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Inhibitory Effect of Curcumin on *WT1* Gene Expression in Patient Leukemic Cells

Songyot Anuchapreeda, Pornngarm Limtrakul[1], Pattra Thanarattanakorn[2], Somjai Sittipreechacharn[2], and Prasit Chanarat

Division of Clinical Microscopy, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand, [1]Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand, and [2]Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

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Leukemias are common worldwide. Wilms'tumor1 (WT1) protein is highly expressed in leukemic blast cells of myeloid and lymphoid origin. Thus, WT1 mRNA serves as a tumor marker for leukemias detection and monitoring disease progression. Curcumin is well known for its anticancer property. The objective of this study was to investigate the effect of curcumin on *WT1* gene expression in patient leukemic cells. The leukemic cells were collected from 70 childhood leukemia patients admitted at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand, in the period July 2003 to February 2005. There were 58 cases of acute lymphoblastic leukemia (ALL), 10 cases of acute myeloblastic leukemia (AML), and 2 cases of chronic myelocytic leukemia (CML). There were 41 males and 29 females ranging from 1 to 15 years old. Leukemic cells were cultured in the presence or absence of 10 mM curcumin for 48 h. WT1 mRNA levels were determined by RT-PCR. The result showed that curcumin reduced *WT1* gene expression in the cells from 35 patients (50%). It affected the *WT1* gene expression in 4 of 8 relapsed cases (50%), 12 of 24 cases of drug maintenance (50%), 7 of 16 cases of completed treatment (44%), and 12 of 22 cases of new patients (54%). The basal expression levels of *WT1* gene in leukemic patient cells as compared to that of K562 cells were classified as low level (1-20%) in 6 of 20 cases (30%), medium level (21-60%) in 12 of 21 cases (57%), and high level (61-100%) in 17 of 23 cases (74%). In summary, curcumin decreased WT1 mRNA in patient leukemic cells. Thus, curcumin treatment may provide a lead for clinical treatment in leukemic patients in the future.

Key words: Curcumin, WT1, Leukemic patient cells, K562 Cell line

INTRODUCTION

Leukemias comprise a group of clonal diseases characterized by an accumulation of abnormal blood cells which are thought to derive from a single cell in the marrow that has undergone a genetic alteration. Leukemia is the most common childhood cancer. The highest incidence of leukemia in Thailand is in the North region, especially in Lampang province (Age-standardized world incidence rate (ASR) = 6.4 in males and ASR = 3.5 in females) in the years 1995 to 1997. In Chiang Mai province, the ASRs were 4.0 in males and 3.8 in females in the same years (Sriplung *et al.*, 2003). From data on leukemia patients in Maharaj Nakorn Chiang Mai Hospital in the period 2000-2002, there were 603 leukemia patients (144 childhood cases, 79 males and 65 females; and 459 adult cases, 255 males and 204 females) (Lorvidhaya *et al.*, 2002). Malignant transformation occurs as a result of the accumulation of genetic mutation in cellular genes. Some cases of mutations in oncogenes have provided useful molecular markers for monitoring the course of disease during treatment. The abl translocation in chronic myelogenous leukemia is a good example. The detection of overexpression in specific oncogenes or tumor suppressor genes provides information that is useful in the diagnosis of leukemia and prognosis of the disease. The overexpression of Wilms' tumor (WT1)

Correspondence to: Songyot Anuchapreeda, Division of Clinical Microscopy, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand
Tel: 66-53-949237, Fax: 66-53-945066
E-mail: songyot@chiangmai.ac.th

protein in leukemia is a good example. WT1 is expressed in stem cells of the bone marrow, but not in normal mature blood cells (Fraizer *et al.*, 1995; Patmasiriwat *et al.*, 1996). Many previous studies demonstrated that *WT1* gene is highly expressed in leukemic blast cells of myeloid and lymphoid origin, and thus WT1 messenger RNA provides a novel tumor marker for detection of minimal residual disease of leukemias and for monitoring disease progression of myelodysplasyic syndromes. It had been demonstrated before that the *WT1* gene is expressed in the leukemic cell lines K562 and HL60, and that differentiation of these cells in culture is accompanied by downregulation of WT1 protein levels (Phelan *et al.*, 1994; Sekiya *et al.*, 1994). K562 cells, which were derived from CML blastic crisis and expressed high levels of WT1, had been treated with nocodazole (40 ng/mL) to arrest synchronously the cell cycle (Yamagami *et al.*, 1996).

The *WT1* gene is defined as a tumor suppressor gene in childhood renal tumor. However, the wild-type *WT1* gene is highly expressed in leukemic blast cells. The *WT1* gene is highly expressed in various types of leukemias [acute myeloblastic leukemia (AML), acute lymphoblastic leukemia (ALL) and chronic myelocytic leukemia (CML)]. Miwa *et al.* (1992) and Miyagi *et al.* (1993) examined *WT1* gene expression in leukemias using Northern blot analysis and detected *WT1* gene expression in some cases of AML, ALL, and CML in the accelerated phase or blast crisis. Inoue *et al.* (1994) provided a new insight into the significance of *WT1* gene expression in leukemias by quantifying the expression levels of *WT1* gene by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). In all leukemia samples examined, including AML, ALL, and CML, significant levels of *WT1* gene expression were found, and the average levels were approximately 1,000 and 100,000 times higher than those in normal bone marrow or peripheral blood cells, respectively (Sugiyama *et al.*, 2001). Moreover, Bergmann *et al.* (1997) reported a correlation between the WT1 mRNA levels and prognosis. Taken together, all these findings demonstrate that the WT1 mRNA is a novel tumor marker in leukemic blast cells of almost all leukemias and its expression level is a new prognostic factor for acute leukemia.

The function of WT1 protein, which is a zinc finger protein having domains characteristic of transcription factors, is not well understood. The level of expression is highest in leukemias with immature phenotypes (Miwa *et al.*, 1992; Miyagi *et al.*, 1993; Inoue *et al.*, 1994). Expression of WT1 is downregulated during differentiation of leukemic cell lines. So it appears to play an important role in cell proliferation of blast cells.

Curcuminoids are natural phenolic coloring compounds found in rhizomes of *Curcuma longa* Linn, a member of Zingiberaceae or ginger family, commonly called turmeric. Curcuminoid content in turmeric varies from 1 to 5% of fresh turmeric rhizome, and has been identified as the major yellow pigment in turmeric. It has been widely used as a spice, to color cheese and butter, as a cosmetic, and in some medicinal preparations (Govindarajan, 1990; Ammon and Wahl, 1991). Curcuminoids include curcumin (curcumin I), demethoxycurcumin (curcumin II), and bisdemethoxycurcumin (curcumin III). All commercial curcuminoids sold as "curcumin" (Sigma-Aldrich, ICN, GNC, etc.) are mixtures of the three curcuminoids. Curcumin has a wide range of biological and pharmacological activities including antioxidant (Kunchandy and Rao, 1990; Soudamini and Kuttan, 1989; Kuo *et al.*, 1996) and anti-inflammatory properties (Ammon and Wahl, 1991), anti-mutagenic activity *in vitro* (Nagabhushan *et al.*, 1987), anti-carcinogenic effects (Rao *et al.*, 1995; Limtrakul, *et al.*, 1997, 2001), hypocholesterolemic effects in rats (Rao *et al.*, 1970), hypoglycemic effects in humans (Srinivasan, 1972), and an MDR modulator (Anuchapreeda *et al.*, 2002). The safety of curcumin has been studied in various animal models (Qureshi *et al.*, 1992), and it is clear that turmeric is not toxic even at high doses in laboratory animals. A single feeding of a 30 percent turmeric diet to rat did not produce any toxic effects. In a 24 h acute toxicity study, mice were fed dosages of 0.5, 1.0, and 3.0 g/kg of turmeric extract. There was no increase in mortality compared to controls in either study. A 90 day treatment with turmeric extract resulted in no significant weight gain (Shankar *et al.*, 1980).

Due to its wide range of biological and pharmacological effects and lack of toxicity in animal models, curcumin was selected for study in leukemia. This paper aims to examine the effect of curcumin on *WT1* gene expression in patient leukemic cells.

MATERIALS AND METHODS

Materials

Commercial grade curcumin (77% curcumin, 17% demethoxycurcumin and 3% bisdemethoxycurcumin), MTT dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide), and DMSO (dimethylsulfoxide) were purchased from Sigma-Aldrich (St Louis, MO, U.S.A.). RPMI 1640, SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA polymerase reagent, TRIzol® reagent, Penicillin-Streptomycin, L-glutamine, and primers were purchased from Invitrogen' Life Technology (Carlsbad, CA, U.S.A.).

Cell line and patient leukemic cells culture conditions

The erythroid leukemic cell line (K562) was a generous gift from Dr. Chaisuree Supawilai (Research Institute for Health Sciences, Chiang Mai, Thailand). This cell line was

cultured in RPMI 1640 medium containing 10% fetal calf serum, 1 mM L-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin. This cell line was maintained in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37°C. When the cells reached confluency, they were harvested and plated for consequent passages or for curcumin treatments.

The patient leukemic cells were collected from bone marrow (BM) of 70 leukemia patients at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. There were 58 cases of acute lymphoblastic leukemia (ALL), 10 cases of acute myeloblastic leukemia (AML), and 2 case of chronic myelocytic leukemia (CML). There were 41 males and 29 females from 1 to 15 years old. Each cell type of leukemia was identified by French-American-British (FAB) Cooperation Group classification. The state of treatment of leukemia patients was divided into four classes: completed treatment; relapsed case; drug maintenance; and new case. Leukemic cells were washed twice with phosphate-buffered saline (PBS). After that, red blood cells were lysed by hypotonic buffer solution. Cells were cultured in completed RPMI 1640 medium in the presence and absence of 10 μM curcumin for 48 h in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37°C. K562 cell line was used as a positive control cell in this experiment because of its high expression level of WT1 mRNA.

The effects of curcumin on cell growth were observed by examining morphology of cultures with an inverted phase contrast microscope. The MTT test was used throughout all experiments to check cell viability.

MTT assay

Cell survival was determined by using the MTT assay as described (Alley *et al.*, 1988). The MTT assay was performed by plating cells in 96-well plates (3.0×10^5 cells/well) in 100 mL medium, and incubating at 37°C for 24 h before curcumin treatment. After 24 h, various curcumin concentrations in medium were added (100 μL) and incubated for another 24 h. This study, curcumin stock was dissolved in DMSO. The metabolic activity in each well was determined by the MTT assay and compared to untreated cells. Briefly, after removal of 100 μL medium, MTT stock dye solution was added (15 μL/100 μL medium) to each well, and the plate was incubated at 37°C in 5% CO_2 atmosphere. After 4 h, DMSO (200 μL) was added to each well and mixed thoroughly to dissolve the dye crystals. The absorbance at a wavelength of 540 nm was measured with an ELISA plate reader with a reference wavelength of 630 nm. The fractional absorbance was calculated by the following formula:

$$\%\text{ Cell survival} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \times 100$$

RNA extraction and quantitative RT-PCR

Total RNAs of K562 cells and patient leukemic cells were isolated by TRIzol® reagent according to the manufacturer's instructions. RNaseOUT™ was added to the RNA extraction products for RNA protection (40 units/20 μL of reaction mixture). The amount of RNA was determined by OD measurement at λ = 260 (one OD unit = 40 μg/mL). RT-PCR was performed using SuperScript™ III One-step RT-PCR System with Platinum® *Taq* DNA polymerase reagent. For WT1, the sense primer sequence for exon 7 used was 5'-GGCATCTGAGACCAGTGAGAA-3', and the antisense primer for exon 10 sequence used was 5'-GAGAGTCAGACTTGAAAGCAGT-3', corresponding to residues 780-800 and residues 1232-1253, respectively, of the published cDNA sequence (Oji *et al.*, 1999). cDNA was synthesized from 1 μg of total RNA at 60°C for 30 min and denatured at 94°C for 2 min. PCR amplification was performed for 30 cycles of sequential denaturation (94°C, 1 min), annealing (60°C, 1 min), and extension (72 °C, 1 min). Using these primers, PCR yields gave a 474-bp product. β-actin gene expression, used as an internal control of the RNA amount, was carried out by using the sense primer sequence 5'-CAGAGCAAGAGAGGCATCCT-3' and the antisense primer sequence 5'-TTGAAGGTC-TCAAACATGAT-3' corresponding to residues 216-235 and residues 405-424, respectively, which yield a 201-bp product. cDNA was synthesized at 55°C for 30 min and denatured at 94°C for 2 min. PCR amplification was performed for 30 cycles of sequential denaturation (94°C, 1 min), annealing (55°C, 1 min), and extension (72°C, 1 min). For negative control, water was amplified a total of 30 cycles to detect possible contamination. A total 15 μL of each PCR product was analyzed on a 1% agarose gel electrophoresis, visualized with ethidium bromide staining (2 μg/mL), and quantitated using a scan densitometry (BIO-RAD, Richmond, CA, U.S.A.). The K562 cDNAs were used as a positive control in every experiment.

Data and statistical analysis

After curcumin treatment of patient leukemic cells, the values of *WT1* gene expression were compared with vehicle control of the same sample. An inhibitory effect was defined as one in which the difference between treatment and control was ≥3%. Data were the mean ± standard deviation of mean from triplicate samples of three independent experiments. Differences between the means were analyzed by one-way ANOVA analysis of variance. Probability values $p<0.05$ were considered to be a statistical significance.

RESULTS

The cytotoxicity of curcumin on the leukemic cells (K562

cell line) was determined by MTT assay. Curcumin exhibited a cytotoxicity effect on K562 cells with the inhibitory concentration at 50% (IC_{50}) of approximately 20 μg/mL (54.3 μM). For WT1 protein and WT1 mRNA detection, IC_{20} was used for curcumin treatment. The IC_{20} was 6.25 mg/mL (17 μM) as shown in Fig. 1.

The effect of curcumin on patient leukemic cells was determined by RT-PCR. The 70 samples of patient leukemic cells were cultured with 10 μM curcumin for 48 h. Cell viability after curcumin treatment was about 86 ± 13% by MTT assay. This study of *WT1* gene expression in patient leukemic cells in four classes of leukemia patients (relapsed cases, drug maintenance, completed treatment, and new cases) found that relapsed cases showed the highest level of WT1 mRNA (55.4 ± 42.1), followed by new cases (48.8 ± 37.4). Completed treatment and drug maintenance showed lower WT1 mRNA levels than those of the other two groups (37.6 ± 39.5 and 36.7 ± 33.7) (Fig. 2). However, the difference was not statistically significant. When *WT1* gene expression was analyzed by leukemic cell type (ALL, AML, and CML) it was found that the ALL group showed the highest level of WT1 mRNA (45.1 ± 37.8), followed by AML (38.8 ± 35.2), and CML (27.0 ± 28.3) (Fig. 3). Again, the difference was not statistically significant.

The effect of curcumin on *WT1* gene expression in patient leukemic cells was determined by RT-PCR. The amounts of WT1 mRNA after ethidium bromide staining were measured by scan densitometer. This revealed that in 35 cases (50%) WT1 mRNA levels were decreased by curcumin treatment, including 12 of 22 new patients (54%), 4 of 8 relapsed cases (50%), 12 of 24 drug maintenance cases (50%), and 7 of 16 completed treatment cases (44%), as shown in Fig. 4 and Table I. Curcumin-responsive leukemic cell samples were ALL (31 of 58; 53%), AML (3 of 10; 30%), and CML (1 of 2; 50%), as shown in Table I. Curcumin affected both males and females with the values of 44% and 59% of total samples, respectively. *WT1* gene expression in these 70 patients was divided into 3 levels, relative to expression in K562 control, low level (1-20%), medium level (21-60%) and high level (61-100%). The leukemic cells of six patients

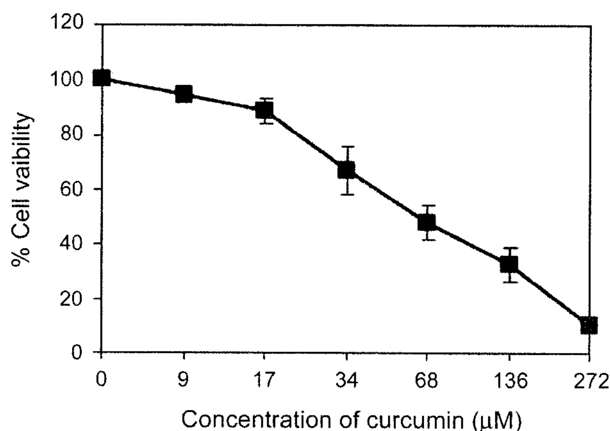

Fig. 1. Cytotoxicity of curcumin on K562 cell line. Cells (3×10^5 cells/well), in 200 μL medium were grown in the presence of 0.4% DMSO (vehicle control) or various concentrations of curcumin for 48 h. The numbers of viable cells were determined by MTT assay. Viable cells were expressed as a percentage of vehicle control. Each point represents the mean ± SD of three independent experiments performed in triplicate.

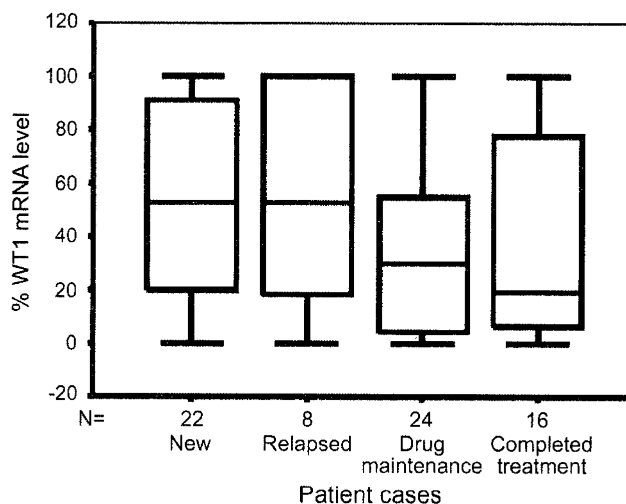

Fig. 2. *WT1* gene expression in patient leukemic cells (70 samples) in groups of leukemic patients; new cases, relapse cases, drug maintenance, and completed treatment. The WT1 mRNAs were determined by RT-PCR and quantitated by scan densitometer. The relative levels of *WT1* gene expression in each patient cell was defined in K562 cell line as 100% expression).

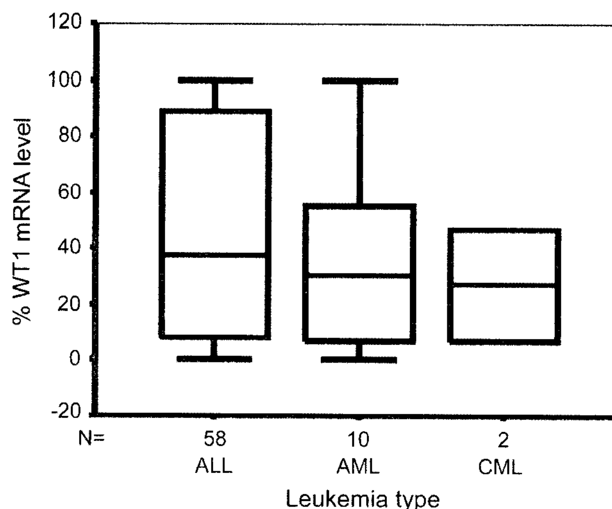

Fig. 3. *WT1* gene expression in patient leukemic cells (70 samples) in groups of patient leukemia types; ALL, AML, and CML. The WT1 mRNAs were determined by RT-PCR and quantitated by scan densitometer. The relative levels of *WT1* gene expression in each patient cell was defined in K562 cell line as 100% expression.

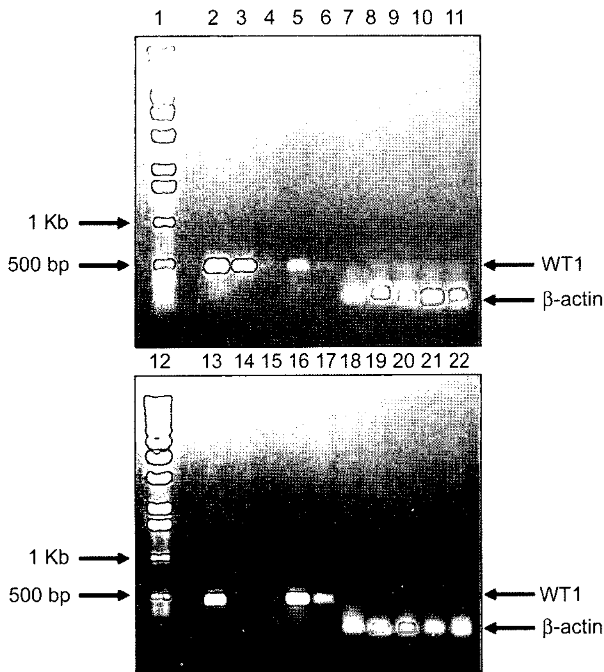

Fig. 4. Gel electrophoresis analysis of the effects of curcumin treatment on WT1 mRNA in 4 samples of leukemic patient cells. mRNA levels following treatment with 10 μM curcumin for 48 h were determined by RT-PCR. The PCR products (474 bp WT1 and 201 bp β-actin) were run in 1% agarose gel. Lanes No.1 and 12 = Markers; No.2 and 13 = K562 cell line (Positive control); No.3 = sample 1; No.4 = sample 1 + curcumin; No.5 = sample 2; No.6 = sample 2 + curcumin; No.7-11 = β-actin of lane No. 2-6; No.14 = sample 3; No.15 = sample 3 + curcumin; No. 16 = sample 4; No.17 = sample 4 + curcumin; No.18-22 = β-actin of lane No. 13-17.

did not express *WT1* gene. WT1 mRNA levels were decreased by curcumin treatment in 6 of 20 in the low level group (30%), 12 of 21 in the medium level group (57%), and 17 of 23 in the high level group (74%), as shown in Fig. 5 and Table I. In the low level group, new cases and completed treatment showed the highest numbers of samples in which *WT1* gene expression was inhibited by curcumin (33%), followed by drug maintenance (17%), and relapsed cases (17%). Within the medium level group drug maintenance (58%) had the highest number of samples, followed by new cases (17%), completed treatment (17%), and relapsed cases (8%). Within the high level group, new cases showed the highest number of leukemia samples (47%), followed by drug maintenance (23%), completed treatment (18%), and relapsed cases (12%) (Table I).

DISCUSSION

In the research for new compounds as candidates for anticancer drugs, dietary plants, for example, turmeric, chili, ginger, pepper, and garlic are of central interest in Thailand. Curcumin, a major active component of the food flavor turmeric (*Curcuma longa* Linn.) consists of three major active ingredients; curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcumin displays numerous biological properties, including antioxidant and anti-inflammatory effects, as well as antimutagen and anticancer properties. Moreover, curcumin also inhibits oncogene

Table I. Samples with WT1 mRNA levels decreased by curcumin in different clinical patient groups

WT1 mRNA level	Patient groups	Fraction of total samples with WT1 mRNA level decreased by curcumin (%)			Fraction of total	Percent of total
		ALL	AML	CML	samples*	samples*
0	New case	0/1	-	-	-	-
	Completed treatment	0/1	-	-	-	-
	Drug maintenance	0/2	0/1	-	-	-
	Relapsed case	0/1	-	-	-	-
Low (1-20%)	New case	2/4 (50)	0/1 (0)	-	2/6	33
	Completed treatment	2/6 (33)	0/1 (0)	-	2/6	33
	Drug maintenance	0/5 (0)	0/1 (0)	1/1 (100)	1/6	17
	Relapsed case	1/1 (100)	-	-	1/6	17
Medium (21-60%)	New case	1/5 (20)	1/2 (50)	-	2/12	17
	Completed treatment	2/3 (67)	-	-	2/12	17
	Drug maintenance	7/8 (88)	-	0/1 (0)	7/12	58
	Relapsed case	-	1/2 (50)	-	1/12	8
High (61-100%)	New case	7/8 (87)	1/1 (100)	-	8/17	47
	Completed treatment	3/5 (60)	-	-	3/17	18
	Drug maintenance	4/5 (80)	-	-	4/17	23
	Relapsed case	2/3 (67)	0/1 (0)	-	2/17	12

*Samples in this case are total samples with decreased WT1 mRNA after curcumin treatment.

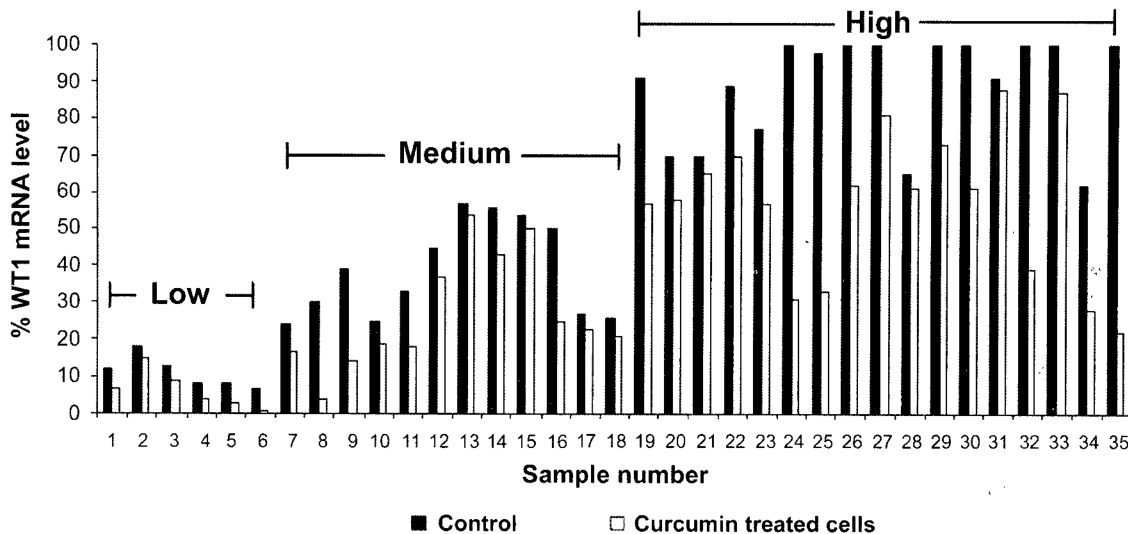

Fig. 5. Sample analysis of the effects of curcumin treatment on WT1 mRNA in low, medium, and high level samples of leukemic patient cells in the 35 samples of leukemic patient cells that were decreased by curcumin treatment. The WT1 and β-actin mRNA levels following treatment with 10 μM curcumin for 48 h were determined by RT-PCR. The PCR products (474 bp WT1 and 201 bp β-actin) were run in 1% agarose gel. The bands were quantitated by scan densitometer. *WT1* gene expression was measured and normalized to β-actin expression. The relative levels of *WT1* gene expression in each patient cell was defined in K562 cell line as 100% expression.

expression. The anticancer properties of curcumin have been described by many researchers, including our group. This inhibitory effect by curcumin regulated a wide variety of genes that require AP1 and NFkB activation which promote cell proliferation and cell differentiation. WT1 protein had been reported to play an important role in early hematopoiesis and controls cell differentiation. It is absent in mature blood cells (Fraizer *et al.*, 1995; Patmasiriwat *et al.*, 1996).

These transcription factors are regulated by protein kinase C (PKC), which also regulates WT1 protein by phosphorylation at C-terminal domain (Ye *et al.*, 1996), and in turn regulates cell proliferation in leukemic cells. Duvoix found that curcumin inhibited GSTP1-1 mRNA as well as protein which correlated to the apoptotic effect on K562 cell line (Dovoix *et al.*, 2003). We found by MTT assay that curcumin affects the proliferation of several cancer cell lines, including Hep-2 (human larynx cancer), PC-9 and PC-14 (human lung cancer), Hep-1 (mouse hepatoma), and F-25 (mutate H-ras transfected NIH mouse fibroblast) (Limtrakul *et al.*, 1999). In the K562 cell line curcumin showed an inhibitory with an effect IC_{50} of 54 μM. This was less toxic than its effect on HL60 and U937 cell lines with 19 μM and 24 μM, respectively (8.8 μg/mL and 7.2 μg/mL, respectively) (data not shown). The cytotoxic effect of curcumin in both cell lines presented at a concentration more than 8 μM (3 μg/mL) at IC_{20}. This result is consistent with Kuo's report that curcumin induced apoptosis in HL60 cell line at a concentration as low as 3.5 μg/mL (Kuo *et al.*, 1996). Recently the WT1 gene, one marker in leukemia, was shown to be over-expressed in leukemic cells, K562, and HL60 by RT-PCR (Wu *et al.*, 1995).

In this research, we focused on the inhibition by curcumin of the WT1 mRNA levels using RT-PCR. This is the first report of the effects of curcumin on WT1 mRNA levels in patient leukemic cells.

WT1 mRNA serves as a tumor marker for leukemia detection and monitoring disease progression. In the present study, the WT1 expression levels significantly increased at relapse compared with drug maintenance and completed treatment. This is similar to earlier studies with *WT1* expression in which levels significantly increased at relapse compared with those at the time of diagnosis (Tamaki *et al.*, 1996). For AML, the lowest levels of WT1 expression were observed in the differentiated leukemias, M4 and M5. The effect of curcumin, at the same concentration as K562 treatment (10 μM), on patient leukemic cells from bone marrow showed the inhibitory effect by 50% of total samples. See Fig. 2 and 3.

Curcumin had effects in all types of leukemic cells; ALL, AML, and CML (53, 30, and 50%, respectively), but could not be concluded that curcumin affected ALL more than the others. Curcumin affected females 25% more than males. Curcumin affected the *WT1* gene expression in all four classes of clinical cases. Thus curcumin may be applicable to all cases. *WT1* gene expression was divided into three groups according to levels (low, medium, and high levels). Curcumin decreased WT1 mRNA in all three of these groups of patient leukemic cells. It is an important new result that curcumin produced a greater decrease of WT1 mRNA levels in the high-and medium-level groups

than it had in the low-level group. In the case of high *WT1* gene expressive level, curcumin significantly inhibited *WT1* gene expression ($p<0.05$). Curcumin slightly inhibited *WT1* gene expressive level in the low WT1 expression group (1-20%), and medium WT1 expression group (21-60%), however the data are not significantly different. The inhibitory effect of curcumin on WT1 expression is obviously demonstrated in patient leukemic cells with high expressive level of *WT1* gene (61-100%).

In summary, modulating effect of curcumin on *WT1* gene expression are not dependent on different leukemic types (AML, ALL, and CML), clinical treatment state (new cases, relapsed cases, drug maintenance, and completed treatment), and sex while *WT1* mRNA levels in leukemic patients seem to play role on modulating effects of curcumin on the *WT1* gene expression. These findings may be important for leukemia treatment in the future, since curcumin can potentially be used as a chemotherapeutic agent. This research may lead to clinical trials in the future.

ABBREVIATION

Age-Standardized Rate (ASR) is a summary measure of the cancer incidence rate that a population will have if it has a standard age structure. It is calculated first by estimating the age-specific incidence rates and then applying these rates to the standard population. The world standard population is used in this study. It is expressed in units of incidence per 100,000 population. Standardization is necessary when comparing several populations that differ with respect to age, because age has a powerful influence on the risk of cancer.

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