日本財団補助金による
1999 年度日中医学協力事業報告書

－在留中国人研究者研究助成－

2000 年 3 月 3 日

財団法人 日中医学協会
理事長 中島 章 殿

研究室で撮影した本人のスナップ写真、及び発表論文のコピーを添付

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研究テーマ 骨髄異形成症候群の病態と治療に関する基礎的研究

2．本年度の研究業績

(1) 学会・研究会等においての口頭発表 (有) ・ 無 (学会名・内容)

学会名：第 62 回日本血液学会総会
一般演題示説（ポスター）テーマ：トロンボポエチン（TPO）の骨髄異形成症候群（MDS）芽球増殖に対する効果。

(2) 学会誌等に発表した論文 (有) ・ 無 (雑誌名・論文名)

雑誌名：Stem Cells
論文名：Effect of thrombopoietin on proliferation of blasts from patients with myelodysplastic syndromes.（印刷中）

3．今後の研究計画

骨髄異形成症候群の病態と治療に関しての研究を続けて行きたいと思います。

4．研究指導者の意見

羅　善順君は日本医科大学第三内科(血液内科)にて血液学の研究を非常に勤勉に行い、その成果を別記のごとく日本医学会分科会である日本血液学会総会で発表し、さらにその内容を米国のピアレビュー誌である"Stem Cells"に投稿して受理され、現在印刷中であります。このように貴財団補助金を極めて有意義に使用し、着実な業績をあげております。

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5．研究報告

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研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

論文発表に当っては、日中医学協会－日本財団補助金による旨を明記して下さい。

トロンボポエチン(TPO)の骨髄異形成症候群(MDS)芽球増殖に対する効果

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要旨

MDS 芽球に対する TPO の刺激効果を検討した。患者細胞を TPO 存在下で培養すると芽球数は High-risk MDS（RAEB 以上）および白血病化 MDS で増加したが、Low-risk MDS では増加しなかった。芽球数増加は芽球細胞周期の刺激を伴っていた。芽球の TPO 受容体mRNA 発現と TPO 反応性とは関連がなかった。芽球が TPO に反応する例は血清 LDH 値が高値であった（P=０．００３６）。このデータから High-risk MDS および白血病化 MDS の臨床適応には病勢の進行がおきる可能性があり注意が必要と考えられるが High-risk MDS については特に LDH 高値例にその Risk が高いように思われる。一方 Low-risk MDS については今回の in vitro の検討では増殖刺激効果を認めなかったが実際の臨床適用においては慎重さが必要と思われる。

Effect of Thrombopoietin on Proliferation of Blasts from Patients with Myelodysplastic Syndromes

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Running head: TPO and MDS blasts

Key Words. *Thrombopoietin* · *Myelodysplastic syndromes* · *Blast proliferation*

ABSTRACT

Thrombopoietin (TPO), a major cytokine involved in megakaryocytopoiesis/thrombopoiesis, may be effective for treatment of the thrombocytopenia associated with myelodysplastic syndromes (MDS). However, it has been unclear whether TPO stimulates proliferation of MDS blasts, as observed in *de novo* acute myeloid leukemia. This study examined this concern. When marrow cells from 37 MDS cases were cultured with or without recombinant human PEGylated TPO, TPO increased the blast number (stimulation index $\geqq$ 1.5) in nine of 16 high-risk MDS cases (refractory anemia with excess blasts (RAEB) and RAEB in transformation) and four of 10 cases with MDS transformed to acute leukemia (MDS-AL) but none of 11 cases with low-risk MDS (refractory anemia (RA) and RA with ringed sideroblasts). When the cell cycle of cultured cells was determined by three-color flow cytometry, TPO activated the cell cycle of MDS cells (causing a decrease in G0-phase cells) in most of the cases whose blast number increased in response to TPO. Reverse transcription-polymerase chain reaction analysis detected TPO receptor mRNA in purified blasts from all six cases examined, irrespective of the response of their blasts to TPO in culture. Analysis of the patients' characteristics identified a high serum lactate dehydrogenase (LDH) value as being associated with blast proliferation in high-risk MDS cases ($p = 0.0036$). We conclude that TPO stimulates *in vitro* proliferation of blasts from a fraction of MDS patients. High-risk MDS patients, especially those who have a high serum LDH value, and MDS-AL patients should be monitored with particular care in clinical trials of TPO for MDS.

INTRODUCTION

Myelodysplastic syndromes (MDS), which are malignant disorders of hematopoietic progenitors, show various degrees of anemia, neutropenia and thrombocytopenia. Manifestations caused by the cytopenia, such as infections and bleeding, and transformation to acute leukemia are the major causes of death in MDS. No effective therapy for MDS has been established yet, except for allogeneic stem cell transplantations, which are available for a limited number of patients [1]. Hematopoietic growth factors (HGFs), such as granulocyte colony-stimulating factor (G-CSF), may be effective in preventing infections in neutropenic patients [2]. Thrombopoietin (TPO, mpl-ligand), a major HGF involved in the growth and development of megakaryocytes and platelet production [3-5], is a candidate for treatment of thrombocytopenia in MDS, and a clinical trial of TPO for MDS is now being conducted in Japan.

TPO receptor (TPO-R, c-mpl) is expressed mainly on platelets, megakaryocytic cells and hematopoietic progenitors [6, 7] in normal humans. Data from patients with acute myeloid leukemia (AML) indicate that blasts from a high proportion of AML patients have TPO-R mRNA [8, 9] and blasts from a fraction of these patients proliferate in response to TPO *in vitro* [10, 11]. Therefore, the clinical effect of TPO in AML patients should be carefully monitored. Regarding MDS, such analysis has been rare. By using Northern blotting, Bouscay *et al.* [12] detected TPO-R mRNA in mononuclear cells (MNCs) from the bone marrow or peripheral blood of 19 of 58 MDS patients. The same group also reported that TPO increased ^{3}H-thymidine uptake by marrow MNCs,

which consisted of various cell types responding to TPO, and enhanced cluster formation (probably derived from the MDS clone) induced by combined stimulus with interleukin 3 (IL-3), IL-6, erythropoietin, granulocyte-macrophage colony-stimulating factor and stem cell factor (SCF), in some MDS cases [13]. We have previously detected TPO-R protein, which was able to bind with and degrade TPO, on blasts from a patient with MDS transformed to acute leukemia (MDS-AL) [14].

In this study, we performed *in vitro* studies to examine whether TPO increases the blast number and activates the cell cycle of blasts from MDS patients. We also sought a correlation between the proliferative response of blasts to TPO and various background characteristics of MDS patients and examined TPO mRNA expression by purified blasts from some patients.

MATERIALS AND METHODS

Subjects

Twenty-six MDS patients, who were diagnosed by the standard criteria [15], and 11 MDS-AL patients were the subjects in this study (Table 1). For this study, we classified refractory anemia (RA) and RA with ringed sideroblasts (RARS) as a low-risk MDS and RA with excess blasts (RAEB) and RAEB in transformation (RAEB-t) as a high-risk MDS.

Cell Preparation

Heparinized bone marrow cells were obtained from the patients after they

gave informed consent. MNCs were separated by gradient centrifugation, using Histopaque-1077 (Sigma Chemical Co.; St. Louis, MO). To examine TPO-R mRNA expression, blasts were purified from six cases using immunomagnetic beads conjugated with anti-CD34 antibody (Dynal A.S.; Oslo, Norway) as described previously [16]. The purity of the blasts was 97% or more when determined for Wright-Giemsa-stained cytospin preparations.

Blast Proliferation Assay

The marrow MNCs were suspended in RPMI-1640 medium containing 10% fetal calf serum (FCS) (5 x 10^5 cells/ml) and cultured at 37°C in air containing 5% CO_2 in the presence of recombinant human PEGylated TPO (Kirin Brewery Co.; Tokyo) or a mixture of recombinant human hematopoietic growth factors (HGFs), i.e., 100 ng/ml of G-CSF, IL-3 and SCF (Kirin Brewery Co.). This HGF mixture (HGFs/Mix) is a powerful stimulator of MDS cells, as described previously [17]. The cells were also cultured without any HGFs as a negative control in each case. After the culture, the nucleated cell counts (NCC) were determined with a hemocytometer, and the percentage of blasts was determined for Wright-Giemsa-stained cytospin preparations. Then the blast number was calculated from these data. The stimulation index (SI) was calculated by dividing the number of blasts in TPO- or HGFs/Mix-stimulated cultures by the number in the negative control culture.

In preliminary experiments, the marrow MNCs were cultured with different concentrations of TPO for various lengths of time. In cases whose blast number

increased in response to TPO, the maximum increase was observed at 100 ng/ml of TPO, and the SI values were comparable between 48-h culture and 96-h culture. Although the viability of cells was maintained for up to 48-h of culture (≧ 97% by the trypan blue exclusion test), the viability decreased when the cells were cultured for 96-h in some cases. Thus, 100 ng/ml of TPO and 48-h culture were used as the experimental conditions in this study. To examine the reproducibility of this assay, cell cultures not supplemented with any HGF were set in duplicate for 10 cases, and each duplicate culture was arbitrarily labeled A or B at the initiation of culture. When the blast number after the culture was determined, the coefficient of variation, calculated from the mean ± SD of $I \times II^{-1}$, was 14.1% (I = the blast number in culture A of each case, and II = the blast number in culture B of each case).

Cell Cycle Analysis

For this analysis, we selected high-risk MDS cases, whose blasts were positive for CD13. The CD13-positivity of the blasts was identified with anti human CD13 monoclonal antibody (Becton Dickinson; San Jose, CA) using an immunomagnetic beads method, as described in our previous report [17]. Briefly, the marrow MNCs were reacted with anti-human CD13 monoclonal antibody, followed by incubation with immunomagnetic beads coated with anti-mouse IgG antibody. The incubated cells were subjected to cytospin preparation and Wright-Giemsa staining. Cases were selected if more than 50% of the blasts were bound to the beads. The reasons for using CD13 to identify the cells to be

analyzed are described in detail in the Discussion. MDS-AL cases whose marrow MNCs contained more than 90% blasts were also subjected to the cell cycle analysis.

The cell cycles of the marrow MNCs cultured for 48 h were determined by three-color flow cytometry, as described previously in detail [17, 18]. Briefly, cells were labeled with anti-human CD13 monoclonal antibody conjugated with fluorescein (this step was omitted for the MDS-AL cases whose marrow MNCs contained more than 90% blasts). After washing, the cells were incubated in a buffer containing 0.004% saponin (Wako Chemical; Osaka, Japan). Then, the DNA and RNA in the cells were stained with 7-aminoactinomycin D (Sigma Chemical Co.) and pyronin Y (Polysciences; Warrington, PA), respectively. The cell cycle (G_0-, G_1-, S-, and G_2/M-phases) of the fluorescein-stained cells (or non-stained cells in the MDS-AL cases) was analyzed with a FACScan flow cytometer using CellFIT and Lysis II softwares (Becton Dickinson). In these analyses, lymphocytes in freshly isolated peripheral blood MNCs from normal volunteers were used as an internal standard for G_0 cells, because a majority of unstimulated lymphocytes are -by definition- G_0 cells [19, 20]. The coefficient of variation of this cell cycle analysis method was determined by us and reported previously [17].

The indexes of change in the G_0 cell percentage (G_0IC) and the S cell percentage (SIC) were calculated with the following formulas: G_0IC = G_0 cell percentage in experimental culture x G_0 cell percentage in control culture^{-1} x 10^2, SIC = S cell percentage in experimental culture x S cell percentage in control

culture^{-1} x 10^2.

Detection of TPO-R mRNA by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

RNA was extracted from the purified blasts or three cell lines by the acid guanidinium thiocyanate-phenol-chloroform method [21]. The cell lines used were Dami cells (American Tissue Culture Collection; Rockville, MD) which express TPO-R mRNA, and K562 and Jurkat cells (RIKEN Cell Bank; Ibaraki, Japan) which do not express TPO-R mRNA [22].

RT-PCR for TPO-R mRNA was performed using an RNA PCR Kit (Ver. 2.1; Takara Shuzo; Otsu, Japan) as described previously [23]. In brief, one μg of total RNA was reverse-transcribed to cDNA in a final volume of 20 μl using 1 mM each of four deoxyribonucleotide 5' triphophates, 20 U RNase inhibitor, 2.5 μM of the random 9 mers, and 5 U AMV reverse transcriptase XL. The reaction was performed for 10 min at 30°C, 30 min at 42°C, 5 min at 99°C and 5 min at 5°C, and the product was subjected to PCR. PCR was performed in a final volume of 100 μl containing 0.2 μM of the sense primer (nucleotide 843, 5'-TGGAGATGCAGTGGCACTTG-3'), 0.2 μM of the anti-sense primer (nucleotide 1029, 5'-TGATGTCTGGGGTGTCAAGA-3'), and 2.5 U of Taq polymerase. Amplification was performed for 35 cycles (denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min). Eight-μl aliquots of the products were analyzed by 2% agarose gel electrophoresis. The presence of intact mRNA was verified by concomitant amplification of β-actin mRNA.

Measurement of Plasma TPO Concentration

Heparinized peripheral blood was drawn from the patients after obtaining informed consent, and the plasma was immediately separated and stored at -20°C until use. Plasma TPO concentrations were determined with a sensitive sandwich enzyme-linked immunosorbent assay, as described in our previous report [14].

Statistical Analysis

Differences between two groups of data for continuous variables were evaluated using the Mann Whitney-U test. Three or more groups of data for continuous variables were compared by ANOVA, and when the results were significant these groups of data were further compared with each other by Scheffe's test. Differences in categorical variables were evaluated using the chi-square test. Definitions of cytogenetic subgroups and prognostic subgroups by the international prognostic scoring system (IPSS) were the same as the reported criteria [24]. Overall survival was calculated from the day of diagnosis until death. The progression-free survival of high-risk MDS was calculated from the day of diagnosis until disease progression (transformation to RAEB-t or MDS-AL in RAEB cases and to MDS-AL in RAEB-t cases). Kaplan-Meier product limit estimates were performed to determine these survivals. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Effects of TPO on proliferation of MDS blasts in culture

First, we examined whether TPO increases the number of MDS blasts in the cultures. The change in the blast number in response to TPO or the HGFs/Mix, which is expressed as the SI of the blast number, are presented in Table 1. When the data for low-risk MDS, high-risk MDS and MDS-AL were compared, the increase in the blast number induced by TPO differed significantly among the three groups ($p = 0.0355$ by ANOVA, $p = 0.0119$ for low-risk MDS vs high-risk MDS, $p = 0.0646$ for low-risk MDS vs MDS-AML). The increase in the blast number induced by the HGFs/Mix did not differ significantly among the three patient groups ($p = 0.7262$ by ANOVA).

When a significant increase in the number of MDS blasts was arbitrarily defined as SI $\geqq$ 1.5, the TPO and HGFs/Mix induced a significant increase in 13 cases (35.1%) and 33 cases (89.2%), respectively, among the total of 37 cases. Regarding the MDS subtype, a positive response to TPO (SI $\geqq$ 1.5) was observed only in the high-risk MDS and MDS-AL cases (seven of the 11 RAEB cases, two of the five RAEB-t cases and four of the 10 MDS-AL cases), but not in any of the 11 low-risk MDS cases.

To explore whether the increase in the number of MDS blasts is accompanied by cycle activation of quiescent blasts (Go-phase blasts), we performed cell cycle analysis of the cultured cells. To obtain reliable results, only cases whose bone marrow samples contained a substantial proportion of blasts (high-risk MDS and MDS-AL) were included in this cell cycle analysis. The cell

cycle data of the examined cases are summarized in Table 2, while the indices of the cell cycle (GoIC and SIC) of each case are presented in Table 1.

Figure 1 illustrates the indices of the cell cycle in patients whose blast number increased in response to TPO (SI $\geqq$ 1.5) and in patients whose blast number did not increase (SI < 1.5). TPO decreased the GoIC and increased the SIC in most cases belonging to the former patient group. Further, cell cycle activation was more marked in the former patient group compared with the latter patient group (SIC $\geqq$ 200 was observed in seven of the 12 cases with SI $\geqq$ 1.5 but not in any of the six cases with SI < 1.5, $p = 0.0167$; GoIC $\leqq$ 50 was observed in five of the 12 cases with SI $\geqq$ 1.5 but not in any of the six cases with SI < 1.5, $p = 0.0628$).

Correlation between increase in blast number in response to TPO and clinical and biological variables

Because TPO induced blast proliferation in a fraction of the high-risk MDS and MDS-AL cases, we examined whether there were any patient background characteristics which correlated with the TPO-induced blast proliferation (Table 3). Regarding the high-risk MDS cases, only the LDH value differed significantly between the cases whose SI was 1.5 or more and the cases whose SI was less than 1.5 ($p = 0.0036$, Fig. 2). Regarding the MDS-AL cases, there was no characteristic which differed between the two groups divided according to the SI value.

The overall survival and progression-free survival did not differ between

these two groups in the high-risk MDS cases or the MDS-AL cases ($p > 0.2$ for all analyses).

Expression of TPO-R transcripts in MDS blasts

We were able to purify blasts from six cases (Cases 16, 18, 21, 26, 27 and 36) and subject them to RT-PCR analysis for TPO-R mRNA. Although the *in vitro* proliferation response of the blasts to TPO differed among these cases (SI of blast number: $\geqq$ 1.5 in three cases and < 1.5 in other three cases), TPO-R mRNA was detected in blasts from all six cases (Fig. 3).

DISCUSSION

Although a clinical trial of TPO for thrombocytopenia in MDS is being conducted, there are no data which clarify whether TPO induces blast proliferation *in vitro* and/or disease progression *in vivo* in MDS patients. A prior report showed that TPO increased ^{3}H-thymidine uptake by bone marrow MNCs in some high-risk MDS cases [13]. However, because the marrow MNCs consist of various types of cells and TPO may stimulate myeloid and erythroid cells as well as megakaryocytic cells [25-27], it is unknown whether TPO induced ^{3}H-thymidine uptake by MDS blasts in that prior report. In this study, we showed that TPO increased the blast number (SI $\geqq$ 1.5) in *in vitro* liquid culture in nearly half of cases with high-risk MDS and MDS-AL but not in any of 11 cases with low-risk MDS. In contrast, a combination of HGFs (HGFs/Mix) increased the blast number in most cases, including the low-risk MDS cases. In these

experiments, we used a short-term culture (48 h), because viable MDS blasts were not able to be maintained well in longer culture. Therefore, it remains unknown whether long-term stimulus by TPO induces blast proliferation in low-risk MDS cases. Nevertheless, our data suggest that the risk of blast proliferation by TPO is higher in high-risk MDS and MDS-AL than in low-risk MDS.

The increase in blast number by TPO indicates that TPO induces proliferation of blasts in the active cell cycle and/or TPO induces G0-phase blasts to enter the cell cycle and proliferate. Thus, we also examined whether TPO modulates the cell cycle of MDS cells by using the recently developed flow cytometric analysis method [28]. In applying this method, we employed an anti-CD13 antibody, which reacts not only with MDS blasts but also with more mature myeloid cells and monocytic cells, to identify the cells to be analyzed instead of other relatively blast cell-specific antibodies, i.e., anti-CD34 and anti-c-kit antibodies. Our main reason for using anti-CD13 antibody was that the expressions of CD34 and c-kit are restricted to a low percentage of blasts in a limited number of MDS patients [29]. On the contrary, a majority of blasts in most MDS patients express CD13 [29]. Moreover, we previously showed that the cell cycle did not differ significantly between CD13-positive cells and purified blasts in an MDS case [17]. Our present results also showed that the cell cycle of examined cells from both MDS-AL (whose MNCs consisted of more than 90% blasts in this study) and high-risk MDS cases was stimulated by TPO in most cases whose blast number increased in response to TPO. Taken together, the

present data indicate that TPO induces Go-phase blasts to enter the cell cycle and proliferate, at least in some high-risk MDS and MDS-AL cases. In Cases 14 and 36, although their blast number increased in response to TPO, this was not accompanied by a decrease in Go-phase cells. It is probable that in such cases only cycling blasts were stimulated by TPO. In the meantime, it is known that AML blasts produce HGFs which stimulate blast proliferation in some cases [30]. Therefore, another point of interest is whether MDS blasts also produce HGFs which cooperate with TPO in stimulating blast proliferation in our cases. However, we currently have no data elucidating this point.

Identification of patient characteristics which correlate with blast proliferation in response to TPO is essential for safe clinical application of TPO. Our analysis identified the serum LDH value as a predictive factor for the blast response to TPO in high-risk MDS. Other serum enzymes, such as GOT, GPT and ALP, were not associated with the blast response to TPO in the present subjects (data not shown). Although the mechanism responsible for this association remains unknown, we conclude that patients with high-risk MDS who have a high serum LDH value should be monitored with particular care in clinical trials of TPO for MDS.

Data regarding the expression of TPO-R by MDS blasts are sparse. By using Northern blot analysis for marrow MNCs, Bouscay *et al.* [12] detected an elevated TPO-R mRNA level in 11 of 26 patients with RAEB or RAEB-t but a normal TPO-R mRNA level (faint band by blotting, as observed in the normal marrow samples) in all 14 low-risk MDS. In the present study, we obtained

purified MDS blasts to minimize contamination by mRNA from other cells. Because the number of purified blasts was small, we used RT-PCR instead of a ligand binding assay or Northern blotting to examine TPO-R expression. TPO-R mRNA was detected in blasts from all six patients but not in control cells which had been reported to have no TPO-R mRNA. It is noted that the presence of TPO-R mRNA was not related with an *in vitro* blast response to TPO in our subjects. This finding is consistent with the data for *de novo* AML, in which an *in vitro* response of blasts to TPO is not significantly associated with the TPO-R mRNA expression by the blasts [10, 11].

The data generated in this study will be useful for planning clinical trials of TPO for MDS and monitoring patients in such trials. We hope that, in the near future, appropriate use of TPO will contribute to the treatment of this intractable disease.

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Table 1. Effects of TPO and HGFs/Mix on blast number and cell cycle indices of MDS cells from each case

Case No.	Disease	Age/ Sex	Increase in blast number (SI)		Indices of cell cycle			
					HGFs/Mix		TPO	
			HGFs/Mix	TPO	GoIC	SIC	GoIC	SIC
1	RA	75/M	5.00	1.00				
2	RA	70/M	1.95	0.90				
3	RA	77/F	4.50	1.30				
4	RA	82/F	0.50	1.00				
5	RA	60/F	1.47	0.33				
6	RA	70/M	5.64	1.07				
7	RA	58/M	4.90	1.33				
8	RA	27/F	4.00	1.00				
9	RA	65/M	6.17	1.25				
10	RA	74/F	9.44	0.62				
11	RARS	75/M	5.18	1.45				
	Subtotal		4.4 ± 2.5	1.0 ± 0.3				
12	RAEB	78/M	2.83	1.50	23.0	220.2	17.9	280.2
13	RAEB	65/M	8.14	4.86				
14	RAEB	68/M	2.34	1.50	169.5	454.3	116.4	271.7
15	RAEB	53/M	1.33	1.00	NC*	160.7	NC*	63.2
16	RAEB	72/F	3.80	1.07				
17	RAEB	87/F	5.50	4.38	14.6	6233.3	83.7	633.3
18	RAEB	61/M	3.92	1.35	34.6	164.2	116.1	103.4
19	RAEB	80/M	5.79	2.86	41.0	204.7	82.6	158.4
20	RAEB	64/M	3.43	2.57	50.8	288.1	31.4	202.4
21	RAEB	70/M	2.67	1.49				
22	RAEB	60/M	4.33	2.87	9.2	256.4	30.3	344.8
23	RAEB-t	74/M	2.20	1.45				
24	RAEB-t	48/M	1.74	1.11	40.0	116.0	153.3	100.4
25	RAEB-t	76/M	1.66	1.47				
26	RAEB-t	77/M	10.50	6.00	19.1	185.2	35.5	198.5
27	RAEB-t	59/M	5.50	4.50	48.5	166.4	67.0	99.1
	Subtotal		4.1 ± 2.5	2.5 ± 1.6				
28	MDS-AL	77/M	1.52	1.48	26.4	134.4	64.6	115.9
29	MDS-AL	66/M	1.42	0.75				
30	MDS-AL	65/F	3.12	1.32	52.4	295.2	75.7	64.5
31	MDS-AL	70/M	1.74	0.76				
32	MDS-AL	69/M	1.90	2.62	69.2	229.4	115.4	92.6
33	MDS-AL	48/M	7.94	6.14	51.1	395.8	68.5	201.4
34	MDS-AL	70/M	3.38	1.46	81.2	140.0	82.8	109.2
35	MDS-AL	69/M	7.30	2.06	3.9	219.2	36.4	212.1
36	MDS-AL	51/M	5.00	2.38	86.9	155.8	92.9	160.3
37	MDS-AL	72/M	2.40	1.40				
	Subtotal		3.6 ± 2.4	2.0 ± 1.6				

Subtotals of low-risk MDS, high-risk MDS and MDS-AL are the mean ± SD. Blanks mean no analysis was done. SI, G0IC and SIC were calculated using the formulas described in **Materials and Methods**.

*The indices could not be calculated because the percentage of Go-phase cells in the control culture was 0.

Table 2. Summary of effects of TPO and HGFs/Mix on cell cycle of MDS cells

		Cell cycle (%)			
Disease	Stimulator	G_0	G_1	S	$G_2 + M$
High-risk MDS	None	10.0 ± 9.0	73.8 ± 11.1	10.2 ± 7.1	4.6 ± 4.8
	TPO	7.8 ± 10.6	72.4 ± 13.4	14.8 ± 6.6	4.5 ± 4.0
	HGFs/Mix	4.0 ± 4.0	66.6 ± 7.4	20.5 ± 6.2	9.0 ± 5.5
MDS-AL	None	24.8 ± 28.7	78.9 ± 12.7	11.8 ± 10.2	4.1 ± 3.5
	TPO	18.2 ± 22.4	69.9 ± 21.2	15.6 ± 14.1	6.4 ± 6.3
	HGFs/Mix	14.7 ± 21.4	60.1 ± 22.7	21.6 ± 14.1	10.4 ± 15.2

Data are mean ± SD.

Table 3. Correlation between blast proliferation response to TPO and clinical and biological variables

	High-risk MDS		MDS-AL	
	SI ≧ 1.5	SI < 1.5	SI ≧ 1.5	SI < 1.5
Age	70.9 ± 9.9	64.8 ± 4.2	59.3 ± 11.3	70.0 ± 4.3
Leukocytes (x 10^9/l)	5.8 ± 4.2	10.8 ± 17.0	22.8 ± 29.4	28.3 ± 33.4
Neutrophils (x 10^9/l)	2.6 ± 2.3	5.9 ± 9.1	10.8 ± 17.4	11.5 ± 21.1
Circulating blasts (x 10^9/l)	0.19 ± 0.31	0.83 ± 2.01	2.62 ± 2.24	12.69 ± 13.57
Hemoglobin (g/dl)	7.8 ± 1.7	7.7 ± 1.6	6.8 ± 0.9	6.3 ± 0.8
Platelets (x 10^9/l)	89.4 ± 176.5	45.0 ± 43.2	33.0 ± 29.2	64.3 ± 40.0
Bone marrow blast (%)	14.2 ± 6.8	16.5 ± 7.5	54.6 ± 24.4	62.1 ± 32.4
Serum LDH (IU/l)[a]	816 ± 312	363 ± 152	960 ± 723	718 ± 352
Serum TPO (fmol/ml)	2.4 ± 2.0	1.5 ± 1.4	2.2 ± 3.1	1.0 ± 0.4
Cytogenetic subgroup[b]	3/0/5/1	4/1/2/0	1/1/2/0	1/0/2/3
IPSS[c]	0/1/3/4/1	0/1/0/5/1		

High-risk MDS cases and MDS-AL cases were divided into two groups, respectively, according to the proliferation response of blasts to TPO, i.e., the SI value of the blast number.

Data at the date of experiment are shown as the mean ± SD or case number.

aSignificantly different between the two groups in the high-risk MDS ($p = 0.0036$).

bGood/intermediate/poor/no data.

cLow/Int-1/Int-2/High/no data.

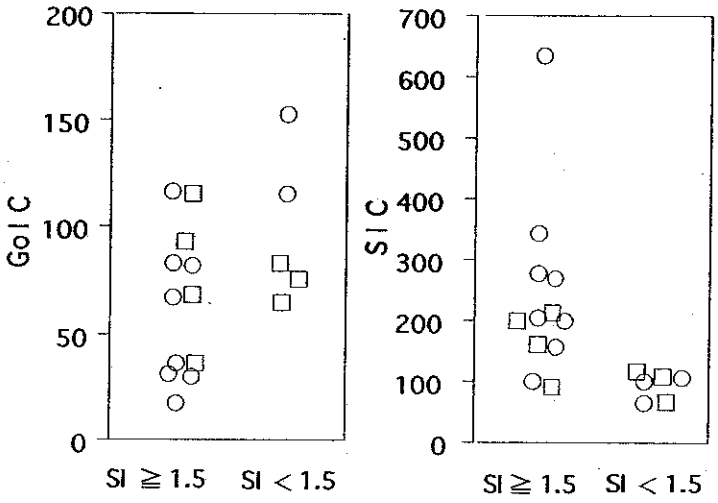
Cytogenetic subgroup and IPSS were defined according to the previous report (24).

FIGURE LEGENDS

Figure 1. TPO-induced cell cycle change (GoIC and SIC) in the cases whose SI of the blast number was $\geqq$ 1.5 and in the cases whose SI was < 1.5. Circles indicate high-risk MDS and squares indicate MDS-AL.

Figure 2. Serum LDH values of the high-risk MDS cases whose SI of the blast number was $\geqq$ 1.5 and the cases whose SI was < 1.5. Serum LDH values were significantly different between the two groups ($p = 0.0036$).

Figure 3. RT-PCR analysis of TPO-R mRNA (t) and β-actin mRNA (a) for MDS blasts and cell lines. M, D, K and J indicate markers, Dami cells, K562 cells and Jurkat cells, respectively. The numbers indicate the case numbers. The SI of the blasts was $\geqq$ 1.5 in Cases 21, 18, and 16 and < 1.5 in the other three cases.


200
150
100
50
0
GoIC
SI ≧ 1.5
SI < 1.5
700
600
500
400
300
200
100
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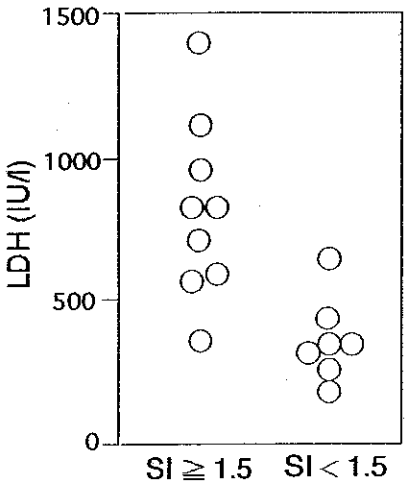
SIC
SI ≧ 1.5
SI < 1.5


1500
1000
500
0
LDH (IU/l)
SI ≧ 1.5
SI < 1.5