Discussion

With the improvement of supportive care for allogeneic HSCT, transplant-related mortality has decreased.[4,8] However, disease relapse still remains the most important factor interfering with the success of allogeneic HSCT for MDS. The reported relapse rate after allogeneic HSCT for MDS ranges from 23 to 48%.[1–8] These reported relapse rates correspond to all MDS patients, including those with refractory anemia or refractory anemia with ringed sideroblasts, and thus the relapse rate is reported to be much higher (41–67%) in patients with advanced MDS (with excessive blasts).[1–8] Therefore, a reduction in post-transplant disease relapse in advanced MDS patients could directly improve transplant outcomes. G-CSF has been reported to increase the susceptibility of leukemic cells to cytarabine *in vitro* by recruiting quiescent leukemic cells into the cell cycle.[9–11] In this context, several reports have shown the efficacy of the combination of G-CSF with cell-cycle-specific chemotherapeutic agents such as cytarabine in refractory myeloid malignancies.[12–15] In a randomized trial, it has been shown that addition of G-CSF to cytarabine-based induction chemotherapy for AML patients significantly contributes to a higher rate of disease-free survival owing to the reduced rate of relapse.[15] In an HSCT setting, we previously reported the results of 14 patients in two institutes, who underwent allogeneic HSCT from an HLA-identical sibling after being conditioned with TBI and G-CSF-combined high-dose cytarabine; in these cases, a high disease-free survival rate of 67.7% was demonstrated.[16] In the present long-term follow-up study conducted at a single institute, which included a greater number of patients with advanced MDS including patients who received grafts from unrelated donors, the 5-year disease-free survival rate was 72.2% with a relapse rate of only 16.0%. This relapse rate is somewhat lower than those reported in the studies reported by other investigators.[1–8] Furthermore, non-relapse mortality rate, which could affect the survival rate, was identical to that in the other report.[20] Therefore, together with the results of our previous report, the present results strongly suggest that a conditioning regimen including G-CSF-combined high-dose cytarabine could effectively reduce disease relapse and contribute to a better survival rate in patients with advanced MDS after allogeneic HSCT.

The previously reported studies used TBI or busulfan plus cyclophosphamide as a myeloablative conditioning regimen for allogeneic HSCT from an alternative donor.[1–8] In the present study, 10 patients received HSCT from a serologically HLA-matched unrelated donor, and hematopoietic engraftment was successfully achieved in these patients, suggesting that our regimen without cyclophosphamide could provide sufficient immunosuppressive effects to allow sustained engraftment even in HSCT from an unrelated donor.

We conclude that TBI with G-CSF-combined high-dose cytarabine is a promising conditioning regimen of allogeneic HSCT for patients with advanced MDS and that it does not increase regimen-related toxicities. Future randomized study is required to evaluate the efficacy of combining G-CSF with cytarabine to reduce the incidence of post-transplant disease relapse.

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standard test for what remains a clinical dilemma, distinguishing AA from hMDS. This is in part, we believe, because CD34 numbers, although statistically different between the disease groups, showed considerable overlap in several studies. Rather than attempting to 'reinvent the wheel,' we believe that our results build upon past studies by providing clinical outcomes that improve the ability to classify patients with either disease state. When doing this, we find no overlap in marrow CD34 numbers between AA and hMDS, and thus believe quantifying marrow CD34 numbers should be further tested as a standard approach for separating the two disorders. Furthermore, these findings also suggest that patients with normal cytogenetics and low CD34 + percentages are at a low risk of disease progression and may be managed in a conservative manner in the absence of life-threatening cytopenias.

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Reply to Matsui *et al.*

Leukemia (2006) **20**, 2042. doi:10.1038/sj.leu.2404395; published online 14 September 2006

I did appreciate the reply letter from Dr Matsui *et al.*,[1] which I believe clarifies most of the issues I had raised in reference to their recent paper.[2] However, their reply, which refers to 'a potential confounding factor in their (our) analysis[3] may have come from reliance on the FAB guidelines to accurately diagnose patients', is still somewhat misleading. Although the presence of a cytogenetic abnormality is useful in confirming a suspected diagnosis of myelodysplastic syndrome (MDS), its present is not mandated by either FAB or WHO 2001 guidelines. The diagnostic criteria for MDS in both classifications largely overlap. Therefore, the assumption that we might have 'misclassified' cases because of the use of FAB (the only system available in 1996) is incorrect.

I completely agree with Dr Matsui *et al.*[1] that the enumeration of bone marrow CD34 positive cells should be further tested, possibly in a randomized prospective way, in order to conclusively confirm its validity for separating the two disorders. The fact that the 'CD34 approach' has not become standard of practice up to now, I suspect, it is also due to the usual interlaboratory variability which plagues immunohistochemistry laboratories in general. As this is not likely to change in the near future, the best approach to this difficult differential diagnosis remains, in my judgment, a comprehensive one inclusive of morphology, immunohistology, flow cytometry and cytogenetics. Marrow cytogenetics still maintains its major prognostic role in this group of hypoplastic marrow disorders.

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Quantitative RT-PCR analysis of sphingolipid metabolic enzymes in acute leukemia and myelodysplastic syndromes

Leukemia (2006) **20**, 2042–2046. doi:10.1038/sj.leu.2404386; published online 7 September 2006

Sphingolipids and their metabolites recently appeared as a potent class of regulators of cell proliferation, survival as well as apoptosis.[1] The ceramide/S1P rheostat has been proposed as the model to determine the cell's fate.[2] This rheostat is being increasingly recognized as a critical element in tumor cell proliferation and chemotherapy. Sphingomyelinase is responsible for the first step of the sphingomyelin-catabolic pathway and produces ceramide, which figures mostly as the proapoptic

factor in response to various reagents including anti-cancer drug or radiation. On the contrary, sphingosine kinase (SPHK) is the enzyme that produces sphingosine 1–phosphate (S1P) from sphingosine. S1P binds to five G-protein coupled receptors called S1P receptors. S1P promotes cell survival or motion as the first or second cellular messenger in response to various agonists. Therefore, enzymes in this pathway provide potential targets for new anti-cancer drugs.

Interestingly, overexpression of SPHK1 is thought to be oncogenic, and renders transfected cells chemoresistant.[2] SPHK1 mRNA was significantly higher in various cancer tissues than in their normal counterparts.[3] In prostate cancer cell lines, we reported the inverse relationship between SPHK1 level and anti-cancer drug sensitivity.[4] The quantity of each cellular sphingolipid metabolite was thought to be determined by the complex balance between each metabolic enzyme activity and substrate. However, no analysis of gene expression of sphingolipid metabolizing enzymes including SPHK1 of acute leukemia or related diseases has been reported.

In the present study, we performed quantitative RT-PCR assay to measure the mRNA levels of nine major enzymes involved in the sphingolipid metabolic pathway including sphingosine kinase 1 (SPHK1), sphingosine kinase 2 (SPHK2), acid sphingomyelinase (ASMase), neutral sphingomyelinase 2 (NSMase2), acid ceramidase (ACDase), sphingosine 1–phosphate lyase (SPL), sphingosine 1–phosphate phosphatase 1 (SPP1), glucosyl ceramide synthase (GlcCer Syn), sphingomyelin synthase 1 (SM Syn). Multidrug-resistant gene (MDR) and BCL2 were also measured to examine the chemoresistance gene expression in the current samples.

Quantitative PCR was performed with Power SYBR Green master mix (Applied Biosystems, Foster City, CA, USA) in duplicate using primer sets described in Table 1, and ABI PRISM 7000 sequence detection systems (Applied Biosystems) were used for the measurement. ABL gene expression was measured as the internal control with Taqman probe as shown in Table 1 according to the recommendation by Beillard *et al.*[5] The specificity of PCR product was confirmed in the preliminary experiments using cell lines. Standard curve was created using cDNA fragment of each enzyme produced by the PCR method and then inserted into the cloning vector. The relative gene expression level was calculated as the ratio of each gene expression/ABL gene expression.

After obtaining informed consent, bone marrow cells were collected from 19 patients with acute leukemia and 60 patients with myelodysplastic syndromes (MDS) (28 RA (refractory anemia), 21 RAEB (refractory anemia with excessive blast) and 11 RAEB-t (RAEB in transformation) according to FAB classification) mostly at their initial diagnosis or before any treatment. For the normal control, bone marrow samples for the disease staging were used from 11 patients with non-Hodgkin's lymphoma without bone marrow invasion. Mononuclear cells were collected and RNA was extracted. The first strand cDNA was prepared using the Super Script First-Strand System (Invitrogen). CML-BC and Ph_1 + ALL samples were omitted because the ABL gene was used as the internal control of RT-PCR assay. Patient characteristics are provided as the Supplementary Data.

Figure 1 shows the message levels of sphingolipid metabolic enzymes as well as BCL2 and MDR. We mainly focused on the statistical difference between AL and normal control by using one-way factorial analysis of variance and multiple comparison test (Bonferroni/Dunn's method). Statistical analysis was performed using Microsoft Excel software and Stat view version 5 (SAS Institute Inc., Cary, NC, USA). We used RNA from total mononuclear cells of bone marrow aspirates instead of purified hematopoietic stem cells or blast cells. Therefore, the heterogeneity of bone marrow component might have affected our results.

Among enzymes examined, AL also showed noticeable increases of SPHK1 message as compared to normal population. Some AL showed more than 2 log order higher SPHK1 gene expression as compared to the normal control. SPHK2 did not show significant differences between groups analyzed. It is also of note that SPHK1 expression of RAEB-t is also significantly higher than normal, although we could not see significant differences in SPHK1 gene expression between RAEB and normal control. There was no correlation between the SPHK1 gene expression level and abnormal karyotypes with poor prognosis such as seven monosomy or complex abnormality (data not shown), suggesting that SPHK1 gene expression is independent from karyotype abnormality. Although we did not make a sequential analysis of the same MDS patient, SPHK1 might be a candidate of the surrogate marker of AL and MDS, because its expression gradually increased during the progression of MDS and high in AL.

Okazaki's group[6] reported the correlation between chemoresistance and the increase of glucosylceramide synthase and spingomyelin synthase in leukemia cell lines and a small clinical sample. The increase of these enzyme activities might decrease the cellular ceramide level. However, the significance of these findings has not been repeated by others. In our analysis, the message levels of these genes did not show any significant difference between AL and normal control. ASMase, ACDase and SPL were not different between AL and normal control.

Interestingly, NSMase2 was decreased in AL, RAEB and RA samples as compared to normal control. Among SMases, NSMase2 has been cloned recently and was reported to play as a growth suppressor linking confluence to the G0/G1 cell cycle checkpoint.[7] The decrease of NSMase2 in AL is an unexpected and novel finding. Considering the sphingolipid rheostat model, either the increase of SPHK1 or the decrease of NSMase2 gene expression results in the decrease of ceramide/S1P ratio in leukemia blast cells, which might stimulate cell proliferation or survival. Although the regulatory mechanism of ASMase gene expression was reported in cell lines, the transcriptional regulation of NSMase2 has not been clarified yet, and is an interesting topic for future analysis. Statistical significance was also observed in SPP1 only between AL and normal control but not between MDS and normal control. As for SPL and SPP1, which convert S1P to phosphoethanolamine and palmitaldehyde, or sphingosine, respectively, we only observed the increase in SPP1 message but not SPL. The increase of SPP1 expression is also thought to modulate the ceramide/S1P rheostat, however, the significance of this finding remains to be determined.

As to other well-known chemoresistance genes, MDR showed no significant difference between AL and normal control. On the contrary, statistical significance was observed in BCL2 between AL and normal, but not between MDS and normal control. We analyzed the relationship between SPHK1 and MDR or SPHK1 and BCL2 gene expression. In AL, there was no relationship between SPHK1 gene expression level and two well-recognized genes of chemoresistance, MDR and BCL2, of the same sample (precise data not shown). This could suggest that SPHK1 is not always located downstream of BCL2 in AL.

Furthermore, it was also revealed that there are no correlationships between SPHK1 and NSMase2, between SPHK1 and SPP1 gene expression, or between SPHK1 and SPHK2 gene expression.

Table 1 Primer sets of quantitative RT-PCR

Hugo gene nomenclature	*chromosome*	*Alternate name*	*GenBank accession no.*	*Amplicon location relative to transcription start (bp)*	*% GC*	*Forward primer sequence*	*Reverse primer sequence*	*Annealing Temperature*
SPHK1	17	Sphingosine kinase 1	NM_021972	+821/+1060	58.6	TCCTGGCACTGCTGCACTC	TAACCATCAATTCCCCATCCAC	61.0
SPHK2	19	Sphingosine kinase 2	NM_020126	+26/+186	70.9	AGCAGCAGGACCAGAGGCCA	GGTGAGGGCAAAGCGTGGG	67.0
SPL	10	Sphingosine-1-phosphate lyase 1	NM_003901	+794/+934	51.5	TGGAGGTGGATGTGCGGGCAA	CCCAGACAAGCGTCGACATGAAG	62.0
SPP1	4	Sphingosine-1-phosphate phosphatase 1	NM_030791	+637/+772	44.9	ACCGCCATCCCCATTTCT	AGGAATCCAGCAATAATATCCAG	59.0
ASMase	11	Acid sphingomyelinase	NM_000543	+1073/+1220	56.0	AAGCCCTGCGCACCCTCAGAA	CCTGAAGCTCCCCCACCAGCC	64.0
NSMase2	2	Neutral sphingomyelinase II	NM_018667	+1535/+1645	60.0	ACTTTGATAACTGCTCCTCTGAC	TTCGTGTCCAGCAGAGTACC	63.0
ACDase	8	Acid ceramidase	NM_177924	+875/+968	47.8	GATATTGGCCCCAGCCTACTTT	ACCCTGCTTAGCATCGAGTTCA	60.0
GlcCer synthase	9	Glucosylceramide synthase	NM_003358	+144/+341	37.5	CAAGCTCCCAGGTGTCTCTCTTC	GATTAATGCCAACTTTTTTACCACCTA	64.0
SM synthase1	10	Sphingomyelin synthase 1	NM_147156	+757/+894	48.4	GAAGCCCAACTGCGAAGAATAA	AGAGTCGCCGAGG GGAATAC	60.0
MDR	7	Multidrug resistance 1	NM_000927	+1141/+1349	48.7	AGTGGGCACAAACCAGATAA	CTGTCCATCAACACTGACCA	63.0
BCL2	18	BCL2 (B-cell lymphoma type 2)	NM_000633	+337/+583	61.7	GCCGAGATGTCCAGCCAG	AGTTCCACAAAGGCATCCCA	62.0
ABL	22	PCR primer	Sense			CCCAACCTTTTCGTTGCACTGT		
			Antisense			CGGCTCTCGGAGGAGACGTAGA		
		Taqman probes	Sense			ACTAAAGGTGAAAAGCTCCGGGTC-FITC		
			Antisense			LCRed640-TAGGCTATAATCACAATGGGGAATGG		

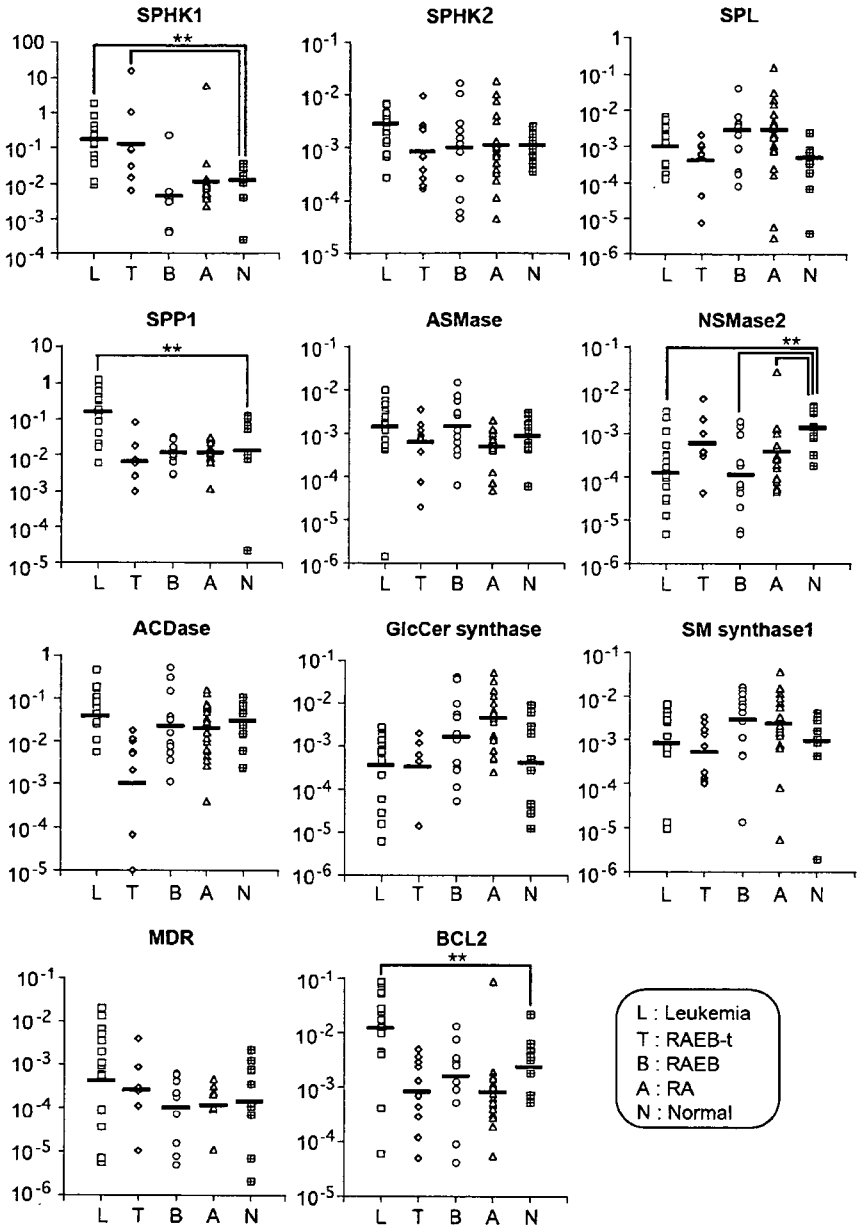

Figure 1 Relative message expression levels of sphingolipid metabolic enzymes in AL, MDS and normal control. Quantitative RT-PCR was performed with bone marrow RNA. The relative expression level was calculated with the enzyme gene expression/ABL gene expression level and was shown in the log scale. The classification of MDS was according to the FAB classification. Horizontal short bar denotes the mean value of the group. Statistical significances were analyzed by using one-way factorial analysis of variance and multiple comparison test (Bonferroni/Dunn's method). *Means $P<0.01$.

Apoptosis induction can be suitable for the treatment of diseases such as malignant tumors. The trials of novel inhibitors of SPHK1 are based on the hypothesis that the modification of ceramide/S1P rheostat induces apoptosis of malignant tumor cells and enhances chemosensitivity. Actually, a synthetic compound with SPHK inhibitor activity can induce apoptosis in tumor cells even with multidrug resistance.[3] As we[4] recently reported that SPHK1 activity is a chemotherapy sensor in prostate cancer cells, and overexpression of SPHK1 has been reported in solid tumors,[3] it is of interest to know whether this observation can be applied to hematological malignancies, especially acute leukemia and MDS. Such data are important because the relapse after intensive chemotherapy is still an unsolved problem in leukemia treatment, and new remedies are urgently required. Our data showed AL or RAEB-t cases with much higher SPHK1 gene expression compared to normal. Therefore, it is suggested that the development of new SPHK1 inhibitor is also beneficial for AL patients whose SPHK1 gene expression (and probably SPHK enzyme activity) is enhanced.

Bonhoure *et al.*[8] reported that sustained SPHK1 overexpresion can render HL60 cells chemoresistant by decreasing the cellular ceramide level and that a novel SPHK1 inhibitor, F-12509a, could recover chemosensitivity. Considering the molecular target of chemotherapy, enzyme inhibitors are more practical than agents for enzyme activation (NSMase2 in our case). Inhibitor of SPHK1 is almost at the stage of clinical investigation. In the present study, we could analyze only one time point of patients (mostly at their first diagnosis before chemotherapy) and could not measure enzyme activities due to the paucity of samples. The measurement of SPHK1 gene expression and/or SPHK enzyme activity of each patient might be necessary to assess the efficacy of enzyme inhibitors in future clinical settings. Sequential analysis of the same patients will also add further information.

The localization and activation of enzymes are also important factors to determine the final cellular ceramide/S1P rheostat. SPHK1 was reportedly activated by phosphorylation by agonists and translocated to membranes.[2] Therefore, further analysis of enzyme activation is necessary to conclude firmly that sphingolipid metabolizing enzymes such as SPHK1 are a novel and promising molecular target for acute leukemia chemotherapy.

Taken together, this is, to our knowledge, the first report of a gene expression profile of major sphingolipid metabolizing enzymes of AL and MDS using quantitative RT-PCR, It documents the increase of SPHK1 gene expression in AL and RAEB-t and the decrease of NSMase2 gene expression in AL and RAEB, suggesting that sphingolipid metabolizing enzymes such as SPHK1 could be a novel target for the chemotherapy of AL.

Acknowledgements

We express sincere thanks to Professor S Nakamura and other members of the Department of Laboratory Medicine, Nagoya University School of Medicine for their assistance. This work was partially supported by the Health and Labor Sciences Research Grants for the Research on Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

A dual-color FISH assay distinguishes between ELL and MLLT1 (ENL) gene rearrangements in t(11;19)-positive acute leukemia

Leukemia (2006) **20**, 2046–2050. doi:10.1038/sj.leu.2404371; published online 14 September 2006

Cytogenetics is an important factor in hematologic disease classification and prognostication. However, accurate cytogenetic diagnosis depends on a number of parameters including overall mitotic index, the presence of dividing cells from the abnormal cell population, chromosome morphology and band-length resolution. As a result, chromosome aberrations found in patients with leukemia may be missed or inaccurately interpreted when the banding resolution is at or below 400 bands per haploid set. In view of these well-recognized limitations, fluorescence *in situ* hybridization (FISH) has proven to be particularly useful for the characterization and identification of known or suspected chromosome aberrations in neoplastic studies.[1]

Patients with t(11;19) leukemias have one of two known 19p breakpoints resulting in gene rearrangement with the mixed lineage leukemia protein (*MLL*) gene on 11q23. The eleven-nineteen lysine-rich leukemia (*ELL*) gene at 19p13.1 is rearranged in individuals with t(11;19) acute myeloid leukemia,[2] whereas the myeloid/lymphoid or mixed-lineage leukemia

Difference in clinical features between Japanese and German patients with refractory anemia in myelodysplastic syndromes

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Several reports indicate that there might be differences in clinical features between Asian and Western myelodysplastic syndrome (MDS) cases. We analyzed refractory anemia (RA) in French-American-British (FAB) classification cases diagnosed in Japan and Germany to perform a more exact comparison between Asian and Western MDS types. In the first step, we analyzed agreement of morphologic diagnosis between Japanese and German hematologists. Blood and bone marrow slides of 129 patients diagnosed with FAB-RA, FAB-RA with ringed sideroblasts (RARS), or aplastic anemia were selected randomly and evaluated separately by each group. The agreements of diagnoses according to FAB and World Health Organization (WHO) classifications were 98.4% and 83.8%, respectively. Second, we compared clinical features between 131 Japanese and 597 German patients with FAB-RA. Japanese patients were significantly younger than German patients. Japanese patients had more severe cytopenias. However, prognosis of Japanese patients was significantly more favorable than that of German patients. Japanese patients had a significantly lower cumulative risk of acute leukemia evolution than did German patients. Frequency of WHO-RA in Japanese patients with FAB-RA was significantly higher than that in German patients. In conclusion, our results indicate that the clinical features of Japanese patients with FAB-RA differ from those of German patients. (Blood. 2005;106:2633-2640)

Introduction

Myelodysplastic syndromes (MDSs) are acquired clonal stem cell disorders characterized by ineffective hematopoiesis with myelodysplasia[1] and are associated with a high risk of progression to acute leukemias.[2] MDSs are very heterogeneous in terms of their morphology, clinical features, and survival.[3] Refractory anemia (RA) according to the French-American-British (FAB) classification is generally classified as a low-risk group in MDS,[4] comprising 30% to 40% of all MDS cases. It was reported that the International Prognostic Scoring System (IPSS) was useful for assessing the prognosis in the whole group of patients with MDS according to the FAB classification.[5] According to the World Health Organization (WHO) classification,[6] most patients with FAB-RA are classified as refractory cytopenia with multilineage dysplasia (RCMD) or, less frequently, as WHO-RA. It was reported that patients with WHO-RA had more favorable prognoses than did patients with RCMD.[7-9]

There are several reports indicating possible differences in clinical features between Western MDS types and Eastern MDS types. It has been reported that the median age of Japanese patients with MDS is 60 years.[10] The median age of patients with MDS in Korea and Thailand and the mean age of those in Central Africa were reported to be 57,[11] 56,[12] and 57 years,[13] respectively. However, large MDS studies from Western countries showed a median or mean age of 68 to 73 years.[5,14-16] We have reported that the clinical features of RA with excess of blasts (RAEB) or RAEB in transformation according to FAB classification seemed to be similar between Japanese and Western patients.[17] However, previous reports indicate that Japanese patients with MDS have a lower frequency of RA with ringed sideroblasts (RARS) according to FAB classification and a higher frequency of FAB-RA than the Western IPSS study[17,18] and that there are different prognostic factors between Japanese and Western patients with MDS.[10,17] From cytogenetic analysis, it was indicated that the frequency of Japanese MDS with isolated del(5q) was lower than that in the IPSS study.[18] We additionally reported that patients with FAB-RA demonstrated favorable outcomes compared with those of the IPSS study.[17] We consider that there are different clinical features between Asian and Western patients with low-risk MDS. In the present study, after conducting an interobserver morphologic variation study for diagnosis of MDS subgroups between the Japanese and German hematologists, we compared

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Submitted January 6, 2005; accepted June 12, 2005. Prepublished online as *Blood* First Edition Paper, June 21, 2005; DOI 10.1182/blood-2005-01-0040.

Supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (no. 16639013) (I.J.) and Kompetenznetzwerk "Akute und Chronische Leukämien" des Bundesforschungsministeriums.

A.M. designed the research, performed morphologic analyses, collected the data, analyzed the data, and wrote the manuscript; U.G. and I.J. designed the research, performed morphologic analyses, collected the data, and analyzed the data; M.T. designed the research, performed morphologic analyses, and analyzed the data; M.I. collected the data, performed morphologic analyses, and analyzed the data; M.B. designed the research and analyzed the data; M.M., A.K., S.K., and M.A. performed morphologic analyses and collected the data; and Y.M., H.T., and M.S. collected the data.

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in detail the clinical features of Japanese and German patients in FAB-RA.

Patients and methods

Patients

A total of 728 consecutive patients (Japan, 131 cases; Germany, 597 cases) with a diagnosis of primary RA according to FAB classification (FAB-RA) were included in this retrospective analysis. Japanese patients were diagnosed at the Saitama Medical School Hospital, Nagasaki University Hospital or affiliated hospitals in Japan between April 1976 and January 1997. German patients were diagnosed at the Department of Hematology, Oncology, and Clinical Immunology of the Heinrich-Heine University in Germany between January 1973 and December 2002. Patients who had previously been treated with antineoplastic drugs or ionizing radiation were excluded from the study. Informed consent was provided according to the Declaration of Helsinki. This study was performed according to the guideline of the institutional review board of the Saitama Medical School.

Interobserver variation study

Hematologic examinations were performed using standard methods (peripheral blood [PB] and bone marrow [BM] Wright-Giemsa or May-Giemsa stained films). PB and BM differential counts were performed on 100 and 500 cells, respectively. Evaluations of bone marrow cellularity were performed using the specimens of BM trephine biopsy and/or clot section.

In the first step, we reviewed all the training slides of FAB-RA (25 Japanese and 20 German cases) by Japanese and German hematologists separately. After this training review, the first joint review meeting for morphologic consensus was performed by 4 Japanese and 4 Germany hematologists for 4 days in February 2002 at Heinrich-Heine-University. At the first joint review, we mainly discussed evaluation of dysplasia and diagnosis using the training slides.

In the second step, the slides of 129 patients (110 FAB-RA, 7 FAB-RARS, 12 aplastic anemia [AA] diagnosed by Japanese or German groups) were selected randomly and were evaluated for morphologic diagnosis according to FAB and WHO classifications by the Japanese and German groups separately in each country. Patients with FAB-RA were reclassified to WHO subgroups according to the criteria of a previous German report.[7] After this separate review, the second joint review meeting for morphologic consensus was performed by 4 Japanese and 4 German hematologists for 4 days in October 2004 at Heinrich-Heine-University. The observers were blinded to the clinical and laboratory data, including cytogenetics, until finishing this separate review. Diagnoses of FAB classification or AA were performed using only morphologic findings. Concerning diagnoses of WHO classification, morphologic and cytogenetic findings were used. In the second joint review meeting, the concordance rate of morphologic diagnosis according to the FAB and WHO classifications between Japanese and German hematologists was analyzed, and we discussed cases whose diagnoses did not agree between Japanese and German hematologists in this separate review.

Cytogenetic analysis

Cytogenetic analyses were performed with a trypsin-Giemsa banding technique on BM cells from aspirates. Ordinarily 20 to 30 metaphases were examined. Cytogenetic aberrations were grouped according to the IPSS publication.[5]

Clinical studies

Comparisons of the clinical features and the prognostic factors between 131 Japanese and 597 German patients with FAB-RA were analyzed. Patients were followed for overall survival (OS) and leukemic progression through June 2004 in Japanese cases and July 2003 in German cases. OS was measured from the date of diagnosis until death from any cause, the date of stem cell transplantation, or until the last patient contact. Leukemic progression was measured from the date of diagnosis until the date of diagnosis of acute leukemia. We classified causes of death into 3 subtypes: MDS related (MDS death), MDS unrelated (non-MDS death), and unclear cause (unclear death). MDS death was defined as acute leukemia, infection, bleeding, or heart failure resulting from anemia or iron overload. Non-MDS death was defined as causes of certain independence from MDS. Unclear death was defined as causes of death without any obvious MDS-related sign and without causes of certain independence from MDS. We also measured survival period censored non-MDS death or unclear death (modified survival). Measurements of modified survival were censored at the date of death in patients with non-MDS death or unclear death.

Statistical methods

The chi-square test and the nonparametric Mann-Whitney test were used to compare the proportions of patients and continuous data, respectively. The Kaplan-Meier method was used to generate the estimate of cumulative probabilities of OS, modified survival, and cumulative risk of acute leukemia evolution. The difference in the cumulative probabilities within subcategories of patients was compared using a 2-sided log-rank test. Age- and sex-adjusted effects of clinical parameters on outcomes were performed with use of 2 different models of multivariate Cox proportional hazards regression. Model A included age category, sex, dichotomized peripheral blood counts, and chromosome category of IPSS. Model B included age category, sex, and IPSS score. An examination for interaction between parameters was performed with the inclusion of interaction terms into each model. The effects of clinical parameters were evaluated as hazard ratios and their 95% confidence intervals. The interobserver concordance was evaluated using the simple κ coefficient. A 2-sided P value of less than .05 was considered to be statistically significant. All statistical analyses were performed with the use of SAS software (version 8.2; SAS Institute, Cary, NC), and all graphic presentations were performed with the use of StatView (version 5.0; SAS Institute).

Results

Morphologic consensus

Of the 129 cases reviewed, the agreement of morphologic diagnosis according to FAB classification between Japanese and German hematologists was 98.4%. A significant concordance was achieved while using FAB classification (κ, 0.94; $P < .001$). There were 2 cases whose diagnoses did not agree between Japanese and German hematologists by separate review. One case was diagnosed as AA by the Japanese group, but the diagnosis by the German group was FAB-RA. The final diagnosis of this case as AA was reached by consensus among the Japanese and German groups by joint review. Another case was diagnosed as RAEB by the Japanese group, but the diagnosis by the German group was FAB-RA. The Japanese hematologists judged that percentage of BM blasts of this patient was slightly higher than 5%. Each group performed morphologic examination again. As a result, the blasts percentage was judged to be less than 5%. The final diagnosis of this case as FAB-RA was reached by consensus among the Japanese and German groups by joint review. Of the 110 FAB-RA and 7 FAB-RARS cases reviewed for WHO classification, the agreement of morphologic diagnosis according to WHO classification between Japanese and German hematologists was 83.8%. A significant concordance was achieved while using WHO classification (κ, 0.73; $P < .001$).

Comparison of clinical features and prognostic factors between Japanese and German patients with FAB-RA

Clinical and laboratory features at the time of diagnosis. The age of Japanese patients with FAB-RA was significantly younger

Table 1. Laboratory features at the time of diagnosis and clinical features in patients with RA classified according to the FAB criteria

	Japan, n = 131	Germany, n = 597	P
Sex, male/female	70/61	309/288	.73
Age, y	57 (12-88)	71 (7-93)	< .001
Neutrophil count, × 10^9/L	1.58 (0.05-10.24)	1.98 (0.06-23.00)	< .001
Hemoglobin concentration, g/L	84 (25-143)	94 (30-169)	.002
Platelet count, × 10^9/L	41 (4-390)	127 (2-1540)	< .001
2- or 3-lineage cytopenias, %*	68	39	< .001
Abnormal karyotype, %	29	53	< .001
Median survival, mo	175	40	< .001

Values for presentation characteristics are given as median and range (in parentheses) where applicable.

*Cytopenia according to IPSS: hemoglobin concentration less than 100 g/L, absolute neutrophil count less than 1.5 × 10^9/L, platelet count less than 100 × 10^9/L.

than that of German patients with FAB-RA ($P < .001$). The sex ratios were not significantly different between the 2 countries. Japanese patients with FAB-RA had significantly lower absolute neutrophil counts (ANCs), lower hemoglobin (Hb) concentrations, lower platelet (PLT) counts, and higher frequency of 2 or 3 lineage cytopenias according to the IPSS definition than did German patients with FAB-RA (Table 1). Cytogenetic analysis was performed in 102 Japanese and 199 German patients. In the Japanese FAB-RA group, the frequency of cytogenetic abnormalities was 30 patients (29%). In contrast, cytogenetic abnormalities were found in 105 (53%) of the German patients with FAB-RA. Japanese patients with FAB-RA had a significantly lower frequency of cytogenetic abnormalities than did German patients with FAB-RA. The subgroups of cytogenetic abnormalities according to IPSS are summarized in Table 2. The distribution of the cytogenetic subgroups according to IPSS showed no significant difference between Japanese and German patients with FAB-RA. Japanese patients with FAB-RA had a significantly lower frequency of FAB-RA associated with an isolated del(5q) cytogenetic abnormality (5q− syndrome) than did German patients with FAB-RA. Japanese patients with FAB-RA were highly categorized into the intermediate-1 (INT-1) risk subgroup, whereas German patients were equally categorized into the low-risk and INT-1 risk subgroups. The frequency of patients with intermediate-2 (INT-2) risk was low in both countries (Table 3).

Prognosis. Follow-up periods ranged from 1 to 292 months (median, 69 months) in Japanese patients with FAB-RA. Follow-up periods in German patients with FAB-RA ranged from 0 to 313 months (median, 13 months). During the follow-up period, 50 Japanese patients and 252 German patients died, and 10 Japanese patients and 56 German patients transformed to acute leukemia. Japanese patients showed a significantly lower cumulative risk of acute leukemia evolution than did German patients (Figure 1). Concerning causes of death, German patients were classified as 153 cases of MDS death (50 acute leukemia, 25 bleeding, 64 infection, 14 heart failure), 24 cases of non-MDS death, and 75 cases of unclear death. Japanese patients were classified as 40 cases of MDS death (11 acute leukemia, 9 bleeding, 19 infection, 1 heart failure), 7 cases of non-MDS death, and 3 cases of unclear death. In both OS and modified survival, all Japanese patients with FAB-RA had a more favorable prognosis than did all German patients with FAB-RA (OS median survival: Japan, 175 months; Germany, 40 months; $P < .001$; modified survival median survival: Japan, 202 months; Germany, 73 months; $P < .001$) (Figure 2A). In OS, for those aged 60 years or younger, Japanese patients with FAB-RA had a more favorable OS than did German patients with FAB-RA (median survival: Japan, 217 months; Germany, 66 months; $P < .001$) and for those aged older than 60 years, Japanese patients with FAB-RA had a more favorable OS than did German patients with FAB-RA (median survival: Japan, 59 months; Germany, 35 months; $P = .025$). In modified survival, for those aged 60 years or younger, Japanese patients with FAB-RA had a more favorable modified survival than did German patients with FAB-RA (median survival: Japan, > 292 months; Germany, 108 months; $P < .001$). However, for those aged older than 60 years, Japanese patients with FAB-RA did not show a more favorable modified survival than did German patients with FAB-RA (median survival: Japan, 102 months; Germany, 69 months; $P = .46$) (Figure 2B-C).

Prognostic factors. In Japanese patients with FAB-RA, the clinical variables of age older than 60 years and Hb concentration less than 70 g/L were significantly correlated with OS. Sex, Hb concentration less than 100 g/L, PLT count fewer than 100 × 10^9/L, ANC fewer than 1.5 × 10^9/L, cytopenias (2 or 3 lineages), and IPSS cytogenetic subgroups were not significantly correlated with OS (Table 3). In German patients with FAB-RA, age older than 60 years, Hb concentration less than 100 g/L, PLT count fewer than 100 × 10^9/L, cytopenias (2 or 3 lineages), and IPSS cytogenetic subgroups were significantly correlated with OS. Sex and ANC fewer than 1.5 × 10^9/L were not significantly correlated with OS (Table 3). The IPSS cytogenetic subgroups and IPSS subgroup were significantly correlated with cumulative risk of acute leukemia evolution in Japanese patients with FAB-RA (Table 3). The other clinical variables in Table 3 were not significantly correlated with cumulative risk of acute leukemia evolution. ANC fewer than 1.5 × 10^9/L, PLT count fewer than 100 × 10^9/L, cytopenias (2 or 3 lineages), IPSS cytogenetic subgroups, and IPSS subgroup were significantly correlated with cumulative risk of acute leukemia evolution in German patients with FAB-RA. Age, sex, and Hb concentrations were not significantly correlated with cumulative risk of acute leukemia evolution (Table 3).

In the age- and sex-adjusted multivariate analyses for OS, there was no clinical parameter that associated with OS in Japanese patients in all models, whereas cytopenias (especially, thrombocytopenia and anemia) and poor IPSS cytogenetic subgroup, and INT-1 and INT-2 IPSS risk subgroups retained as significantly adverse clinical parameters for OS in German patients. For acute leukemia evolution, poor IPSS cytogenetic subgroup and INT-2 IPSS risk subgroup were retained as significant parameters in the cumulative risk of acute leukemia evolution in Japanese patients after age and sex adjustment, whereas in German patients ANC fewer than 1.5 × 10^9/L was no longer associated with acute

Table 2. Cytogenetic findings at the time of diagnosis in patients with RA classified according to the FAB criteria

	Japan, n = 102	Germany, n = 199
Good, no. (%)	79 (77.5)	143 (71.8)
Normal	72	94
−Y	1	4
del(5q)	3	39
del(20q)	3	6
Intermediate, no. (%)	15 (14.7)	31 (15.6)
Poor, no. (%)	8 (7.9)	25 (12.6)
Complex (3 or more abnormalities)	5	16
Chromosome 7 anomalies	3	9

Intermediate indicates other abnormalities not listed in good and poor classifications.

Table 3. Univariate analysis of overall survival and cumulative risk of acute leukemia in patients with RA classified according to the FAB criteria

Variable, by country of origin	No. of patients	Percentile of OS (mo)			Percentile of cumulative risk of AML (mo)			
		75%	50%	P	10%	25%	50%	P
Japanese patients								
Age, y								
60 or younger	72	114	217	< .001	NR	NR	NR	.12
Older than 60	59	18	59		51	NR	NR	
Sex								
Male	70	42	176	.85	74	NR	NR	.53
Female	61	53	129		104	NR	NR	
Neutrophil count								
Fewer than 1.5 × 10^9/L	63	52	157	.84	51	NR	NR	.16
At least 1.5 × 10^9/L	68	53	176		NR	NR	NR	
Hemoglobin concentration								
Less than 100 g/L	81	52	114	.24	92	NR	NR	.95
At least 100 g/L	50	53	202		38	NR	NR	
Hemoglobin concentration								
Less than 70 g/L	45	23	100	.01	104	NR	NR	.81
At least 70 g/L	86	62	202		92	NR	NR	
Platelet count								
Fewer than 100 × 10^9/L	109	52	175	.37	92	NR	NR	.35
At least 100 × 10^9/L	22	54	109		14	NR	NR	
Cytopenia (IPSS)								
0/1	42	53	202	.84	NR	NR	NR	.83
2/3	89	52	157		92	NR	NR	
Chromosome (IPSS)								
Good	79	76	175	.17	104	NR	NR	< .001
Intermediate	15	19	NR		NR	NR	NR	
Poor	8	27	102		4	37	NR	
IPSS*								
Low	21	76	202	.29	NR	NR	NR	< .001
INT-1	73	52	175		104	NR	NR	
INT-2	8	27	102		4	22	NR	
German patients								
Age, y								
60 or younger	133	26	66	< .001	13	91	NR	.85
Older than 60	461	14	35		21	136	173	
Sex								
Male	309	16	41	.92	21	78	173	.41
Female	288	16	43		19	NR	NR	
Neutrophil count								
Fewer than 1.5 × 10^9/L	162	14	43	.54	17	52	173	.014
At least 1.5 × 10^9/L	301	16	37		25	NR	NR	
Hemoglobin concentration								
Less than 100 g/L	337	9	30	< .001	14	136	NR	.18
At least 100 g/L	217	23	57		42	173	NR	
Hemoglobin concentration								
Less than 90 g/L	235	8	29	< .001	17	136	NR	.15
At least 90 g/L	319	20	51		40	173	NR	
Platelet count								
Fewer than 100 × 10^9/L	207	9	23	< .001	11	50	136	< .001
At least 100 × 10^9/L	339	23	53		35	NR	NR	
Cytopenias (IPSS)								
0/1	288	23	55	< .001	63	NR	NR	< .001
2/3	188	7	22		10	28	136	
Chromosome (IPSS)								
Good	143	27	66	< .001	25	NR	NR	< .001
Intermediate	31	26	44		10	91	91	
Poor	25	7	16		4	14	52	
IPSS*								
Low	82	43	82	< .001	NR	NR	NR	< .001
INT-1	78	12	31		10	27	91	
INT-2	11	4	7		2	5	52	

Variables are defined in Tables 1 and 2.
OS indicates overall survival; AML, acute myeloid leukemia; NR, not reached.
*Low indicates 0; INT-1, 0.5-1.0; and INT-2, 1.5-2.0, according to IPSS score.

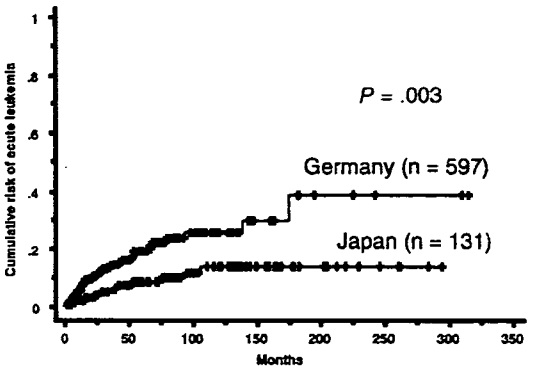

Figure 1. Cumulative risk of acute leukemia evolution of patients with FAB-RA. Japanese patients had a lower cumulative risk of acute leukemia evolution than did German patients ($P = .003$).

leukemia evolution, but other parameters in the univariate analyses were retained as poor prognostic factors (Table 4).

WHO classification. The original diagnoses according to the WHO classification by each group in the present series show that the frequency of WHO-RA in Japanese patients with FAB-RA (73%) was significantly higher than in the German patients with FAB-RA (24%) ($P < .001$). In Japanese patients, patients with WHO-RA were significantly younger and had significantly lower PLT counts than did patients with RCMD. The OS of Japanese patients with WHO-RA was significantly more favorable than that of Japanese patients with RCMD (Table 5). The OS of all Japanese patients with WHO-RA was significantly more favorable than that of all German patients with WHO-RA (Figure 3A). For those aged 60 years or younger, the OS of Japanese patients with WHO-RA was significantly more favorable than that of German patients with WHO-RA. However, for those older than 60 years, Japanese patients with WHO-RA did not show a more favorable OS than did German patients with WHO-RA (Figure 3B-C). Frequencies of poor karyotype according to IPSS in Japanese patients with WHO-RA and RCMD were 4% and 20%, respectively. Japanese patients with WHO-RA had a lower cumulative risk of acute leukemia evolution than did Japanese patients with RCMD (10% cumulative risk: WHO-RA, not reached; RCMD, 38 months; 25% cumulative risk: RCMD, 104 months; $P = .018$).

Discussion

Different clinical features between Asian and Western patients with MDS have been reported by several studies.[10,17] However, these data are based on local series of patients. Speculation about certain differences is problematic because there might be differences in the interpretation of dysplasia in blood and bone marrow by different observers. The present study aimed to characterize the racial features of Western and Asian MDS cases. We thought that an assessment of interpretation of morphologic findings and definition of diagnostic criteria was warranted to check that the diagnoses by the Japanese group were in line with those of the German group, before comparing the clinical features between Japanese and German patients with FAB-RA. In the present study, the agreement of morphologic diagnosis between Japanese and German hematologists was 98.4%. It was confirmed that the diagnoses according to FAB classification or AA were not different between the Japanese and German groups. After morphologic consensus was obtained at the first joint review meeting, we performed this separate review. The concordance rate according to the FAB classification of morphologic diagnosis between Japanese and German hematologists was thus excellent. However, the subjects of this separate review were only FAB-RA, FAB-RARS, and AA cases that had already been diagnosed by the Japanese or German groups. We think that it is most difficult to distinguish FAB-RA and disorders

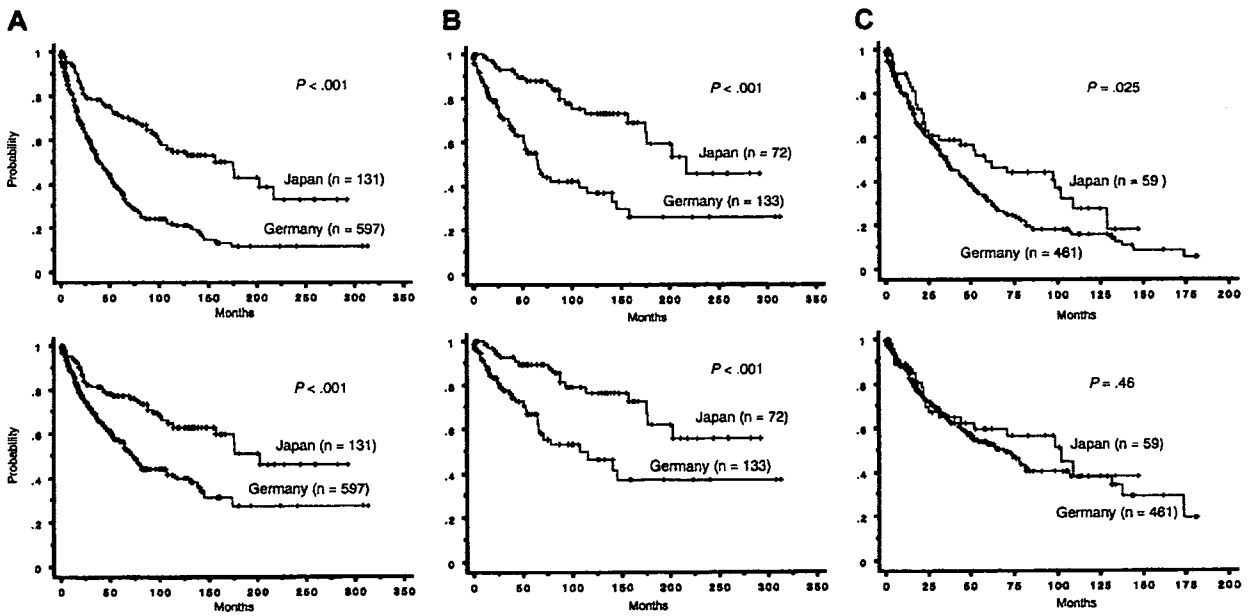

Figure 2. Cumulative survival of patients with FAB-RA. (Top) Overall survival (OS). (Bottom) Modified survival. (A) In all patients with FAB-RA, Japanese patients had a more favorable prognosis than did German patients in OS ($P < .001$). Japanese patients had a more favorable prognosis than did German patients in modified survival ($P < .001$). (B) In patients aged 60 years or younger, Japanese patients had a more favorable prognosis than did German patients in OS ($P < .001$). Japanese patients had a more favorable prognosis than did German patients in modified survival ($P < .001$). (C) In patients aged older than 60 years, Japanese patients had a more favorable prognosis than did German patients in OS ($P = .025$). Japanese patients did not show a more favorable prognosis than did German patients in modified survival ($P = .46$).

Table 4. Multivariate analysis of parameters that affected overall survivors and acute leukemia evolution in patients with RA classified according to the FAB criteria

Characteristic, by model	Overall survival		Leukemic transformation	
	Japanese HR (95% CI)	German HR (95% CI)	Japanese HR (95% CI)	German HR (95% CI)
Model A				
Age older than 60 y	5.1 (2.6-9.9)*	2.2 (1.5-3.0)*	1.6 (0.4-6.4)	1.7 (0.9-3.3)
Sex, male	1.2 (0.7-2.2)	1.0 (0.8-1.4)	1.6 (0.4-7.1)	1.2 (0.7-2.1)
ANC fewer than 1.5 × 10^9/L	1.2 (0.7-2.2)	1.0 (0.7-1.3)	2.0 (0.5-8.2)	1.7 (0.9-3.1)
Platelet count fewer than 100 × 10^9/L	1.3 (0.6-2.7)	1.9 (1.4-2.5)*	0.4 (0.1-1.9)	2.2 (1.2-4.1)*
Hemoglobin concentration less than 100 g/L	1.5 (0.8-2.8)	1.8 (1.4-2.4)*	1.0 (0.2-3.9)	1.9 (1.1-3.5)*
Chromosome (IPSS), intermediate	1.5 (0.6-3.6)	1.1 (0.6-1.9)	1.5 (0.2-14)	2.3 (0.9-5.6)
Chromosome (IPSS), poor	1.4 (0.5-4.2)	2.8 (1.6-4.9)*	11.9 (2.4-59)*	6.6 (2.8-16)*
Model B				
Age older than 60 y	4.6 (2.5-8.7)*	2.1 (1.5-2.9)*	1.7 (0.5-6.3)	1.6 (0.8-3.0)
Sex, male	1.1 (0.6-2.0)	1.2 (0.9-1.6)	1.7 (0.4-6.7)	1.2 (0.7-2.1)
IPSS, INT-1	1.0 (0.5-1.8)	1.4 (1.0-2.0)*	1.0 (0.2-4.7)	3.1 (1.6-5.7)*
IPSS, INT-2	1.6 (0.5-4.7)	4.0 (2.0-8.0)*	8.6 (1.7-43)*	9.5 (3.2-27)*

Model A included age category, sex, dichotomized peripheral blood counts, and chromosome category of IPSS. Model B included age category, sex, and IPSS score.

HR indicates hazard ratio; 95% CI, 95% confidence interval; ANC, absolute neutrophil count; IPSS, International Prognosis Score System; INT-1, Intermediate-1; INT-2, intermediate-2.

*Statistically significant hazard ratio.

with secondary dysplasia (collagen diseases, viral infectious diseases, and liver cirrhosis, etc). If we included these diseases in the present separate review, the concordance rate of morphologic diagnosis would likely have been lower.

Our results indicate that the clinical features of Japanese FAB-RA cases differ from those of German cases. Comparing Japanese and German FAB-RA cases we found that the median age of Japanese patients with FAB-RA was lower than that of German patients with FAB-RA. The population pyramids (negative growth type) and life expectancies (Japan, 80.7 years; Germany, 77.4 years) at 2000 in Japan and Germany are almost the same.[19] Therefore, we think that this difference of median age is real. Furthermore, Japanese patients with FAB-RA had more pronounced cytopenia, especially more severe thrombocytopenia, and a higher frequency of pancytopenia or bicytopenia, as compared with German patients with RA. Also the cytogenetic characteristics differed between Japanese and German RA cases. Although there was no difference in the distribution of cytogenetic subgroups according to IPSS, the frequency of chromosomal abnormalities was lower in Japanese patients with RA; notably that of isolated del (5q) was lower in Japan. Toyama et al[18] and Matsushima et al[20] reported that Japanese patients with MDS had a lower frequency of isolated del (5q) than Western reports (2.0% and 1.5%, respectively). Morel et al[21] and Greenberg et al[5] reported that the frequencies of isolated del (5q) in all MDS cases were 4.7% and 5.9%, respectively. The majority of patients with 5q− syndrome are diagnosed as FAB-RA at diagnosis. If the percentage that patients with FAB-RA compared with all MDS is assumed to be 35%, that of 5q− syndrome in the present German patients with FAB-RA becomes 6.9% of all MDS. Although this frequency of 5q− syndrome present in German patients with FAB-RA was slightly higher than the reports of Morel et al[21] and Greenberg et al,[5] we believe that the result of the present study supported Japanese previous reports.

Table 5. Laboratory features at the time of diagnosis and clinical features in Japanese patients with WHO-RA and RCMD classified according to the WHO criteria

	WHO-RA, n = 96	RCMD, n = 32	*P*
Sex, male/female	53/43	15/17	.41
Age, y	55 (12-86)	66 (16-88)	.038
Neutrophil count, × 10^9/L	1.62 (0.26-4.69)	1.28 (0.05-10.24)	.76
Hemoglobin concentration, g/L	87 (30-143)	71 (25-140)	.094
Platelet count, × 10^9/L	38 (4-246)	127 (13-390)	.026
2- or 3-lineage cytopenias, %*	67	75	.38
Abnormal karyotype, %	24	36	.26
Median survival, mo	176	52	.023

Values for presentation characteristics are given as median and range (in parentheses) where applicable.

*Cytopenia according to IPSS: hemoglobin concentration less than 100 g/L, absolute neutrophil count less than 1.5 × 10^9/L, platelet count less than 100 × 10^9/L.

In OS, regardless of age, Japanese patients with FAB-RA had a more favorable prognosis than did their German counterparts. In modified survival, for those aged 60 years or younger, Japanese patients with FAB-RA had a more favorable modified survival than did German patients with FAB-RA. Therefore, we believe that the favorable prognosis of younger patients with FAB-RA (≤ 60 years) is certain. In modified survival, for those older than 60 years, Japanese patients with FAB-RA did not show a more favorable modified survival than did German patients with FAB-RA. Therefore, the prognostic difference between Japan and Germany may result from the characteristics of young Japanese patients with FAB-RA (≤ 60 years).

Characteristics of Japanese patients with WHO-RA were younger and had lower PLT counts. These characteristics were similar to those of MDS responders for immunosuppressive therapy (IST) in a report by Molldrem et al.[22] The response rate for IST from a Japanese report[23] was higher than from Western reports.[22,24] However, only 8 Japanese cases received IST and only 3 responded in our present study. We think that a large-scale study is necessary to establish the relationship between Japanese WHO-RA and response for IST.

In the present study, IPSS was useful for assessing OS in German FAB-RA cases but not in Japanese FAB-RA cases. This was mainly due to the lack of a significant correlation between the number and degree of cytopenias and OS in Japanese patients with FAB-RA. In the IPSS publication, the researchers reported that cytopenias (2 or 3 lineages) were related with poor survival. In this study, however, Japanese patients showed more favorable prognoses despite possessing more pronounced cytopenia. Management of thrombocytopenia seems to be similar between Japan and

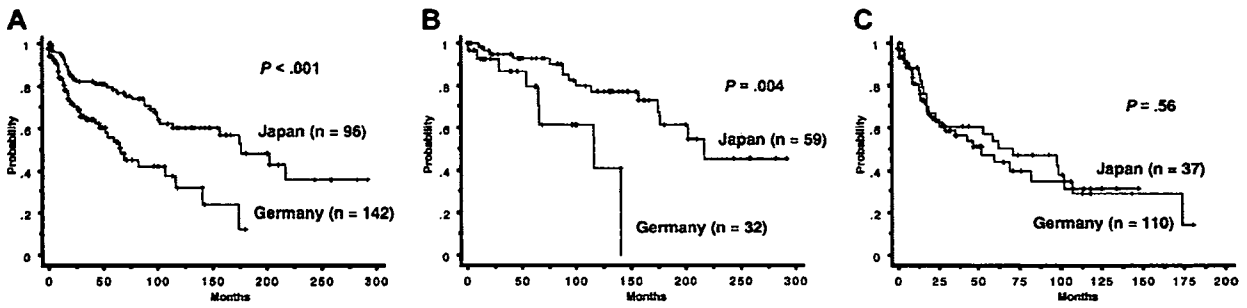

Figure 3. Cumulative overall survival of patients with WHO-RA. (A) Among all patients with WHO-RA, Japanese patients had a more favorable prognosis than did German patients ($P < .001$). (B) In patients aged 60 years or younger, Japanese patients had a more favorable prognosis than did German patients ($P = .004$). (C) In patients aged older than 60 years, Japanese patients did not show a more favorable prognosis than did German patients ($P = .56$).

Germany. Concerning the prognostic effect of Hb concentration, the threshold was different between Japanese and German patients with FAB-RA. Most of the Japanese patients with Hb concentrations greater than 70 g/L had no symptoms related to anemia and did not require red cell transfusion. In fact, most Japanese patients with Hb concentration lower than 70 g/L had received red cell transfusion. In contrast, most German patients with Hb concentration lower than 90 g/L had received red cell transfusion. We presumed that the cause of the different prognostic Hb concentration thresholds by Japanese and German patients may be related to these red cell transfusion procedures. We also presume that the difference in Hb concentration used as a threshold for red cell transfusion may be related to the different general characteristics among races rather than the different characteristics of FAB-RA between Asian and Western patients with FAB-RA. The Italian guideline recommends that all patients with Hb concentration lower than 80 g/L should receive red cell transfusion.[25] Japanese patients with FAB-RA with Hb concentration greater than 70 g/L do not usually require regular red cell transfusion. We compared Japanese patients with RA with Hb concentrations greater than 100 g/L and those with Hb concentrations of 70 to 100 g/L. In fact, the latter group (70-100 g/L) did not differ in clinical course from patients with Hb concentrations greater than 100 g/L ($P = .86$). Moreover, Japanese patients with Hb concentrations of 70 to 100 g/L had a significantly more favorable prognosis than those with Hb concentrations lower than 70 g/L ($P = .039$) (Figure 4). This result indicates that the Hb threshold below which transfusion should be recommended may be different between Asian and Western patients with FAB-RA.

We think that our results concerning the prognostic OS effect of chromosomal findings may be insufficient and may include some problematic issues. In particular, the observation periods of Japanese patients with poor karyotype according to IPSS may be problematic. Four of 8 Japanese patients with poor karyotype are surviving. However, the observation periods of the 2 surviving patients were insufficient (1 and 6 months, respectively). Concerning acute leukemia evolution, the effect of chromosomal findings was not different between Japanese and German patients. We think that the prognostic effect on OS of chromosomal findings may not be different between Japanese and German patients, if sufficient observation periods for Japanese patients with poor karyotype are available.

We made great efforts to achieve morphologic consensus in the present study. The original diagnoses according to FAB and WHO classifications were not different between the Japanese and German groups. In the present series, the original diagnoses according to the WHO classification by each group show the frequency of WHO-RA in Japanese patients to be higher than that in German patients. In Japanese patients, the prognosis of patients with WHO-RA was more favorable than that of patients with RCMD, and patients with WHO-RA had a lower cumulative risk of acute leukemia evolution than did patients with RCMD. In a previous report of a German group,[7] the same results had been reported. This finding indicates that one reason for the better prognosis of Japanese patients may be the different distribution of subgroups by WHO classification between Asian and Western patients with FAB-RA, namely a higher frequency of patients with WHO-RA in Japan. In Japanese patients, patients with WHO-RA were younger and had lower PLT counts than did patients with RCMD, significantly. Furthermore, the prognosis of Japanese patients with WHO-RA was significantly more favorable than that of Japanese patients with RCMD. For those aged 60 years or younger, the prognosis of Japanese patients with WHO-RA was significantly more favorable than that of German patients with WHO-RA. However, for those older than 60 years, Japanese patients with WHO-RA did not show a more favorable prognosis than did German patients with WHO-RA. These findings in young Japanese patients with WHO-RA ($\leq$ 60 years) might indicate the differences in clinical features between Japanese and German patients with FAB-RA.

This is the first report to compare clinical features between Asian and Western patients with FAB-RA after confirming a morphologic consensus. Our results indicate that the clinical features of Japanese FAB-RA cases differ from those of German cases. These differences are not due to the different interpretation of morphologic features by different observers. Several guidelines[25,26] have been published in Western countries. To adapt these Western guidelines to Asian patients, some modifications may be required, taking into account ethnic characteristics.

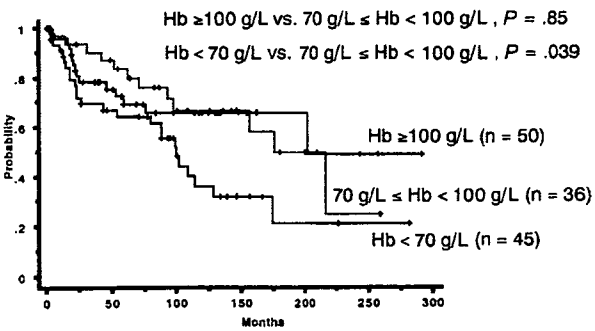

Figure 4. Cumulative overall survival of Japanese patients with FAB-RA. The group with hemoglobin concentration of 70 to 100 g/L showed no significant prognostic difference from the group with hemoglobin greater than 100 g/L in patients with FAB-RA ($P = .85$). The group with hemoglobin concentrations of 70 to 100 g/L had a more favorable prognosis than did the group with hemoglobin concentrations lower than 70 g/L in patients with FAB-RA ($P = .039$).

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Leukemia Research xxx (2008) xxx–xxx
Leukemia Research
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Letter to the Editor

Mutation analysis of AML1 gene in pediatric primary myelodysplastic syndrome and juvenile myelomonocytic leukemia

To the Editor,

Chromosomal translocation of *t*(8;21) is frequently found in patients with acute myeloid leukemia-M2. In 1991, Miyoshi et al. first sequenced the AML1 gene on chromosome 21, which is a frequent target of chromosomal translocation associated with leukemia [1]. They found three forms of the gene transcript, which they named AML 1a (250 amino acids), AML 1b (453 amino acids) and AML 1c (480 amino acids) [2]. The three proteins share a Runt domain encoded within exons 3 through 5 of the AML1 gene. AML 1 protein is one of the alpha subunits of the transcription factor polyomavirus enhancer binding protein 2 (PEBP2). The alpha subunit binds DNA via a Runt domain. AML 1b and 1c also have a long C-terminal region encoded by exons 6, 7B and 8, which also encode a transcription activation domain. The Runt domain is located between the 50th and 177th amino acids of the AML1b protein [3].

We examined bone marrow DNA specimens from eight patients – five boys and three girls – ranging in age from 5 days to 13 years, with primary myelodysplastic syndrome (MDS) or juvenile myelomonocytic leukemia (JMML) for mutations in exons 3 through 8 by PCR-SSCP and direct sequencing (predicted PCR products <207 bases), or standard PCR and direct sequencing (predicted PCR products >207 bases). Six patients suffered from JMML and two suffered from refractory anemia. The DNA specimens were extracted from paraffin-embedded clot materials that had been stocked by the MDS Committee of the Japanese Society of Pediatric Hematology. The PCR primers used in this study and the predicted PCR products for each primer pair are listed in Table 1. Single nucleotide polymorphisms detected are listed in Table 2. The two mutations were not identified in bone marrow DNA specimens from healthy volunteers. A silent mutation was found in one JMML patient, whereas a missense mutation causing amino acid transposition was detected in one patient with refractory anemia. The transposition occurred at only the 3rd amino acid from the Runt domain of the AML1 protein. It is possible that this amino acid transposition influenced the Runt domain because aspartic acid is acidic and asparagine is relatively neutral.

AML-1 gene mutations have been frequently observed in patients with secondary MDS [4–6]. Harada et al. reported that 17% of adult patients with primary MDS showed AML-1 gene mutation, compared with half of adult patients with secondary MDS. Moreover, they mentioned that 8% of adult primary MDS showed mutations in the Runt domain, whereas in 9% the mutations occurred in the C-terminal region [5]. The two mutations detected in our study did not occur in the Runt domain of the AML-1 gene. The rate of mutation of the AML-1 gene in patients with pediatric primary MDS or JMML (2/8 = 25%) was higher than that in adult patients, although most cases of primary MDS, both adults and children, do not have AML-1 gene mutation(s) and the number of cases we studied was lower than those of adult MDS examined by Harada et al. [5]. Our data indicate that AML-1 gene mutation may be more closely related to the pathological backgrounds of pediatric primary MDS or JMML than to

Table 1
Oligonucleotide primers used for amplification of the AML1 gene and the predictable products

Exon	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (°C)	Predictable PCR products
Exon 3	CAAGCTAGGAAGACCGACCC	TGCAGGGTCCTAACTCAATC	61	440 bases
Exon 4	ACTTCGACCGACAAACCTGA	CCTGATGTCTGCATTTGTCC	63	186 bases
Exon 5	GTGTACCAGCCCCAAGTGGA	GCCACCAACCTCATTCTGTT	63	178 bases
Exon 6	GCAGTGGGCTCCATCTGGTA	CTGATCTCTTCCCTCCCTCC	64	279 bases
Exon 7A	CCACATTCTGCCTTCCTCAT	TTTCTCCCTGGTCACACATG	63	193 bases
Exon 7B	AGAATGTGTTTTCAAGTGGC	GACCTTTCTGATTCTCTTCA	55	207 bases
Exon 8	TGACCTACAGCGAGATCCTG	CCGCAACCTCCTACTCACTT	63	684 bases

0145-2126/$ – see front matter
doi:10.1016/j.leukres.2007.09.004

Please cite this article in press as: Masunaga A, et al., Mutation analysis of AML1 gene in pediatric primary myelodysplastic syndrome and juvenile myelomonocytic leukemia, Leuk Res (2008), doi:10.1016/j.leukres.2007.09.004

Table 2
Cases and mutation characteristics of the patients with pediatric primary MDS or JMML

Age (years)	Gender	Genome mutation of AML 1b	Amino acid mutation of AML 1b protein (single-letter amino acid codes)
13	GIRL	142 G>A	48 D>N
2.9	BOY	441 T>G	147 T>T

DDBJ accession number: D43968.

that of adult primary MDS. It remains to be clarified whether the relation of the AML1 gene to pediatric primary MDS and JMML is different from that to adult primary MDS or not.

Acknowledgements

This work is not supported financially.

Contributions: Atsuko Masunaga was involved in summary, histological diagnosis, PCR and SSCP analysis; Toshiyuki Mitsuya in histological diagnosis; Tsuyoki Kadofuku in PCR and SSCP analysis, direct sequencing; Sanju Iwamoto in PCR and SSCP analysis; Akira Miyazaki in PCR and SSCP analysis; Atsushi Manabe in cytological, hematological and clinical diagnoses; Yuji Zaike in collection and custody of the paraffin-embedded clot materials; Masahiro Tsuchida in cytological, hematological and clinical diagnoses and Tatsutoshi Nakahata in cytological, hematological and clinical diagnoses.

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4 September 2007

International Journal of
HEMATOLOGY

The Effect of Anabolic Steroids on Anemia in Myelofibrosis with Myeloid Metaplasia: Retrospective Analysis of 39 Patients in Japan

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Received June 21, 2006; received in revised form January 16, 2007; accepted January 22, 2007

Abstract

Between 1999 and 2005, 285 patients received new diagnoses of myelofibrosis with myeloid metaplasia (MMM) in Japan. Anemic symptoms were present in 162 patients, and hemoglobin (Hb) concentrations were <10 g/dL in 197 patients. Fifty-five MMM patients were treated with anabolic steroids, and their effect on anemia during MMM was evaluated in 39 patients. A "good" response was defined as an Hb increase of ≥1.5 g/dL, cessation of transfusion dependence, and an Hb concentration of >10 g/dL maintained for at least 8 weeks. A "minimum" response was defined as an Hb increase of ≥1.5 g/dL and transfusion independence for at least 8 weeks. Both good and minimum responses were considered "favorable." Favorable responses were achieved in 17 patients (44%, 8 good and 9 minimum responses). None of the pretreatment variables, such as the lack of transfusion dependence, a higher Hb concentration at the start of treatment, or the absence of cytogenetic abnormalities, were associated with a response to anabolic steroid therapy. Adverse events associated with anabolic steroid therapy were moderate and transient. Two patients required definitive withdrawal of treatment. Thus, anabolic steroids are well tolerated and effective for the treatment of anemia in a subset of MMM patients.
Int J Hematol. 2007;85:338-343. doi: 10.1532/IJH97.06135

Key words: Myelofibrosis with myeloid metaplasia; Anabolic steroids; Anemia; Therapy

1. Introduction

Myelofibrosis with myeloid metaplasia (MMM) is a clonal stem cell disorder that is characterized by the fibrosis, osteosclerosis, and angiogenesis of bone marrow stromal cells [1]. Jak2 [2,3], an essential tyrosine kinase that transduces cytokine signals by binding to cytokine receptors, is mutated in approximately 40% of MMM patients [4-7]. Jak2 is constitutively activated in such cases in the absence of cytokine stimulation, leading to autonomous cell growth [5]. Consequently, the number of megakaryocytes increases, and there is excess production of cytokines, including transforming growth factor β1 and osteoprotegerin, which stimulate bone marrow stromal cells to induce myelofibrosis and osteosclerosis [8-10]. Because MMM is a stem cell disease, conventional drug therapies are often ineffective and only palliative. Allogeneic hematopoietic stem cell transplantation is the only known curative treatment [11-14]. MMM occurs more often in elderly people, however, and MMM prognoses vary widely, with survival times ranging from only months to more than a

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