and Hochhauser D: Mechanisms of multidrug resistance in cancer treatment. Acta Oncol *31*: 205-213, 1992.

5 Higgins CF: Multiple molecular mechanisms for multidrug resistance transporters. Nature *446*: 749-757, 2007.

6 Modok S, Mellor HR and Callaghan R: Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer. Curr Opin Pharmacol *6*: 350-354, 2006.

7 Han K, Kahng J, Kim M, Lim J, Kim Y, Cho B, Kim HK, Min WS, Kim CC, Lee KY, Kim BK and Kang CS: Expression of functional markers in acute nonlymphoblastic leukemia. Acta Haematol *104*: 174-180, 2000.

8 van der Kolk DM, Vellenga E, Scheffer GL, Muller M, Bates SE, Scheper RJ and de Vries EG: Expression and activity of breast cancer resistance protein (BCRP) in *de novo* and relapsed acute myeloid leukemia. Blood *99*: 3763-3770, 2002.

9 Dalton WS, Crowley JJ, Salmon SS, Grogan TM, Laufman LR, Weiss GR and Bonnet JD: A phase III randomized study of oral verapamil as a chemosensitizer to reverse drug resistance in patients with refractory myeloma. A Southwest Oncology Group study. Cancer *75*: 815-820, 1995.

10 Kourti M, Vavatsi N, Gombakis N, Sidi V, Tzimagiorgis G, Papageorgiou T, Koliouskas D and Athanassiadou F: Expression of *multidrug resistance 1 (MDR1), multidrug resistance-related protein 1 (MRP1), lung resistance protein (LRP),* and *breast cancer resistance protein (BCRP)* genes and clinical outcome in childhood acute lymphoblastic leukemia. Int J Hematol *86*: 166-173, 2007.

11 Suvannasankha A, Minderman H, O'Loughlin KL, Nakanishi T, Ford LA, Greco WR, Wetzler M, Ross DD and Baer MR: Breast cancer resistance protein (BCRP/MXR/ABCG2) in adult acute lymphoblastic leukaemia: Frequent expression and possible correlation with shorter disease-free survival. Br J Haematol *127*: 392-398, 2004.

12 Shen H, Xu W, Luo W, Zhou L, Yong W, Chen F, Wu C, Chen Q and Han X: Up-regulation of *mdr1* gene is related to activation of the MAPK/ERK signal transduction pathway and YB-1 nuclear translocation in B-cell lymphoma. Exp Hematol *39*: 558-569, 2011.

13 Sui H, Zhou S, Wang Y, Liu X, Zhou L, Yin P, Fan Z and Li Q: COX-2 contributes to P-glycoprotein-mediated multidrug resistance *via* phosphorylation of c-JUN at Ser63/73 in colorectal cancer. Carcinogenesis *32*: 667-675, 2011.

14 Imai Y, Ohmori K, Yasuda S, Wada M, Suzuki T, Fukuda K and Ueda Y: Breast cancer resistance protein/ABCG2 is differentially regulated downstream of extracellular signal-regulated kinase. Cancer Sci *100*: 1118-1127, 2009.

15 Zhu MM, Tong JL, Xu Q, Nie F, Xu XT, Xiao SD and Ran ZH: Increased JNK1 signaling pathway is responsible for ABCG2-mediated multidrug resistance in human colon cancer. PLoS One *7*: e41763, 2012.

16 Tomiyasu H, Watanabe M, Goto-Koshino Y, Fujino Y, Ohno K, Sugano S and Tsujimoto H: Regulation of expression of *ABCB1* and *LRP* genes by mitogen-activated protein kinase/extracellular signal-regulated kinase pathway and its role in generation of side population cells in canine lymphoma cell lines. Leuk Lymphoma *54*: 1309-1315, 2013.

17 Yamazaki H, Nishida H, Iwata S, Dang NH and Morimoto C: CD90 and CD110 correlate with cancer stem cell potentials in human T-acute lymphoblastic leukemia cells. Biochem Biophys Res Commun *383*: 172-177, 2009.

18 Nishida H, Yamazaki H, Yamada T, Iwata S, Dang NH, Inukai T, Sugita K, Ikeda Y and Morimoto C: CD9 correlates with cancer stem cell potentials in human B-acute lymphoblastic leukemia cells. Biochem Biophys Res Commun *382*: 57-62, 2009.

19 Tomiyasu H, Goto-Koshino Y, Takahashi M, Fujino Y, Ohno K and Tsujimoto H: Quantitative analysis of mRNA for 10 different drug resistance factors in dogs with lymphoma. J Vet Med Sci *72*: 1165-1172, 2010.

20 Beillard E, Pallisgaard N, van der Velden VH, Bi W, Dee R, van der Schoot E, Delabesse E, Macintyre E, Gottardi E, Saglio G, Watzinger F, Lion T, van Dongen JJ, Hokland P and Gabert J: Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) – a Europe Against Cancer Program. Leukemia *17*: 2474-2486, 2003.

21 Yang JM, Vassil AD and Hait WN: Activation of phospholipase C induces the expression of the *multidrug resistance* (*MDR1*) gene through the RAF-MAPK pathway. Mol Pharmacol *60*: 674-680, 2001.

22 Zhang Z, Knoepp SM, Ku H, Sansbury HM, Xie Y, Chahal MS, Tomlinson S and Meier KE: Differential expression of FAK and PYK2 in metastatic and non-metastatic EL4 lymphoma cell lines. Clin Exp Metastasis *28*: 551-565, 2011.

23 Tomiyasu H, Yuko G-K, Yasuhito F, Koichi O and Hajime T: Epigenetic regulation of the *ABCB1* gene in drug-sensitive and drug-resistant lymphoid tumour cell lines obtained from canine patients. Vet J 2013. in press DOI: http://dx.doi.org/10.1016/j.tvjl.2013.10.022

24 El Azreq MA, Naci D and Aoudjit F: Collagen/β1 integrin signaling up-regulates the ABCC1/MRP-1 transporter in an ERK/MAPK-dependent manner. Mol Biol Cell *23*: 3473-3484, 2012.

Received October 19, 2013
Revised November 2, 2013
Accepted November 4, 2013