Biochemical and Biophysical Research Communications 448 (2014) 430–436

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Increased expression of sorcin is associated with multidrug resistance in leukemia cells via up-regulation of MDR1 expression through cAMP response element-binding protein

Nobuyuki Yamagishi [a,b,*], Ryota Nakao [a], Rumi Kondo [a], Mai Nishitsuji [a], Youhei Saito [a], Takahisa Kuga [a], Takumi Hatayama [a], Yuji Nakayama [a]

[a] Department of Biochemistry & Molecular Biology, Division of Biological Sciences, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan
[b] Radioisotope Center, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan

ARTICLE INFO

Article history:
Received 18 April 2014
Available online 2 May 2014

Keywords:
Sorcin
Multidrug-resistance
MDR1/P-glycoprotein
CREB

ABSTRACT

Sorcin, a 22 kDa Ca^{2+} binding protein, was first identified in a vincristine-resistant Chinese hamster lung cell line, and was later demonstrated to be involved in the development of multidrug-resistance (MDR) phenotypes in a variety of human cancer cell lines. However, the exact role of sorcin in MDR cells is yet to be fully elucidated. Here we explored the role of sorcin in the development of MDR in leukemia cells, and revealed that the expression level of sorcin was directly correlated to the expression of MDR1/P-glycoprotein (P-gp). In addition, it was shown that sorcin induced the expression of MDR1/P-gp through a cAMP response element (CRE) between −716 and −709 bp of the *mdr1/p-gp* gene. Furthermore, overexpression of sorcin increased the phosphorylation of CREB1 and the binding of CREB1 to the CRE sequence of *mdr1/p-gp* promoter, and induced the expression of MDR1/P-gp. These findings suggested that sorcin induces MDR1/P-gp expression markedly through activation of the CREB pathway and is associated with the MDR phenotype. The new findings may be helpful for understanding the mechanisms of MDR in human cancer cells, prompting its further investigation as a molecular target to overcome MDR.

1. Introduction

Multidrug-resistance (MDR) phenotypes are a major obstacle to the successful treatment of cancer [1]. Although the mechanism of MDR in cancer cells is polygenetic and yet to be completely understood, it is often associated with increased expression of ATP-binding cassette transporters including ABCB1, also known as MDR1/P-glycoprotein (P-gp), which extrude the internalized drugs from the cells [2–4]. Therefore, various approaches have been reported to overcome MDR, including pharmacological inhibition of MDR1/P-gp and modulation of endogenous regulators of the *mdr1/p-gp* gene [5]. To date, several transcriptional factors have been proposed to be involved in *mdr1/p-gp* gene expression, including NF-IL6 and NF-κB [6,7]. However, the regulation of *mdr1/p-gp* gene expression is not fully understood. In addition, inhibition of these ATP-binding cassette transporters does not always result in the reversal of chemoresistance in patients [8], and the absence of MDR1/P-gp overexpression in some cancer cases also indicates that there might be other mechanisms responsible for MDR in cancer cells [9,10].

Sorcin is a 22 kDa Ca^{2+} binding protein belonging to the penta-EF-hand protein family, and functions a Ca^{2+} sensor which regulates the activity of ryanodine receptor RyRs, Na^{+}/Ca^{2+} exchanger NCX, and voltage-dependent L-type Ca^{2+} channel [11–13]. Previous evidence suggests the involvement of sorcin in the drug resistance of human cancer. Indeed, sorcin was first identified in a vincristine-resistant Chinese hamster lung cell line [14], and was later demonstrated to be overexpressed in several MDR cell lines [15–17]. A direct correlation between the expression of sorcin and the MDR phenotype has also been reported in human leukemia and gastric carcinoma cells [18–21]. However, the exact mechanisms of MDR mediated by sorcin in cancer cells remain elusive.

Here, we showed that sorcin induces MDR1/P-gp expression markedly through the cAMP response element in *mdr1/p-gp* promoter. The new findings may be helpful for understanding the mechanisms of MDR and prompting its further investigation as a molecular target to overcome MDR.

* Corresponding author at: Department of Biochemistry & Molecular Biology, Division of Biological Sciences, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan. Fax: +81 75 595 4770.
E-mail address: yamagisi@mb.kyoto-phu.ac.jp (N. Yamagishi).

http://dx.doi.org/10.1016/j.bbrc.2014.04.125
0006-291X/

2. Materials and methods

2.1. Cell culture and transfection

African green monkey kidney COS-7 cells, human leukemia K562 cells and their MDR-phenotype cell lines, K562/ADR cells and K562/VIN cells, respectively, were provided by RIKEN BRC through the National Bio-resource Project of MEXT, Japan.

To obtain sorcin-overexpressing cells, K562 cells (1×10^6 cells) were transfected with 15 μg pcDNA-SRI and 60 μl DMRIE-C reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. At 48 h after transfection, the cells were seeded in 96-well plates (50–500 cells/well) and maintained in complete medium containing 400 μg/ml geneticin (Nacalai Tesque, Kyoto, Japan) for 3 weeks to select geneticin-resistant cells. The surviving cell clones were isolated and grown in complete medium containing 200 μg/ml geneticin.

For knockdown of sorcin expression, 10 pmol sorcin siRNA (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or RISC-free control siRNA (Dharmacon, Chicago, IL, USA) was transfected into K562/ADR cells or K562/VIN cells using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's instructions.

2.2. Plasmid construction

To construct the expression plasmid for sorcin (pcDNA-SRI), sorcin cDNA was amplified from the total RNA of K562/ADR cells by reverse transcriptase-polymerase chain reaction. Then, the PCR products were subcloned in the mammalian expression vector pcDNA3.1(+) (Invitrogen).

Plasmids pGL4-MDR(-906), pGL4.29[luc2P/CRE], and pGL4.11 [luc2P] were purchased from Promega (Madison, WI, USA). A series of 5' deletions of the human *mdr1/p-gp* promoter sequence was generated by self-ligation of the DNA sequence made by PCR using pGL4-MDR(-906) as the template DNA. The substitution construct of pGL4-MDR(-906mtCRE) was made using a QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions.

2.3. Proteomic analysis

Proteomic analysis was performed as described previously [22]. Briefly, each protein extract (700 μg protein) was loaded onto dry immobilized pH gradient strips (17 cm, pH3-10 nonlinear; Bio-Rad Laboratories, Hercules, CA, USA) under conditions of passive rehydration. To check for reproducibility, the experiment was repeated at least five times using independently purified samples. Differentially expressed spots were excised and digested with trypsin, and the resultant peptides were extracted and analyzed using a Voyager-DE MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA). Proteins were identified with peptide mass fingerprinting using an MS-Fit search engine.

2.4. Western blotting

Aliquots (20 μg protein) of cell extracts were subjected to SDS–polyacrylamide gel electrophoresis, then transferred onto nitrocellulose membranes by electrotransfer. Immunodetection was performed with a chemiluminescent detection method (Clarity Western ECL Substrate; Bio-Rad Laboratories).

2.5. Measurement of cell death rate

Cells (5×10^5 cells/35 mm dish) were treated with adriamycin for 24 h or with vincristine for 48 h in RPMI1640 medium supplemented with 10% fetal bovine serum. Then, the cells were harvested, centrifuged at 400×*g* for 5 min, and re-suspended with phosphate-buffered saline (PBS) containing 0.2% trypan blue. After 1 min, the cells were transferred to a hemocytometer, and viable (phase bright) and nonviable (blue) cells were counted, respectively.

2.6. Drug accumulation assay

At 48 h after transfection with sorcin siRNA to K562/ADR cells or K562/VIN cells, the cells were collected and exposed to 1 μM Rhodamine123 (Rho123) in the medium at 37 °C for 2 h. After washing twice with ice-cold PBS, the cells were resuspended with ice-cold PBS, and the cellular accumulation of Rho123 was determined using a flow cytometer or a fluorescence microscope.

2.7. Reporter gene activity assay

COS-7 cells in 24-well plates (7×10^4 cells/well) were transfected with 1.5 μg reporter plasmid and 0.75 μg pcDNA-SRI or pcDNA3.1(+) vector by lipofection using DMRIE-C reagent according to the manufacturer's instructions (Invitrogen). At 48 h after transfection, the cells were lysed with the cell culture lysis reagent (Promega) and aliquots of the cell extracts underwent measurement of luciferase activity as described previously [23].

2.8. Binding CREB1 to the promoter region of mdr1/p-gp gene

COS-7 cells in 6-well plates (7×10^5 cells/well) were transfected with 2 μg pFN21-CREB1 (obtained from Promega) and 2 μg pcDNA-SRI by lipofection. CREB1-binding assay to the promoter region of *mdr1/p-gp* gene was performed by using HaloCHIP system (Promega) in accordance with the manufacturer's instructions. Recovered DNA were analyzed by PCR (35 cycles, 94 °C 15 s, 60 °C 15 s, 72 °C 30 s) using a set of specific primers for the *mdr1/p-gp* gene between −841 and −577 (forward 5'-GTTGGCAGT AAATATGGAAG-3', reverse 5'-CTGAGTTACATCTGTTCATC-3') and intron 3 of the *mdr1/p-gp* gene (forward 5'-TTCAGTCCACTGAAAG GGAC-3', reverse 5'-CAGTGTGCTTTCGACTACTC-3').

3. Results

3.1. Expression of sorcin is directly related to the MDR phenotype in K562 cells

In the present study, we used two different levels of MDR leukemia cell lines derived from drug-sensitive K562 cells, K562/ADR cells and K562/VIN cells. In agreement with previous studies [24–26], K562/ADR cells and K562/VIN cells exhibited about 10- and >100-fold resistance to adriamycin and vincristine, respectively, compared to their parental K562 cells (Fig. S1). To identify proteins that exhibited the expression related to the MDR phenotype, total proteins extracted from parental K562 cells, K562/ADR cells and K562/VIN cells were separated by 2-DE (Fig. S2). About 500 spots in each image were matched and quantified using PDQuest analyzing software, and at least six protein spots had an expression that related to the MDR phenotype in K562 cells. The proteins were analyzed by peptide mass fingerprinting after in-gel digestion with trypsin, and were identified as vimentin, sorcin, glutathione-*S*-transferase P1, peroxiredoxin 2, Hsp27, and DJ-1 (Table S1). Among these proteins, we focused on sorcin because the role of sorcin in MDR cells remains elusive, although this protein has been suggested to be involved in the development of the MDR phenotype in a variety of human cancer cell lines [18–20].

To examine the functional role of sorcin upregulation in cells with the MDR phenotype, sorcin expression in K562/ADR cells was down-regulated using sorcin-specific siRNA. As shown in Fig. 1A, the transfection of sorcin siRNA, but not by control siRNA, significantly reduced endogenous sorcin expression by approximately 75% in K562/ADR cells, and the cells down-regulating sorcin expression were more sensitive to adriamycin and vincristine. Similar results were also obtained by another non-overlapping sorcin siRNA (Fig. S3).

To further dissect the contribution of sorcin to the MDR phenotype, we next established stable K562 cell clones that overexpressed sorcin. Here, we used two sorcin-overexpressing K562 cell lines, cl.14 and cl.23. Sorcin-overexpressing cells showed more resistance to adriamycin and vincristine than control cells transfected with empty pcDNA3.1(+) vector (Fig. 1B). Furthermore, similar results were also obtained in COS-7 cells that transiently overexpressed sorcin (data not shown). These data confirmed that sorcin is an important protein associated with the development of MDR in leukemia cells, as described previously [18,19].

3.2. Sorcin modulates the expression of MDR1/P-gp at the transcriptional level

MDR1/P-gp is a multidrug efflux pump which reduces intracellular drug accumulation, and its overexpression represents one of the major mechanisms that contribute to the MDR phenotype. As it was shown that overexpression of sorcin is closely associated with amplification of the *mdr1/p-gp* gene [27], we next examined the association of MDR1/P-gp with the sorcin-mediated MDR phenotype. Transfection of sorcin siRNA, but not by control siRNA,

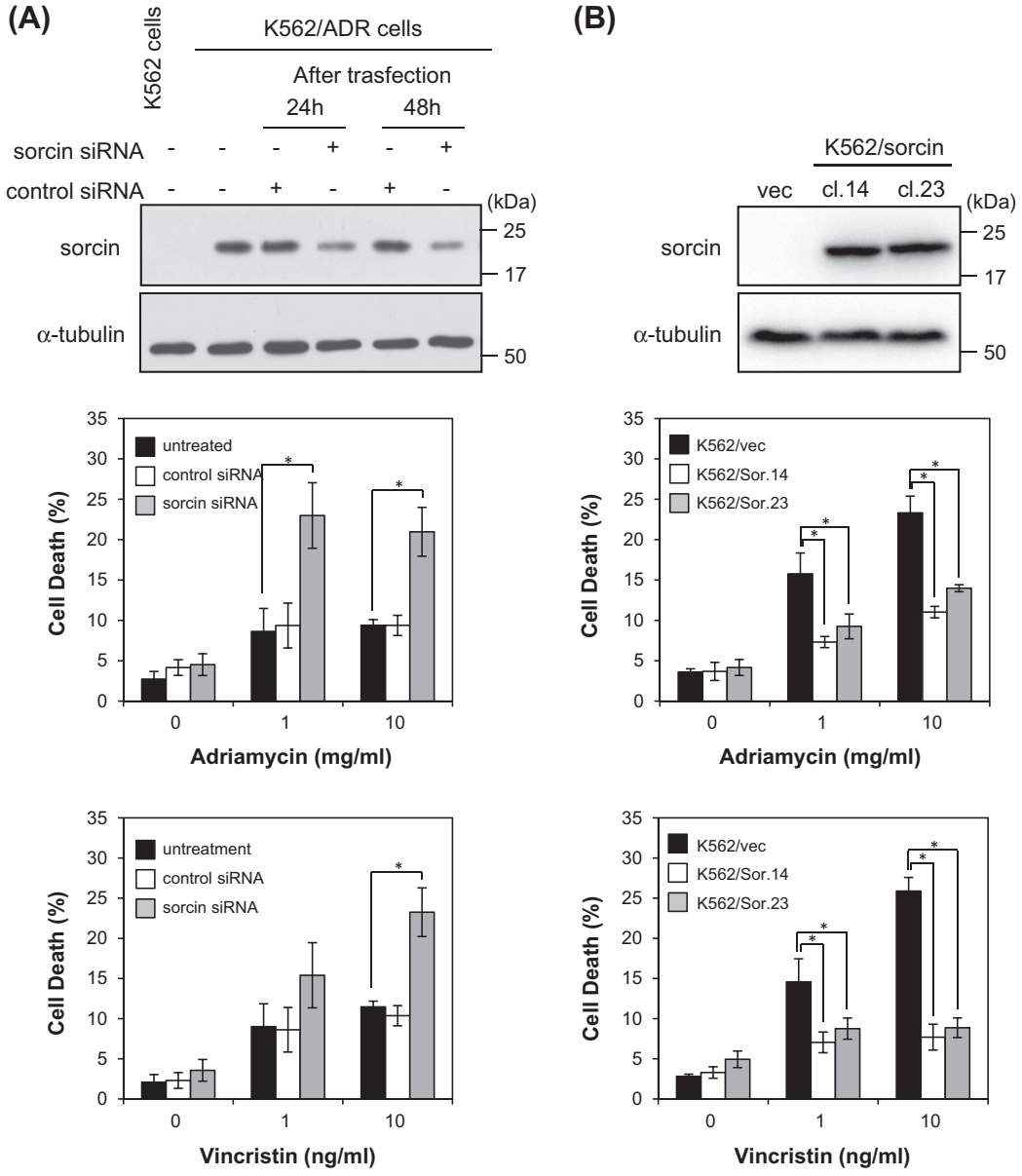

Fig. 1. Sorcin is directly related to the MDR phenotype in K562 cells. (A) Effect of downregulation of sorcin expression on the sensitivity to adriamycin and vincristine. Expression of sorcin in K562/ADR cells after treatment with sorcin siRNA or RISC-free control siRNA was determined by Western blotting. At 48 h after transfection, the cells were incubated for another 24 h with various doses of adriamycin or for another 48 h with various doses of vincristine, and then cell viability was determined by the trypan blue excretion assay. (B) Effect of overexpression of sorcin expression on the sensitivity to adriamycin and vincristine. Sorcin-overexpressing cell clones (cl.14 and cl.23) were incubated for 24 h with various doses of adriamycin or for 48 h with various doses of vincristine, and then cell viability was determined by the trypan blue excretion assay. Each value is the mean ± S.D. of four independent experiments. Asterisk indicates significance at $p < 0.01$.

significantly reduced endogenous MDR1/P-gp expression in K562/VIN cells (Fig. 2A). The intercellular accumulation of Rho123 was also increased by the knock-down of sorcin (Fig. 2B and C). Furthermore, the effect of sorcin on *mdr1/p-gp* promoter activity was analyzed using cells containing a construct of the human *mdr1/p-gp* promoter fused to a luciferase reporter gene, and we found that overexpression of sorcin markedly enhanced *mdr1/p-gp* promoter activity (Fig. 3A). These results suggest that sorcin enhanced MDR1/P-gp expression at the transcriptional level.

3.3. Sorcin modulates the expression of MDR1/P-gp through the cAMP response element between −716 and −709 bp of mdr1/p-gp gene

To elucidate the mechanism by which sorcin induces the expression of MDR1/P-gp, a series of *mdr1/p-gp* 5'-promoter deletion-luciferase constructs was generated and transiently co-transfected into COS-7 cells with the sorcin expression plasmid. As shown in Fig. 4A, although sorcin enhanced the *mdr1/p-gp* promoter deleted up to −728 bp, further deletion of the *hsp70* promoter up to −568 bp abolished the responsiveness to sorcin. In addition, we found a putative cAMP response element (CRE) between −716 and −709 bp of the *mdr1/p-gp* gene through transcription factor database searches. When the pGLMDR(-906mCRE) construct, in which four mutated nucleotides within the CRE sequence were transiently transfected into COS-7 cells with the sorcin expression plasmid, activation of the *mdr1/p-gp* promoter by sorcin was not observed (Fig. 3A). In addition, overexpression of sorcin enhanced the luciferase activity of pGL4.29[luc2P/CRE], which contains a CRE that drives the transcription of the luciferase reporter gene (Fig. 3B).

To further examine whether CREB1 bound to the *mdr1/p-gp* promoter *in vivo*, the CREB1-binding assay was performed using the HaloCHIP system (Fig. 3C). Binding of CREB1 to the region between −841 and −577 bp of the *mdr1/p-gp* promoter was observed in the cells that overexpressed sorcin. On the other hand, CREB1 did not bind to intron 3 of the *mdr1/p-gp* gene regardless of sorcin overexpression, suggesting that sorcin seemed to enhance CREB1 binding to CRE between −716 and −709 bp of the *mdr1/p-gp* gene, resulting in the induction of MDR1/P-gp expression.

3.4. Overexpression of sorcin stimulates the phosphorylation of CREB1 at Ser133

To elucidate whether activation of CREB1 was associated with the MDR phenotype, we next examined the phosphorylation of CREB1 at Ser133 in MDR cells compared to their parental K562 cells (Fig. 4A). Phosphorylated CREB1 was expressed at a higher level in MDR cells than in their parental K562 cells. In addition, we examined whether sorcin stimulated the phosphorylation of CREB1 at Ser133, and found that the phosphorylation of CREB1 was markedly increased by the overexpression of sorcin in COS-7 cells (Fig. 4B). Thus, sorcin seemed to enhance the expression of MDR1/P-gp through activation of the CREB pathway and was associated with the MDR phenotype.

4. Discussion

Sorcin, a 22 kDa Ca^{2+} binding protein that is widely distributed in mammalian tissue [11], is associated with the MDR phenotype in a variety of human cancer cell lines [15–21]. However, the exact mechanisms of MDR mediated by sorcin in cancer cells have been poorly studied. Here, we showed that overexpression of sorcin markedly induced MDR1/P-gp expression, while knockdown of sorcin induced the up-regulation of MDR-1/P-gp expression. These results are in good agreement with those reported by He et al. [21], in which overexpression of sorcin in gastric cancer cells resulted in increased drug resistance with up-regulation of MDR-1/P-gp expression, while inhibition of sorcin expression led to reversal

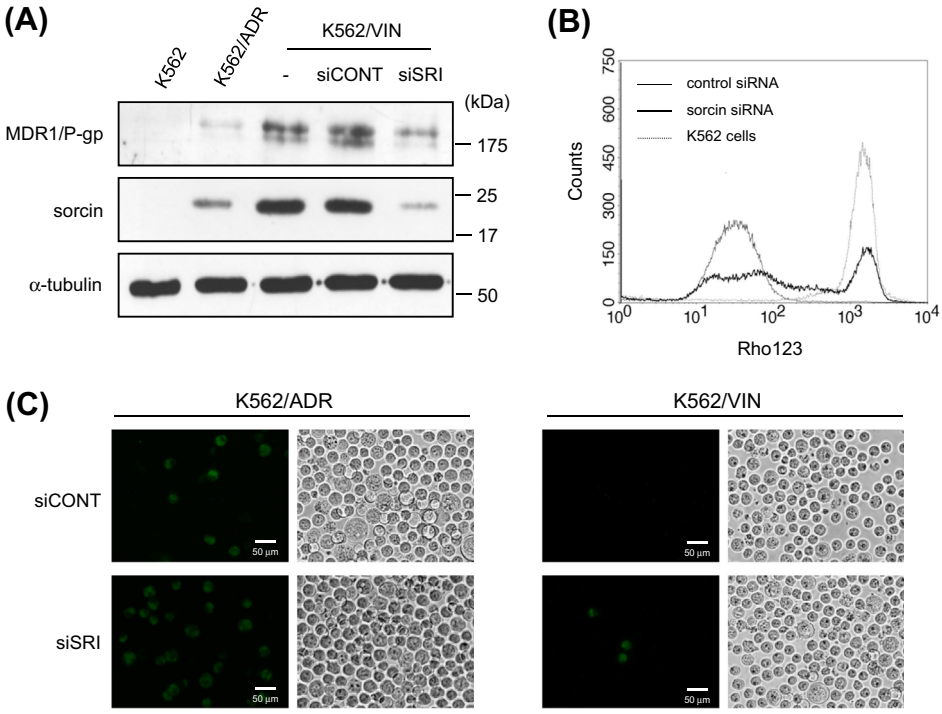

Fig. 2. Sorcin modulates the expression of MDR1/P-gp. (A) K562/VIN cells were transfected with sorcin siRNA or RISC-free control siRNA. At 48 h after transfection, the cells were harvested and analyzed for the expression of sorcin and MDR1/P-gp with α-tubulin as a loading control by Western blotting using their respective antibodies. (B, C) K562/ADR and K562/VIN cells were transfected with sorcin siRNA or RISC-free control siRNA. At 48 h after transfection, the cells were collected and exposed to 1 μM Rho123 in RPMI1640 medium at 37 °C for 2 h. The cellular accumulation of Rho123 was determined using a flow cytometer (B) or directly observed by a fluorescence microscope (C).

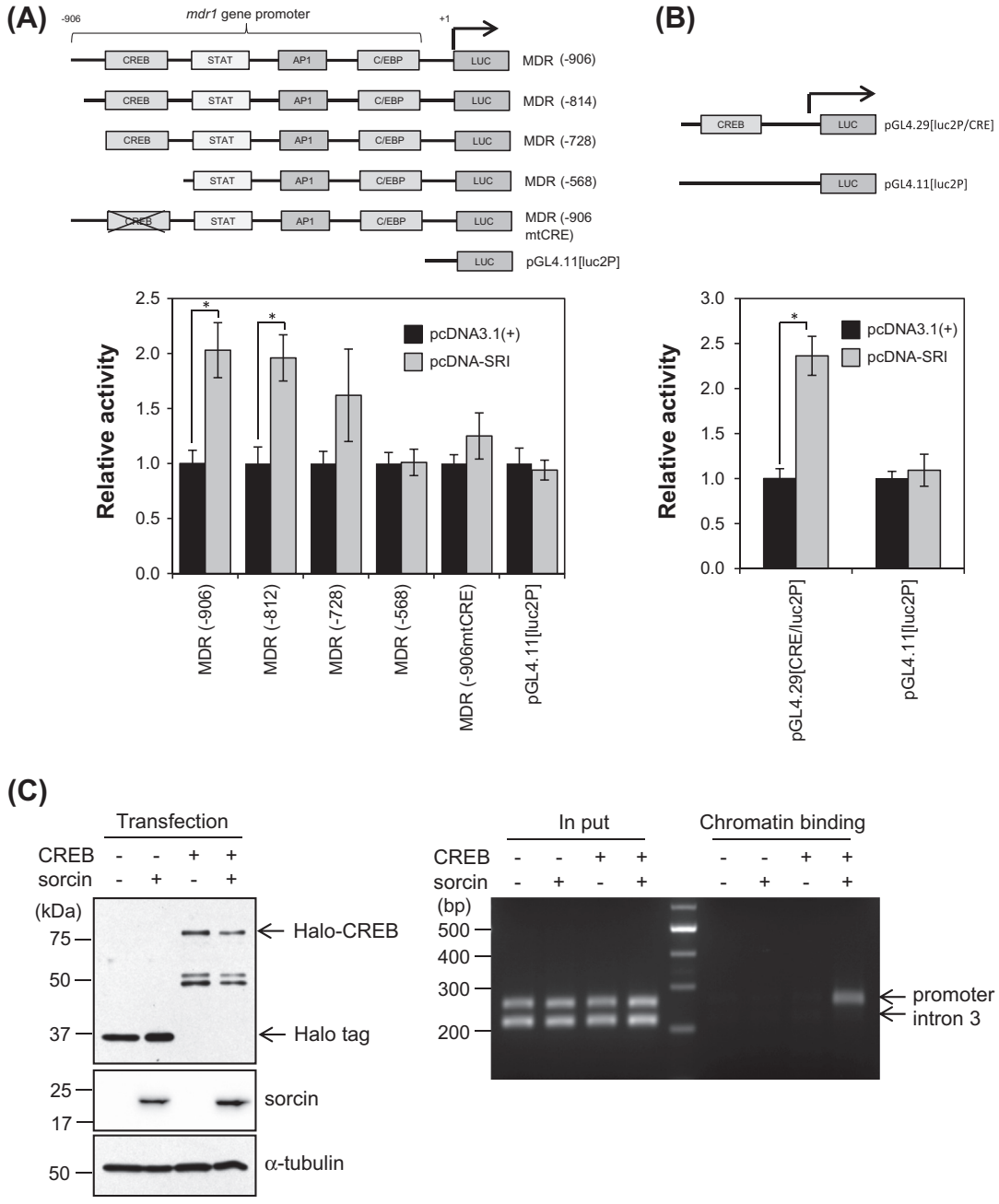

Fig. 3. Sorcin modulates the expression of MDR1/P-gp through the cAMP response element between −716 and −709 bp of *mdr1/p-gp* gene. (A, B) The expression plasmid for sorcin was co-transfected to COS-7 cells with the *mdr1/p-gp* promoter reporter constructs as described above. At 48 h after transfection, the cells were harvested, and luciferase activities were measured. Relative activity is expressed as a ratio to that of cells co-transfected with pcDNA3.1(+) vector. Values are the means ± SD of four independent experiments, and asterisks represent significant differences at $p < 0.01$. (C) COS-7 cells were co-transfected with pFN21-CREB1 and pcDNA-SRI. At 48 h after transfection, the cells were harvested, and the expression of sorcin and Halo-tagged CREB1 was confirmed (*left*). CREB1-binding assay to the promoter region of the *mdr1/p-gp* gene was performed using the HaloCHIP system (*right*). The *input* represents 5% of the material used in the binding assay.

of drug resistance. Furthermore, we found that sorcin enhanced the phosphorylation of CREB1 at Ser133 and the promoter activity of the *mdr1/p-gp* gene through CREB1.

Overexpression of MDR1/P-gp, a multidrug efflux pump which reduces intracellular drug accumulation, represents one of the major mechanisms that contribute to acquisition of the MDR phenotype. To date, a number of transcriptional factors have been proposed to be involved in *mdr1/p-gp* gene expression, including NF-IL6 and NF-κB [6,7]. The induction of MDR expression was also modulated by various protein kinases, including protein kinase A (PKA) [28,29] and protein kinase C [30]. In the present study, we showed that a putative CRE sequence between −716 and −709 bp of the *mdr1/p-gp* gene is necessary for sorcin-induced expression of the *mdr1/p-gp* gene. In addition, overexpression of sorcin stimulated the CRE-driven expression of the luciferase reporter gene. These results suggest that sorcin induces MDR1/P-gp expression markedly through activation of the CREB pathway. The CREB and closely related factors are activated by PKA [31]. We also found that the phosphorylation of CREB1 and MDR1/P-gp expression were markedly increased by the overexpression of sorcin. Therefore, sorcin seems to induce MDR1/P-gp expression through the activation of CREB1 by PKA.

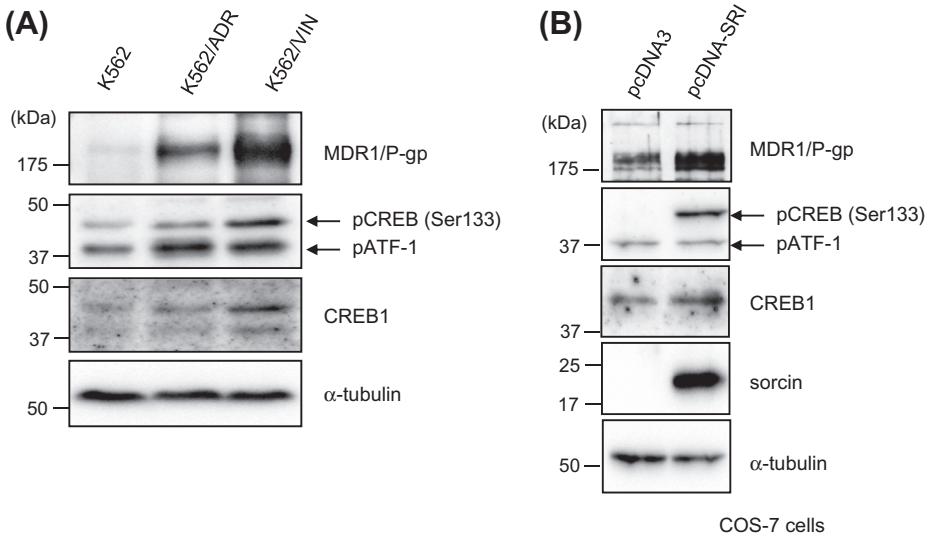

Fig. 4. Overexpression of sorcin stimulates the phosphorylation of CREB1 at Ser133. (A) Cell extracts (20 μg proteins) were separated by SDS–PAGE, and analyzed for the expression of phosphorylated CREB1 (Ser133), CREB1, MDR1/P-gp and sorcin with α-tubulin as a loading control by Western blotting. (B) COS-7 cells were transfected with pcDNA-SRI or pcDNA3.1(+) vector. At 48 h after transfection, the cells were harvested and analyzed for the expression of phosphorylated CREB1 (Ser133), CREB1, MDR1/P-gp and sorcin with α-tubulin as a loading control by Western blotting.

Amplification of the gene that encodes sorcin is thought to be closely associated with amplification of the *mdr/p-gp* gene [27]. However, to date, it has been suggested that overexpression of sorcin is not always observed in conjunction with *mdr/p-gp* gene amplification and/or overexpression. Beyer-Sehlmeyer et al. [15] demonstrated increased expression of sorcin in MDR tumor cell lines of hematopoietic origin without a concomitant increase in the expression of MDR/P-gp. Studies using vincristine-resistant HOB1 lymphoma cell lines revealed that the sorcin gene was amplified upon exposure of parental HOB1 cells to a high concentration of vincristine, but this phenomenon was not related to *mdr/p-gp* gene amplification in drug-resistant cells [32,33]. More recently, Hu et al. [34] demonstrated that sorcin overexpression exhibited a close relationship with chemoresistance, but down-regulation of sorcin could not alter MDR/P-gp expression and function in MDR cell lines, K562/A02 and MCF-7/A02 cells. These results were not consistent with the present data. Although the reasons for these differences remain to be clarified, the mechanism of sorcin and its correlation with MDR-1/P-gp might be different in different cancer cells.

Previously, it was suggested that modulation of Ca^{2+} homeostasis is a crucial step in the regulation of the cell response to stress conditions and in favoring drug resistance in tumors [35,36]. In addition, the intracellular Ca^{2+} level may play a role in the development of chemoresistance, as suggested by clinical trials with verapamil, a Ca^{2+} channel blocker which selectively enhances drug cytotoxicity in MDR cell lines [37]. As it is well known that sorcin is a calcium-binding protein and can bind and thereby sequester up to 10% of cytosolic Ca^{2+}, sorcin may play an important role in the emergence of MDR by regulating intracellular Ca^{2+}. Indeed, Maddalena et al. [38] recently reported that sorcin induces a drug-resistant phenotype in human colorectal cancer by modulating Ca^{2+} homeostasis. In addition, other reports have suggested a correlation between sorcin and apoptosis-related proteins [18,34]. Furthermore, Hu et al. [39] recently demonstrated that down-regulation of sorcin inhibits epithelial-to-mesenchymal transition (EMT), which is thought to be responsible for resistance to chemotherapy and cancer cell metastasis. Thus, sorcin might contribute to several mechanisms responsible for MDR in human cancer, such as regulating drug efflux, modulating apoptosis and controlling EMT.

In conclusion, we found here that sorcin induces MDR1/P-gp expression markedly through the CREB pathway and is associated with the MDR phenotype in leukemia cells. These data are valuable for further study of the mechanism of MDR in human cancer, and also provide some new clues for developing more effective MDR reversal treatments.

Acknowledgments

This study was supported in part by the Program for the Strategic Research Foundation at Private Universities (2008-2012) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and Kyoto Pharmaceutical University Fund for the Promotion of Scientific Research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.04.125.

References

[1] M. Hwang, L. Moretti, B. Lu, HSP90 inhibitors: multi-targeted antitumor effects and novel combinatorial therapeutic approaches in cancer therapy, Curr. Med. Chem. 15 (2008) 1192–1208.
[2] C.P. Wu, A.M. Calcagno, S.V. Ambudkar, Reversal of ABC drug transporter-mediated multidrug resistance in cancer cells: evaluation of current strategies, Curr. Mol. Pharmacol. 1 (2008) 93–105.
[3] J. Robert, A.K. Larsen, Drug resistance to topoisomerase II inhibitors, Biochimie 80 (1998) 247–254.
[4] S.V. Ambudkar, S. Dey, C.A. Hrycyna, M. Ramachandra, I. Pastan, M.M. Gottesman, Biochemical, cellular, and pharmacological aspects of the multidrug transporter, Annu. Rev. Pharmacol. Toxicol. 39 (1999) 361–398.
[5] G. Szakács, J.K. Paterson, J.A. Ludwig, C. Booth-Genthe, M.M. Gottesman, Targeting multidrug resistance in cancer, Nat. Rev. Drug Discov. 5 (2006) 219–234.
[6] N.J. Combates, R.W. Rzepka, Y.N. Chen, D. Cohen, NF-IL6, a member of the C/EBP family of transcription factors, binds and trans-activates the human MDR1 gene promoter, J. Biol. Chem. 269 (1994) 29715–29719.
[7] G. Zhou, M.T. Kuo, NF-kappaB-mediated induction of mdr1b expression by insulin in rat hepatoma cells, J. Biol. Chem. 272 (1997) 15174–15183.

[8] D.R. Ferry, H. Traunecker, D.J. Kerr, Clinical trials of P-glycoprotein reversal in solid tumours, Eur. J. Cancer 32A (1996) 1070–1081.
[9] K. Fan, D. Fan, L.F. Cheng, C. Li, Expression of multidrug resistance-related markers in gastric cancer, Anticancer Res. 20 (2000) 4809–4814.
[10] Y. Takebayashi, S. Akiyama, S. Natsugoe, S. Hokita, K. Niwa, M. Kitazono, T. Sumizawa, A. Tani, T. Furukawa, T. Aikou, The expression of multidrug resistance protein in human gastrointestinal tract carcinomas, Cancer 80 (1998) 661–666.
[11] M.B. Meyers, V.M. Pickel, S.S. Sheu, V.K. Sharma, K.W. Scotto, G.I. Fishman, Association of sorcin with the cardiac ryanodine receptor, J. Biol. Chem. 270 (1990) 26411–26418.
[12] M.B. Meyers, T.S. Puri, A.J. Chien, T. Gao, P.H. Hsu, M.M. Hosey, G.I. Fishman, Sorcin associates with the pore-forming subunit of voltage-dependent L-type Ca^{2+} channels, J. Biol. Chem. 273 (1998) 18930–18935.
[13] C. Zamparelli, N. Macquaide, G. Colotti, D. Verzili, T. Seidler, G.L. Smith, E. Chiancone, Activation of the cardiac Na(+)-Ca(2+) exchanger by sorcin via the interaction of the respective Ca(2+)-binding domains, J. Mol. Cell. Cardiol. 49 (2010) 132–141.
[14] M.B. Meyers, J.L. Biedler, Increased synthesis of a low molecular weight protein in vincristine-resistant cells, Biochem. Biophys. Res. Commun. 99 (1981) 228–235.
[15] G. Beyer-Sehlmeyer, W. Hiddemann, B. Wörmann, J. Bertram, Suppressive subtractive hybridisation reveals differential expression of serglycin, sorcin, bone marrow proteoglycan and prostate-tumour-inducing gene I (PTI-1) in drug-resistant and sensitive tumour cell lines of haematopoetic origin, Eur. J. Cancer 35 (1999) 1735–1742.
[16] Y.X. Yang, Z.Q. Xiao, Z.C. Chen, G.Y. Zhang, H. Yi, P.F. Zhang, J.L. Li, G. Zhu, Proteome analysis of multidrug resistance in vincristine-resistant human gastric cancer cell line SGC7901/VCR, Proteomics 6 (2006) 2009–2021.
[17] Y. Qu, Y. Yang, B. Liu, W. Xiao, Comparative proteomic profiling identified sorcin being associated with gemcitabine resistance in non-small cell lung cancer, Med. Oncol. 27 (2010) 1303–1308.
[18] J. Qi, N. Liu, Y. Zhou, Y. Tan, Y. Cheng, C. Yang, Z. Zhu, D. Xiong, Overexpression of sorcin in multidrug resistant human leukemia cells and its role in regulating cell apoptosis, Biochem. Biophys. Res. Commun. 349 (2006) 303–309.
[19] Y. Zhou, Y. Xu, Y. Tan, J. Qi, Y. Xiao, C. Yang, Z. Zhu, D. Xiong, Sorcin, an important gene associated with multidrug-resistance in human leukemia cells, Leuk. Res. 30 (2006) 469–476.
[20] Y. Yang, Z. Chen, G. Zhang, H. Yi, Z. Xiao, A subcelluar proteomic investigation into vincristine-resistant gastric cancer cell line, J. Cell. Biochem. 104 (2008) 1010–1021.
[21] Q. He, G. Zhang, D. Hou, A. Leng, M. Xu, J. Peng, T. Liu, Overexpression of sorcin results in multidrug resistance in gastric cancer cells with up-regulation of P-gp, Oncol. Rep. 25 (2011) 237–343.
[22] N. Yamagishi, T. Ueda, A. Mori, Y. Saito, T. Hatayama, Decreased expression of endoplasmic reticulum chaperone GRP78 in liver of diabetic mice, Biochem. Biophys. Res. Commun. 417 (2012) 364–370.
[23] N. Yamagishi, H. Fujii, Y. Saito, T. Hatayama, Hsp105beta upregulates hsp70 gene expression through signal transducer and activator of transcription-3, FEBS J. 276 (2009) 5870–5880.
[24] T. Tsuruo, H. Iida, E. Ohkochi, S. Tsukagoshi, Y. Sakurai, Establishment and properties of vincristine-resistant human myelogenous leukemia K562, Gann 74 (1983) 751–758.
[25] T. Tsuruo, H. Iida-Saito, H. Kawabata, T. Oh-hara, H. Hamada, T. Utakoji, Characteristics of resistance to adriamycin in human myelogenous leukemia K562 resistant to adriamycin and in isolated clones, Jpn. J. Cancer Res. 77 (1986) 682–692.
[26] H. Shibata, R. Kanamaru, T. Sato, C. Ishioka, Y. Konishi, A. Ishikawa, A. Wakui, T. Tsuruo, Increase in the level of P-glycoprotein mRNA expression in multidrug-resistant K562 cell lines treated with sodium butyrate is not accompanied with erythroid differentiation, Jpn. J. Cancer Res. 81 (1990) 1214–1217.
[27] A.M. Van der Bliek, M.B. Meyers, J.L. Biedler, E. Hes, P. Borst, A 22-kd protein (sorcin/V19) encoded by an amplified gene in multidrug-resistant cells, is homologous to the calcium-binding light chain of calpain, EMBO J. 5 (1986) 3201–3208.
[28] C. Rohlff, R.I. Glazer, Regulation of multidrug resistance through the cAMP and EGF signalling pathways, Cell. Signal. 7 (1995) 431–443.
[29] C. Rohlff, R.I. Glazer, Regulation of the MDR1 promoter by cyclic AMP-dependent protein kinase and transcription factor Sp1, Int. J. Oncol. 12 (1998) 383–386.
[30] E. Flescher, R. Rotem, Protein kinase C epsilon mediates the induction of P-glycoprotein in LNCaP prostate carcinoma cells, Cell. Signal. 14 (2002) 37–43.
[31] B. Mayr, M. Montminy, Transcriptional regulation by the phosphorylation-dependent factor CREB, Nat. Rev. Mol. Cell Biol. 2 (2001) 599–609.
[32] S.L. Wang, M.F. Tam, Y.S. Ho, S.H. Pai, M.C. Kao, Isolation and molecular cloning of human sorcin a calcium-binding protein in vincristine-resistant HOB1 lymphoma cells, Biochim. Biophys. Acta 1260 (1995) 285–293.
[33] W.P. Lee, Purification, cDNA cloning, and expression of human sorcin in vincristine-resistant HOB1 lymphoma cell lines, Arch. Biochem. Biophys. 325 (1996) 217–226.
[34] Y. Hu, X. Cheng, S. Li, Y. Zhou, J. Wang, T. Cheng, M. Yang, D. Xiong, Inhibition of sorcin reverses multidrug resistance of K562/A02 cells and MCF-7/A02 cells via regulating apoptosis-related proteins, Cancer Chemother. Pharmacol. 72 (2013) 789–798.
[35] M.P. Mattson, Neuronal life-and-death signaling, apoptosis, and neurodegenerative disorders, Antioxid. Redox Signal. 8 (2006) 1997–2006.
[36] Z. Sulová, M. Seres, M. Barancík, L. Gibalová, B. Uhrík, L. Poleková, A. Breier, Does any relationship exist between P-glycoprotein-mediated multidrug resistance and intracellular calcium homeostasis, Gen. Physiol. Biophys. 28 (2009) F89–F95.
[37] M. Giai, N. Biglia, P. Sismondi, Chemoresistance in breast tumors, Eur. J. Gynaecol. Oncol. 12 (1991) 359–373.
[38] F. Maddalena, G. Laudiero, A. Piscazzi, A. Secondo, A. Scorziello, V. Lombardi, D.S. Matassa, A. Fersini, V. Neri, F. Esposito, M. Landriscina, Sorcin induces a drug-resistant phenotype in human colorectal cancer by modulating Ca(2+) homeostasis, Cancer Res. 71 (2011) 7659–7669.
[39] Y. Hu, S. Li, M. Yang, C. Yan, D. Fan, Y. Zhou, Y. Zhang, E. Yagüe, D. Xiong, Sorcin silencing inhibits epithelial-to-mesenchymal transition and suppresses breast cancer metastasis *in vivo*, Breast Cancer Res. Treat. 143 (2014) 287–299.