



# Expression of TRAIL and its receptor DR5 and their significance in acute leukemia cells

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**ABSTRACT.** We investigated the roles of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its receptor death receptor 5 (DR5) in the onset of acute leukemia and changes in their expression during chemotherapy. Bone marrow samples from 16 patients newly diagnosed with acute leukemia were collected before chemotherapy. Bone marrow samples from patients with non-hematologic malignancies served as the control group. Peripheral blood samples of patients with acute leukemia were also collected before chemotherapy and at 1 and 3 days after chemotherapy. Mononuclear cells in the bone marrow and peripheral blood were isolated and used to detect the expression of TRAIL and DR5 by flow cytometry. Compared with mononuclear cells from the control group, mononuclear cells from newly diagnosed patients with acute leukemia showed no significant difference in the expression of TRAIL ( $P > 0.05$ ) but showed significantly increased expression of DR5 ( $P < 0.05$ ). TRAIL and DR5 expression in peripheral blood mononuclear cells after chemotherapy was significantly increased compared to expression before chemotherapy ( $P < 0.05$ ). Patients showing high expression of DR5 had a higher remission rate. One of the mechanisms underlying the treatment of leukemia with chemotherapy drugs may be the induction of TRAIL and DR5, which may promote

apoptosis in leukemia cells. TRAIL-mediated apoptosis is regulated by DR5 expression.

**Key words:** Acute leukemia; Chemotherapy; DR5; TRAIL

## INTRODUCTION

Leukemia is a malignancy with the highest incidence rate in patients younger than 60 years. In recent years, considerable progress has been made in treating leukemia, but recurrence and primary drug resistance remain serious problems. In addition to the well-documented side effects of chemotherapy drugs, the research and development of sensitive and specific new drugs is still very urgent. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), discovered in 1995, is an important regulatory protein for apoptosis in tumor necrosis factor superfamily. Its binding to its specific death receptor (DR) can activate a variety of apoptotic signal pathways and induce apoptosis (Gura, 1977). TRAIL selectively induces apoptosis in cancer cells and has nearly no toxic effects on normal cells. Thus, it is an ideal antineoplastic agent (Pan et al., 1997; Walczak et al., 1999; Ashkenazi, 2002). However, a previous study showed that some cancer cells were resistant to TRAIL, leading to drug tolerance (Thorburn et al., 2008) and reducing the desired efficacy in cancer therapy. Further studies identified 5 receptors for TRAIL, of which only DR4 and DR5 were shown to induce apoptosis. The other 3 receptors, soluble receptor osteoprotegerin, decoy receptor 1 (DCR1), and DCR2, could not transmit the death signal of TRAIL (Testa, 2010). The decoy receptors DCR1 and DCR2 are associated with the treatment of drug tolerance (Truneh et al., 2000; Secchiero et al., 2002; Yerbes et al., 2011). Under normal physiological conditions, TRAIL induces apoptosis mainly through its binding to DR5, as DR5 has a significantly higher affinity to TRAIL than does DR4 (Truneh et al., 2000). Therefore, DR5 plays more important role in transmitting the TRAIL death signal and inducing apoptosis in cancer cells. Thus, we focused on TRAIL and its receptor DR5 in this study to explore the significance of TRAIL and DR5 in the pathogenesis of acute leukemia. TRAIL and DR5 expression was detected using flow cytometry in acute leukemia cells before and after treatment with chemotherapeutic drugs. Our results will be useful for identifying new methods and drugs for treating acute leukemia.

## MATERIAL AND METHODS

### Subjects

Sixteen patient newly diagnosed with acute leukemia (peripheral blood leukocyte count  $\geq 50 \times 10^9/L$ ) were confirmed by morphology, immunology, cytogenetic, and molecular methods. The subjects included 10 males and 6 females, aged from 15 to 58 years. There were 4 cases of acute lymphoblastic leukemia, treated with a chemotherapy VDCLP program, and 12 cases of acute non-lymphocytic leukemia, treated with chemotherapy daunorubicin program. Ten patients with non-hematologic malignancies with nearly normal bone marrow were enrolled in the control group, including 7 males and 3 females, aged from 17-53 years. From patients in the control and acute leukemia groups, 2 mL bone marrow was collected. Additionally, 2 mL peripheral blood samples from patients in the acute leukemia group were

collected before chemotherapy and at 1 and 3 days after chemotherapy. All samples were heparin-anticoagulated and used to isolate mononuclear cells using lymphocyte separation medium (Ficoll, density 1.077). The cell density was then adjusted to  $1 \times 10^7/L$ .

## Flow cytometry

The isolated cells were first incubated with Percp-cy5.5-labeled CD45 monoclonal antibody (BD Biosciences, Franklin Lakes, NJ, USA) and then divided into 3 tubes, 2 of which were further incubated with phycoerythrin-labeled mouse anti-human TRAIL antibody and phycoerythrin-labeled mouse anti-human DR antibody (BD Biosciences). Cell suspensions were incubated with antibodies for 30 min at room temperature in the dark, followed by washing 3 times with phosphate-buffered saline. CD45, TRAIL, and DR expression in the cells was detected by flow cytometry (FacsCaliber, BD Biosciences). Fluorescence intensities for  $1 \times 10^4$  cells were collected for quantitative analysis, and mean fluorescence intensity (MFI) was calculated.

## Determination of the results

Data for the 2 groups are reported as MFI. The MFI of the mononuclear cells and leukemic cells were referred to as T-MFI and L-MFI, respectively.

## Statistical analysis

Statistical analysis was performed using the SPSS v10.0 statistical software (SPSS, Inc., Chicago, IL, USA). Data are reported as means  $\pm$  SD. Comparisons between the 2 groups were carried out using the paired *t*-test.  $\alpha = 0.05$  was considered to be significant.

## RESULTS

### TRAIL and DR5 expression

TRAIL expression in the bone marrow cells of patients with acute leukemia and the control group showed no significant difference ( $P > 0.05$ ), but DR5 expression was significantly elevated in the patient group than that in the control group ( $P < 0.05$ ; Table 1).

**Table 1.** Expression of TRAIL in bone marrow cells before chemotherapy.

Groups	TRAIL	DR5
Acute leukemia	$3.15 \pm 1.12^{\#}$	$12.5 \pm 3.7^*$
Control	$3.22 \pm 0.52$	$8.8 \pm 2.3$

$^{\#}P > 0.05$  and  $^*P < 0.05$  vs control group.

### TRAIL expression was enhanced by chemotherapy

TRAIL expression in the peripheral blood mononuclear cells of all newly diagnosed patients with acute leukemia (T-MFI) was remarkably enhanced at 1 and 3 days after

chemotherapy as compared with that before chemotherapy ( $P < 0.01$ ). In leukemia cells (L-MFI), TRAIL expression showed the same change in MFI in response to chemotherapy ( $P < 0.01$ ; Table 2).

**Table 2.** Expression of TRAIL in peripheral blood mononuclear cells before and after chemotherapy.

Groups	Cases	Time points		
		Day 0	Day 1	Day 3
T-MFI	16	3.20 ± 0.76	3.86 ± 0.88*	3.91 ± 1.22*
L-MFI	16	3.59 ± 1.23	4.05 ± 1.53*	4.34 ± 1.77*

\* $P < 0.01$ , compared with day 0.

### DR5 expression was significantly elevated by chemotherapy

DR expression in the peripheral blood mononuclear cells of patients with acute leukemia was markedly higher than that in the control group, and was further enhanced by chemotherapy ( $P < 0.01$ ). However, DR5 expression showed no significant difference between 1 and 3 days after chemotherapy ( $P > 0.05$ ; Table 3).

**Table 3.** Expression of DR5 in peripheral blood mononuclear cells before and after chemotherapy.

Groups	Cases	Time points		
		Day 0	Day 1	Day 3
T-MFI	16	12.8 ± 4.1	41.3 ± 11.3*	47.6 ± 15.2*
L-MFI	16	14.1 ± 2.8	41.9 ± 8.59*	43.4 ± 7.63*

\* $P < 0.01$ , compared with day 0.

### Relationship between DR expression and clinical therapeutic effects

Twelve patients, whose mean DR5 expression at 1 day after chemotherapy was  $44 \pm 6.3$ , obtained complete remission. One patient showing partial remission had a DR5 expression level of 38.6 at 1 day after chemotherapy and the remaining 4 patients without remission had DR5 expression levels of  $34.2 \pm 4.7$  at 1 day after chemotherapy.

## DISCUSSION

TRAIL can induce apoptosis in most leukemia malignant cells but not kill normal cells (Yerbes et al., 2011). Additionally, it can induce the differentiation of leukemia cells and normal myeloid precursor cells to become monocyte-like, so as to regulate normal hematopoiesis (Secchiero et al., 2002). In this study, we compared TRAIL expression in bone marrow cells between patients with acute leukemia and patients with non-leukemia and found no significant difference between the 2 groups, suggesting that TRAIL also plays a role in regulating apoptosis in normal cells. However, TRAIL itself cannot induce apoptosis of leukemic cells and does not show increased levels with the proliferation of malignant cells. Two receptors of TRAIL, DR4 and DR5, are expressed in leukemia cells and normal cells at varying levels. It has been reported that leukemia cells express higher levels of apoptotic receptors than normal cells, and

that DR5 expression is significantly higher than DR4 (Liu et al., 2003), suggesting that the DR5-mediated signal transduction pathway plays a more important role in TRAIL-induced apoptosis. Our results showed that DR5 expression was significantly higher in patient than in the control group, which is consistent with the above results. This indicates that DR5 plays a more important role in inducing apoptosis in leukemia cells.

Plasilova et al. (2002) found that TRAIL can induce apoptosis in K562, human promyelocytic leukemia cells (HL-60), ML-1, and other leukemia cells. In acute non-lymphoid leukemia patients with hematologically complete remission and patients with lymphoma but not involving the bone marrow, precursor cells in normal bone marrow and normal cord blood do not respond to the treatment with TRAIL. Lee et al. (2003) treated a cell mixture of normal cord blood mononuclear cells and Jurkat cells with TRAIL and found that Jurkat cells were specifically cleared when TRAIL was added, while the normal colony-forming unit-granulocyte/macrophage colony numbers were not reduced. Our results also showed that the mean fluorescence intensity of TRAIL expression in leukemia cells was remarkably enhanced by treatment with chemotherapy drugs, indicating that treatment with chemotherapy drugs may induce the expression of TRAIL and then induce apoptosis.

To investigate the mechanism underlying chemotherapy-induced apoptosis of leukemia cells through TRAIL, we also studied the expression of DR, a receptor for TRAIL, in leukemia cells. DR5 was highly expressed in leukemia cells and was further enhanced by chemotherapy treatment. A previous study showed that the chemotherapy drugs etoposide, cytarabine, and doxorubicin could regulate the expression of DR5 in HL-60 cells and improve the sensitivity of HL-60 cells to TRAIL (Gong and Almasan, 2000). Several other studies showed that when TRAIL was combined with chemotherapy drugs causing DNA damage, TRAIL-induced apoptosis of cancer cells was enhanced, which was mediated by the upregulation of DR5 (Gibson et al., 2000; Nagane et al., 2000). Canestraro et al. (2010) treated HL-60 cells with arsenic trioxide combined with bortezomib and found that combination therapy enhanced the expression of Fas and DR5. Our results showed that DR5 expression in leukemia cells before chemotherapy was significantly higher than that in the control group, indicating that leukemia cells had good activity of TRAIL-induced apoptosis. However, the expression of TRAIL and DR5 was prominently elevated by chemotherapy, although there was no marked difference in the expression of TRAIL in leukemia cells before chemotherapy and in the control group, suggesting that the apoptotic activity of TRAIL and DR5 was chemotherapy-induced. The mechanism may be associated with the apoptotic pathway of TRAIL.

The apoptotic effect of TRAIL on cancer cells is regulated by its membrane receptors. TRAIL binding to its receptor is the first step of the induction of apoptosis (Zhang and Fang, 2005). TRAIL-resistant cancer cells were reported to express higher levels of DCRs on their surface, which can protect cancer cells from TRAIL-induced apoptosis (Dyer et al., 2007). Our results showed that the ability of TRAIL-induced apoptosis was influenced by DR5 and that TRAIL expression in leukemia cells could be increased by chemotherapy drugs, although some cells still failed to initiate the apoptotic pathway. Patients with high expression of DR5 could obtain complete remission, while patients with low DR5 expression showed low response rates. Thus, patients sensitive to chemotherapy drugs can initiate the apoptotic pathway, leading to apoptosis in cancer cells by inducing TRAIL expression and the upregulation of DR5, whereas patients with low expression of DR5 cannot initiate the TRAIL-mediated apoptotic pathway. This is because some drug-resistant cancer cells *in vivo* express decoy receptors, resulting in partial remission or no remission after chemotherapy.

As an ideal new drug for treating cancer, various forms of recombinant TRAIL protein have been successfully developed and entered into Phase II clinical studies. Leucine zipper-TRAIL recombinant (Walczak et al., 1999) is more sensitive against human Jurkat cells and can induce apoptosis in human breast cancer MDA-231 cells, but showed no killing effect against normal mammary epithelial cells and other normal tissue cells. Continuous and systematic administration of large doses of TRAIL to non-human primates showed no clinical and histopathological changes. The safe application of TRAIL in primates is critical for its potential application in humans. TRAIL can induce apoptosis in various tumor cells and has a synergistic effect with chemotherapy. TRAIL has also shown significant efficacy and safety (Ashkenazi et al., 1999; Shi et al., 2005). However, some tumors have inherent tolerance or acquired resistance to TRAIL, limiting the clinical application of TRAIL. However, development of the DR5 monoclonal antibody shows promise. Ichikawa et al. (2001) developed a specific monoclonal antibody TRA-8 for DR5, which can specifically bind to DR5 and exert its killing effects on primary and metastatic hepatocellular carcinoma, but not on the normal liver cells.

With the synthesis of genetically engineered TRAIL and the completion of clinical trials examining recombinant TRAIL, rhTRAIL may increase the sensitivity to chemotherapeutic drugs and reduce the drug dosage and side effects in patients when it is used in the clinical treatment of human malignancies, including leukemia. This treatment will effectively improve the therapeutic effect against leukemia as well as patient outcomes.

### Conflicts of interest

The authors declare no conflict of interest.

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