Kaplan-Meier method, and groups were compared using the log-rank test. The probabilities of the others were estimated based on a cumulative incidence method to accommodate competing risks. Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS, and a Fine and Gray proportional hazards model for the others.

The following variables for multivariate analysis were considered: age (<45 versus ≥45 years), disease status at CBT (standard risk versus high risk), cord blood nucleated cell count ($<2.5 \times 10^7$ versus $\geq 2.5 \times 10^7$/kg), cord blood CD34 + cell count ($<1 \times 10^5$ versus $\geq 1 \times 10^5$/kg), HLA disparities based on antigen level HLA-A and -B and allele level HLA-DRB1 (1 versus 2 versus ≥3), sex compatibility between donor and recipient (female donor to male recipient versus other), year of CBT (1998 to 2005 versus 2006 to 2013), and ABO compatibility between donor and recipient (match versus major mismatch versus minor mismatch versus bidirectional mismatch). The ABO match was considered the reference group in the multivariate analyses.

All statistical analyses were performed with EZR, a graphic user interface for R 2.13.0 [11]. $P < .05$ was considered significant. Analysis of data was performed in August 2013. The median follow-up of surviving patients was 92 months (range, 5 to 181) after CBT in the entire cohort.

RESULTS

The characteristics of patients and cord blood units are shown in Table 1. There were no significant differences among the 4 groups, except for HLA disparities. The major mismatch group contained a slightly higher number of HLA disparities as compared with the minor mismatch group ($P = .07$) or the bidirectional mismatch group ($P = .08$), although these were not statistically significant.

The probability of OS at 5 years significantly differed among the 4 groups in univariate analysis ($P = .03$) (Figure 1A). However, multivariate analysis of mortality adjusting for other variables showed no significant difference between ABO match and major (hazard ratio [HR], 1.20; $P = .62$), minor (HR, .72; $P = .41$), or bidirectional (HR, 1.76; $P = .14$) mismatch (Table 2). In univariate analysis, ABO incompatibility was not associated with cumulative incidence of TRM (Figure 1B) or relapse (Table 2). In multivariate analysis, a trend toward a higher incidence of TRM was observed in the major mismatch compared with the match group, but this was not significant ($P = .05$).

In univariate analysis, there was no significant difference in the cumulative incidence of grades II to IV aGVHD among the 4 groups ($P = .91$) (Figure 1C). In multivariate analysis, a higher number (≥3) of HLA disparities (HR, 1.56; 95% confidence interval [CI], 1.05 to 2.32; $P = .02$), a higher cord blood CD34 + cell count (HR, 1.51; 95% CI, 1.05 to 2.18; $P = .02$), and older year of CBT (HR, 1.85; 95% CI, 1.30 to 2.65; $P < .01$) were associated with a higher incidence of grades II to IV aGVHD, but ABO incompatibility was not associated with the incidence of grades II to IV aGVHD (Table 2). The cumulative incidence of grades III to IV aGVHD significantly differed among the 4 groups in univariate analysis ($P = .02$). However, multivariate analysis adjusting for other variables showed no significant difference in the cumulative incidence of grades III to IV aGVHD between ABO match and major (HR, 2.56; $P = .19$), minor (HR, .59; $P = .56$), or bidirectional (HR, 1.46; $P = .67$) mismatch (Table 2). In univariate analysis, there was no significant difference in the cumulative incidence of extensive cGVHD among the 4 groups ($P = .86$) (Figure 1D). In multivariate analysis, older age (HR, 1.85; 95% CI, 1.06 to 3.23;

Table 1
Characteristics of Patients, Cord Blood Units, and Transplantation

	Total	Match	Major Mismatch	Minor Mismatch	Bidirectional Mismatch	P
Number (%)	191	55 (28)	47 (24)	58 (30)	31 (16)	
Age, yr, median (range)	40 (16-55)	40 (16-55)	40 (16-53)	40 (16-53)	41 (18-52)	.94
Disease type, n (%)						.61
AML	101 (52)	30 (54)	24 (51)	30 (51)	17 (54)	
ALL	45 (23)	17 (30)	10 (21)	11 (18)	7 (22)	
MDS	25 (13)	5 (9)	5 (10)	10 (17)	5 (16)	
CML	11 (5)	1 (1)	4 (8)	4 (6)	2 (6)	
NHL	9 (4)	2 (3)	4 (8)	3 (5)	0 (0)	
Disease status at CBT,* n (%)						.09
Standard risk	79 (41)	24 (44)	17 (36)	30 (51)	8 (25)	
High risk	112 (58)	31 (54)	30 (64)	28 (48)	23 (74)	
Conditioning regimen, n (%)						.36
TBI12Gy+Ara-C/G-CSF+CY	131 (68)	34 (61)	33 (70)	40 (68)	24 (77)	
TBI12Gy+Ara-C+CY	31 (16)	9 (16)	11 (23)	9 (15)	2 (6)	
TBI12Gy+CY	16 (8)	6 (10)	1 (2)	5 (8)	4 (12)	
TBI12Gy+others	13 (6)	6 (10)	2 (4)	4 (6)	1 (3)	
GVHD prophylaxis, n (%)						.10
Cyclosporine A + methotrexate	188 (98)	55 (100)	47 (100)	57 (98)	29 (93)	
Cyclosporine A	3 (1)	0 (0)	0 (0)	1 (2)	2 (6)	
Number of nucleated cells, $\times 10^7$/kg, median (range)	2.43 (1.32-5.69)	2.52 (1.32-5.50)	2.47 (1.65-4.92)	2.38 (1.51-5.69)	2.58 (1.65-5.07)	.79
Number of CD34$^+$cells, $\times 10^5$/kg, median (range)	.92 (.17-7.75)	.88 (.28-3.15)	.93 (.17-1.99)	.91 (.28-7.75)	1.14 (.44-2.84)	.20
HLA disparities,† n (%)						.05
1	23 (12)	4 (7)	7 (14)	8 (13)	4 (12)	
2	106 (55)	32 (58)	16 (34)	37 (63)	21 (67)	
3	57 (29)	17 (30)	23 (48)	12 (20)	5 (16)	
4	5 (2)	2 (3)	1 (2)	1 (1)	1 (3)	
Sex compatibility, n (%)						
Female donor to male recipient	58 (30)	19 (34)	13 (27)	17 (29)	9 (29)	.88
Other	133 (69)	36 (65)	34 (72)	41 (70)	22 (70)	
Year of CBT, n (%)						.58
1998-2005	102 (53)	28 (50)	22 (46)	33 (56)	19 (61)	
2006-2013	89 (46)	27 (49)	25 (53)	25 (43)	12 (38)	

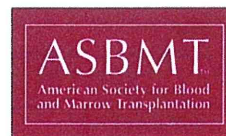
AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia, MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; NHL, non-Hodgkin lymphoma, Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide.

* For disease status at CBT, patients in complete remission (CR) 1 or CR2 without poor prognostic karyotype for AML and ALL, refractory anemia for MDS, chronic phase for CML, and CR1 or CR2 for NHL were classified as standard risk, whereas patients in all other situations were classified as high risk.

† The number of HLA disparities defined as low resolution for HLA-A and -B and high resolution for HLA-DRB1.

Biol Blood Marrow Transplant 20 (2014) 396–401

Single-Unit Cord Blood Transplantation after Granulocyte Colony-Stimulating Factor–Combined Myeloablative Conditioning for Myeloid Malignancies Not in Remission



Takaaki Konuma [1,*], Seiko Kato [1], Jun Ooi [2], Maki Oiwa-Monna [1], Yasuhiro Ebihara [1], Shinji Mochizuki [1], Koichiro Yuji [1], Nobuhiro Ohno [1], Toyotaka Kawamata [1], Norihide Jo [1], Kazuaki Yokoyama [1], Kaoru Uchimaru [1], Shigetaka Asano [3], Arinobu Tojo [1], Satoshi Takahashi [1]

[1] *Department of Hematology/Oncology, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[2] *Department of Hematology/Oncology, Teikyo University School of Medicine, Tokyo, Japan*
[3] *System Medical Biology Laboratory, School of Advanced Science and Engineering, Waseda University, Tokyo, Japan*

Article history:
Received 9 November 2013
Accepted 4 December 2013

Key Words:
Cord blood transplantation
Granulocyte colony-stimulating factor
Acute myelogenous leukemia
Myelodysplastic syndrome
not in remission

A B S T R A C T

High disease burden in myeloablative allogeneic hematopoietic stem cell transplantation is associated with adverse outcomes in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS). Quiescent leukemia stem cells could be induced to enter cell cycle by granulocyte colony-stimulating factor (G-CSF) administration and become more susceptible to chemotherapy. We report on the outcome of unrelated cord blood transplantation (CBT) using a conditioning regimen of 12 Gy total body irradiation, G-CSF–combined high-dose cytarabine, and cyclophosphamide in 61 adult patients with AML or advanced MDS not in remission. With a median follow-up of 97 months, the probability of overall survival and cumulative incidence of relapse at 7 years were 61.4% and 30.5%, respectively. In multivariate analysis, poor-risk cytogenetics and high lactate dehydrogenase values at CBT were independently associated with inferior survival. These data demonstrate that CBT after G-CSF–combined myeloablative conditioning is a promising curative option for patients with myeloid malignancies not in remission.

© 2014 American Society for Blood and Marrow Transplantation.

INTRODUCTION

The prognoses of patients with acute myelogenous leukemia (AML) and advanced myelodysplastic syndrome (MDS) who have not achieved remission after chemotherapy have been poor. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only potentially curative therapy for such patients, high disease burden has been reported to be associated with increased relapse or poor survival rate after allo-HSCT [1-9]. Recently, cord blood (CB) has been considered an acceptable alternative as a source of hematopoietic stem cells in unrelated allo-HSCT for adult patients without HLA-identical related or unrelated donors [9-16]. In comparison with other sources of allo-HSCT, one of the main advantages of using CB for patients with a high disease burden who require urgent transplantation is its rapid and convenient availability. Because it was shown that administration of granulocyte colony-stimulating factor (G-CSF) increased the susceptibility of cell-cycle–specific agent cytarabine in leukemia cells in vitro [17], we administered G-CSF–combined high-dose cytarabine in myeloablative conditioning for allo-HSCT [18,19] and reported that a G-CSF–combined conditioning regimen provided better engraftment and survival results in cord blood transplantation (CBT) for myeloid malignancies [13-16]. The objective of this retrospective study was to confirm the effects of CBT after G-CSF–combined myeloablative conditioning in adult patients with myeloid malignancies not in remission and to identify variables influencing long-term outcomes.

PATIENTS AND METHODS

Patients and Transplantation Procedures

This retrospective study included 61 consecutive adult patients who underwent unrelated transplantation using single-unit CB for AML or advanced MDS not in remission at our institute between 1998 and 2013. Thirty-two patients were included in our previous study [15,16] and extended the follow-up. The diagnoses of AML and MDS were made according to the World Health Organization classification. Advanced MDS was defined as having refractory anemia with excess blasts type 1 or refractory anemia with excess blasts type 2 by World Health Organization classification. Myeloid malignancies not in remission were defined as more than 5% blasts in the bone marrow (BM), or circulating blasts in peripheral blood (PB) or central nervous system. The cytogenetic subgroups were defined according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML [20] and International Prognostic Scoring System criteria for MDS [21]. All patients received 12 Gy total body irradiation (TBI) in 4 divided fractions on days −8 and −7, cytarabine on days −5 and −4 (total dose 12 g/m2, and 3 g/m2 every 12 hours for 2 days) with 5 μg/kg G-CSF (lenograstim) from 12 hours before the first dose of cytarabine to the end of cytarabine dosing, and cyclophosphamide (total dose 120 mg/kg) on days −3 and −2 [15,16]. Fifty-eight patients received cyclosporine (CSP) (3 mg/kg/day) with a short course of methotrexate (15 mg/m2 on day +1 and 10 mg/m2 on days +3 and +6), and 3 patients received CSP only as graft-versus-host disease (GVHD) prophylaxis. CB units were obtained from the Japanese Cord Blood Bank Network. Donor-recipient HLA-matching status was based on antigen level HLA-A and -B and on allele level HLA-DRB1 typing. All patients received similar supportive care and CB units were

Financial disclosure: See Acknowledgments on page 400.
* Correspondence and reprint requests: Takaaki Konuma, Department of Hematology/Oncology, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail address: tkonuma@ims.u-tokyo.ac.jp (T. Konuma).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.12.555

Table 1
Characteristics of Patients, Cord Blood Units, and Transplantation

Characteristic	Value
No. of patients	61
Sex	
Male	36 (59)
Female	25 (41)
Age, median (range), yr	41 (18-55)
CMV serostatus	
Positive	54 (86)
Negative	7 (11)
Disease type	
De novo AML	24 (39)
AML secondary to MDS	24 (39)
Advanced MDS*	13 (21)
Cytogenetics†	
Good	1 (2)
Intermediate	27 (44)
Poor	30 (49)
Unknown	3 (5)
Bone marrow blasts at CBT, median (range), %	17.7 (1.4-86.0)¶
< 25%	39
≥ 25%	22
Peripheral blood blasts at CBT, median (range), %	6.5 (0-68.5)
Absent	12
Present	49
LDH at CBT	
≤ ULN	41 (67)
> ULN	20 (33)
Disease status at CBT‡	
Untreated	31 (51)
Primary refractory	14 (23)
Refractory relapse	16 (26)
Time from diagnosis to CBT, median (range), mo	7 (1-219)
Conditioning regimen	
TBI12Gy+Ara-C/G-CSF+CY	61
GVHD prophylaxis	
CyclosporineA+methotrexate	58 (95)
CyclosporineA	3 (5)
Number of nucleated cells, median (range), $\times 10^7$/kg	2.43 (1.32-5.50)
Number of $CD34^+$cells, median (range), $\times 10^5$/kg	1.03 (.21-2.27)
HLA disparities§	
1	13 (21)
2	32 (52)
3	14 (22)
4	2 (3)

CMV indicates cytomegalovirus; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; CBT, cord blood transplantation; LDH, lactate dehydrogenase; ULN, upper limit of normal; TBI, total body irradiation; Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide; GVHD, graft-versus-host disease; HLA, human leukocyte antigen.
Data presented are n (%) unless otherwise indicated.
* Advanced MDS are defined as having refractory anemia with excess blasts-1 (RAEB-1) or RAEB-2 by WHO criteria.
† The cytogenetic subgroups according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML and International Prognostic Scoring System criteria for MDS.
‡ Untreated was defined as no treatment before conditioning regimen, indicating that the majority of patients with AML secondary to MDS or advanced MDS received CBT as an up-front treatment. Primary refractory was defined as failure to achieve complete remission with induction chemotherapy. Refractory relapse was defined as failure to achieve complete remission with salvage chemotherapy after first or subsequent relapse.
§ The number of HLA disparities, defined as the low resolution for HLA-A and -B and the high resolution for HLA-DRB1.
¶ The 5 patients with less than 5% blasts in the bone marrow included circulating blasts in peripheral blood (n = 3) or central nervous system (n = 2).

selected, as previously reported [15,16]. The institutional review board of the Institute of Medical Science, University of Tokyo approved this study. This study was conducted in accordance with the Declaration of Helsinki.

End Points and Statistical Analysis

The primary study end point was overall survival (OS), defined as time from the date of transplantation to the date of death or last contact. Secondary end points were relapse, including disease progression before engraftment; transplantation-related mortality (TRM); neutrophil and platelet engraftment; acute graft-versus-host disease (aGVHD); and chronic GVHD (cGVHD). Relapse was defined as morphologic evidence of disease in PB, BM, or extramedullary sites. TRM was defined as death during remission. Neutrophil engraftment was defined as the first of 3 consecutive days during which the absolute neutrophil count was at least .5 $\times$ 10^9/L. Platelet engraftment was achieved on the first of 3 days when the platelet count was higher than 50 $\times$ 10^9/L without transfusion support. Both aGVHD and cGVHD were graded according to the previously published criteria [22,23]. The incidence of aGVHD was evaluated in all engrafted patients, whereas the incidence of cGVHD was evaluated in engrafted patients surviving more than 100 days.

The probability of OS was estimated according to the Kaplan-Meier method, and the groups were compared using the log-rank test. The probabilities of relapse, TRM, neutrophil and platelet engraftment, and acute and chronic GVHD were estimated based on a cumulative incidence method to accommodate competing risks [24]. Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS and Fine and Gray proportional hazards model for relapse [25]. The following variables were considered: age (< 45 versus ≥ 45 years), disease type (de novo AML versus AML secondary to MDS versus advanced MDS), cytogenetic risk (other than poor versus poor), proportion of blasts in BM (< 25 versus ≥ 25%), the presence of blasts in PB (absent versus present), lactate dehydrogenase (LDH) at CBT (≤ upper limit of normal versus > upper limit of normal), disease status at CBT (untreated versus primary refractory versus refractory relapse), cord blood nucleated cell count (< 2.5 versus ≥ 2.5 $\times$ 10^7/kg), and HLA disparities based on antigen level HLA-A and -B and allele level

ONCOLOGY LETTERS 6: 323-328, 2013

Effect of *in vivo* administration of reprogramming factors in the mouse liver

AKIRA TOMOKUNI[1], HIDETOSHI EGUCHI[1], HIROMITSU HOSHINO[1], DYAH LAKSMI DEWI[1], SHINPEI NISHIKAWA[1], YOSHIHIRO KANO[1], NORIKATSU MIYOSHI[1], ARINOBU TOJO[2], SEIICHIRO KOBAYASHI[2], NORIKO GOTOH[3], KUNIHIKO HINOHARA[3], NOEMI FUSAKI[4], TOSHIYUKI SAITO[5], HIROSHI SUEMIZU[6], HIROSHI WADA[1], SHOGO KOBAYASHI[1], SHIGERU MARUBASHI[1], MASAHIRO TANEMURA[1], YUICHIRO DOKI[1], MASAKI MORI[1], HIDESHI ISHII[1] and HIROAKI NAGANO[1]

[1]Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871; Divisions of [2]Molecular Therapy and [3]Systems Biomedical Technology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639; [4]DNAVEC Corporation, Tsukuba, Ibaraki 300-2611; [5]Transcriptome Profiling Group, National Institute of Radiological Sciences, Inage-ku, Chiba 263-8555; [6]Biomedical Research Department, Central Institute for Experimental Animals, Miyamae, Kawasaki, Kanagawa 216-0001, Japan

Received January 25, 2013; Accepted May 14, 2013

DOI: 10.3892/ol.2013.1418

Abstract. Cancer is initiated by the transformation of stem cells or progenitor cells via a dedifferentiation process that leads to cancer stem cells; however, the process involves the activation of growth-promoting oncogenes and the inactivation of growth-constraining tumor suppressor genes. The introduction of defined factors, such as those encoded by *c-Myc*, *Sox2*, *Oct3/4* and *Klf4*, in normal somatic cells results in their dedifferentiation into induced pluripotent stem (iPS) cells. We previously reported that these defined factors induced the development of induced multipotent cancer (iPC) cells from gastrointestinal cancer cells by reducing tumor aggressiveness. Previous studies indicated that although reprogramming may be facilitated by *p53* inhibition, gain-of-function oncogenic mutations in *p53* and oncogenic mutations in *Kras*-stimulated tumorigenic activity, and their roles *in vivo* are imperfectly understood. Hence, in the present study, the effect of direct injection of a Sendai virus (SeV) vector encoding four defined factors *in vivo* was studied using various backgrounds of transgenic and knockout mice, and was compared with that of direct injection of microRNAs (miRNAs) diluted with cationic lipid. The *in vivo* imaging data revealed transformation hot spots for *p53* deficiency or conditional activation of mutant *Kras*, and the sizes were concordant with those in immunodeficient NOD/SCID and uPA-NOG mice, as well as larger compared with those in the control mice. Overall, the present data on *in vivo* reprogramming indicated that *Kras* activation may facilitate the effect of cellular reprogramming in normal liver cells, and the effect of *Kras* activation is more apparent than that of tumor suppressor *p53* deficiency. The results also revealed that immunodeficiency may increase the effect of reprogramming, presumably by blocking the immunosurveillance of transformed cells. These findings provide a rationale for further studies to develop a therapeutic approach involving direct *in vivo* reprogramming.

Correspondence to: Dr Hideshi Ishii or Dr Hiroaki Nagano, Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
E-mail: hishii@cfs.med.osaka-u.ac.jp
E-mail: hnagano@gesurg.med.osaka-u.ac.jp

Abbreviations: iPS cells, induced pluripotent stem cells; ES cells, embryonic stem cells; iPC cells, induced multipotent cancer cells; 5-FU, 5-fluorouracil; SeV, Sendai virus; miRNA, microRNA; NOD/SCID mice, NOD.CB17-*Prkdc*^{scid}/J mice; NOG mice, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tmSug}/Jic mice; uPA, urokinase-type plasminogen activator; MMTV, mouse mammary tumor virus; LTR, long terminal repeat

Key words: Kras, *p53*, reprogramming, differentiation, liver

Introduction

The discovery that complete cellular reprogramming may be achieved by introducing the defined transcription factors *c-Myc*, *Sox2*, *Oct3/4* and *Klf4* into terminally differentiated somatic fibroblasts of mouse and human origins was an important breakthrough (1,2). The generation of induced pluripotent stem (iPS) cells by the introduction of defined factors, which are generally expressed in embryonic stem (ES) cells, results in the reconstitution of organs in chimeric mice and contributes to the regeneration of human tissues (3). We previously showed that gastrointestinal cancer cells acquired multipotential differentiation ability upon the introduction of defined factors; the gene expression profiles of mesodermal and ectodermal cells appeared in gastrointestinal cancer cells of endodermal origin

[termed induced multipotent cancer (iPC) cells] (4). Whether the iPC cells were generated via a state of pluripotency remains to be investigated, although the iPC cells expressed ES-like genes and possessed the ability to differentiate from cells of endodermal origin into other endoderm and mesoderm lineages (4). Notably, *in vitro* differentiation resulted in sensitization to therapeutic reagents such as vitamins A and D and the chemotherapeutic agent 5-fluorouracil (5-FU), as well as reduced tumorigenicity, suggesting that altering the cancer cell lineage through reprogramming *in vivo* may be a promising concept for novel and efficient cancer therapy (4). However, at present, there are a limited number of studies concerned with reprogramming *in vivo,* and thus the mechanism involved in reprogramming *in vivo* remains unknown.

Epithelial tumor tissues are composed of various types of mesenchymal cells, such as myofibroblasts, fibroblasts, endothelial cells, lymphocytes, monocytes and macrophages, certain of which are known to be components of a microenvironment (niche). These components are involved in tumorigenesis at the early stages, support cancers cells and provide resistance against exposure to chemotherapeutic reagents. Overall, although it is assumed that mesenchymal cells are important in the process of reprogramming in the complex system *in vivo*, no investigations on how reprogramming factors affect the mesenchymal components have been conducted. To assess this, the effect of direct injection of a Sendai virus (SeV) vector encoding four defined factors into the liver was studied using transgenic and knockout mice with various genetic backgrounds, and the effect was compared with that of direct injection of microRNAs (miRNAs) diluted with cationic lipid. The *in vivo* bioluminescence imaging data revealed transformation hot spots for *p53* (also known as *TP53* in humans and *Trp53* in mice) deficiency or conditional activation of mutant *Kras,* and the sizes were consistent with those in immunodeficient NOD.CB17-*Prkdc*^{scid}/J (NOD/SCID) mice and NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tmSug}/Jic (NOG) mice expressing transgenic urokinase-type plasminogen activator (*uPA)* in the liver (uPA-NOG), as well as larger compared with those in the control mice. The present results suggested that the effect of reprogramming-based, novel therapeutic approaches was enhanced by *Kras* activation. The effect was more apparent with *Kras* activation than with tumor suppressor *p53* deficiency, suggesting a distinct role for the *Kras* pathway in direct reprogramming in the liver. Furthermore, immunodeficiency may increase the effect of reprogramming, presumably by blocking the immunosurveillance of transformed cells.

Subjects and methods

Experimental animals. NOD/SCID mice were purchased from Charles River Japan (Osaka, Japan). All animal experiments were performed with approval from the Animal Experiments Committee of Osaka University. The NOD/SCID mice lack B cells, T cells and the complement system, and possess severely reduced natural killer (NK) cells. More severely immunodeficient uPA-NOG mice were produced by extra-uterine fertilization, resulting in zygotes that expressed transgenic *uPA* in the liver; the extracellular matrix in the liver was modified to activate the hemolytic system, which facilitated xenogeneic engraftment or growth of transformed cells in the present experiment in mice with an immunodeficient background (5). Heterozygous B6.129S4-*Kras*^{tm4Tyj}/J mice (Jackson Laboratory, Bar Harbor, ME, USA), which carry an allele with the most common point mutation whose expression is blocked by the presence of a loxP-flanked stop codon in the ROSA loci, were crossed with B6129-Tg(MMTV-Cre)4Mam/J mice (Jackson Laboratory), which express P1 Cre recombinase under the control of the mouse mammary tumor virus (MMTV) long terminal repeat (LTR) promoter. The MMTV LTR promoter directs a widespread pattern of expression to produce CMV-Cre/*Kras*^{mut} mice; and when expressed in B6.Cg-Tg(Alb-Cre)21Mgn/J mice (Jackson Laboratory), is efficient in achieving liver-specific recombination to produce Alb-Cre/*Kras*^{mut} mice. B6.129S2-*Trp53*^{tm1Tyj}/J mice (Jackson Laboratory), from which a mutant allele was produced by a targeted neo insertion into the *p53* locus, were mated with STOCK Tg(Nanog-GFP, Puro)1 Yam mice, which express the green fluorescent protein under the control of the *Nanog* gene promoter (RIKEN BioResource Center, Tsukuba, Japan), to produce Nanog-GFP/*Trp53*$^{+/-\,(KO)}$ mice. Overall, two immunodeficient mice were used in the experiments, NOD/SCID and uPA-NOG, as well as CMV-Cre/*Kras*^{mut}, Alb-Cre/ *Kras*^{mut} and Nonog-GFP/*Trp53*^{KO} mice. miRNAs were also used to assess the effect.

In vivo administration of viral construct mixture. SeV vectors replicate in the form of negative-sense single-stranded RNA in the cytoplasm of infected cells and do not undergo a DNA phase or integrate into the host genome (6). It was shown that the efficient induction of transgene-free human pluripotent stem cells was achieved using a vector based on SeV, an RNA virus that does not integrate into the host genome; iPS induction could be achieved by the SeV-mediated gene-transfer introduction of the defined transcription factors *c-Myc*, *Sox2*, *Oct3/4* and *Klf4* from terminally differentiated somatic cells (7). A viral construct mixture consisting of: i) 5 μl lentiviral vector and ii) SeV vectors (2.5 μl per each transcription factor) or 10 μl miRNAs was prepared. Co-transfection of the lentiviral luciferase gene was performed to trace the cell populations in which the genes were introduced. The SeV vectors were mixed according to the transcription factors to be introduced, such as SeV vectors encoding *c-Myc*, *Sox2*, *Oct3/4* and *Klf4* (MSOK); *Sox2*, Oct4 and *Klf4* (SOK); or *c-Myc* alone (M). With regard to miRNAs, 60 pmol of double-stranded mature miRNAs (20 pmol of mmu-miR-200c; 5 pmol of mmu-miR-302a, -302b, -302c and -302d; and 10 pmol of mmu-miR-369-3p and -5p) was diluted with 10 μl siPORT (Ambion, Austin, TX, USA). Median laparotomy was performed in each mouse under sevoflurane anesthesia and the viral construct mixture was directly injected into the median lobe of the liver.

In vivo imaging. To trace the behavior of the injected viral construct, the animals were examined at days 14, 21 and 28 using the IVIS Lumina II imaging system (Caliper Life Sciences, Hopkinton, MA, USA) (Fig. 1). Each mouse received luciferin intraperitoneally at 4 mg/kg and was then anesthetized with 2% isoflurane; the mice were left undisturbed for 10 min thereafter. Subsequently, the mice were imaged under the following conditions: Exposure, 2 min; f-stop, 1; binning,

INTERNAL MEDICINE

ORIGINAL ARTICLE

Association between Acute Myelogenous Leukemia and Thrombopoietin Receptor Agonists in Patients with Immune Thrombocytopenia

Yasuo Oshima[1,2], Koichiro Yuji[1], Tetsuya Tanimoto[2,3], Yasushi Hinomura[4] and Arinobu Tojo[1]

Abstract

Objective The development of myeloid malignancies is a concern when administering thrombopoietin receptor (or the myeloproliferative leukemia virus proto-oncogene product, MPL) agonists. Progression from myelodysplastic syndrome (MDS) to acute myelogenous leukemia [AML, 9 (6.12%) AML patients among 147 MDS subjects] was reported in a clinical trial. However, only one (0.15%) case of AML among 653 immune thrombocytopenic purpura (ITP) subjects was reported. Our objective was to determine whether there is currently a safety signal in the FDA files termed Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) for AML in ITP patients who receive MPL agonists.
Methods We conducted a case-controlled study using the FAERS as a source of case and control data. We compared demographic characteristics, such as gender, age and exposure to MPL agonists between AML patients and others among ITP subjects registered between 2002 and 2011.
Results Total of 4,821 ITP subjects were identified, including 62 AML patients. The number of patients treated with romiplostim and eltrombopag was 54 (1.74%) AML patients among 3,102 ITP subjects and nine (1.52%) AML patients among 594 ITP subjects, respectively. It should be noted that all AML patients were exposed to one or more MPL agonists. Another factor associated with AML was male gender.
Conclusion We herein report an association between AML and MPL agonist use in ITP subjects. Due to various biases and the incompleteness of the FAERS data, further studies are warranted to determine whether the detected signal is a real risk. Physicians should not alter their prescribing behaviors based on this single preliminary analysis.

Key words: thrombopoietin receptor agonist, myeloproliferative leukemia virus proto-oncogene product (MPL), immune thrombocytopenia (ITP), FDA adverse event reporting system (FAERS), spontaneous report

(Intern Med 52: 2193-2201, 2013)
(DOI: 10.2169/internalmedicine.52.0324)

Introduction

When human myeloproliferative leukemia virus proto-oncogene product (MPL) was first cloned, it was identified as a proto-oncogene of v-mpl, a truncated form of a cytokine receptor (1). Originally v-mpl was recognized to be a viral oncogene that transforms myeloproliferative leukemia virus-infected hematopoietic progenitors (2). Murine myeloblastic progenitors acquire growth factor independent proliferation on *in vitro* or *in vivo* infection of v-mpl (2-4). Since v-mpl alone is sufficient to promote leukemic transformation, activation of the MPL signal pathway is thought to play a role in leukemogenesis. Due to the mechanisms of action of thrombopoietin receptors (or MPL), one of the concerns regarding the use of these agonists is the potential adverse reaction of myeloid malignancy (5). The risk of progression from myelodysplastic syndromes (MDS) to acute

[1]Institute of Medical Science, the University of Tokyo, Japan, [2]Cancer Institute, Japanese Foundation for Cancer Research, Japan, [3]Internal Medicine, Navitas Clinic, Japan and [4]Japan Pharmaceutical Information Center (JAPIC), Japan
Received for publication February 12, 2013; Accepted for publication May 21, 2013
Correspondence to Dr. Yasuo Oshima, 0-oshima@umin.ac.jp

myelogenous leukemia (AML) has been observed in clinical trials of romiplostim (6). The prescribing information for romiplostim states that a randomized, double-blind, placebo-controlled trial enrolling patients with severe thrombocytopenia and International Prognostic Scoring System (IPSS) low- or intermediate-1-risk MDS was terminated due to the increased number of cases of AML observed in the romiplostim treatment arm. At the time of an interim analysis, among 219 MDS patients randomized 2:1 to receive treatment with romiplostim or a placebo (147 romiplostim: 72 placebo), 11 patients exhibited progression to AML, including nine patients in the romiplostim arm (6.21% [95%CI; 2.83-11.3]) versus two patients in the placebo arm (2.78% [95%CI; 0.34-9.68]). Moreover, Olnes et al. recently reported that as many as three of 25 aplastic anemia patients (12.0%[95%CI; 2.5-31.2%]) treated with eltrombopag developed clonal evolution, including two cases of monosomy 7 and one case of myeloid leukemia (7, 8). Meanwhile, only one case of AML among 653 (0.15% [95%CI; 0.00-0.85]) immune thrombocytopenic purpura (ITP) subjects was reported in a cumulative analysis of clinical trials (http:www.info.pmda.go.jp/shinyaku/P201100020/index.html). Since the risk appeared to be relatively smaller in the ITP patients than in the MDS patients, there were no statistically significant differences between the study drug and placebo groups. Nevertheless, it is noteworthy that the patient who developed AML had been assigned to receive the study drug. Therefore, no statistically significant increases in the incidence of myeloid malignancies have been reported thus far in ITP patients treated with MPL agonists (5).

We routinely run queries to detect signals of adverse reaction risks for whole drugs registered in the Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) and happened to find various potential risks. Some of the detected signals are well known, such as myelosuppression associated with cytotoxic antineoplastic agents or hypoglycemia associated with human insulin or its derivatives. With respect to MPL agonists, we found AML, as well as myelofibrosis, including bone marrow reticulin fibrosis. During the routine queries, not insignificant proportions of patients with myelofibrosis were detected among those receiving romiplostim (2.54%[95%CI; 2.01-3.16]) and eltrombopag (3.81%[95%CI; 2.48-5.57]). An activating mutation of the MPL gene is associated with myelofibrosis rather than AML. In addition, prescriber information for MPL agonists recommends caution regarding the possibility for bone marrow reticulin fibrosis. Therefore, the signals detected for myelofibrosis appear to be relevant. As for AML, it is worth further analyzing the database because MPL agonists are approved for the treatment of ITP, not MDS. We herein further assess the signals of a risk of AML in ITP patients treated with MPL agonists.

Analyzing existing databases, such as the FAERS, can be quickly performed at an affordable cost. Spontaneous reporting databases can be viewed as sources of case and control data for case-controlled studies. However, due to the incompleteness and inconsistencies in spontaneous adverse event reporting, analyses of spontaneous reporting databases are usually regarded as being surrogates for controlled epidemiologic studies. This approach will provide hypotheses to interested scientists. Before conducting conventional controlled epidemiologic research, the use of preliminary and exploratory screening may be helpful, although screening may not yield conclusive results.

Our primary objective was to clarify whether the possibility of the development of AML in patients with ITP is worth further investigation. As a secondary objective, we herein describe the demographic characteristics of the AML patients registered in the database.

Materials and Methods

Study design

A case-controlled study comparing exposure to MPL agonists between patients with myeloid leukemia and a comparison group.

Setting

We used the Japan Pharmaceutical Information Center (JAPIC) AERS database, a version of the FAERS database whose data format was arranged by the JAPIC (Tokyo, Japan). Duplicates and multiple records, a well-known drawback of the FAERS (9), were excluded using a semiautomatic multistep process (10). In order to detect and exclude as many as duplicates as possible in the database, an automated multistep process was applied. The process was carried out using a record-linkage strategy that groups records overlapping in four key fields: event date, patient age and sex and the reporting country, as previously reported (9, 10). Records with three overlaps and just one missing datum were considered to be duplicates.

Subjects

We extracted ITP subjects by searching for their indication for therapeutic drug treatment in the FAERS between 2002 and 2011. The row data handling and basic tabulations were performed as previously reported (11-15). In brief, the codes, "AUTOIMMUNE THROMBOCYTOPENIA," "IDIOPATHIC THROMBOCYTOPENIC PURPURA" or "THROMBOCYTOPENIC PURPURA," coded according to the medical terminology for the safety database, called MedDRA, were assumed to be used to register the ITP subjects. This extraction resulted in the identification of 4,821 ITP subjects after removing duplicate case IDs. Among the ITP subjects, we identified AML patients by searching for the terms, "ACUTE MYELOID LEUKAEMIA," "ACUTE MYELOID LEUKAEMIA (IN REMISSION)," "ACUTE MYELOID LEUKAEMIA AGGRAVATED," "ACUTE MYELOID LEUKAEMIA NOS" and "ACUTE MYELOID LEUKAEMIA RECURRENT," in the adverse reaction fields of the FAERS. Among the 4,281 ITP subjects, there were

Mod Rheumatol (2013) 23:694–704
DOI 10.1007/s10165-012-0709-7

ORIGINAL ARTICLE

Association of hepatitis B with antirheumatic drugs: a case–control study

Yasuo Oshima · Hiroshi Tsukamoto ·
Arinobu Tojo

Received: 13 December 2011 / Accepted: 19 June 2012 / Published online: 18 July 2012
© Japan College of Rheumatology 2012

Abstract
Background Though concern of hepatitis B virus (HBV) reactivation by antirheumatic agents has limited therapeutic opportunities in HBV-infected rheumatoid arthritis (RA) patients, the relative risks (RR) among such agents have not been clarified.
Objective We compared the reporting of antirheumatic-agent-associated hepatitis B.
Patients We assessed 92 hepatitis B cases and 98,069 controls from a population of 98,161 RA patients registered into the US Food and Drug Administration's (FDA's) adverse event database between 2004 and 2010.
Measurements A reporting odds ratio (ROR), a signal suggesting a risk for hepatitis B among antirheumatic agents, was measured.
Results Treatment with corticosteroids [ROR 2.3 (95 % confidence interval 1.3–4.0)], methotrexate [4.9 (3.9–6.0)], rituximab [7.2 (5.3–9.9)], tacrolimus [4.2 (1.5–11.9)], or reporting from Japan [2.2 (1.1–4.2)] were associated with higher signal, whereas adalimumab had a lower ROR [0.2 (0.1–0.4)].
Limitations There are known limitations of spontaneous reporting, such as underreporting, the Weber effect, reporting bias, indication bias, and limited clinical information such as HBV status.
Conclusions Adalimumab's low reporting rate is most likely be due to notoriety. However, the possibility that adalimumab might suppress reactivation of HBV cannot be denied. Until the possibility is clarified in well-designed clinical studies, physicians should use adalimumab cautiously in patients with HBV.

Keywords Hepatitis B · Rheumatoid arthritis · Antirheumatic drug · Adverse event reporting system (AERS) · Spontaneous report

Introduction

Progresses in pathophysiological knowledge, especially in cytokine cascades and their effector cells in rheumatoid arthritis (RA), have brought various developments of new antirheumatic agents. The classes of therapeutic agents directed against specific cytokines or effector cells in the disease process of RA, are: (1) disease-modifying antirheumatic drugs (DMARD), such as methotrexate (MTX), hydroxychloroquine (HCQ), leflunomide (LEF), and sulfasalazine (SSZ); (2) biological DMARDs, such as adalimumab (ADA), etanercept (ETA), infliximab (IFX), and rituximab (RTX); and (3) immunosuppressants, such as tacrolimus (TAC), azathioprine (AZT), cyclosporine (CSA), and mizoribine (MZB). These antirheumatic agents have greatly improved and expanded therapeutic options for RA. However, RA patients infected with hepatitis B virus (HBV) have been excluded from the benefit of therapeutic opportunities with these new agents. Reports about severe hepatitis case with increasing HBV-DNA after methotrexate (MTX) and corticosteroid therapy [1, 2]

Electronic supplementary material The online version of this article (doi:10.1007/s10165-012-0709-7) contains supplementary material, which is available to authorized users.

Y. Oshima (✉) · A. Tojo
The Institute of Medical Science, The University of Tokyo,
4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
e-mail: 0-oshima@umin.ac.jp

H. Tsukamoto
Department of Medicine and Biosystemic Science,
Kyushu University Graduate School of Medical Sciences,
Fukuoka 812-8582, Japan

alerted the medical community about the use of antirheumatic agents in patients with HBV carrier status and proposed an algorithm for assessment and prevention of HBV reactivation in RA patients. The American College of Rheumatology (ACR) made recommendations on the use of DMARDs and biologics based on hepatitis type, Child–Pugh grade, and whether or not antiviral agents to treat hepatitis had been initiated. The college also asked physicians to consider the risks and benefits of all DMARDs [3]. The Japan College of Rheumatology (JCR) more strictly limited the use of tumor necrosis factor-alpha (TNF-α)-blocking biologics and MTX in HBV-carrying RA patients in 2008 (http://www.ryumachi-jp.com/english/index.html).

Serum hepatitis B surface antigen (HBsAg) is infrequent (0.1–0.5 %) in the normal population in the United States and western Europe. However, a prevalence of up to 5–20 % has been found in the Far East and in some tropical countries in patients with Hodgkin's disease, polyarteritis nodosa, and chronic renal disease [4]. Since HBV reactivation was reported not only in HBV carriers but also in RA patients with resolved or past HBV infection [5, 6], and the prevalence of concurrent and resolved HBV infection among RA patients in Japan was reported to be 0.8 % and 25.1 %, respectively [6], approximately one fourth of RA patients appear to be at risk for reactivation of HBV in Japan. As the reasons for restricting the use of certain DMARDs in HBV carriers by the colleges are mainly based on case series, case reports, and reviews of them, and the relative strength of risks still remain to be clarified. Recent reports suggest that screening for HBV infection and careful monitoring during the use of nonbiologic and biologic DMARDs may ameliorate the risk of severe hepatitis [5, 7, 8].

Guideline for the use of immunosuppressants and chemotherapy for malignant neoplasm in patients with HBV carriers is available in Japan [9]. The guidelines are not restricted to antirheumatic agents, but those who want to treat HBV-carrier RA patients may refer to the recommendations mentioned in the guidelines. The JCR also released recommendations regarding immunosuppressant use for RA patients with HBV infection in 2010 (http://www.ryumachi-jp.com/english/index.html). They describe HBV screening and the use of nucleoside analogs prior to immunosuppressive therapies. However, their recommendations lack a description regarding selection of antirheumatic agents. In Japan, one of the HBV-epidemic areas, no useful information appeared to be available for selecting antirheumatic agents for treating HBV-infected patients from the standpoint of relative risk (RR) for HBV reactivation.

There is no doubt that results from prospectively randomized clinical trial yield high-level evidence comparing risk of drugs. In order to assess risk level, prospective intervention studies using randomly assigned nonbiologic DMARDs or biologics to patients selected based on eligibility criteria and standardized assessment of occurrence of hepatitis are, of course, useful. However, ethical limitations and time/cost may make such a study unfeasible. Testing the risk of drugs with concern for severe adverse reactions may not be ethically acceptable. As clinical trials require exhaustive efforts and extensive costs and time, they may not provide timely information with a reasonable cost. Here, we propose the use of an adverse event reporting system (AERS) to rapidly estimate possible risks in these patients, as mentioned elsewhere [10, 11]. Despite limitations of the AERS, it may provide timely information with fewer costs [12, 13]. In this study, we compared reporting odds ratio (ROR) as a signal of risk for HBV reactivation associated with antirheumatic agents use. One can more effectively design clinical trials with less ethical concern to clarify crucial points based on the estimated results from AERS research.

Methods

Study design

A nested case–control analysis of antirheumatic-agent-associated HBV reported to the FDA between January 2004 and December 2010 was conducted. The subcohort study participants were individuals registered in the FDA AERS, with RA as an indication for drug use. Cases and controls were respectively defined as individuals with and without drug-associated HBV among the subcohort. The analyses included the number of unique cases and ROR among antirheumatic agents. In addition to monovariate analyses, a multivariate assessment by unconditional logistic regression was performed.

Datasource

The AERS database was downloaded from the FDA AERS Web page (http://www.fda.gov/), between first quarter 2004 and fourth quarter 2010.

Case identification

Drugs used to treat RA were identified as follows: First, the table for therapeutic indications was searched, which recorded an individual drug identifier (drug code) and corresponding indication for its use. As reported indications in the AERS database are coded according to the Medical Dictionary for Regulatory Activities (MedDRA) (Maintenance and Support Services Organization, Chantilly, VA, USA), we identified drugs with RA as their

PNAS PNAS PNAS

MicroRNA-126–mediated control of cell fate in B-cell myeloid progenitors as a potential alternative to transcriptional factors

Kazuki Okuyama[a], Tomokatsu Ikawa[b], Bernhard Gentner[c], Katsuto Hozumi[d], Ratanakanit Harnprasopwat[e], Jun Lu[f], Riu Yamashita[e], Daon Ha[g], Takae Toyoshima[a], Bidisha Chanda[a], Toyotaka Kawamata[e], Kazuaki Yokoyama[e], Shusheng Wang[h], Kiyoshi Ando[f], Harvey F. Lodish[i], Arinobu Tojo[e], Hiroshi Kawamoto[b], and Ai Kotani[a,j,1]

[a]Division of Hematological Malignancy, Regenerative Medical Science, Tokai University School of Medicine, Kanagawa 259-1193, Japan; [b]RIKEN Research Center for Allergy and Immunology, Kanagawa 230-0045, Japan; [c]San Raffaele Scientific Institute, 20123 Milan, Italy; [d]Department of Immunology, Tokai University School of Medicine, Kanagawa 259-1193, Japan; [e]Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan; [f]Department of Hematology, Tokai University, Kanagawa 259-1193, Japan; [g]Tufts University School of Medicine, Boston, MA 02111; [h]Tulane University, New Orleans, LA 70118; [i]Whitehead Institute for Biomedical Research, Cambridge, MA 02142; and [j]Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), Saitama 332-0012, Japan

Edited by Tasuku Honjo, Graduate School of Medicine, Kyoto University, Kyoto, Japan, and approved May 31, 2013 (received for review January 11, 2013)

Lineage specification is thought to be largely regulated at the level of transcription, where lineage-specific transcription factors drive specific cell fates. MicroRNAs (miR), vital to many cell functions, act posttranscriptionally to decrease the expression of target mRNAs. MLL-AF4 acute lymphocytic leukemia exhibits both myeloid and B-cell surface markers, suggesting that the transformed cells are B-cell myeloid progenitor cells. Through gain- and loss-of-function experiments, we demonstrated that microRNA 126 (miR-126) drives B-cell myeloid biphenotypic leukemia differentiation toward B cells without changing expression of E2A immunoglobulin enhancer-binding factor E12/E47 (E2A), early B-cell factor 1 (EBF1), or paired box protein 5, which are critical transcription factors in B-lymphopoiesis. Similar induction of B-cell differentiation by miR-126 was observed in normal hematopoietic cells in vitro and in vivo in uncommitted murine c-Kit$^+$Sca1$^+$Lineage$^-$ cells, with insulin regulatory subunit-1 acting as a target of miR-126. Importantly, in EBF1-deficient hematopoietic progenitor cells, which fail to differentiate into B cells, miR-126 significantly up-regulated B220, and induced the expression of B-cell genes, including recombination activating genes-1/2 and CD79a/b. These data suggest that miR-126 can at least partly rescue B-cell development independently of EBF1. These experiments show that miR-126 regulates myeloid vs. B-cell fate through an alternative machinery, establishing the critical role of miRNAs in the lineage specification of multipotent mammalian cells.

cell fate decision | lymphopoiesis

Lineage specification is critical in mammalian development, as well as in adult tissue maintenance. In mammals, this developmental hierarchy has been most extensively studied in the hematopoietic system, where well-characterized cell-surface markers allow the purification of distinct cell populations. Lineage specification has been thought to be largely regulated at the level of transcription, where lineage-specific transcriptional factors drive specific cell fates (1–4). Early B-cell factor 1 (EBF1) specifies B-cell differentiation (5), and GATA-3 drives Th2 lineage commitment of CD4 T cells (6). However, regulation of differentiation at the transcriptional level alone does not appear to explain all hematopoietic cell-fate decisions, suggesting the presence of other as-yet-unknown mechanisms for establishing cell fate. Ectopic expression of c-enhancer binding protein-α (c/EBPα) or knock-out of paired box protein 5 (PAX5) in B cells are both capable of reprogramming B cells to macrophages; however, down-regulation of c/EBPα or ectopic expression of PAX5 or E2A immunoglobulin enhancer-binding factor E12/E47 (E2A), both critical transcription factors for B-cell differentiation, fail to reprogram myeloid-committed cells to B cells (7). Therefore, we hypothesized that the developmental fate of mammalian multipotent cells may be guided, at least in part, by a different mechanism of gene regulation, namely, microRNAs (miRNAs).

miRNAs are recently discovered class of small, noncoding RNAs that are 18–24 nt long and that down-regulate target genes at the posttranscriptional level. The majority of miRNA genes are transcribed by RNA polymerase II into long primary (pri) miRNA transcripts, processed by the nuclear nuclease, Drosha, into ~60-bp hairpins, termed precursor (pre) miRNAs, and further cleaved in the cytosol by the Dicer nuclease into mature miRNAs. Mature miRNAs are then incorporated into the multiprotein, RNA-induced silencing complex, exerting post-transcriptional repression of target mRNAs, either by inducing mRNA cleavage, mRNA degradation, or by blocking mRNA translation (8, 9).

Each miRNA is thought to have several target mRNAs, and computational predictions suggest that more than a third of all human genes are targets of miRNAs (10, 11). In animals, miRNAs control many developmental and physiological processes. In *Caenorhabditis elegans*, abnormal expression of certain miRNAs leads to developmental arrest (12). Many studies have revealed specific changes in miRNA expression profiles that correlate with particular human tumor phenotypes (13, 14). In the hematopoietic system, miR-181a down-regulates several phosphatases that regulate the sensitivity of T cells to antigens, and overexpression of miR-181 in hematopoietic stem/progenitor cells significantly increases B-cell production. In addition, overexpression of miR-150 leads to a block in B-cell formation at the proB-to-preB cell transition step by down-regulating c-myb, among other targets (15–18).

Down-regulation of specific miRNAs in certain cancers implies that some miRNAs may act as tumor suppressors. For example, let-7 family members directly down-regulate *Ras* and other protooncogenes. Reduced expression of let-7 family members has been previously characterized in lung cancer (19, 20). On the other hand, increased expression of miR-17–92 and miR-155 often occur in B-cell lymphomas (21), implying that these miRNAs can act as oncogenes (22, 23). Thus, miRNAs are capable of acting as either oncogenes or tumor suppressors.

Author contributions: A.K. designed research; K.O., T.I., B.G., R.H., J.L., R.Y., D.H., T.T., B.C., T.K., and K.Y. performed research; B.G., K.H., S.W., K.A., and H.F.L. contributed new reagents/analytic tools; A.T., H.K., and A.K. analyzed data; and K.O., R.H., and A.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. E-mail: ka102009@tsc.u-tokai.ac.jp.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1220710110/-/DCSupplemental.