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Expression of sorcin predicts poor outcome in acute myeloid leukemia

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Abstract

Using a cDNA microarray 12 differentially expressed genes were identified in a multidrug resistant (MDR) cell line K562/A02. The differential expression of sorcin, which was one of the 12 genes, has been confirmed by Northern blot. To determine the clinical role of sorcin, we have measured its expression in leukemic blast cells of 65 acute myeloid leukemia (AML) patients by reverse transcriptase polymerase chain reaction (RT-PCR). Sorcin overexpression in AML patients was associated with poor clinical outcomes, the complete remission (CR) rate in sorcin− cases was significantly higher than that of sorcin+ cases ($P < 0.001$). Furthermore, sorcin expression in AML patients was positively correlated with mdr1 expression ($r = 0.841$, $P < 0.001$). Combination of sorcin and mdr1 was related to clinical outcome too, cases with sorcin−/mdr1− had best response to induction chemotherapy. Our results indicated that sorcin might be one of the factors that contributes to drug resistance of AML patients.

Keywords: AML; Sorcin; Multidrug resistance; cDNA microarray; RT-PCR

1. Introduction

Multidrug resistance (MDR) is a major obstacle in the chemotherapeutic treatment of cancers, particularly in acute myeloid leukemia (AML). AML lends itself as a model disease for the evaluation of the clinically relevant mechanism of resistance toward anticancer drugs [1,2]. One of the best-characterized drug-resistance mechanisms in AML is the drug efflux mediated by P-glycoprotein (Pgp). Pgp, encoded by *mdr1* gene, functions as an energy-dependent drug efflux pump for natural hydrophobic substrates including anticancer drugs [1,3]. Expression of mdr1 mRNA as well as Pgp have been shown to be associated with worse outcome and taken as an important prognostic indicator in AML [4,5]. But there is evidence suggesting that MDR involves more than Pgp, the reported incidence of mdr1 overexpression in clinically resistant leukemia shows considerable variation [6].

In AML, several other non-Pgp proteins may be associated with MDR phenotypes. One such protein is multidrug resistance protein (MRP). MRP is a member of adenosine triphosphate-binding cassette (ABC) superfamily and mediated resistance to a similar spectrum of anticancer drugs as Pgp. Although, MRP is highly expressed in 26% of AML samples, but this expression does not predict for outcome of induction chemotherapy or survival of the patients. So, investigations of the prognosis relevance of MRP in AML remain inconclusive [1–3,6–8]. Another protein contributes to MDR is lung resistance protein (LRP). LRP is overexpressed in Pgp-negative MDR cell lines, it was reported that LRP predicted for poor outcome in AML and appeared to be another clinically relevant drug resistant marker. LRP may confer resistance by altering drug transport between the cytoplasm and the nucleus [1–3]. But in another study no correlations were observed for LRP with complete remission rate and resistant disease [7]. So, the role of LRP is still under discussion.

Based on the data mentioned above, it is accepted that drug resistance in AML is multifactorial, and we should

Abbreviations: ABC, adenosine triphosphate-binding cassette; AML, acute myeloid leukemia; BCRP, breast cancer resistance protein; CR, complete remission; CsA, cyclosporine; GST, glutathione-*S*-transferase; LRP, lung resistance protein; MDR, multidrug resistance; M-MLV, Moloney murine leukemia virus reverse transcriptase; MRP, multidrug resistance protein; NR, non-remission; Pgp, P-glycoprotein; RT-PCR, reverse transcriptase polymerase chain reaction; sorcin, soluble resistance-related calcium-binding protein; TOP II, topoisomerase II

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combine mdr1/Pgp with other MDR markers to improve the predictions of drug resistant AML patients. To understand the mechanism of MDR, a human leukemic MDR cell line K562/A02 was established by stepwise increase of concentration of adrimycin in medium of K562 cells [9]. In an attempt to investigate new markers of MDR, we selected a cDNA microarray containing 1176 human genes to analyze the mRNAs of an adriamycin-selected MDR cell line K562/A02 and found 12 genes involving it [10]. One of the genes is sorcin, which is significantly upregulated in K562/A02 cells. Sorcin (also named VP19, CP22) gene is amplified in most of the MDR cell lines, but the role of sorcin in MDR is unknown. To determine whether sorcin is a clinically relevant drug resistance gene in AML, we have studied sorcin expression in K562/A02 cells and 65 AML samples, and analyzed its relationship to mdr1 expression and to clinically drug resistance. Here, we report the results of this study.

2. Materials and methods

2.1. Cell lines

K562/A02 is a MDR cell line induced with adrimycin by our research group. It shows resistance not only to adrimycin but also to other structurally unrelated lipophilic cytotoxic drugs, including harringtonine, vincristine, amsacrine, etc. [9]. The cells were cultured in RPMI1640 (Life Technologies) supplemented with 10% fetal bovine serum (Hyclone) and 1 μg/ml adrimycin at 37 °C in a humidified atmosphere containing 5% CO_2 (carbon dioxide). Their parental cells K562 were cultured in the same condition without adrimycin.

2.2. Patient characteristics

Bone marrow aspirates were collected from 65 AML patients with the approval of the Blood Diseases Hospital, Chinese Academy of Medical Sciences. The median age of the patients was 35.4 years (range 6–76); 30 were male, 35 were female; the French–American–British classification [11] were as follows: one was M0, one was M1, 19 were M2, 15 were M3, 14 were M4, nine were M5, two were M6, one was M7, three were HAL. Of these 65 patients, 38 were studied de novo, 14 were studied when achieving complete remission (CR) and 13 were studied at the time of relapse according to standard criteria [12,13]. In induction phase, all patients received standard induction chemotherapy protocols. Treatment consisted of harringtonine 4 mg/m^2 daily on days 1–7, daunorubicin 45 mg/m^2 daily on days 1–3 and cytarabine 200 mg/m^2 daily on days 1–7 (HAD) in 38 patients; 14 patients were given consolidation therapy with daunorubicin plus cytarabine (DA) or etoposide plus cytarabine (EA); 13 patients were treated with idarubicin plus cytarabine (IA) or mitoxamtrone plus cytarabine (MA).

Normal bone marrow aspirates from 10 iron deficiency and two thrombocytopenia and normal peripheral blood from 15 healthy volunteers were used as control. The mononuclear cells were purified by a discontinuous Ficoll-Hypaque density gradient as reported earlier [14].

2.3. RNA Preparation

Exponentially growing cells of K562 and K562/A02 were collected, RNAs were harvested using Trizol reagent (Life Technologies Inc.), as specified by the manufacturer. Then total RNAs were treated with DNase I to exclude DNA contaminations.

2.4. Labeling, hybridization and scanning of microarray

The labeling and hybridization procedures were conducted as the Atlas cDNA Expression Arrays User Manual (PT3140-1, Clontech). Briefly, total RNAs were harvested and converted to ^{32}P-labeled cDNA probes using Moloney murine leukemia virus reverse transcriptase (M-MLV) and [α-^{32}P]dATP with 10× CDS Primer Mix. The hybridization was performed in a mixture of ^{32}P-labeled cDNA probes and 5 ml of prewarmed (at 68 °C) ExpressHyb solution (containing 100 μg/ml heat-denatured sheared salmon sperm DNA) with microarrays (Clontech). Hybridization was conducted overnight with continuous agitation at 68 °C. The filters were washed three times in 200 ml of prewarmed wash solution 1 (2% × SSC, 1% SDS) and one time in prewarmed wash solution 2 (0.1% × SSC, 0.5% SDS) for 30 min with continuous agitation at 68 °C. Finally, the filters were exposed to Kodak BioMax MS X-ray films with an intensifying screen at −70 °C in a plastic wrap.

2.5. Northern blot analysis

Northern blot analyses of differentially expressed genes were performed on K562/A02 cells and their parental cells K562. Total RNA (15 μg per lane) was electrophoresed on a 1% agarose/2.2 M formaldehyole gel and capillary blotted onto a Hybond nylon membrane (Pharmacia Biotech) [15]. Blots were probed overnight at 42 °C with α-^{32}P-labeled probes. Membranes were washed twice in 2% × SSC, 0.1% SDS at room temperature for 5 min each; once in 1% × SSC, 0.1% SDS at 65 °C for 15 min; twice in 0.1% × SSC, 0.1% SDS at 65 °C for 10 min each. Blots were exposed to XAR-5 film (Eastman Kodak) at −80 °C for 12–48 h. The blot labeled with random prime-labeled β-actin was as a control equal loading of RNA. Probe templates were obtained by PCR amplification of the cDNA clones and labeled with α-^{32}P using Random Primer Labeling Kit (Stratagene).

2.6. RT-PCR

Total cellular RNA was isolated from AML blast cells and leukemic cell line K562 and its MDR cell line K562/A02.

Table 1
The primer sequences and conditions for PCR of sorcin, mdr1 and β-actin

Transcripts	Primer sequences (5′–3′)	PCR conditions	Fragment length (bp)
Sorcin	ggt, gat, ctt, tcc, att, ggt, g; tcc, gct, gta, tgg, tta, ctt, tg	30 cycles of 94 °C for 1 min 10 s, 55 °C for 1 min and 72 °C for 1 min	367
mdr1	ccc, atc, att, gca, ata, gca, gg; gtt, caa, act, tct, gct, cct, ca	30 cycles of 94 °C for 40 s, 58 °C for 1 min and 72 °C for 1 min	157
β-Actin	tga, cgg, ggt, cac, cca, cac, tgt, gcc, cat, cta; cta, gaa, gca, ttt, gcg, gtg, gac, gat, gga, ggg	30 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min 10 s	661

RT reaction was prepared in a mixture containing 2 μg of RNAs, 10 mM DTT, 250 mM dNTPs, 0.5 μg/μl RNasin, 2.5 mg/ml random hexamers as primers and 5 μg/μl M-MLV transcriptase and its supplied reaction buffer. About 4 μl of the resulting cDNA was added per 50 μl of PCR mixture containing 3U of Taq polymerase and 5 μl of the reaction buffer, 200 mol/l dNTP and 0.6 mol/l of each 5′ and 3′ primers to sorcin, mdr1 and β-actin, respectively. The primer pairs and conditions for polymerase chain reaction (PCR) of sorcin, mdr1 and β-actin were seen in Table 1. The amplified products were electrophoresed on a 2% agarose gel, visualized by ethidium bromide staining and quantitated by densitometry (Electrophoresis Digital Analysis System and Kodak Digital Science 1D Image Analysis Software, USA).

2.7. Level of sorcin and mdr1 mRNA expression

The level of sorcin expression measured by reverse transcriptase polymerase chain reaction (RT-PCR) was quantified by determining the density of PCR products separated in an ethidium bromide-stained 2% agarose gel. The variations between samples in the cDNA synthesis were normalized by their relative quantities of β-actin. The normalized yield of sorcin relative to β-actin was than compared with that of K562/A02 cells, which were defined as one arbitrary unit. If the normalized expression of sorcin $\geq$1 unit, it was taken as positive expression arbitrarily; if $<$1 unit, it was taken as negative expression. The situation of mdr1 was the same as that of sorcin.

2.8. Statistical analysis

Correlation between sorcin and mdr1, sorcin and clinical outcome were assessed by Chi-squared tests. The SPSS10.0 was used for calculations.

3. Result

3.1. cDNA microarray analysis of differentially expressed genes between K562/A02 and K562 cells

Human 1.2 II Atlas array, which contains 1.176 cDNAs of oncogenes, tumor suppressors, apoptosis related genes, DNA synthesis, transcription factors, receptors, cell–cell communication and cytoskeleton, was used to examine relative gene expression between K562/A02 and K562. Comparative expression analysis revealed 12 genes displaying altered signal intensity (Table 2). Of the 12 differentially expressed genes, seven were decreased in expression in K562/A02, whereas five were increased in K562/A02.

3.2. Northern blot analysis of sorcin and mdr1 expression in K562/A02 cells

To confirm differential gene expression in K562/A02, cDNA or plasmid inserts amplified PCR fragments of sorcin and mdr1 were used to probe total RNA from K562/A02 cells and their parental cells, respectively. Using North-

Table 2
The differentially expressed genes between K562/A02 and K562 cells analyzed by cDNA microarray

Gene name	Intensity		Ratio[a]
	K562/A02	K562	
CCAAT/enhancer binding protein gamma (CEBPG)	14	21	0.3256
Breakpoint cluster region protein (BCR)	31	38	6.2000
EG-Golgi intermediate compartment 53 kDa protein	22	29	5.5000
Apolipoprotein E precursor (APOE)	4	11	0.0678
Steroid 5α reductase 1 (SRD5A1)	13	20	0.3940
Dihydrodiol dehydrogenase + chlordecone reductase	10	17	0.3215
60S ribosomal protein L22 (RPL22)	70	77	0.4698
Ras GTPase-activating-like protein IQGAP1	35	42	3.1818
Sorcin 22 kDa protein (SRI); CP-22	107	114	5.9444
Prothrombin precursor	19	26	0.1159
Lipoprotein-associated coagulation inhibitor	54	61	0.1908
Neurofilament triplet H protein (200 kDa neurofilament protein; NF-H)	22	29	$+\infty$

[a] Ratio is calculated as the intensity of K562/A02 divided by that of K562.

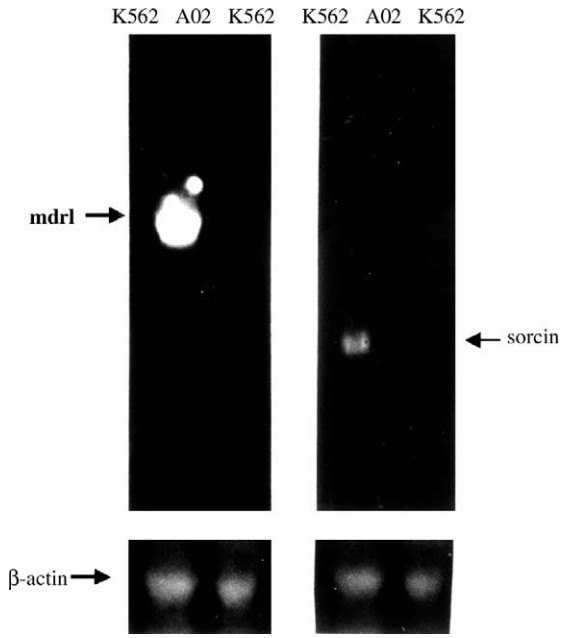

Fig. 1. The expression of sorcin and mdr1 mRNA in K562/A02 and the parental cells identified with Northern blot assay, β-actin as internal control.

ern blot analysis, we found both of sorcin and mdr1 were overexpressed only in K562/A02 cells. β-Actin fragments amplified from cDNA were used to probe total RNA from both of the two cell lines as an internal control(Fig. 1).

3.3. Expression of sorcin mRNA in mononuclear cell samples from AML patients at diagnosis

A total of 27 normal mononuclear cell samples and 65 AML bone marrow aspirates were analyzed by RT-PCR. The sorcin products is 367 bp in size and β-actin housekeeping gene product is 661 bp (Fig. 2). In 27 normal blood specimens no positive expression of sorcin was observed. In the mononuclear cell fractions isolated from 65 bone marrow aspirates 23 (35.4%) positively expressed sorcin ($P < 0.05$), including 11 (28.9%) of de novo AML specimens (AML-d), 3 (21.4%) of complete remission AML specimens (AML-c) and 9 (69.2%) of relapsed AML specimens (AML-r). This suggested AML-r cases showed a significantly higher frequency than AML-d and AML-c cases, $P < 0.01$ and $P < 0.05$, respectively (Table 3). Furthermore, significant heterogeneity of sorcin expression was observed among the FAB categories, as shown in Table 4. Sorcin was positively expressed in M2 (36.8%), M3 (20.0%), M4 (28.6%) and M5 (88.9%) cases. We observed a correlation of sorcin expression with M5 subtype ($P = 0.001$ and 0.006 represent comparison of M5 subtype versus M3 subtype and M4 subtype, respectively). No sorcin expression was observed in M0, M1, M6 and M7 cases partly because of not enough samples.

Table 3
RT-PCR analysis of the sorcin expression in AML samples at diagnosis[a]

Type of samples	Sorcin expression		Total
	Positive	Negative	
AML-d	11 (28.9)	27 (71.1)	38
AML-c	3 (21.4)	11 (78.6)	14
AML-r	9 (69.2)[b]	4 (30.8)	13
Normal samples	0 (0)	27 (100)	27

AML-d is de novo AML patients, AML-c is complete remission AML patients, AML-r is relapsed AML patients.

[a] Values given in parenthesis are in percent.

[b] The positive expression percentage in AML-r is significantly higher than those in AML-d and AML-c, $P < 0.01$ and $P < 0.05$, respectively.

Table 4
Correlation of sorcin expression with FAB subtype in AML

	M0	M1	M2	M3	M4	M5	M6	M7	HAL
Patients	1	1	19	15	14	9	2	1	3
Sorcin expression	0	0	7	3	4	8	0	0	0
Percentage (%)			36.8	20.0	28.6	88.9			

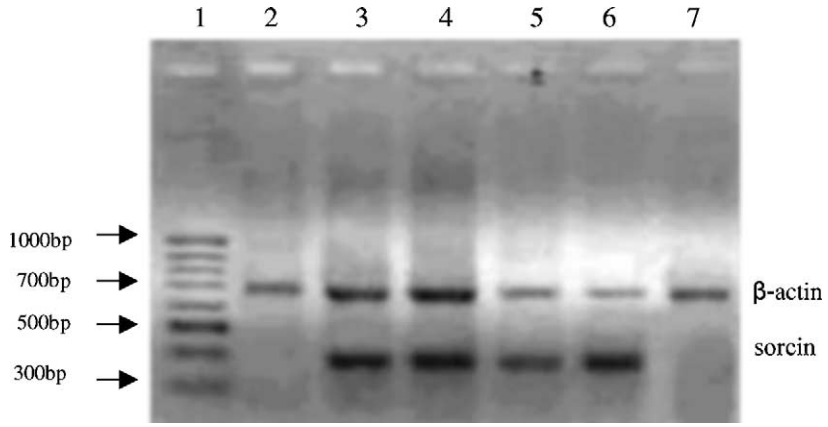

Fig. 2. RT-PCR demonstrative analysis of sorcin expression (367 bp) and β-actin internal control (661 bp). Lane 1: marker; lanes 2 and 3: K562 and K562/A02 cells; lanes 4, 5 and 6: AML-d, AML-c and AML-r; lane 7: healthy volunteers.

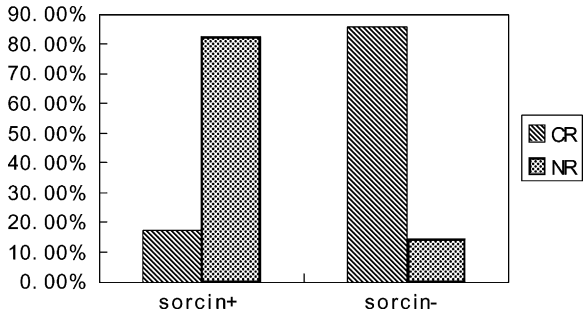

Fig. 3. The relationship of sorcin expression to clinical outcome of induction chemotherapy. After induction phase, 40 of 65 patients acquired CR, the CR rate in sorcin− group (85.71%) was significantly higher than that of sorcin+ group (17.39, $P < 0.001$).

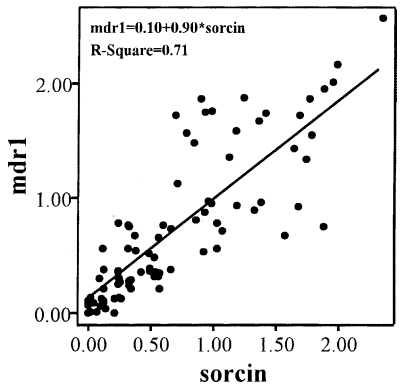

Fig. 4. The correlation between sorcin expression and mdr1 expression by RT-PCR in 65 AML patients, 12 non-AML patients and 15 healthy volunteers. There was a significant positive correlation between sorcin expression and mdr1 expression ($r = +0.841$, $P < 0.001$). The *x*-axis and *y*-axis represented the ratio of sorcin/β-actin and mdr1/β-actin, respectively.

3.4. Sorcin expression and clinical outcome of induction chemotherapy

All the 65 AML patients received chemotherapy and 40 patients acquired CR, 25 patients remained non-remission (NR). The patients with NR showed drug resistance. In order to analyze the relationship between sorcin expression and clinical outcome, we divided the 65 patients into two groups, sorcin+ group (23 cases) and sorcin− group (42 cases; Fig. 3). In sorcin+ group, 4 of 23 patients acquired CR, CR rate was 17.39%; but in sorcin− group 36 cases acquired CR, CR rate was 85.71%, which was significantly higher than that of soricn+ group ($P < 0.001$). This suggested that patients with sorcin− showed better response to induction chemotherapy.

3.5. The relationship of sorcin expression with mdr1 expression

We also detected the expression of mdr1 in 65 AML patients, 12 non-AML patients and 15 healthy volunteers by RT-PCR and found significant positive correlation between sorcin expression and mdr1 expression ($r = 0.841$, $P < 0.001$; Fig. 4).

3.6. The relationship of sorcin/mdr1 status with clinical outcome

Finally, we analyzed the combination of sorcin and mdr1 status in relation to clinical outcome (Table 5). We divided the 65 AML patients into four groups: sorcin+/mdr1+, sorcin+/mdr1−, sorcin−/mdr1+ and sorcin−/mdr1−. In sorcin+/mdr1+ group 1 (7.1%) of 14 patients showed CR, and the CR rate was lower than that of sorcin+/mdr1− group (44.4%) and sorcin−/mdr1+ group (42.8%), but $P > 0.05$. In sorcin−/mdr1− group 3 response to induction chemotherapy was best, 32 (91.4%) of 35 patients showed CR, and the CR rate was significantly higher than that in sorcin+/mdr1+ group ($P < 0.001$). Furthermore, we have observed that sorcin/mdr1 status was correlated with the time to relapse and overall survival. The sorcin−/mdr1− group had a significantly longer time to relapse and overall survival than sorcin+/mdr1+ group ($P < 0.001$).

4. Discussion

MDR is a well-defined phenomenon of cross-resistance of tumor cells to several structurally unrelated chemotherapeutic agents after exposure to a single cytotoxic drug. Several mechanisms have been found to contribute to MDR, including mdr1/Pgp, MRP, LRP, breast cancer resistance protein (BCRP), altered expression of topoisomerase II (TOP II), glutathione-*S*-transferase (GST) and hormone receptors, etc.

1981, Meyers and Biedler [16] firstly identified a low molecular weight protein in a vincristine-resistant Chinese

Table 5
The expression of sorcin and mdr1 in relation to clinical outcome of induction chemotherapy[a]

	Sorcin+/mdr1+	Sorcin+/mdr1−	Sorcin−/mdr1+	Sorcin−/mdr1−
NR	13 (92.9)	5 (55.6)	4 (57.2)	3 (8.6)
CR	1 (7.1)	4 (44.4)	3 (42.8)	32 (91.4)[c]
Time to relapse (month)	9 ± 4.2	17 ± 3.0[b]	16 ± 4.7[c]	30 ± 10.4[c]
Overall survival (day)	86 ± 28.8	156 ± 171.0	168 ± 148.9	780 ± 413.7[c]

[a] Values given in parenthesis are in percentage.
[b] Sorcin+/mdr1− vs. sorcin+/mdr1+ group, $P < 0.05$.
[c] Versus sorcin+/mdr1+ group, $P < 0.001$.

hamster lung cell line DC-3F/VCRd-5L and named it sorcin, soluble resistance-related calcium-binding protein. Sorcin is a cytosolic protein of 22 kDa, which has four typical "E–F hand" structures of calcium-binding sites. More than 50% of the MDR sublines, selected with a wide variety of drugs, overproduced sorcin, including colchicines, actinomycin D, taxol, vinblastine, etoposide, teniposide and adrimycin. These resistant sublines were derived from cells of human, hamster and mouse origin. As long as these cells remains an MDR phenotype, sorcin levels usually remains high [17]. So, sorcin was thought to be one of the factors contributing to MDR. But not all MDR cells overproduce sorcin or amplify the gene, even in the sorcin-overproducing MDR cells the amounts of sorcin was not in direct proportion to the degree of resistance [18,19]. When transfected of human sorcin cDNA purified from HOB1/VCR1.0 cells into parental HOB1 lymphoma cells, no significant MDR phenotype was observed [20]. Some researchers thought sorcin was overexpressed only temporally in the development of MDR and called it "passenger gene", the amplification of sorcin genes may be accidental consequences resulting from the proximity to those genes of Pgp [21,22].

K562/A02 has been established for 10 years and still shows stable MDR phenotype [9,10]. Northern blot analysis revealed both of sorcin and mdr1 were overexpressed in K562/A02. Lee [15] established a series of MDR cell lines with different degree of resistance to vincristine: HOB1/VCR0.01, HOB1/VCR0.1, HOB1/VCR0.5, HOB1/VCR0.75 and HOB1/VCR1.0. mdr1 was amplified increasingly in these cell lines and reached plateau amplification in HOB1/VCR0.5 cells. But sorcin was amplified only in HOB1/VCR1.0 cells. This suggested that sorcin gene was not amplified secondarily to *mdr1* gene, but under the pressure of high concentration of vincristine. It suggests that sorcin may not be a "passenger gene", but might contribute to MDR by direct or indirect interaction with Pgp and affect its function.

To investigate the clinical significance of sorcin in AML patients, we have detected the expression of sorcin in leukemic cell lines and mononuclear cells from 65 AML patients by Northern Blot and RT-PCR. We found the relapsed cases showed higher frequency (69.2%) than de novo cases and CR cases at diagnosis ($P < 0.01$ and <0.05, respectively). Interestingly, the M5 AML cases in this study showed the highest frequency of sorcin expression among AML subtypes, the significance and mechanism of the high frequency sorcin expression in M5 subtype remains to be determined. Moreover, we found significant positive correlations between sorcin expression and clinical outcome, the CR rate in sorcin− cases (85.71%) was significantly higher than that of sorcin+ cases (17.39%; $P < 0.001$). According to standard criteria [13], the bone marrows of the patients who achieved CR contained less than 5% blast cells. Our results suggested that sorcin be one of the factors contribute to drug resistance of AML patients. Furthermore, we have studied the correlation between sorcin and mdr1 in AML patients and found that the expression of sorcin and mdr1 are significantly positively correlated ($r = 0.841$, $P < 0.001$). It is also reported that sorcin always co-amplifies with Pgp genes [18,19,22]. These observations suggest that sorcin may be involved in MDR as a co-factor of mdr1/Pgp. As a co-factor of Pgp, the role of sorcin in MDR is speculated as below [19]: Pgp is phosphorylated, and the phosphorylation may be controlled in part by phosphatase. Phosphatase catalyzes Pgp dephosphorylation. While the activity of phosphatase is mediated by cellular calcium. Sorcin, as a calcium-binding protein, once overexpressed, binds at least 10% of the total cellular calcium which results in the decreasing of cellular calcium. The activity of phosphatase is inhibited, the phosphorylation of Pgp increase and more cellular drugs will be pumped out. This hypothesis remains to be confirmed.

Furthermore, we have studied sorcin expression in other MDR tumor cell lines and observed interesting results. In leukemic MDR cell line HL60/ADR, sorcin was positively expressed, but in its parental cell line HL60, no sorcin expression could be observed just like the results observed from K562/A02 and K562 cells. While in solid MDR tumor cells KB_{V200} and the parental KB cells sorcin were not expressed in both of them. From another two pairs of solid tumor cells, we observed sorcin expression, but no significantly differential expressions could be observed from MDR cells and their parental cells, such as $A549^{DDP}$ and A549, MCF-7/ADR and MCF-7 (data not shown). It seems that sorcin is a specific MDR indicator of AML.

Drug resistance in AML is multifactorial. The prognostic value of a single marker is limited. Combination of several markers may improve the sensitivity and specificity of determination. From our results, we found that prognosis of AML patients is best in the absence and worst in the presence of sorcin. This suggested that sorcin has prognostic relevance in AML. Sorcin is an independent marker or a co-marker of mdr1.

Knowledge of the clinically active mechanisms of drug resistance might result in new treatment strategies. At present there are several MDR modulators in different stages of clinical trials, such as verapamil, cyclosporine (CsA). Most of them reverse Pgp-mediated MDR [23]. But drug resistance in AML is multifactorial, the blockade to a single mechanism might be insufficient for important clinical outcome. Our results warrant the pharmaceutical development of resistance modifiers of sorcin function. It 1s reasonable to take sorcin as a target to design MDR modifiers which will prove a clinical reversal in AML. Combination of modifiers target Pgp with modifiers target sorcin will be more effective for clinical applications.

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