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# Expression of survivin gene in apoptosis induced by dexamethasone in CEM cells

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**Abstract**: **Objective** The precise mechanism of glucocorticoid-induced apoptosis has not yet been elucidated. Survivin, a member of the inhibitors of apoptosis protein family, correlates with inhibition of apoptosis, proliferation, angiogenesis and multiple drugs resistance. This study aimed to investigate the variation of the survivin gene expression in apoptosis induced by dexamethasone (Dex) in the human T-lineage acute lymphoblastic leukemia (ALL) cell line, CEM-WT cells. **Methods** The logarithmically growing CEM cells cultured *in vitro* (cell density $2 \times 10^5$/mL) were exposed to 0.1, 0.5, 1, 5, and 10 μM Dex, then were collected 24, 48 and 72 hrs later. Untreated CEM cells were used as Controls. The cell viability was determined by trypan blue dye exclusion. Apoptosis was evaluated by morphology and flow cytometry. Survivin protein and gene were analyzed by Western Blot and RT-PCR. **Results** CEM cells growth was obviously inhibited by 0.1, 0.5, 1, 5, and 10 μM Dex from 48 hrs. The inhibition effect was dose- and time-dependent. CEM cells treated with Dex (≥5 μM) exhibited typical apoptotic features. The apoptosis increased after 5 μM Dex treatment in a time-dependent manner, with the apoptosis percentage increasing from 14.9% (12 hrs) to 46.2% (48 hrs). Compared with that of the Control group, the expression of survivin protein was down-regulated, with the expression rate of 54.6%, 45.5%, 15.8% and 9.7% respectively at 12, 24, 48 and 72 hrs after 5 μM Dex treatment. 5 μM Dex treatment also resulted in a decrease of survivin mRNA expression. The survivin mRNA expression was 76.4%, 67.3%, 55.0%, 49.9%, 38.3% and 18.3% of the Control respectively at 6, 12, 24, 48 and 72 hrs after Dex treatment. **Conclusions** Apoptosis induced by Dex in CEM cells is associated with downregulation of the survivin expression.

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**Key words**: Dexamethasone; Leukemia cell; Apoptosis; Survivin

## survivin 基因在地塞米松诱导 CEM 细胞凋亡过程中的变化

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[**摘 要**] **目的** 糖皮质激素诱导白血病细胞凋亡的确切机制尚不清楚。存活素(survivin)是凋亡抑制蛋白(inhibitors of apoptosis protein,IAPs)家族的成员,与凋亡抑制、肿瘤细胞增殖、血管形成及耐药正相关。该研究探讨地塞米松诱导急性淋巴白血病细胞系 CEM 凋亡过程中 survivin 基因的表达。**方法** 将在体外培养的对数生长期的 CEM 细胞浓度调至 $2 \times 10^5$ 个/mL,接种于 24 孔培养板中,用终浓度分别为 0.1,0.5,1,5,10 μM 地塞米松处理,以不加任何药物的 CEM 细胞作为对照组,培养后 24,48,72 h 取样。台盼蓝拒染法测定细胞活力,流式细胞仪解析地塞米松诱导 CEM 细胞凋亡,Western Blot 、RT-PCR 方法分别检测 survivin 蛋白和基因的表达。**结果** 0.1,0.5,1,5,10 μM 地塞米松于 48 h 开始明显抑制 CEM 细胞的生长,抑制效果呈时间、剂量依赖方式。随着地塞米松剂量增大,凋亡细胞比例逐渐增加。5 μM 地塞米松处理 12 ~ 48 h,凋亡细胞比例从 14.9% 升至46.2%。5 μM 地塞米松处理降低了 survivin 蛋白表达,12 h 降至对照组的 54.6%,24 h 降至 45.5%,48 h 降至 15.8%,72 h 降至 9.7%。survivin mRNA 表达在 5 μM 地塞米松处理后也被下调,处理后 6 h 降至对照组的 76.4%,12 h 降至67.3%,24 h 降至 55.0%,36 h 降至 49.9%,48 h 降至 38.3%,72 h 降至 18.3%。**结论** 地塞米松诱导 CEM 细胞凋亡与下调 survivin基因表达有关。

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[**关 键 词**] 地塞米松;白血病 CEM 细胞;凋亡;survivin

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Glucocorticoids have occupied a central role in the treatment of hematological malignancies, due to their ability to induce apoptosis in neoplastic lymphoid cells[1,2]. The response to initial glucocorticoid monotherapy serves as a prognostic marker and an indicator of potential treatment stratification[3]. But the precise mechanism by which glucocorticoids induce apoptosis has not yet been elucidated. Survivin, a member of the inhibitor of apoptosis protein family, is over-expressed in many cancers and is considered to play an important role in inhibition of apoptosis. Expression of survivin correlates with proliferation[4], angiogenesis[5] and multiple drugs resistance[6,7]. Its expression is an unfavourable prognostic factor in several cancers. In this study the expression of survivin gene was detected in order to illuminate the apoptotic mechanism induced by dexamethasone (Dex).

## Materials and methods

### Drugs and chemicals

The following reagents were obtained from these sources: RPMI-1640 from GIBCO (USA); Dex, PI, RNAse and AKP from Sigma (USA); mouse antihuman polyclonal antiserum against survivin from Santa Cruz (USA); the alkaline phosphatase-labeled antimouse secondary antibodies from Beijing Zhongshan Biotechnology Corporation; TRIzol from Promega Corporation (USA); RT-PCR kit from TaKaRa Biotechnology Co. Ltd (Dalian).

### *In vitro* cell cultures

The human T-lineage acute lymphoblastic leukemia (ALL) cell line, CEM-WT, was kindly provided by Tianjin Institute of Hematology. The CEM cells were incubated in a 37°C and 5% $CO_2$ saturated humidity atmosphere, with the RPMI-1640 medium supplemented with 10% fetal bovine serum and gentamicin(12 U/mL). Logarithmically growing cells were used for this experiment.

### Detection of CEM cells growth

CEM cells (cell density $2 \times 10^5$/mL) were exposed to 0.1, 0.5, 1, 5, and 10 μM Dex, and then were collected 24, 48 and 72 hrs later. Untreated CEM cells were used as Controls. Cell viability was determined by trypan blue dye exclusion.

### Detection of apoptosis

CEM cells treated with 0.1, 0.5, 1, 5, and 10 μM Dex were observed by light microscopy after Wright-Giemsa-staining cytospin treatment. $1 \times 10^6$ cells were harvested, washed twice with ice-cold PBS, and fixed in 70% ethanol overnight at 4°C. After being washed with PBS, the cells were incubated with 200 μg/mL of RNase for 30 minutes at 37°C, and were then stained in the dark with 20 μg/mL of propidium iodide for 30 minutes. The DNA content was analyzed by flow cytometry.

### Western Blot analysis

Western Blot analysis was done following a previous method[8]. Cells ($1 \times 10^7$ cells/condition) were pelleted by centrifugation, lysed in a buffer containing 10 mmol/L Tris-HCl (pH = 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA (pH = 8.0), 0.1% SDS, 1% Triton-100, 100 μg/mL PMSF and 2 μg/mL Aprotitin, followed by sonication for 1 hr at 4°C and then centrifugation at 15 000 rpm for 30 minutes at 4°C. Protein was quantified by the Lowry assay. Equal amounts of protein (50 μg/lane) were separated by 15% SDS-PAGE separating gel and electroblotted onto nitrocellulose. The blots were blocked in Tris-buffered saline containing Tween 20 (TBS-T) / 5% milk for 1 hr, washed twice with TBS-T, and incubated overnight at 4°C with the appropriate dilution of primary survivin antibody (1:500). The blots were washed with TBS-T and incubated with an alkaline phosphatase -labeled antimouse secondary antibody diluted appropriately in TBS-T/5% milk for 2 hrs at room temperature. After incubation, blots were developed with AKP assay reagents. The intensities of bands indicated by Western Blot were quantified by densitometry.

### Reverse transcription-PCR technique

Survivin gene was detected following Ambrosini's method[9]. β-actin was used as an internal control. The upstream primer of survivin was 5′-GGACCACCG-CATCTCTACAT -3′ and the downstream one was 5′-GCACTTTCTTCGCAGTTTCC-3′. Its amplified product was 338 bp. The upstream primer of β-actin was 5′-CACCCTGTGCTGCTCACCGAGGCC- 3′ and 5′- CCA-CACAGATGACTTGCGCTCAGG -3′ for the downstream primer. Its amplified product was 690 bp.

Total RNA ($1 \times 10^6$ cells/condition) was extracted by Trizol reagent following the manufacturer's instructions. Their purity was examined by gel electrophoresis and measured by spectrophotometry. Synthesis of single-stranded cDNA according to " First Strand cDNA synthesis Kit". Survivin PCR was carried for 32 cycles: 95 ℃/ 40 s, 55 ℃/ 1 min, and 72 ℃/ 1 min using a GeneAmp PCR System 9600. PCR products were electrophoresed in 2% agarose gels, stained in 0.5 μg/mL ethidium bromide and photographed. The survivin mRNA expression level was determined by the ratio of electrophoresis scanning results of survivin and β-actin. It was analyzed by EB-Kodak digital system.

### Statistical analysis

The data were presented as the mean value of three independent determinations and were analyzed using T-test.

## Results

### Growth inhibition by Dex

CEM cell growth was obviously inhibited by 0.1, 0.5, 1, 5, and 10 μM Dex from 48 hrs ($P<0.05$). The inhibition effect was dose- and time-dependent ($P<0.05$) (Figure 1). The 50% inhibition concentration (IC50) at 24, 48 and 72 hrs was 9.8, 0.9 and 0.4 μM, respectively.

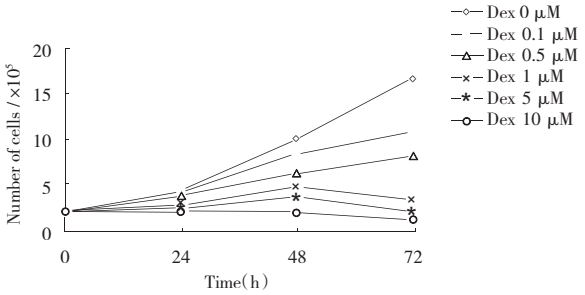


**Figure 1 Effect of Dex on CEM cell growth.** CEM cell growth was obviously inhibited by 0.1, 0.5, 1, 5, and 10 μM Dex from 48 hrs in a dose- and time-dependent manner.

### Induction of apoptosis by Dex

CEM cells treated with Dex (≥ 5 μM) exhibited typical apoptotic features such as cell shrinkage, nuclear condensation and formation of apoptotic bodies (Figure 2). Flow cytometric analysis revealed that apoptosis was increased after 5 μM Dex treatment in a time-dependent manner, with the apoptosis percentage of 14.9%, 16.9%, 26.2% and 46.2%, respectively at 12, 24, 36 and 48 hrs after Dex treatment.

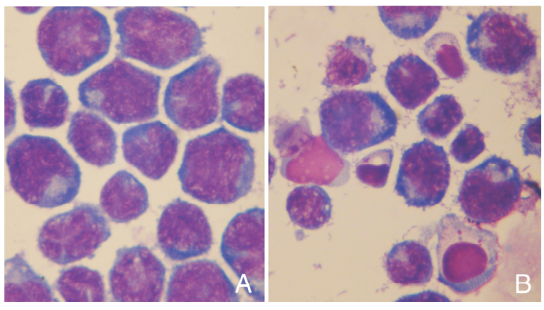


**Figure 2 Morphological changes of CEM cells after Dex treatment (R-G staining ×1 000).** **A**: The cells of the Control group were clearly presented with normal shape and density. **B**: The cells after 5μM Dex treatment for 48 hrs exhibited cell shrinkage, nuclear condensation and formation of apoptotic bodies.

### Effect of Dex on survivin gene expression

5 μM Dex treatment resulted in a decrease of survivin mRNA expression. The survivin mRNA expression was 76.4%, 67.3%, 55.0%, 49.9%, 38.3% and 18.3% of the Control respectively at 6, 12, 24, 48 and 72 hrs after Dex treatment (Figure 3).

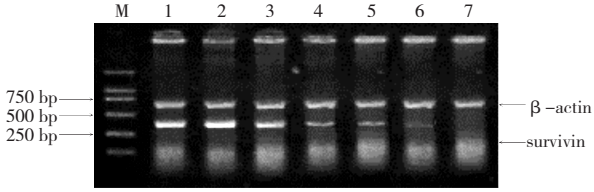


**Figure 3 Effect of Dex treatment on survivin mRNA expression.** 5 μm Dex treatment resulted in a decrease of survivin mRNA expression in a time-dependent manner. 1: Control group; 2: 6 hrs after treatment; 3:12 hrs after treatment; 4: 24 hrs after treatment; 5: 36 hrs after treatment; 6: 48 hrs after treatment; 7: 72 hrs after treatment; M: Marker.

### Effect of Dex on survivin protein

5 μM Dex treatment also decreased the survivin protein expression. The survivin protein expression was 54.6%, 45.5%, 15.8%, and 9.7% of the Control respectively at 12, 24, 48 and 72 hrs after Dex treatment (Figure 4).

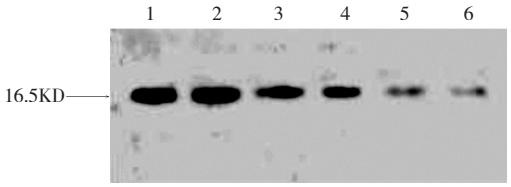


**Figure 4 Effect of Dex treatment on survivin protein expression.** 5 μm Dex treatment decreased the survivin protein level in a time-dependent manner. 1: Control group; 2: 6 hrs after treatment; 3: 12 hrs after treatment; 4: 24 hrs after treatment; 5: 48 hrs after treatment; 6: 72 hrs after treatment.

### Discussion

Glucocorticoids have been used for the treatment of leukemia and lymphoma for over 50 years. The effects of glucocorticoids on lymphoid cells are dramatic, including the induction of G1 cell cycle arrest and apoptosis. Treatment with 5 μM Dex for 12 hrs resulted in typical apoptosis on morphology. At the same time, the apoptosis percentage was increased from 14.9% (12 hrs) to 46.2% (48 hrs). This study demonstrated that glucocorticoids are effective in the treatment of leukemia through inducing apoptosis in CEM cells. But the mechanism remains unclear. The IAP family is a relatively new group of apoptosis regulating proteins, which consists of a number of proteins that can bind to

and inhibit caspases. Eight human IAP family members have been identified so far. They are c-IAP1, c-IAP2, XIAP, NAIP, survivin, apollon, ML-IAP/livin and ILP-2. The survivin gene, the smallest IAP member, spans 14.7 kb and encodes a protein of 142 amino acids, with a molecular weight of approximately 16.5 kDa. Survivin is present during fetal development and malignant tumors but is undetectable in terminally differentiated adult tissues. Following the analysis of human transcriptomes, survivin transcription is in the fourth place in malignant tumor[10]. Tamm[11] has reported survivin was expressed in all 60 cancer cell lines analyzed, with highest levels in breast, lung cancers, hematologyic cell lines such as CCRF-CEM, K562 and MOLT-4. The previous data suggested that the survivin expression in cancer is associated with cancer progression, poor prognosis, drug resistance, and shorter survival of the patient. This study has shown that the survivin expression increased in CEM cells, suggesting that increased survivin expression is associated with antiapoptosis and proliferation of CEM cells.

Glucocorticoids are effective for the treatment of leukemia through inducing leukemia cells apoptosis. The changes of the survivin expression in apoptosis remain unknown. Previous studies [8] demonstrated that the survivin expression was down-regulated in bufalin-induced apoptosis of HL-60 cells . Moriai [12] has also reported that the survivin expression in HL-60 cells was decreased after treatment with ATRA and TNF for 5 days. Wang [13] has reported that the survivin antisense RNA can enhance taxol-induced apoptosis in leukemia cell line HL-60. This study also showed that the survivin mRNA and protein in CEM cells were decreased after Dex treatment. It is suggested that the survivin downregulation may be associated with apoptosis of CEM cells induced by Dex. This finding may be also helpful to the research on the mechanism of Dex resistance.

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