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Level of DNA topoisomerase IIα mRNA predicts the treatment response of relapsed acute leukemic patients

Yan-Hua Wang[a], Minoko Takanashi[b], Kazue Tsuji[a], Norina Tanaka[a], Masayuki Shiseki[a], Naoki Mori[a], Toshiko Motoji[a,*]

[a] Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan
[b] Blood Preparation Department, Japanese Red Cross Tokyo Blood Center, Tokyo, Japan

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ABSTRACT

The DNA topoisomerase IIα (Topo IIα) is known as a target enzyme for many chemotherapeutic agents. We investigated the Topo IIα mRNA expression by real-time RT-PCR in 37 paired samples at diagnosis and at relapse of acute leukemic patients in relation to drug sensitivity and clinical outcome. The Topo IIα levels in leukemic blasts at relapse were significantly higher than that at diagnosis, especially in ALL. The increase in the Topo IIα level at relapse was significant in cases which could not achieve a second remission, but not significant in cases which achieved a second remission. These results suggest that the change of Topo IIα expression in leukemic blasts at relapse may predict therapeutic responsiveness.

1. Introduction

In acute leukemia, although clinical outcomes have been improved due to recent advances in chemotherapy, relapses are still preventing the long-term survival of patients. The mechanisms of relapse and the accompanying resistance of leukemic blast cells to anti-leukemic treatment have not been fully clarified. The multidrug resistance (MDR) phenomenon has been shown to be composed of many factors including the *mdr1 gene*/P-glycoprotein (P-gp), DNA topoisomerases (Topo), lung resistance-related protein, multidrug resistance-associated protein, glutathione-S-transferase and so on [1,2].

Topo are essential nuclear DNA-binding enzymes that control and regulate cellular functions such as DNA replication, repair, gene transcription and cell division during cell proliferation [3]. Topo II is the only enzyme able to cleave and religate double-stranded DNA, and plays a major role in the remodeling of chromatin during mitosis in cell cycles [4,5]. In human cells, two distinct isoenzymes of Topo II have been identified based on differences in molecular weight, pattern of expression, protein structure and function. Topo IIα has been shown to have a critical role in drug sensitivity, while Topo IIβ has not [6–8]. Topo IIα has been known as the main target for a variety of anti-leukemic agents including anthracyclines and epipodophyllotoxins. The cytotoxicity of Topo IIα targeting drugs is thought to be a consequence of the stabilization of the Topo II–DNA complexes, which leaves DNA breaks and leads to apoptosis [9], though the exact mechanisms remain elusive. The expression of Topo IIα also varies in different phases of the cell cycle [10–12]. There are some reports on Topo IIα gene expression of leukemic blasts at relapse in comparison to that at onset from clinical samples, but a controversy remains whether quantitative alteration of Topo IIα occurs in relapsing acute leukemia [7,13–15].

To investigate the significance of Topo II in relapse of leukemia and relapse related drug resistance, we have examined Topo IIα mRNA relative levels with real-time quantitative reverse transcriptase-polymerase chain reaction (real-time RT-PCR), which is a sensitive and highly reliable method for detecting the enzyme quantitatively, in paired samples at diagnosis and at relapse of adult acute leukemic patients. We have also evaluated the correlation between the Topo IIα, Topo IIβ mRNA level, drug sensitivity to the Topo IIα-mediated agent daunorubicin (DNR), and the clinical therapeutic response in these patients.

2. Materials and methods

2.1. Cell line

The human myelogenous leukemic cell line K562, which is known to express Topo IIα [16], was used to make standard curves for real-time RT-PCR in this study. The cells were cultured in RPMI1640 medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal calf serum (FCS; Life Technologies, Grand Island, NY). When the leukemic cells were in a logarithmic proliferation phase, they

* Corresponding author. Tel.: +81 3 3353 8112; fax: +81 3 3353 8970.
E-mail address: motoji@dh.twmu.ac.jp (T. Motoji).

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were collected and washed with phosphate-buffered saline (PBS), and stored at −80 °C until use.

2.2. Patients and chemotherapy

Thirty-seven patients were studied after written informed consent was given both at diagnosis and at the time of the first relapse; 19 were *de novo* acute myelocytic leukemia (AML), 4 were AML from myelodysplastic syndrome (AML-MDS), and 14 were acute lymphocytic leukemia (ALL). The median age of the patients was 51 years (range 16–71 years). All samples were taken before chemotherapy both at diagnosis and at relapse. The characteristics of patients are summarized in Table 1. The diagnosis of *de novo* AML and its subtypes was determined according to the French–American–British classification [17]. All patients received chemotherapy including Topo IIα inhibitors at diagnosis, primarily in accordance with the Japan Adult Leukemia Study Group (JALSG) protocols [18–27]. The response to treatment was assessed after two courses of induction chemotherapy. Complete remission (CR) was judged when the normocellular bone marrow showed less than 5% leukemic blast cells and the peripheral blood counts recovered to a normal level. The period of time from when the patients achieved CR to relapse varied from 9 to 48 months. At relapse, 18 out of 23 AML and 13 out of 14 ALL patients received the re-induction treatment which, except for 8 patients, included Topo IIα inhibitors. The other six patients did not receive any treatment due to their poor performance status at relapse. The second CR was only achieved in 7 out of the 18 AML and 4 out of the 13 ALL patients.

2.3. Separation of leukemic blast cells

Mononuclear cells were separated through Ficoll-Conray density gradient centrifugation (density 1.077 g/ml) from bone marrow and/or peripheral blood samples at the initial diagnosis and at the relapse of the disease. The leukemic blast cells

Table 1
Clinical data and Topo IIα mRNA levels in paired blast cells samples of acute leukemia.

Patients			Treatment regimen		Clinical outcome		Topo IIα mRNA level ($\times 10^{-3}$)	
No.	Type	Age/Sex	Diagnosis	Relapse	Diagnosis	Relapse	Diagnosis	Relapse
1	AML M1	51/M	IDA, AraC[a]	NT	CR	–	1.9	2.0
2	AML M1	29/F	IDA, AraC[a]	DNR, AraC	CR	CR	9.6	43.7
3	AML M1	62/F	IDA, AraC[a]	DNR, AraC	CR	NR	17.1	204.7
4	AML M1	63/F	DNR, BH-AraC, 6-MP, PSL[b]	MIT, BH-AraC, VP-16	CR	CR	24.1	51.2
5	AML M2	68/F	DNR, BH-AraC, 6-MP, PSL[b]	NT	CR	–	28.2	6.2
6	AML M2	65/M	DNR, BH-AraC, 6-MP, PSL[b]	MIT, BH-AraC, VP-16	CR	CR	210.3	163.5
7	AML M2	43/M	DNR, BH-AraC, 6-MP, PSL[b]	ACR, AraC	CR	NR	40.0	2.5
8	AML M2	52/M	DNR, BH-AraC, 6-MP, PSL[b]	IDA, AraC	CR	NR	0.0	33.9
9	AML M2	57/F	IDA, AraC[a]	HD-AraC	CR	NR	4.8	59.7
10	AML M2	57/F	IDA, AraC[a]	HD-AraC	CR	CR	8.8	8.4
11	AML M2	40/F	DNR, AraC[c]	MIT, BH-AraC, VP-16	CR	CR	22.6	37.4
12	AML M3	37/M	IDA, AraC, ATRA[d]	As_2O_3	CR	CR	140.8	133.5
13	AML M4	21/M	IDA, AraC[a]	MIT, HD-AraC	CR	NR	10.1	66.8
14	AML M4	43/M	IDA, AraC[a]	HD-AraC	CR	CR	116.9	40.9
15	AML M4	57/F	IDA, AraC[a]	MIT, BH-AraC, VP-16	CR	NR	71.4	98.7
16	AML M4	50/F	IDA, AraC[a]	VCR, PSL	CR	NR	72.7	168.9
17	AML M5a	47/M	IDA, AraC[a]	DNR, HD-AraC	CR	NR	22.1	47.9
18	AML M5a	54/M	IDA, AraC[a]	MIT, AraC	CR	NR	0.9	1.0
19	AML M5a	65/M	IDA, AraC[a]	DNR, HD-AraC	CR	NR	13.1	2.2
20	AML-MDS	69/F	IDA, AraC[e]	NT	CR		29.1	8.4
21	AML-MDS	50/M	IDA, AraC[e]	MIT, BH-AraC, VP-16	CR	NR	7.1	27.6
22	AML-MDS	65/F	IDA, AraC[e]	NT	CR		95.9	41.2
23	AML-MDS	71/M	IDA, AraC[e]	NT	CR		7.1	58.1
24	ALL	55/F	VCR, ADM, CPM, MTX, PSL[f]	VCR, DNR, MTX, AraC, L-asp, 6-MP, PSL[h]	CR	NR	23.8	103.9
25	ALL	21/M	VCR, ADM, CPM, MTX, PSL[f]	MIT, HD-AraC	CR	NR	45.7	137.9
26	ALL	46/M	VCR, ADM, CPM, MTX, PSL[f]	VCR, PSL	CR	NR	16.4	64.4
27	ALL	32/F	VCR, ADM, CPM, MTX, PSL[f]	VCR, DNR, MTX, AraC, L-asp, 6-MP, PSL[h]	CR	NR	11.2	74.0
28	ALL	32/M	VCR, ADM, CPM, MTX, PSL[f]	NT	CR		32.5	141.3
29	ALL	16/M	VCR, ADM, CPM, MIT, PSL[f]	VCR, ADR, CPM, L-asp, PSL[i]	CR	CR	53.1	16.7
30	ALL	25/M	VCR, ADM, CPM, MTX, PSL[f]	HD-AraC	CR	NR	12.6	9.6
31	ALL	31/F	VCR, ADM, CPM, MTX, PSL[f]	VCR, DNR, CPM, L-asp, PSL[g]	CR	NR	26.9	53.4
32	ALL	66/F	VCR, ADM, CPM, MTX, PSL[f]	VCR, PSL	CR	NR	20.2	19.9
33	ALL	65/F	VCR, ADM, CPM, MTX, PSL[f]	VCR, DNR, L-asp, PSL	CR	NR	15.3	11.6
34	ALL	57/F	VCR, ADM, CPM, MTX, PSL[f]	DNR, HD-AraC	CR	CR	60.9	86.5
35	ALL	32/F	VCR, ADM, CPM, MTX, PSL[f]	VCR, DNR, ADM, CPM, DEX[g]	CR	CR	26.5	27.8
36	ALL Ph1$^+$	39/M	VCR, DNR, CPM, STI571, PSL[g]	VCR, ADM, CPM, MTX, DEX, AraC, STI571[j]	CR	CR	41.2	105.7
37	ALL Ph1$^+$	63/F	VCR, DNR, CPM, STI571, PSL[g]	VCR, DNR, PSL[j]	CR	NR	21.2	34.5

Abbreviations: ACR, aclarubicin; ADM, doxorubicin; AraC, cytarabine; ATRA, tretinoin; BH-AraC, behenoylcytarabine; CPM, cyclophosphamide; DNR, daunorubicin; DEX, dexamethasone; HD-AraC, high-dose AraC; IDA, idarubicin; L-asp, L-asparaginase; 6-MP, 6-Mercaptopurine; MIT, mitoxantrone; MTX, methotrexate; PSL, prednisolone; VCR, vincristine; VP-16, etoposide; STI571, imatinib mesilate; CR, complete remission; NR, no response; NT, no treatment.

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