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Role of microRNAs in glucocorticoid-resistant B-cell precursor acute lymphoblastic leukemia

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Abstract. Acute lymphoblastic leukemia (ALL) is the most common malignant disorder in children and intensive combination therapy has markedly improved patient prognosis. However, efficacy of the treatment still fails in 10-15% of patients. Glucocorticoids (GCs) such as prednisone and dexamethasone (DEX) are essential drugs used for ALL chemotherapy, and the response to GC treatment is a strong independent factor of ALL prognosis. In the present study, we examined the mechanism of GC resistance of B-cell precursor ALL (BCP-ALL). As determined by RT-qPCR and western blot analyses, GC treatment upregulated glucocorticoid receptor (GR) protein and Bcl-2-interacting mediator of cell death (BCL2L11, BIM) protein expression, resulting in apoptosis of a GC-sensitive BCP-ALL cell line, but not of a GC-resistant BCP-ALL cell line as shown by flow cytometry. GR was downregulated in a DEX-resistant BCP-ALL cell line which was induced by treatment of cells with increasing concentrations of DEX. Importantly, expression levels of miR-142-3p and miR-17~92 cluster were upregulated in the BCP-ALL cell line with acquired DEX resistance as examined by RT-qPCR. Our results suggest that interference of miR-142-3p and miR-17~92 may overcome the resistance of BCP-ALL to GCs.

Introduction

Acute lymphoblastic leukemia (ALL) is the most commonly diagnosed malignancy in children, accounting for approximately 30% of all pediatric cancers worldwide (1). Advances in the treatment of children with ALL have led to 5-year disease-free survival rates exceeding 85% (2). However, children with ALL cells that show resistance to glucocorticoids (GCs) have a significantly poorer treatment outcome when compared with patients with ALL cells that are sensitive to GCs (3-6). The underlying mechanisms of this phenomenon are not yet clear. Therefore, there is an immediate need to elucidate the mechanisms of GC resistance and thereby explore novel therapeutic strategies to reverse GC resistance, which will help achieve complete treatment potential and significantly improve prognosis.

GCs are widely used in antileukemia therapy, due to their extreme pro-apoptotic effects on lymphoblasts. The physiologic and pharmacologic actions of GCs are mediated by the GC receptor (GR), which consists of three isoforms: GRα, GRβ and GRγ (7). In spite of GC binding to all three isoforms, only GRα mediates appropriate GC signaling, whereas GRβ and GRγ prevent the GC/GR complex from binding to DNA (8). Thus, these two latter forms of GRs inhibit GC activity and play an important role in GC resistance (9).

Bcl-2-interacting mediator of cell death (BCL2L11, BIM) is a pro-apoptotic BH3-only member of the B-cell leukemia/lymphoma (BCL2) family, and plays a critical role in the development of the lymphoid system. Three major alternative transcription variants, BIM-EL, BIM-L and BIM-S, which are formed by alternative splicing within exon 2, induce apoptosis through a pro-apoptotic BH3 domain that is encoded exclusively by exon 4 of the *BIM* gene (10). BIM is a critical mediator of GC-induced cell death of normal lymphocytes and ALL cells and is upregulated upon GC stimulation (11).

MicroRNAs (miRNAs) belong to a class of endogenously expressed non-coding single-stranded RNAs of 18-24 nucleotides and induce gene silencing by binding to target mRNAs with partial complementarity (12,13). miRNAs play a significant regulatory role in GC resistance by several mechanisms such as: i) Post-transcriptionally modulating GR mRNA translation thereby affecting GR levels; ii) altering the receptor-isoform ratio; or iii) controlling activity of other transcriptional factors. Involvement of miRNAs in the GR transcriptional pathways have been studied. High levels of miR-142-3p resulted in low levels of GRα protein expression, thus leading to resistance of T-leukemic cells to GCs (14). It has also been reported that miR-124 induces resistance

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Abbreviations: GC, glucocorticoid; GR, glucocorticoid receptor; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; DEX, dexamethasone

Key words: glucocorticoid, glucocorticoid receptor, miR-142-3p, miR-17~92, B-cell precursor acute lymphoblastic leukemia

to GC treatment by targeting GR in ALL (15). Aberrantly expressed miR-130b (16) and miR-21 (17) exhibited similar expression pattern with respect to GRα transcripts inhibiting GC-induced apoptosis in multiple myeloma.

On the contrary, several studies have investigated the mechanisms that contribute to the upregulation of BIM and induction of apoptosis in lymphocytes. A recent report demonstrated that GC treatment induced the expression of pro-apoptotic protein BIM via downregulation of the miR-17~92 cluster (18). The loss of miR17 family expression and concomitant increases in the miR17 target BIM were found to occur in GC-sensitive ALL cells but not in GC-resistant ALL cells (19). Furthermore, two miRNAs, miR-142-3p and miR-17-5p, are computationally predicted to be closely related to GC resistance in pediatric ALL (20).

In the present study, we demonstrated that GC treatment upregulated GR protein and BIM protein expression, and induced apoptosis in a GC-sensitive B-cell precursor ALL (BCP-ALL) cell line. However, GR protein and BIM protein levels were not upregulated in a GC-resistant BCP-ALL cell line. Expression levels of the miR-17~92 cluster and miR-142-3p were upregulated in a GC-resistant BCP-ALL cell line which was induced by increasing concentrations of GC treatment. Our data suggest that continuous GC treatment leads to elevated expression of the miR-17~92 cluster and miR-142-3p, with concomitant downregulation of GR.

Materials and methods

Cell lines, culture conditions and reagents. BCP-ALL cell lines, 697 (cat. no. CRL-7433; ATCC, Manassas, VA, USA), MB-YU (21) and REH (cat. no. ACC22; DSMZ, Braunschweig, Germany) were used in the present study. Cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (Wako Pure Chemical Industries, Osaka, Japan) containing 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C in humidified air with 5% CO_2. Dexamethasone (DEX) was obtained from Sigma-Aldrich/Merck KGaA (Darmstadt, Germany) and dissolved in PBS. The following antibodies were used in this study: Glucocorticoid receptor (GR; cat. no. sc-899; Santa Cruz Biotechnology, Heidelberg, Germany), polyclonal rabbit anti-BIM (cat. no. ADI-AAP-330; Stresgen, Farmingdale, NY, USA), anti-cleaved PARP (cat. no. 5625; Cell Signaling Technology, Danvers, MA, USA), anti-caspase-3 (M097-3; MBL Int Corp., Woburn MA, USA) and anti-actin (cat. no. A5316; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The secondary antibodies conjugated with horseradish peroxidase, goat anti-mouse IgG (H+L) (cat. no. 31430) and goat anti-rabbit IgG (cat. no. 31460) were obtained from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Induction of DEX-resistant BCP-ALL cells. To induce acquired DEX-resistance, aliquots of parental cells were seeded into 25 cm^2 culture bottles and cultured in RPMI-1640 medium supplemented with 10% FBS and increasing concentrations of DEX (from 0.1 nM to 1 μM). Fresh medium with DEX was changed every 48 h. Cells were transferred into new culture bottles every 7 days. We continued this process while observing cell death every day, and performing cell counting regularly by using an Invitrogen Cell Counter (Thermo Fisher Scientific, Inc.). Cells were treated with increasing concentrations of DEX after two rounds of cell counting showed a consistent increase in cell number. Thus, after 4 to 12 weeks, DEX-resistant sublines were obtained that grew stably in DEX (1 μM)-containing medium and these resistant cell lines were named 697DR and MB-YUDR.

Cell proliferation and viability assays. Cell proliferation was measured using a conventional hemocytometer counting chamber. A trypan blue exclusion test was conducted to assess cell viability, where the relative percentages of live and dead cells were quantified using a hemocytometer.

Apoptosis assay. Detection and quantification of hypodiploid DNA content of apoptotic cells was performed by flow cytometric analysis after staining cells with propidium iodide (PI) as previously described (22). The apoptotic cell nuclei (sub-G1 peak in the DNA fluorescence histogram) were counted using CellQuest software version 3.0 (BD Biosciences, San Jose, CA, USA). Data are expressed as the percentage of the sub-G1 fraction.

RNA isolation, reverse transcription and quantitative real-time PCR (RT-qPCR). Total RNA was isolated using RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA). RNA samples were resuspended in RNase-free water and quantified by measuring absorbance at 260 and 280 nm. cDNA was synthesized using the SuperScript® VILO™ cDNA Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.). The mRNA expression levels of *BIM* and *GRα* were analyzed using *GAPDH* as a reference gene. RT-qPCR was performed using QuantiFast SYBR® Green PCR (Qiagen, Inc.). The primers used are listed in Table I. The reaction mixtures were incubated at 95˚C for 5 min, followed by 45 cycles of 95˚C for 15 sec and 57˚C for 30 sec in a Stratagene RT-qPCR instrument (Agilent Technologies, Inc., Santa Clara, CA, USA). The data were analyzed using the ΔCq (cycle threshold) method.

Analysis of miRNAs by RT-qPCR. Total miRNAs were isolated from ALL cell lines using the miRNeasy Mini Kit (Qiagen, Inc.). The miScript SYBR® Green PCR kit (Qiagen, Inc.) and miScript Primer Assays (Qiagen, Inc.) were used on a real-time PCR system to quantify the plasma miRNAs. Qiagen miScript primers (hsa-miR-142-3p, hsa-miR-17-5p, hsa-miR-18a-5p, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-20a-5p and hsa-miR-92-3p) used are listed in Table II. The data were analyzed using the ΔCq (cycle threshold) method.

Western blot analysis. Western blotting was performed with whole-cell extracts prepared by lysing cells ($1x10^7$) in lysis buffer (95% Laemmli sample buffer and 5% 2-mercaptoethanol). Protein concentration was determined using BCA protein assay reagents (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The proteins (20 μg/lane) were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel by electrophoresis followed by semi-dry transfer onto PVDF membranes (Invitrogen; Thermo Fisher Scientific, Inc.). Transferred PVDF membranes

Table I. Primers sequences of GRα, Bim and GAPDH.

Gene	Primer	Base sequence
GRα	Forward primer	5'-CGGTCTGAAGAGCCAAGAG-3'
	Reverse primer	5'-CAGCTAACATCTCGGGGAAT-3'
Bim	Forward primer	5'-CGATCCTCCAGTGGGTATTTCTCT-3'
	Reverse primer	5'-ATACCCTCCTTGCATAGTAAGCG-3'
GAPDH	Forward primer	5'-GAAGGTGAAGGTCGGAGTC-3'
	Reverse primer	5'-GAAGATGGTGATGGGATTTC-3'

Table II. miScript primer sequences.

miRNA primer	Base sequence
hsa-miR-142-3p	5'-UGUAGUGUUUCCUACUUUAUGGA-3'
hsa-miR-17-5p	5'-CAAAGUGCUUACAGUGCAGGUAG-3'
hsa-miR-18a-5p	5'-UAAGGUGCAUCUAGUGCAGAUAG-3'
hsa-miR-19a-3p	5'-UGUGCAAAUCUAUGCAAAACUGA-3'
hsa-miR-19b-3p	5'-UGUGCAAAUCCAUGCAAAACUGA-3'
hsa-miR-20a-5p	5'-UAAAGUGCUUAUAGUGCAGGUAG-3'
hsa-miR-92a-3p	5'-UAUUGCACUUGUCCCGGCCUGU-3'

were pretreated with 5% non-fat dry milk in TBST (10 mM Tris-HCl, pH 7.6, 137 mM NaCl, 0.1% Tween-20) and incubated with the primary antibody (dilution 1:1,000-3,000) at 4˚C overnight. The membrane was then washed 3 times with TBST and incubated with horseradish peroxidase-conjugated secondary antibody (dilution 1:1,000-3,000) for 1 h at room temperature. After washing three times again, antibodies bound to protein blots were detected by using Western Lightening Chemiluminescence Reagent Plus (PerkinElmer, Inc., Waltham, MA, USA) and visualized on LAS-3000 Mini (Fujifilm, Snizuoka, Japan).

Statistical analysis. Results are expressed as mean ± standard deviation of at least three independent experiments performed in triplicate. To test differences between two populations the unpaired Student's t-test was applied. To test the differences among more than two populations, one-way ANOVA was performed followed by Dunnett's post hoc test. Differences were considered to be statistically significant at $P<0.05$.

Results

DEX induces apoptosis in cell line 697 but not in cell line REH. To examine whether DEX induces cell death in BCP-ALL cells, two human BCP-ALL cell lines, 697 and REH were treated with DEX at increasing concentrations from 0.1 nM to 1 μM. DEX strongly induced cell apoptosis in a time- and dose-dependent manner in the 697 cell line (Fig. 1A). However, the REH cell line was completely resistant to DEX (Fig. 1B). The cell proliferation of 697 and REH cells upon DEX treatment was examined. Fifty percent of DEX-sensitive 697 cells showed cell death and the proliferation of cells which survived was completely inhibited by 100 nM DEX treatment. However, higher concentrations of DEX (>1 μM) did not suppress the proliferation of DEX-resistant REH cells.

DEX upregulates glucocorticoid receptor and BIM expression and induces apoptosis in the 697 cell line. RT-qPCR and western blot analyses were performed to detect the effect of DEX on the expression levels of GR and BIM in DEX-sensitive and -resistant cell lines. GRα and BIM exhibited differential expression of mRNA and protein between the DEX-sensitive and -resistant cell line. DEX treatment upregulated both mRNA and protein expression levels of GRα in the DEX-sensitive 697 cell line, but not in the DEX-resistant REH cell line (Figs. 2A, C and 3). After 6 h of DEX treatment, mRNA and protein expression levels of BIM were upregulated in the DEX-sensitive 697 cell line, but not in the DEX-resistant REH cell line (Figs. 2B, D and 3). Western blot analysis was performed using caspase-3 and cleaved PARP antibodies to further validate that cells had undergone apoptosis. As shown in Fig. 3, activation of caspase-3 and cleavage of PARP were observed in the DEX-sensitive 697 cell line in a time-dependent manner, but not in the DEX-resistant cell line REH.

Long-term exposure induces DEX resistance in BCP-ALL cells. To explore acquired DEX resistance in the 697 cell line, cells were treated with increasing concentrations of DEX (from 0.1 nM to 1 μM). DEX-sensitive 697 cells that grew stably in a low DEX concentration underwent apoptosis at 100 nM DEX (Fig. 4B and C). However, 697 cells that grew stably in a high DEX concentration did not undergo apoptosis even at 1 μM DEX (Fig. 4D and E). After incubation with DEX for 4 to 12 weeks, acquired DEX-resistant sublines (697DR and MB-YUDR) could proliferate stably in RPMI-1640 medium with 10% FBS in the presence of DEX (10 μM). The DEX-resistant sublines (697DR and MB-YUDR) (Figs. 4F and 5B) exhibited marked resistance to DEX compared to the parental cells (697 and MB-YU) (Figs. 4A, F and 5). RT-qPCR and western blot analyses showed that GRα mRNA and

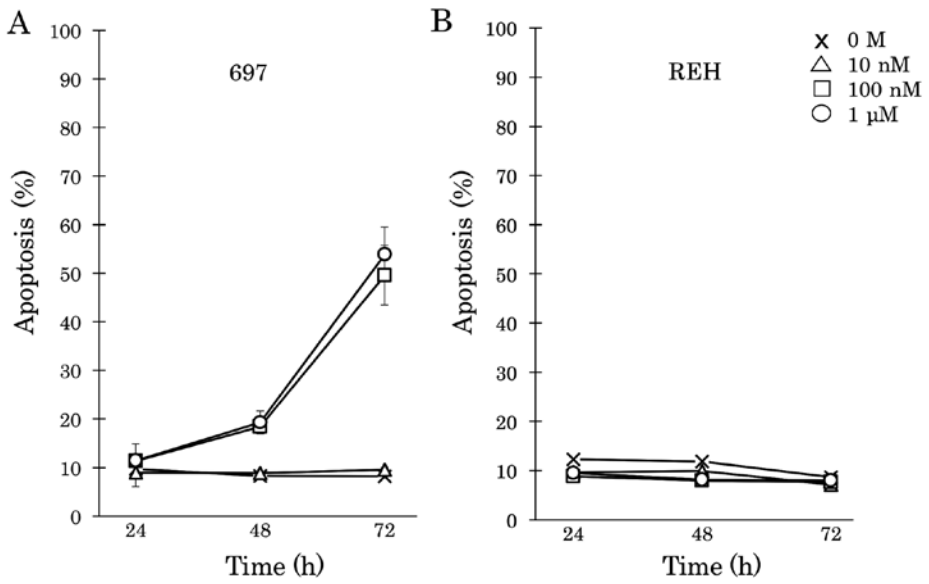

Figure 1. Evaluation of DEX-induced apoptosis in ALL cell lines. ALL cell lines, (A) 697 and (B) REH, were treated with 0, 10, 100 nM and 1 μM of DEX for 24, 48 and 72 h. Apoptotic progression was monitored via flow cytometric analysis of propidium iodide-stained nuclei. The results are expressed as the percentage of positive mean values ± standard deviation (SD) for three independent experiments. DEX, dexamethasone; ALL, acute lymphoblastic leukemia.

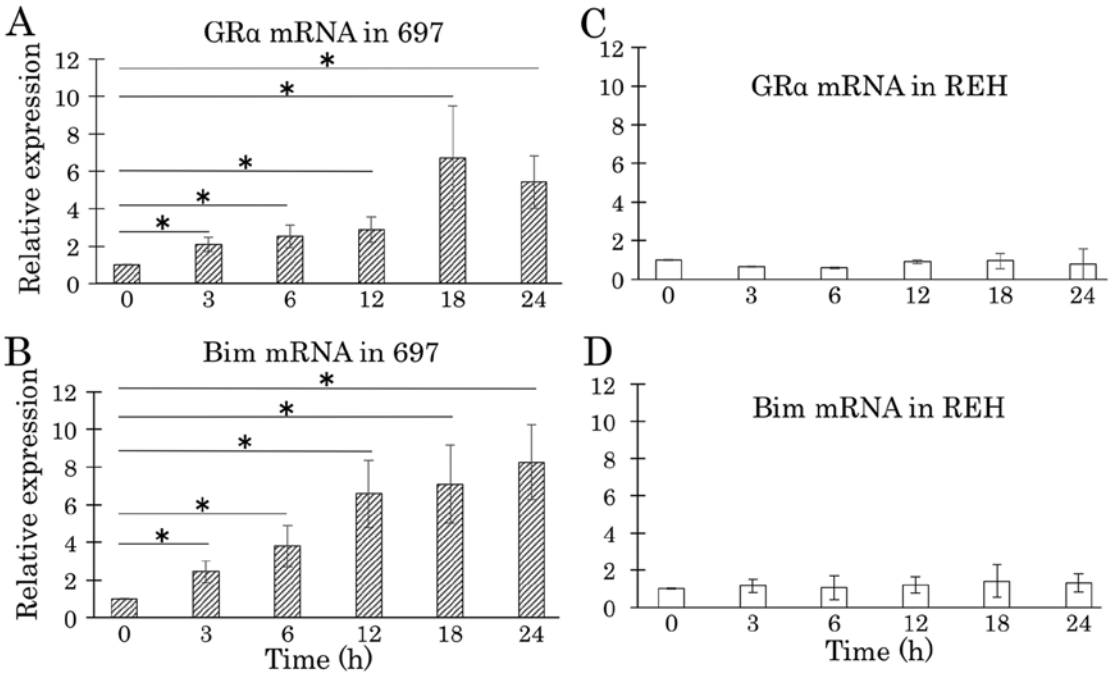

Figure 2. Relative expression of *GRα* and *BIM* mRNA in ALL cell lines upon DEX treatment. ALL cell lines, 697 and REH, were treated with 100 nM of DEX at the indicated time points and the mRNA levels of *GRα* and *BIM* were determined by RT-qPCR. (A) *GRα* mRNA expression in the 697 cell line. *P<0.05. (B) BIM mRNA expression in the 697 cell line. *P<0.05. (C) *GRα* mRNA expression in the REH cell line. (D) *BIM* mRNA expression in the REH cell line. The results are expressed as the percentage of positive mean values ± standard deviation (SD) for three independent experiments. DEX, dexamethasone; ALL, acute lymphoblastic leukemia; BIM, Bcl-2-interacting mediator of cell death; GR, glucocorticoid receptor.

GRα protein were downregulated in both 697DR (Fig. 6) and MB-YUDR cell lines (Fig. 7).

Expression of GR and BIM in DEX-resistant BCP-ALL cell lines by DEX treatment. RT-qPCR was performed to detect mRNA expression levels of *GRα* and *BIM* in DEX-resistant BCP-ALL cell lines in response to 100 nM DEX treatment. Although DEX treatment upregulated mRNA expression levels of *GRα* and *BIM* in the DEX-sensitive 697 cells in a time-dependent manner, mRNA expression levels of *GRα* and *BIM* were not altered by DEX treatment in 697DR cells (Fig. 8A and B).

Expression of miR-142-3p and miR-17~92 in DEX-resistant BCP-ALL cell lines. miR-142-3p regulates the expression of

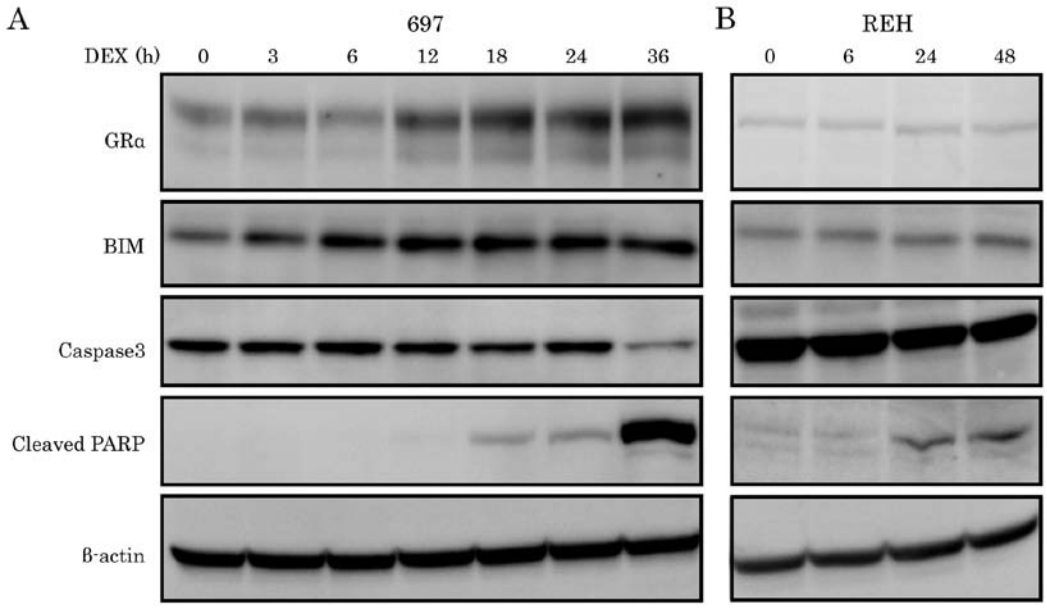

Figure 3. Protein expression levels of GRα, BIM, caspase-3 and cleaved PARP in ALL cell lines upon DEX treatment. ALL cell lines, (A) 697 and (B) REH, were treated with 100 nM of DEX at the indicated time points and the protein expression levels of GRα, BIM, caspase-3 and cleaved PARP were determined by western blotting. DEX, dexamethasone; ALL, acute lymphoblastic leukemia; BIM, Bcl-2-interacting mediator of cell death; GR, glucocorticoid receptor.

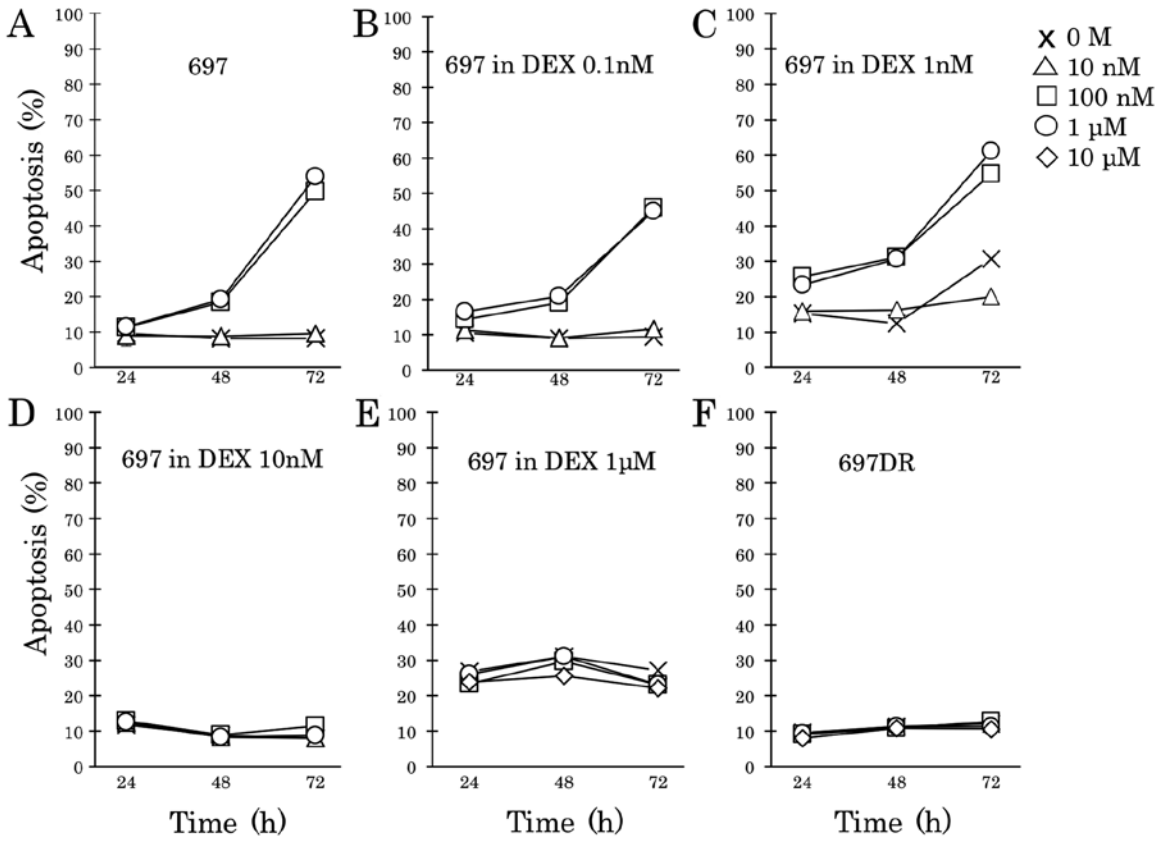

Figure 4. Development of DEX-resistant BCP-ALL cell lines. Aliquots of parental cells were seeded into 25 cm^2 culture bottles, and cultured in RPMI-1640 medium supplemented with 10% FBS and treated with increasing concentrations of DEX (from 0.1 nM to 1 μM). Fresh medium with DEX was changed every 48 h. Cells were transferred into new culture bottles every 7 days. We continued this process while observing cell death every day, and performing cell counting regularly by using Invitrogen Cell Counter. Cells were treated with increasing concentrations of DEX after two rounds of cell counting and showed a consistent increase in cell number. (A) Parental 697 cells were treated with 0 M, 10, 100 nM, 1 and 10 μM of DEX for 24, 48 and 72 h. Apoptotic progression was monitored via flow cytometric analysis of propidium iodide-stained nuclei. (B) 697 cells that grew stably in 0.1 nM DEX were treated with 0 M, 10, 100 nM, 1 and 10 μM of DEX for 24, 48 and 72 h. Apoptotic progression was monitored. (C) 697 cells that grew stably in 1 nM DEX were treated with 0 M, 10, 100 nM, 1 and 10 μM of DEX for 24, 48 and 72 h. Apoptotic progression was monitored. (D) 697 cells that grew stably in 10 nM DEX were treated with 0 M, 10, 100 nM, 1 and 10 μM of DEX for 24, 48 and 72 h. Apoptotic progression was monitored. (E) 697 cells that grew stably in 1 μM DEX were treated with 0 M, 10, 100 nM, 1 and 10 μM of DEX for 24, 48 and 72 h. Apoptotic progression was monitored. (F) 697DR cells were treated with 0 M, 10, 100 nM, 1 and 10 μM of DEX for 24, 48 and 72 h. Apoptotic progression was monitored. BCP-ALL, B-cell precursor acute lymphoblastic leukemia; DEX, dexamethasone.

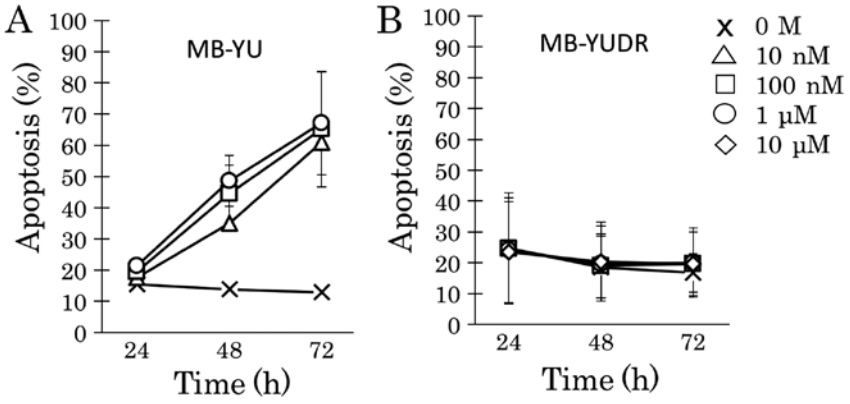

Figure 5. Evaluation of DEX-induced apoptosis in BCP-ALL cell lines. DEX-sensitive BCP-ALL cell line (A) MB-YU and acquired DEX-resistant BCP-ALL cell line (B) MB-YUDR, were treated with 0 M, 10, 100 nM, 1 and 10 μM DEX for 24, 48 and 72 h. Apoptotic progression was monitored via flow cytometric analysis of propidium iodine-stained nuclei. The results are expressed as the percentage of positive mean values ± standard deviation (SD) for three independent experiments. BCP-ALL, B-cell precursor acute lymphoblastic leukemia; DEX, dexamethasone.

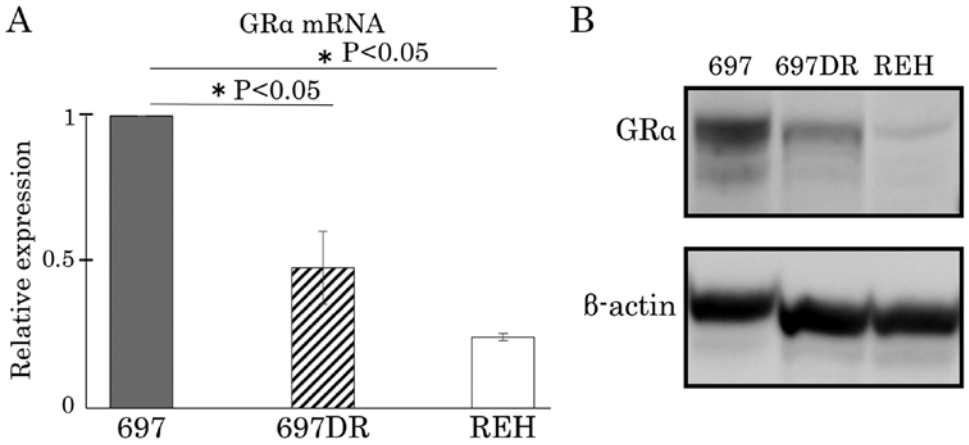

Figure 6. Expression of *GRα* mRNA and protein in 697, 697DR and REH cells. (A) The expression of *GRα* mRNA in 697, 697DR and REH cells was determined by RT-qPCR. The results are expressed as the percentage of positive mean values ± standard deviation (SD) for three independent experiments. *P<0.05. (B) GRα protein levels in 697, 697DR and REH cells were detected by western blotting. GR, glucocorticoid receptor.

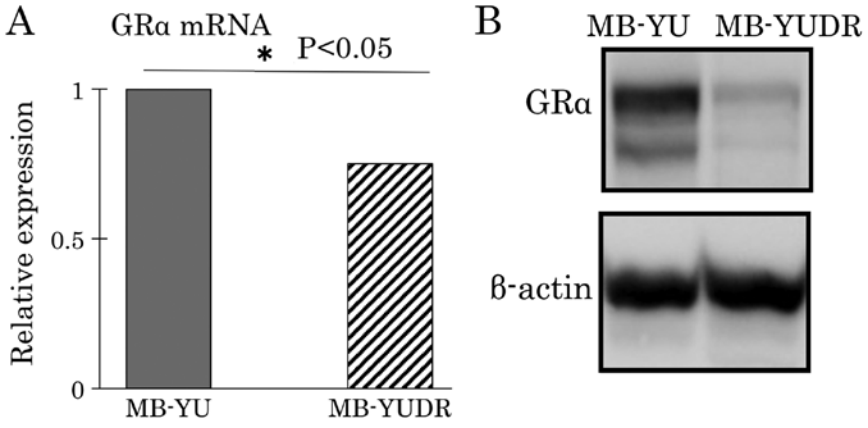

Figure 7. Expression of GRα mRNA and protein in MB-YU and MB-YUDR cells. (A) The expression of GRα mRNA in MB-YU and MB-YUDR cells was determined by RT-qPCR. The results are expressed as the percentage of positive mean values ± standard deviation (SD) for three independent experiments. *P<0.05. (B) The GRα protein levels in MB-YU and MB-YUDR cells were detected by western blotting. GR, glucocorticoid receptor.

GRα (14) and the expression level of miR-17~92 is an important regulator of GC-induced apoptosis (18). Expression levels of miR-142-3p and miR-17~92 were examined by RT-qPCR. miR-142-3p and miR-17~92 were upregulated in acquired DEX-resistant 697DR cells (Fig. 9A) and MB-YUDR cells (Fig. 9B). Although miR-17-5p, miR-18a-5p and miR-19a-3p

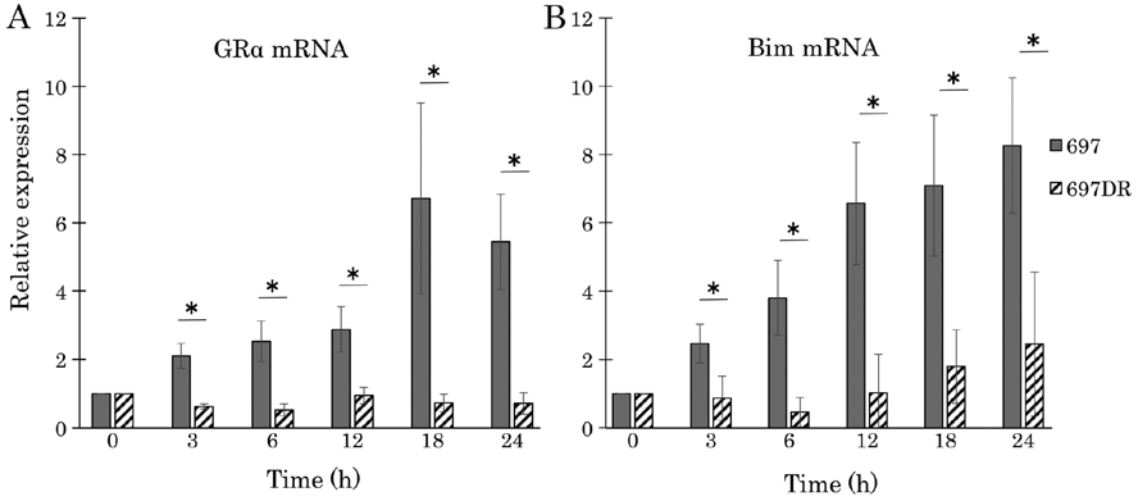

Figure 8. Relative expression of *GRα* and *BIM* mRNA in DEX-resistant BCP-ALL following DEX treatment. DEX-sensitive BCP-ALL 697 cells and acquired DEX-resistant BCP-ALL 697DR cells were treated with 100 nM of DEX at the indicated time points and the mRNA levels of *GRα* and *BIM* were determined by RT-qPCR. (A) *GRα* mRNA expression in 697 cells and 697DR cells. (B) *BIM* mRNA expression in 697 cells and 697DR cells. The results are expressed as the percentage of positive mean values ± standard deviation (SD) for three independent experiments. *P<0.05. BCP-ALL, B-cell precursor acute lymphoblastic leukemia; DEX, dexamethasone; BIM, Bcl-2-interacting mediator of cell death; GR, glucocorticoid receptor; DEX, dexamethasone.

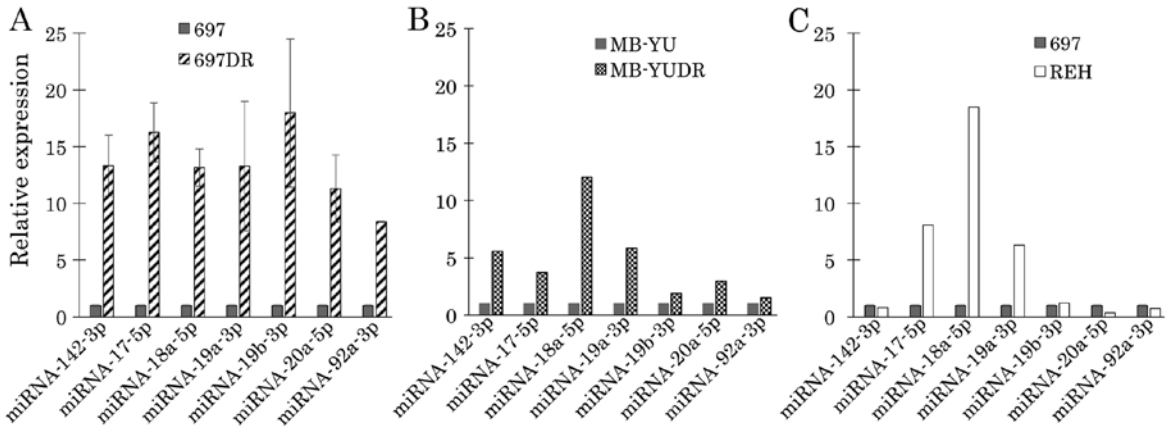

Figure 9. Expression of miR-142-3p and miR-17~92 cluster in 697, 697DR, MB-YU, MB-YUDR and REH cell lines. (A) The relative expression of miRNAs in 697 and 697DR cells was detected by RT-qPCR. (B) The relative expression of miRNAs in MB-YU and MB-YUDR cells was detected by RT-qPCR. (C) The relative expression of miRNAs in 697 and REH cells was detected by RT-qPCR. The results are expressed as the percentage of positive mean values ± standard deviation (SD) for three independent experiments.

were upregulated in DEX-resistant cell line REH, miR-142-3p, miR-19b-3p, miR-20a-5p and miR-92a-3p were not upregulated (Fig. 9C).

Discussion

Although the mechanisms regulating glucocorticoid (GC) resistance are being unraveled, resistance to GCs still remains a major hurdle in the treatment of B-cell precursor acute lymphoblastic leukemia (BCP-ALL). In the present study, it was demonstrated that GC-sensitive BCP-ALL cells exhibit upregulation of the GRα gene and protein expression in response to GC treatment. However, GRα mRNA and protein expression was downregulated in the GC-resistant cell line (REH) and dexamethasone (DEX)-resistant cell lines (697DR and MB-YUDR). It is controversial whether GC resistance occurs at the level of the GR (23). Previous research has shown that there was no significant difference in GR mRNA and protein expression when comparing GC-resistant and GC-sensitive leukemia cells (24,25). However, our observations are consistent with the notion that low cellular levels of functional GR in leukemia cells decreases sensitivity to GCs (26). Although GCs induce auto upregulation of GR expression in GC-sensitive ALL cells (27), GCs give rise to therapeutic resistance to themselves with high GR-β levels that cause an imbalance in the GR-α/β ratio (28). In addition to downregulated receptors, miRNA-mediated gene dysfunction may relate to GC resistance. miR-142-3p decreased GRα protein expression by directly targeting the 3'-untranslated region of *GRα* mRNA, leading to GC resistance (14). Furthermore, Lv *et al* found that the miR-142-3p inhibitor effectively reversed GC resistance to GC sensitivity, due to the resultant increase in GRα expression (14). Although miR-142-3p was found to affect the expression of GRα protein

but not *GRα* mRNA in Jurkat cells (14), both GRα mRNA as well as protein were decreased in our study. This disparity could be due to differences between T-ALL and BCP-ALL cells. miR-142-3p may regulate the expression of GRα at the transcriptional level in BCP-ALL. Although both GRα mRNA and protein were downregulated in DEX-resistant REH cells (Fig. 6), miR142-3p was not upregulated (Fig. 9B). It has been reported that DNA methylation status could affect GC-sensitivity (29) and mutation in the *GR* gene decreased sensitivity to GCs (30). Therefore, low cellular levels of GRα due to DNA methylation or *GR* gene mutations may result in GC resistance in REH cells.

Upregulation of BIM, a pro-apoptotic member of the B-cell lymphoma-2 family, is an important mediator of GC-induced apoptosis. It was recently identified that GR binding at the intronic region of the *BIM* gene enhances BIM transcription (11). We observed that BIM mRNA and protein were upregulated by DEX stimulation in the GC-sensitive ALL cell line. However, both GR and BIM were downregulated in GC-resistant ALL cell line. These findings support that the absence of GR binding at the BIM intronic region was associated with *BIM* gene silencing and DEX resistance (11). The miR-17~92 cluster, containing six individual miRNAs, has been strongly implicated in hematopoietic malignancies (31). Overexpression of miR-17~92 causes BIM reduction, resulting in the inhibition of induction of apoptosis (32). We observed upregulation of miR-17~92 in acquired DEX-resistant BCP-ALL cell lines (697DR and MB-YUDR). miR17-5p, miR18a-5p and miR19a-3p were upregulated in DEX-resistant REH cells. Our data are consistent, therefore, with a model in which DEX-resistance is mediated through the sequential upregulation of miR-17 and downregulation of BIM. GCs are known to downregulate miR-17~92 expression resulting in elevation of BIM mRNA and protein levels (18). Since the promotor of miR-17~92 is a prime target of GCs (19), its binding to the miR-17~92 promotor region might be altered in our DEX-resistant cells. The BET (bromodomain and extraterminal domain) proteins, such as BRD4, directly regulate miR-17~92 expression by binding to the miR-17~92 promoter (33). Therefore, the expression level of BET protein might be upregulated in DEX-resistant cells. This might be another mechanism of developing GC resistance, which needs further investigation.

Although intracellular levels of miR-142-3p and miR-17~92 cluster were increased in GC-resistant BCP-ALL cells, it is not known whether miR-142-3p and miR-17~92 cluster directly regulate GR expression and GC sensitivity. Thus, further study in overexpression and/or downregulation of these microRNAs should be carried out to confirm the role of miR-142-3p and miR-17~92 cluster in GC resistance. Our data may provide an additional predictive marker in BCP-ALL. A large cohort of patient samples should be analyzed together with the *in vivo* response to therapy.

There are several other microRNAs that have been shown to modulate GC sensitivity in lymphoid malignancies. Downregulation of miR-128b and miR-221 has been implicated in GC resistance (34). miR-182 functions in promoting resistance to GCs in lymphoblastic malignancies via negative regulation of FOXO3A (35). With increasing miRNA expression patterns emerging into the vision of researchers, they are worth more investigation as promising predictive biomarkers for chemotherapy effectiveness and disease prognosis.

In conclusion, GC resistance is associated with GR reduction and increased intracellular levels of miR-142-3p and miR-17~92 cluster. This is the first report to show that elevation of miR-142-3p and miR-17~92 plays an important role in acquired GC resistance of BCP-ALL. The potential of miR-142-3p and miR-17~92 as critical therapeutic targets for BCP-ALL requires further investigation.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

NS, YK and MH conceived and designed the study. NS performed the experiments and organized all data. NS, YK, RH, MM, TI, DN and MH analyzed and interpreted the data. NS, YK and MH wrote the paper. NS, YK, RH, MM, TI, DN and MH reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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