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Wnt3a stimulates maturation of impaired neutrophils developed from severe congenital neutropenia patient-derived pluripotent stem cells

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Edited by George Q. Daley, Children's Hospital Boston, Boston, MA, and accepted by the Editorial Board January 4, 2013 (received for review October 1, 2012)

The derivation of induced pluripotent stem (iPS) cells from individuals of genetic disorders offers new opportunities for basic research into these diseases and the development of therapeutic compounds. Severe congenital neutropenia (SCN) is a serious disorder characterized by severe neutropenia at birth. SCN is associated with heterozygous mutations in the neutrophil elastase [elastase, neutrophil-expressed (ELANE)] gene, but the mechanisms that disrupt neutrophil development have not yet been clarified because of the current lack of an appropriate disease model. Here, we generated iPS cells from an individual with SCN (SCN-iPS cells). Granulopoiesis from SCN-iPS cells revealed neutrophil maturation arrest and little sensitivity to granulocyte-colony stimulating factor, reflecting a disease status of SCN. Molecular analysis of the granulopoiesis from the SCN-iPS cells vs. control iPS cells showed reduced expression of genes related to the wingless-type mmtv integration site family, member 3a (Wnt3a)/β-catenin pathway [e.g., lymphoid enhancer-binding factor 1], whereas Wnt3a administration induced elevation lymphoid enhancer-binding factor 1-expression and the maturation of SCN-iPS cell-derived neutrophils. These results indicate that SCN-iPS cells provide a useful disease model for SCN, and the activation of the Wnt3a/β-catenin pathway may offer a novel therapy for SCN with ELANE mutation.

apoptosis | unfolded protein response | SCN disease model

Severe congenital neutropenia (SCN) is a heterogeneous bone marrow (BM) failure syndrome characterized by severe neutropenia at birth, leading to recurrent infections by bacteria or fungi (1). SCN patients reveal an arrest in neutrophil differentiation in the BM at the promyelocyte or myelocyte stage (1), as well as a propensity to develop myelodysplastic syndrome and acute myeloid leukemia (2). Current treatment by high-dose granulocyte-colony stimulating factor (G-CSF) administration induces an increase in the number of mature neutrophils in the peripheral blood of most SCN patients (3). Although this treatment is curative for the severe infections, there is a concern that high-dose G-CSF may increase the risk of hematologic malignancy in these individuals (4).

Several genetic mutations have been identified in SCN patients. Approximately 50% of autosomal-dominant SCN cases were shown to have various heterozygous mutations in the gene encoding neutrophil elastase [elastase, neutrophil-expressed (ELANE)] (5, 6), a monomeric, 218-amino acid (25 kDa) chymotryptic serine protease (7) that is synthesized during the early stages of primary granule production in promyelocytes (8, 9). However, the mechanism(s) causing impaired neutrophil maturation in SCN patients remains unclear due to the current lack of an appropriate disease model.

Results and Discussion

In the present study, we generated induced pluripotent stem (iPS) cells from the BM cells obtained from an SCN patient with a heterologous ELANE gene mutation (exon 5, 707 region, C194X) (SCN-iPS cells) to provide the basis for an SCN disease model. The patient who donated BM cells recurrently suffered from severe infections without exogenous G-CSF administration, but the G-CSF administration once a week prevented his repeated infection. The SCN-iPS cells continued to show embryonic stem cell morphology after >20 passages and also expressed pluripotent markers (Fig. S1*A*). The silencing of exogenous genes and the capability to differentiate into three germ layers by teratoma formation were confirmed for each of the three SCN-iPS cell clones (Fig. S1 *B* and *C*). Furthermore, the same ELANE gene mutation that was present in the patient persisted in the SCN-iPS cells (Fig. S1*D*). The SCN-iPS cells, as well as control iPS cells that were generated from healthy donors, had the normal karyotype (Fig. S1*E*) (10, 11) and no mutations in the mutation-sensitive region of the G-CSF receptor gene (12).

We first compared the hematopoietic differentiation from SCN-iPS cells with that from control iPS cells that were generated from healthy donors. SCN-iPS and control iPS cells were cocultured with a 15-Gy-irradiated murine stromal cell line (the AGM-S3 cell line), as reported (13). After 12 d, the cocultured cells were harvested, and the $CD34^+$ cells separated from these cells (SCN-iPS–$CD34^+$ and control iPS–$CD34^+$ cells, respectively) were cultured in a hematopoietic colony assay by using a cytokine mixture (*Materials and Methods*). The number and size of the erythroid (E) and mixed-lineage (Mix) colonies derived from SCN-iPS–$CD34^+$ cells (1×10^4 cells) were nearly identical to those of the corresponding colonies derived from control iPS–$CD34^+$ cells (E colonies: SCN-iPS cells, 11.0 ± 3.0, and control iPS cells, 11.4 ± 3.9; Mix colonies: SCN-iPS cells, 25.1 ± 7.2, and control iPS cells, 17.4 ± 4.0) (Fig. 1 *B* and *C* and Fig. S2 *A* and *B*). However, the number of myeloid colonies derived from SCN-iPS–$CD34^+$ vs. control iPS–$CD34^+$ cells was significantly lower (SCN-iPS cells, 47.4 ± 19.5; control iPS cells, 127.8 ± 17.9; $P < 0.01$), and the size of the colonies was also smaller (Fig. 1 *A*

Author contributions: T.H., Y.E., K.Y., S.M., S.Y., Y.F., K. Tani, H.N., M.K., and K. Tsuji designed research; T.H., Y.M., K.N., and K.Y. performed research; T.H., Y.E., Y.M., K.N., K.Y., K.U., N.N., S.M., S.Y., M.N., and K. Tsuji analyzed data; and T.H., Y.E., and K. Tsuji wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. G.Q.D. is a guest editor invited by the Editorial Board.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1217039110/-/DCSupplemental.

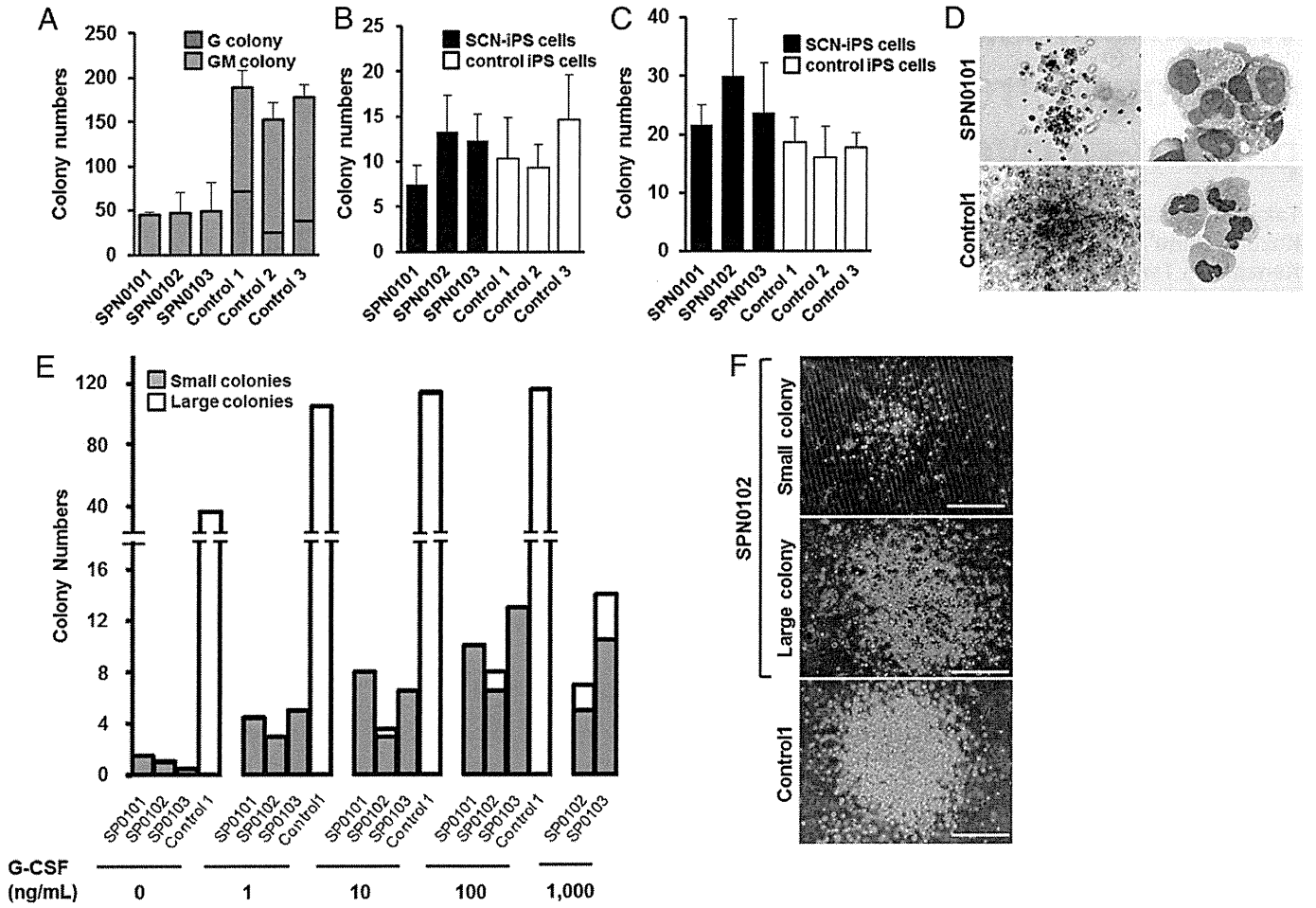

Fig. 1. Impaired neutrophil development from SCN-iPS cells. (*A*–*C*) A hematopoietic colony assay was performed by using 1×10^4 $CD34^+$ cells derived from three SCN-iPS cell clones (SPN0101, SPN0102, and SPN0103) and three control iPS cell clones (controls 1, 2, and 3) in the presence of a cytokine mixture. Colonies were sorted as myeloid (*A*), erythroid (*B*), and mixed-lineage (Mix) (*C*). Data are shown as mean ± SD. (*D*) Photographs of colonies (*Left*; 100×) and cells in a GM colony (*Right*; 400×; May–Grünwald–Giemsa staining). (*E*) A hematopoietic colony assay with dose escalation of G-CSF was performed by using 1×10^5 $CD34^+$ cells derived from SCN-iPS and control iPS cells. Filled and open bars indicate small colonies consisting of <100 cells and large colonies consisting of >100 cells, respectively. Data are shown as the average of three independent experiments. (*F*) Photographs of a small colony derived from SCN-iPS cells (SPN0102) in the presence of 10 ng/mL G-CSF, large colonies derived from SCN-iPS cells in the presence of 1,000 ng/mL G-CSF, and large colonies derived from control iPS cells (control 1) in the presence of 10 ng/mL G-CSF. (Scale bars, 200 μm.)

and *D*). In particular, only a few SCN-iPS cell-derived granulocyte (G) colonies—myeloid colonies consisting of only granulocytes—were detected (Fig. 1*A*). SCN-iPS cell-derived granulocyte–macrophage (GM) colonies—myeloid colonies consisting of macrophages/monocytes with/without granulocytes—contained a few immature myeloid cells in addition to macrophages/monocytes, whereas control iPS cell-derived GM colonies included a substantial number of mature, segmented, and band neutrophils (Fig. 1*D*).

We also found that Mix colonies derived from SCN-iPS cells, but not control iPS cells, contained immature myeloid cells and few mature neutrophils (Fig. S2 *C* and *D*). Next, we conducted a hematopoietic colony assay using various concentrations of G-CSF alone instead of the cytokine mixture to examine the G-CSF dose dependency of neutrophil differentiation from SCN-iPS and control iPS–$CD34^+$ cells. For all concentrations of G-CSF used (1–1,000 ng/mL), the SCN-iPS cell-derived myeloid colonies were significantly lower in number and smaller in size than the control iPS cell-derived myeloid colonies (Fig. 1*E*). Myeloid colony formation from control iPS cells reached a plateau at ~1–10 ng/mL G-CSF, whereas the number and size of those from SCN-iPS cells gradually increased with increasing concentrations of G-CSF. However, the values observed for SCN-iPS cells did not reach those for the control iPS cells, even at the highest dose of G-CSF used (1,000 ng/mL). Furthermore, large colonies consisting of >100 cells derived from SCN-iPS cells were only found with higher concentrations of G-CSF (Fig. 1*F*). Thus, granulopoiesis initiated from SCN-iPS cells was relatively insensitive to G-CSF, reflecting the inadequate in vivo response of neutrophils to G-CSF in SCN patients (14, 15). Therefore, these results support the applicability of the SCN-iPS cells established herein as a disease model for SCN.

To examine neutrophil development from SCN-iPS cells in more detail, SCN-iPS and control iPS–$CD34^+$ cells (1×10^4 cells each) were cocultured in suspension with AGM-S3 cells in the presence of neutrophil differentiation medium (*SI Materials and Methods*). The number of nonadherent cells derived from SCN-iPS–$CD34^+$ cells was lower than that from control iPS–$CD34^+$ cells on day 14 of culture (SCN-iPS cells, $9.77 \times 10^4 \pm 1.65 \times 10^4$ cells; control iPS cells, $52.48 \times 10^4 \pm 23.13 \times 10^4$ cells; $P < 0.05$) (Fig. 2*A*). The proportion of mature neutrophils among the nonadherent cells was also significantly lower for SCN-iPS cells relative to control iPS cells on day 14 (SPN-iPS cells, 15.53% ± 4.33%; control iPS cells, 71.285 ± 3.30%; $P < 0.05$) (Fig. 2 *B* and *C*), indicating that myeloid cells derived from SCN-iPS cells revealed the maturation arrest in the neutrophil development. We then examined a possibility that the maturation arrest in SCN-

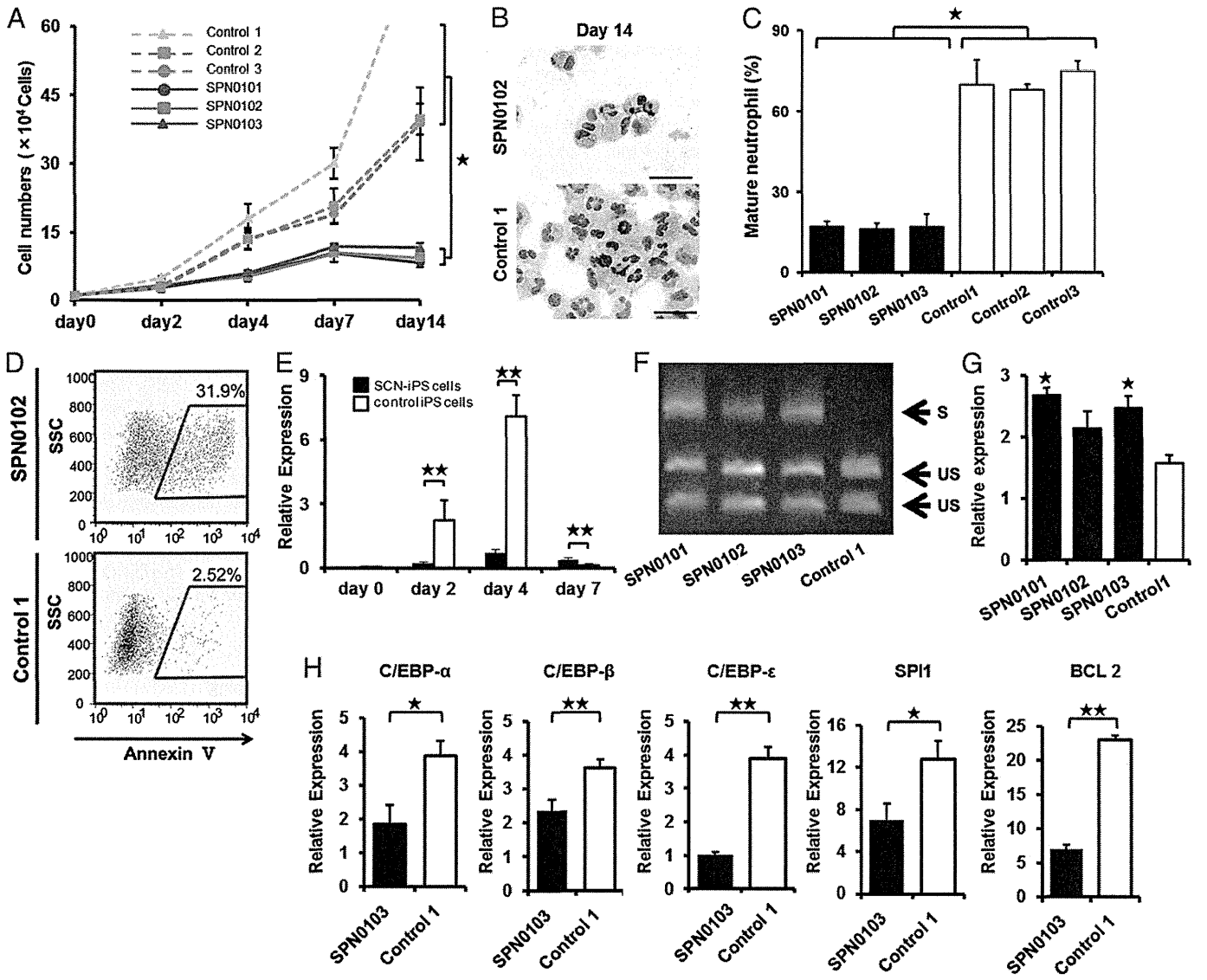

Fig. 2. Analysis of impaired neutrophil development from SCN-iPS cells. (*A*) Total number of nonadherent cells in the suspension culture of 1×10^4 $CD34^+$ cells derived from SCN-iPS and control iPS cells. Data are shown as mean ± SD. $^*P < 0.01$. (*B*) Photographs of nonadherent cells derived from SCN-iPS (SPN0103) and control iPS cells (control 1) on day 14 of culture (400×; May–Grünwald–Giemsa staining; scale bars, 50 μm.) (*C*) Filled and open bars show the proportion of mature neutrophils among the cells derived from SCN-iPS (filled bars) and control iPS (open bars) cells on day 14 of suspension culture. Data are shown as mean ± SD. $^*P < 0.05$. (*D*) Flow cytometric analysis of annexin V expression on cultured cells from SCN-iPS cells (SPN0102) or control iPS cells (control 1) on day 7. (*E*) Sequential qRT-PCR analysis of the relative expression of ELANE mRNA [ELANE/hypoxanthine–guanine phosphoribosyltransferase (HPRT) expression]. Data obtained from independent experiments using three SCN-iPS cell clones (SPN0101, SPN0102, and SPN0103) and three control iPS cell clones are shown as mean ± SD. $^{**}P < 0.01$. (*F* and *G*) $CD34^+$ cells derived from SCN-iPS or control iPS cells were cultured in neutrophil differentiation medium (see text). On day 7, nonadherent cells were collected and analyzed. (*F*) Representative gel showing spliced (S) and unspliced (US) XBP-1 bands on day 7. (*G*) qRT-PCR analysis of the relative mRNA expression (target/HPRT expression) of BiP on day 7. Data are shown as mean ± SD. $^*P < 0.05$; different from control 1). (*H*) qRT-PCR analysis of the relative mRNA expression (target / HPRT expression) of C/EBP-α, C/EBP-β, C/EBP-ε, SPI1, and BCL2 genes in non-adherent cells derived from SCN-iPS cells (filled bars, SPN0103) and control iPS cells (open bars, control 1) on day 2 of suspension culture. Data are shown as the mean ± the s.d. ($^{**}P < 0.01$, $^*P < 0.05$).

iPS cell-derived myeloid cells might be caused by their apoptosis. In flow cytometric analysis, SCN-iPS cell-derived myeloid cells contained a significantly higher proportion of annexin V-positive cells than control iPS-derived myeloid cells on day 7 of culture, suggesting that the maturation arrest in myeloid cells derived from SCN-iPS cells might be caused by their apoptosis (Fig. 2*D*).

We next examined ELANE mRNA expression levels in nonadherent cells derived from SCN-iPS vs. control iPS cells (Fig. 2*E*). ELANE expression was significantly lower in nonadherent cells derived from SCN-iPS vs. control iPS cells on days 2 and 4 of culture ($P < 0.01$), as reported (16, 17). However, the former was a little higher than the latter on day 7 ($P < 0.01$). This result may be explained by the existence of SCN-iPS cell-derived myeloid cells arrested at an early stage along the neutrophil differentiation pathway even on day 7 of culture. We also examined the expression of proteinase 3 and azurocidin, which comprise a family of closely related genes encoding neutrophil granule proteins along with ELANE, and found these genes were more highly expressed on day 4 (Fig. S3).

It has been reported that induction of the endoplasmic reticulum stress (ER) response and the unfolded protein response (UPR) has been advanced as a potential explanation for the molecular pathogenesis of SCN (18, 19). Thus, we examined activation of the UPR by X-box binding protein 1 (XBP-1) mRNA splicing on day 7. As shown in Fig. 2*F*, SPN-iPS cells induced XBP-1 mRNA splicing. We also found the up-regulation of BiP

(also known as GRP78 or HSPA5) (Fig. 2*G*). These results suggested that ER stress response and UPR might be involved in the pathogenesis in SCN.

To examine further the differences in gene expression between the two cell types, a microarray analysis was carried out by using $CD34^+$ cells derived from SCN-iPS and control iPS cells (three clones of each) in suspension culture on day 2. At this early time point, differences in cell number and morphology were not yet readily discernible between SCN-iPS and control iPS cells, as shown in Fig. 2*A*. However, the microarray analysis revealed a differential expression of various genes between the two cell types. Transcription factor genes, which were related to neutrophil development [e.g., CCAAT/enhancer-binding protein (C/EBP)-α (20), C/EBP-β (21), C/EBP-ε (22), and SPI1 (also known as PU.1) (23)], were all down-regulated in SCN-iPS cells. B-cell chronic lymphocytic leukemia/lymphoma 2, which regulates cell death under ER stress through the core mitochondrial apoptosis pathway (24), was also down-regulated (Fig. 3*A*). These findings were confirmed by quantitative reverse-transcriptional PCR (qRT-PCR), as shown in Fig. 2*H*.

Notably, the down-regulation of the genes in SCN-iPS cells related to and regulated by the wingless-type mmtv integration site family, member 3a (Wnt3a)/β-catenin pathway [e.g., Wnt3a, lymphoid enhance-binding factor (LEF)-1, BIRC5 (also known as survivin), and cyclin D1] was also uncovered by microarray analysis and qRT-PCR (Fig. 3 *A–C* and Fig. S4). Therefore, we examined the effect of enhancement of Wnt3a/β-catenin signaling by exogenous Wnt3a addition on the neutrophil development of $CD34^+$ cells derived from SCN-iPS and control iPS cells. Although Wnt3a did not stimulate the survival, proliferation, and differentiation of $CD34^+$ cells derived from both iPS cells in the absence of cytokines stimulating myelopoiesis including G-CSF, the addition of Wnt3a to the neutrophil differentiation medium induced a dose-dependent increase in the percentage of mature neutrophils among the cultured cells, as shown in Fig. 3 *D* and *E*. Furthermore, when Wnt3a was added concurrently with 1,000ng/mL G-CSF, the proportion of mature neutrophils increased more than it did with Wnt3a or 1,000 ng/mL G-CSF alone, reaching a value comparable with that observed for control iPS cells (Fig. 4 *A* and *B*).

The reduced expression of LEF-1 (as regulated by the Wnt3a/β-catenin pathway) reportedly plays a critical role in the defective maturation of neutrophils in SCN patients (25). Therefore, we next examined LEF-1 mRNA expression in SCN-iPS–$CD34^+$ cells cultured in the presence of Wnt3a, G-CSF (1,000 ng/mL), or both. Wnt3a and G-CSF both enhanced LEF-1 mRNA expression, but the most significant increase was observed in the presence of Wnt3a plus G-CSF. LEF-1 expression in SCN-iPS–$CD34^+$ cells in response to Wnt3a plus G-CSF was almost the same as that in control iPS–$CD34^+$ cells (Fig. 4*C*). These results substantiate the importance of LEF-1 in neutrophil development and the pathogenesis of SCN, as shown (25). Moreover the

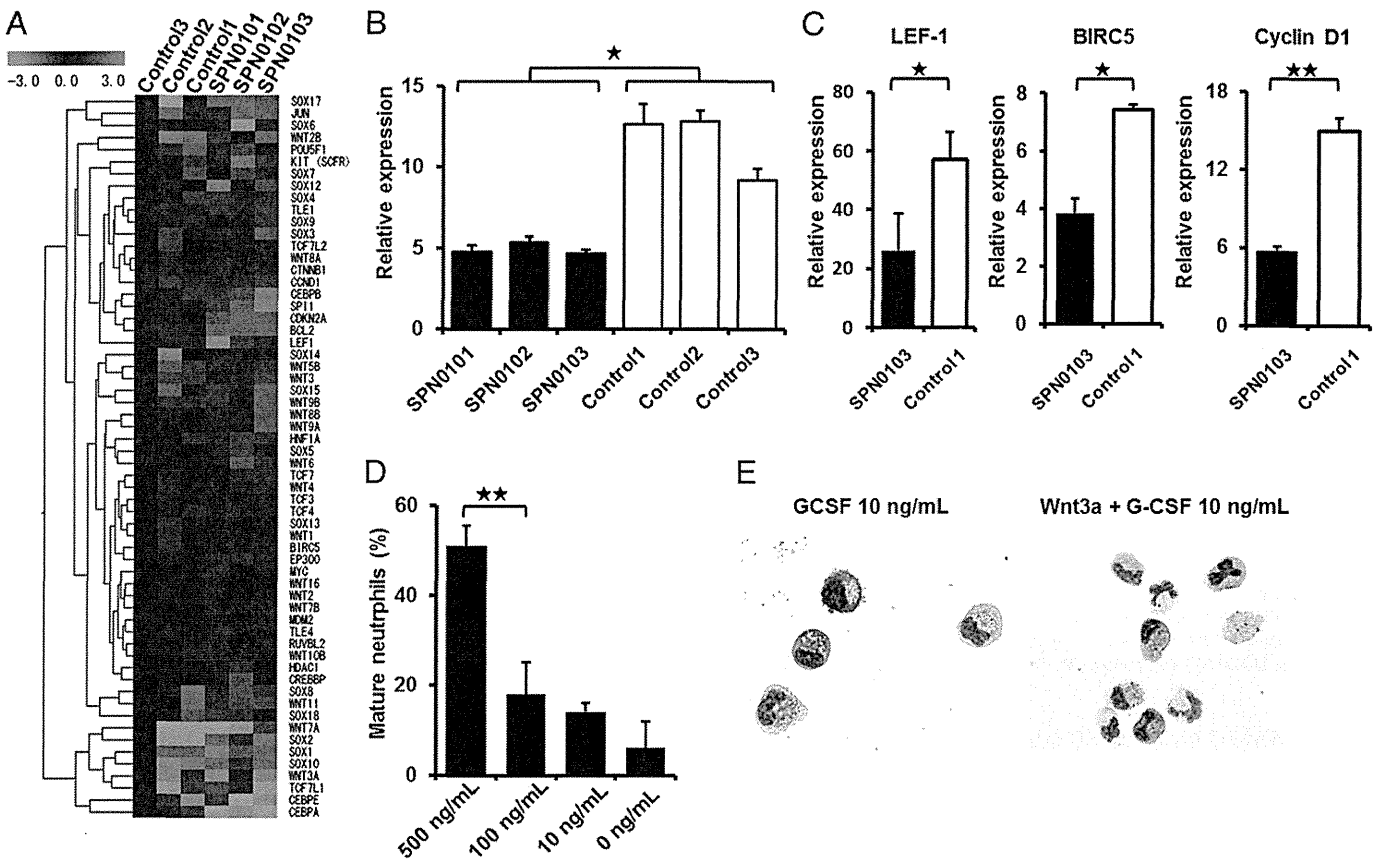

Fig. 3. Effects of Wnt3a on neutrophil development from SCN-iPS cells. (*A*) Heat map showing differential gene expression among SCN-iPS and control iPS cells on day 2. Red, high gene expression; blue, low gene expression compared with gene expression in control 3. (*B*) qRT-PCR analysis of the relative mRNA expression (target/HPRT expression) of Wnt3a on day 2. Filled and open bars indicate experiments using SCN-iPS cells (SPN0101, SPN0102, and SPN0103) and control iPS cells (controls 1, 2, and 3), respectively. Data are shown as mean ± SD. $^*P < 0.05$. (*C*) qRT-PCR analysis of the relative expression (target/HPRT expression) of genes regulated by the Wnt3a/β-catenin pathway (LEF-1, survivin, and cyclin D1) in SCN-iPS cells (filled bars, SPN0103) vs. control iPS cells (open bars, control 1) on day 2 of suspension culture. Data are shown as mean ± SD. $^{**}P < 0.01$; $^*P < 0.05$. (*D*) Proportion of mature neutrophils among the cells derived from SCN-iPS cells (SPN0102) on day 14 of suspension culture with dose escalation of Wnt3a. Data are shown as mean ± SD. $^{**}P < 0.01$. (*E*) Photographs of nonadherent cells on day 7 of suspension culture with or without Wnt3a (500 ng/mL) (400×; May–Grünwald–Giemsa staining).

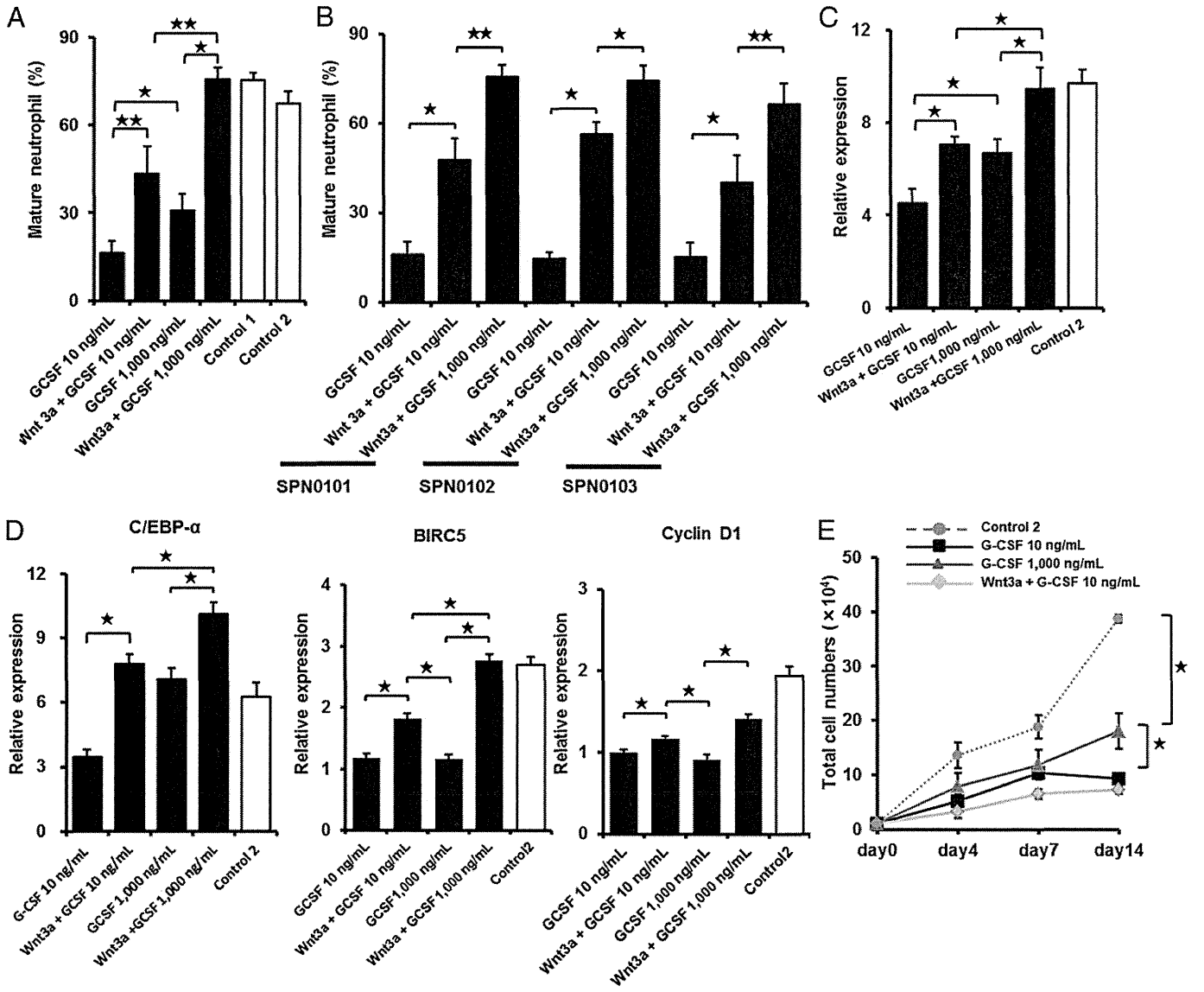

Fig. 4. Effects of Wnt3a in combination with high-dose G-CSF. (*A*) Filled and open bars show the proportion of mature neutrophils among the cells derived from SCN-iPS cells (SPN0101) on day 14 of suspension culture in the presence of neutrophil differentiation medium containing 10 ng/mL G-CSF (GCSF 10 ng/mL); 500 ng/mL Wnt3a and 10 ng/mL G-CSF (Wnt3a+GCSF 10 ng/mL); 1,000 ng/mL G-CSF (GCSF 1,000 ng/mL); or 500 ng/mL Wnt3a and 1,000 ng/mL G-CSF (Wnt3a + GCSF 1,000 ng/mL); and that from control iPS cells (controls 1 and 2) cultured in the neutrophil differentiation medium containing 10 ng/mL G-CSF, respectively. Data are shown as mean ± SD. $^{**}P < 0.01$; $^{*}P < 0.05$. (*B*) The proportion of mature neutrophils among the cells derived from three SCN-iPS cell clones (SPN0101, SPN0102, and SPN0103) on day 14 of suspension culture in the presence of neutrophil differentiation medium containing 10 ng/mL G-CSF (GCSF 10 ng/mL); 500 ng/mL Wnt3a and 10 ng/mL G-CSF (Wnt3a+GCSF 10 ng/mL); or 500 ng/mL Wnt3a and 1,000 ng/mL G-CSF (Wnt3a + GCSF 1,000 ng/mL). Data are shown as mean ± SD. $^{**}P < 0.01$; $^{*}P < 0.05$. (*C*) Filled and open bars show the relative expression (target/HPRT expression) of LEF-1 mRNA in SCN-iPS cells (SPN0101) on day 2 of suspension culture in the presence of differentiation medium containing the same combinations of Wnt3a and G-CSF as shown in *A* and that from control iPS cells (control 2), respectively. Data are shown as mean ± SD. $^{**}P < 0.01$; $^{*}P < 0.05$. (*D*) Filled and open bars show the relative expression (target/HPRT expression) of C/EBP-α, BIRC5, or cyclin D1 mRNA in SCN-iPS cells (SPN0101) on day 2 of suspension culture in the presence of differentiation medium containing the same combinations of Wnt3a and G-CSF as shown in *A* and that from control iPS cells (control 2), respectively. Data are shown as mean ± SD. $^{**}P < 0.01$; $^{*}P < 0.05$. (*E*) Total cell numbers of nonadherent cells in suspension cultures of 1×10^4 $CD34^+$ cells derived from control iPS cells (control 2; red broken line) and SCN-iPS cells (SPN0101) in the presence of neutrophil differentiation medium (black line) and those from SCN-iPS cells in the presence of neutrophil differentiation medium containing 500 ng/mL Wnt3a (yellow line) or 1,000 ng/mL G-CSF (black line). Data are shown as mean ± SD. $^{**}P < 0.05$.

administration of Wnt3a led to up-regulate C/EBP-α, cyclin D1, and BIRC5/survivin in addition to LEF-1 in the presence of G-CSF (Fig. 4*D*). These results suggested that the up-regulation of LEF-1 expression might promote granulopoiesis by increasing the expressions of cyclin D1, BIRC5/survivin, and C/EBP-α and its binding to LEF-1 in accordance with the previous report (25). Interestingly, Wnt3a did not stimulate the proliferation of myeloid cells, whereas 1,000 ng/mL G-CSF did to a certain extent (Fig. 4*E*). Hence, Wnt3a was capable of stimulating the maturation of impaired neutrophils in the presence of G-CSF, but not the proliferation of myeloid cells from SCN-iPS cells.

Importantly, aside from providing new insights into the mechanisms behind impaired neutrophil development in SCN patients, the present study demonstrates that agents activating the Wnt3a/β-catenin pathway are potential candidates for new drugs for SCN with mutations in the ELANE gene. Because endogenous G-CSF is readily increased in SCN patients (26), these activating agents may be viable alternatives to exogenous G-CSF treatment.

Materials and Methods

Additional information is available in *SI Materials and Methods*.

Generation of Human iPS Cells. BM fibroblasts from a patient with SCN and skin dermal fibroblasts from a healthy donor were acquired after obtaining informed consent after getting the approval by the Ethics Committee of the Institute of Medical Science, University of Tokyo, in accordance with the Declaration of Helsinki. The SCN patient presented with a heterozygous mutation in the ELANE gene in the 707 region of exon 5. SCN-iPS cells were established from the SCN-BM fibroblasts by transfection with the pMX retroviral vector, as described (10). This vector expressed the human transcription factors OCT3/4, SOX2, KLF4, and c-MYC. Control iPS cell clones, control 1 (TkDN4-M) and control 3 (201B7), were gifts from K. Eto and S. Yamanaka (Kyoto University, Kyoto), respectively (10, 11). Control 2 (SPH0101) was newly generated from another healthy donor's skin dermal fibroblasts by using the same methods.

Hematopoietic Colony Assay. A hematopoietic colony assay was performed in an aliquot of culture mixture, which contained 1.2% methylcellulose (Shin-Etsu Chemical), 30% (vol/vol) FBS, 1% (vol/vol) deionized fraction V BSA, 0.1 mM 2-mercaptoethanol (2-ME), α-minimum essential medium, and a cytokine mixture consisting of 100 ng/mL human stem cell factor (hSCF) (Wako), 100 ng/mL fusion protein 6 [FP6; a fusion protein of interleukin (IL)-6 and IL-6 receptor] (a gift from Tosoh), 10 ng/mL human IL-3 (hIL-3) (a gift from Kirin Brewery), 10 ng/mL human thrombopoietin (hTPO) (a gift from Kirin Brewery), 10 ng/mL human G-CSF (a gift from Chugai Pharmaceutical), and 5 U/mL human erythropoietin (a gift from Kirin Brewery). For dose escalation experiments, various concentrations (0, 1, 10, 100, and 1,000 ng/mL) of G-CSF were used instead of the cytokine mixture described above. Colony types were determined according to established criteria on day 14 of culture by in situ observations under an inverted microscope (IX70; Olympus) (27).

Suspension Culture and Neutrophil Differentiation Assay. $CD34^+$ cells (1×10^4 cells) were cocultured with irradiate confluent AGM-S3 cells in neutrophil differentiation medium containing Iscove's modified Dulbecco's medium, 10% FBS, 3 mM L-glutamine, 1×10^{-4} M 2-ME, 1×10^{-4} M nonessential amino acids solution, 100 ng/mL hSCF, 100 ng/mL FP6, 10 ng/mL hIL-3, 10 ng/mL hTPO, and 10 or 1,000 ng/mL human G-CSF. Wnt3a (10, 100, or 500 ng/mL) (R&D) was then added. The medium was replaced with an equivalent volume of fresh medium every 4 d. Living, nonadherent cells were counted following 0.4% trypan blue staining.

PCR primer. All primer sets used in this study are shown in Table S1.

Statistical Analysis. All data are presented as mean ± SD. $P < 0.05$ was considered significant. Statistical analyses were performed by using Prism software (GraphPad).

ACKNOWLEDGMENTS. We thank the individual with SCN who participated in this study; K. Eto for providing control iPS cells (control 1; TkDN4-M); S. Yamanaka for providing control iPS cells (control 3; 206B7); and E. Matsuzaka and S. Hanada for technical assistance. This work was supported by in part by Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT) Grants-in-Aid (to Y.E.) and Project for Realization of Regenerative Medicine (MEXT) Grants-in-Aid (to K.Tsuji).

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Int J Hematol (2013) 98:74–88
DOI 10.1007/s12185-013-1364-2

ORIGINAL ARTICLE

Incidence and survival rates of hematological malignancies in Japanese children and adolescents (2006–2010): based on registry data from the Japanese Society of Pediatric Hematology

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Received: 18 December 2012 / Revised: 7 May 2013 / Accepted: 7 May 2013 / Published online: 24 May 2013

Abstract Neither accurate incidence nor survival data for pediatric patients with hematological malignancies (HM) have been available in Japan to date. Incidence of patients under 20 years of age, who were diagnosed with HM from 2006 to 2010, and their two-year survival rate (2y-OS) were obtained from disease registry data maintained by the Japan Society of Pediatric Hematology (JSPH). A total of 5,287 cases of HM were identified during this period. Acute lymphoblastic leukemia (ALL, 46.6 %) showed the highest incidence, followed by acute myeloid leukemia (AML, 16.7 %), non-Hodgkin lymphoma (NHL, 11.9 %), and histiocytosis (11.8 %). ALL, AML and histiocytosis were common in younger patients aged 1–4, while NHL tended to occur more frequently in older patients aged 5–14. The 2y-OS of HM was 91.6 %, with that for the most common B-precursor ALL rising to 96.2 %. The 2y-OS for M3 AML, lymphoblastic-B-precursor or diffuse large B cell NHL, Hodgkin lymphoma, myeloproliferative disorders, and Langerhans cell histiocytosis was >95 %. There were no gender differences in prognosis, while infants (88.0 %) and adolescents aged 15–19 (90.6 %) tended toward a poorer prognosis. This is the first report to describe incidence and survival times from the nationwide JSPH disease registry. More precise data with longer follow-up is needed.

Keywords Hematological malignancies · Children · Registry data · Survival

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Introduction

Until now, knowledge of the incidence of pediatric hematological malignancies in Japan has relied on registration at the Research Program for the Treatment of Chronic Pediatric Diseases of Specified Categories [1, 2] which is the epidemiological research done by the research and investigation section of the Ministry of Health, Labour and Welfare, and population-based cancer registries [3–6]. Because of the quality problems, all these available data is far from a comprehensive, systematic investigation of pediatric hematological diseases across the country, and the precision of the data gained thereby is limited. Furthermore, while the progress in treatment and supportive care for the last several decades have led to improve treatment outcome [7–14], the absence of nationwide data not only for the incidence but also for survival prognoses of

pediatric hematological malignancies has made it difficult to judge whether these advances in medical science have contributed to the welfare of children across the country.

In order to resolve these issues, the Japanese Society of Pediatric Hematology (JSPH), which unified with the Japan Society of Pediatric Oncology to the Japanese Society of Pediatric Hematology/Oncology since January, 2012, began a registry of newly diagnosed hematological diseases including non-malignant diseases partly in conjunction with the Japanese Society of Hematology in 2006, and planned complementary research into the prognoses (outcomes regarding dead or alive) as part of a research project intended to grasp the total number of pediatric patients with hematological diseases. This is the first report to describe survival times for the nationwide patients with pediatric hematological malignancies as well as the incidence of them from the JSPH disease registry [15, 16].

Materials and methods

The disease registry survey was conducted on the treatment facilities, where JSPH members are working and also preregistered to JSPH Disease Registry Project. Using electronic or paper-based survey forms, the participating facilities voluntarily and continuously registered the cases of patients below the age of 20, who were diagnosed as hematological malignancies or benign hematological disorders after 2006. As for the electronic registration, E-DMS online by the e-Trial Co., Ltd. was used until December 2011 when it was replaced by Patient Data Organizing System (Ptosh) developed by the National Hospital Organization Nagoya Medical Center Clinical Research Center in collaboration with a non-profit organization, Organization for Supporting Clinical Research (NPO-OSCR). For fax registrations, the disease registry data were sent to the Data Management Department of NPO-OSCR. The database prepared for the registry was coordinated with the registry for epidemiological researches/clinical trials organized by Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) in an attempt to "unify the registration" so as to provide more convenience to the participating facilities and to prevent non-registration.

In order to maintain the uniformity of the diagnoses concerning diseases to be registered, JSPH Disease Registry Committee prepared a guideline for diagnosis, to which the participants were requested to conform [17]. Acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), rare leukemia, myelodysplastic syndromes and/or myeloproliferative neoplasms (MDS and/or MPN), or transient abnormal myelopoiesis associated with Down syndrome (Down-TAM), non-Hodgkin (NHL) and Hodgkin lymphomas (HL), histiocytosis including Langerhans cell histiocytosis (LCH) and hemophagocytic lymphohistiocytosis (HLH), other lymphoproliferative disorders (LPD) and other hematological malignancies were defined as the hematological malignancies to be registered. Underlying diseases, pathological/immunological/cytogenetic characteristics, pathogenetic forms (primary/secondary), and other natures of diseases were also recorded.

When a patient is affected by multiple diseases, each disease was registered as one entry. Patients' genders, places of residence at the initial diagnosis, dates of birth, dates of diagnosis, etc., were registered as the basic patient information. The outcomes of the respective diseases (alive or death), along with the diagnosed disease information, were recorded up to the end of May and were registered for every calendar year.

The registered data were compiled according to diagnoses, diagnosed years, genders, age categories (0, 1–4, 5–9, 10–14, and 15–19) and residential areas at diagnosis (Hokkaido, Tohoku, Kanto-Koshinetsu, Tokai-Hokuriku, Kinki, Chugoku-Shikoku, and Kyushu-Okinawa) to indicate the numbers of cases, respectively. A crude incidence rate is the number of new cases by diagnoses in a gender/age-specified populations under age 20 as of each diagnosed year, expressed as the number of hematological malignancies per 100,000 population at risk. Overall survival (OS) was defined as the length of time from the diagnosis of hematological malignancies to death from any cause. Patients were censored at the time of loss of follow-up or June 15, 2011. OS was estimated using the Kaplan–Meier method, and 2-year survival rate (2y-OS) was measured with a 95 % confidence interval (95 % CI) using Greenwood's formula. All statistical analyses were carried out using the SAS software Release 9.1 (SAS Institute Inc., Cary, NC, USA).

This registry project is operated upon obtaining the approval of JSPH Clinical Research Review Committee, followed by consents from the head of each participating institute.

Results

Numbers of registered institutions and cases

The number of institutions registered had increased by 16 from 223 institutions of the 2007 survey to 239 by the time of the 2011 survey (including the 4 institutions which had withdrawn during this period), with participation from 47 prefectures throughout Japan. Registration of cases with hematological malignancies was conducted by 187 (78.2 %) among the 239 institutions. Since retrospective registration was allowed for cases diagnosed since 2006, increases in the number of registered cases were found up

to 3 years in plateau after the diagnoses. A total of 5,287 cases were registered as hematological malignancies from 2006 to 2010, and the numbers by year were 2006: 967 cases, 2007: 1,053 cases, 2008: 1,116 cases, 2009: 1,081 cases and 2010: 1,070 cases.

Incidences

The results of broadly classifying hematological malignancies into the disease groups of ALL, AML, MDS and/or MPN, NHL, HL, histiocytosis, LPD, other hematological malignancies and Down-TAM, and tabulating by the year of diagnosis are shown in Table 1. A total of 5,287 cases were registered in 5 years, which was an annual incidence of hematologic malignancies of 4.5 cases per 100,000 people. The greatest number of cases was ALL with 2,464 cases (46.6 %), followed by AML with 891 cases (16.9 %), NHL with 628 cases (11.9 %) and histiocytosis with 624 cases (11.8 %), and this trend remained nearly constant without any dependence on the year of diagnosis. On the other hand, the number of cases reported as Down-TAM in 2010 had increased by about 1.7 times from the average number of cases reported in previous years. In addition, there were a large number of cases reported as other LPD in 2007, about two times more than in other years. The number of registered cases including rare leukemia (36 cases), other LPD (51 cases) and other hematological malignancies (6 cases) were small.

Table 2 shows the number of registrations by gender, age category, and residential areas at diagnosis for each disease group classification.

In the tabulation of ALL by the immunophenotypic classification, the most part was accounted for by B-precursor ALL with 2,110 cases (85.6 %), followed by T-ALL with 1,269 cases (10.9 %) and mature B-ALL with 60 cases (2.4 %). The peak incidence of ALL occurs between 1 and 4 years of age. In addition, the incidence of ALL is slightly higher among male children than female children, and this difference is consistent regardless of the classification by immunophenotype. Genetic abnormalities in 2,464 cases with ALL included 281 cases (11.4 %) with hyperdiploid karyotype over 50 chromosomes, 247 cases (10.0 %) with ETV6-RUNX1, 135 cases (5.5 %) with MLL rearrangement, 113 cases (4.6 %) with E2A-PBX1, 106 cases (4.3 %) with BCR-ABL1 gene rearrangement, 29 cases (1.2 %) with t(v;8q24) and 583 cases (23.7 %) with other abnormalities.

In the tabulation of AML by FAB classification, overall the greatest number was M2 with 218 cases (24.5 %), followed by M7 with 212 cases (21.5 %), M5 with 124 cases (13.9 %) and M4 with 112 cases (12.6 %). The distribution by age category showed the greatest numbers of M2 for ages 5–9 and 10–14 years (the peak of incidence during age 10–14 years), but for ages 0 and 1–4 years the incidence of M7 was extremely high (the peak of incidence during age 1–4 years), making up almost half of the incidences. Half of patients diagnosed with M7 AML were associated with Down syndrome ($n = 114$), corresponding to 94.2 % of 121 AML patients with Down syndrome. No clear difference in the number of cases of disease was found between the genders.

Table 1 Numbers of cases and incidence rates of hematological malignancies in Japanese children and adolescents, diagnosed between 2006 and 2010

Disease	Total (%)	Crude incidence rate[a]	Year of diagnosis				
			2006	2007	2008	2009	2010
Acute lymphoblastic leukemia	2,464 (46.6)	2.1	444	506	532	504	478
Acute myeloid leukemia	891 (16.9)	0.8	167	165	184	193	182
Rare leukemia	36 (0.7)	0.0	9	7	4	10	6
Myelodysplastic syndrome and/or myeloproliferative neoplasms	296 (5.6)	0.3	61	60	46	54	75
Non-Hodgkin lymphoma	628 (11.9)	0.5	118	129	138	137	106
Hodgkin lymphoma	107 (2.0)	0.1	19	21	24	14	29
Histiocytosis	624 (11.8)	0.5	114	108	138	128	136
Transient abnormal myelopoiesis associated with Down syndrome	184 (3.5)	0.2	26	37	37	31	53
Other hematological malignancies	6 (0.1)	0.0	0	0	5	1	0
Other lymphoproliferative disorders	51 (1.0)	0.0	9	20	8	9	5
Hematological malignancies, Total	5,287 (100.0)	4.5	967	1,053	1,116	1,081	1,070

[a] Crude incidence rate is the number of new cases by diagnoses in a gender/age-specified populations under age 20 as of each diagnosed year, expressed as the number of hematological malignancies per 100,000 population at risk

Table 2 Numbers of incidences of hematological malignancies according to gender, age category, and residential areas at diagnosis in Japanese children and adolescents, diagnosed between 2006 and 2010

Disease	Subtype	*n* (%)	*n* (%)	Gender, *n* (%)		Age, *n* (%)					Residential areas at diagnosis, *n* (%)						
				Male	Female	0	1–4	5–9	10–14	15–19	Hokkaido	Tohoku	Kanto-Koshinetsu	Tokai-Hokuriku	Kinki	Chugoku-Shikoku	Kyushu-Okinawa
Acute lymphoblastic leukemia		2,464 (46.6)		1,411 (57.3)	1,053 (42.7)	108 (4.4)	1044 (42.4)	711 (28.9)	499 (20.3)	102 (4.1)	105 (4.3)	192 (7.8)	971 (39.4)	347 (14.1)	415 (16.8)	190 (7.7)	244 (9.9)
	B-precursor		2,110 (85.6)	1,151	959	102	979	580	372	77	83	168	829	306	350	164	210
	Mature B		60 (2.4)	38	22	5	9	24	20	2	4	2	22	6	11	7	8
	T cell		269 (10.9)	206	63	1	46	101	99	22	15	19	112	34	46	18	25
	Unknown		25 (1.0)	16	9	0	10	6	8	1	3	3	8	1	8	1	1
Acute myeloid leukemia		891 (16.9)		451 (50.6)	440 (49.4)	109 (12.2)	306 (34.3)	168 (18.9)	240 (26.9)	68 (7.6)	32 (3.6)	69 (7.7)	348 (39.1)	121 (13.6)	134 (15.0)	78 (8.8)	109 (12.2)
	M0		33 (3.7)	15	18	4	7	10	7	5	1	4	15	4	4	0	5
	M1		73 (8.2)	35	38	2	17	19	28	7	4	4	30	12	10	6	7
	M2		218 (24.5)	109	109	3	37	73	88	17	11	17	78	28	35	20	29
	M3, M3v		70 (7.9)	37	33	2	12	17	29	10	1	2	22	11	17	7	10
	M4, M4Eo		112 (12.6)	57	55	12	29	20	38	13	3	12	37	19	21	8	12
	M5a, M5b		124 (13.9)	63	61	28	33	16	39	8	6	9	47	15	20	13	14
	M6a, M6b		14 (1.6)	8	6	0	7	3	3	1	0	1	6	1	2	2	2
	M7		212 (23.8)	104	108	55	150	2	3	2	6	17	99	28	19	16	27
	Unknown		35 (3.9)	23	12	3	14	8	5	5	0	3	14	3	6	6	3
Rare leukemia		36 (0.7)		24 (66.7)	12 (33.3)	5 (13.9)	8 (22.2)	6 (16.7)	14 (38.9)	3 (8.3)	2 (5.6)	2 (5.6)	14 (38.9)	4 (11.1)	4 (11.1)	5 (13.9)	5 (13.9)
Myelodysplastic syndrome (MDS) and/or Myeloproliferative neoplasms (MPN)		296 (5.6)		171 (57.8)	125 (42.2)	42 (14.2)	73 (24.7)	66 (22.3)	92 (31.1)	23 (7.8)	13 (4.4)	10 (3.4)	127 (42.9)	40 (13.5)	43 (14.5)	34 (11.5)	29 (9.8)
	MPN		111 (37.5)	62	49	2	13	33	57	6	4	3	46	17	19	13	9
	MDS/MPN		49 (16.6)	32	17	27	18	1	1	2	2	2	23	7	5	3	7
	MDS		136 (45.9)	77	59	13	42	32	34	15	7	5	58	16	19	18	13
Non-Hodgkin lymphoma		628 (11.9)		446 (71.0)	182 (29.0)	7 (1.1)	96 (15.3)	237 (37.7)	240 (38.2)	48 (7.6)	26 (4.1)	45 (7.2)	218 (34.7)	107 (17.0)	107 (17.0)	56 (8.9)	69 (11.0)
	Lymphoblastic-T-precursor		136 (21.7)	100	36	0	14	48	63	11	8	14	51	21	24	8	10
	Lymphoblastic-B-precursor		71 (11.3)	42	29	5	20	31	12	3	5	4	25	14	7	9	7
	Burkitt		154 (24.5)	128	26	0	30	75	43	6	3	9	52	28	30	14	18
	Diffuse large B cell		121 (19.3)	80	41	0	12	42	50	17	4	9	35	17	22	17	17
	Anaplastic large cell		100 (15.9)	71	29	0	13	31	49	7	4	5	35	21	17	7	11

Table 2 continued

Disease	Subtype	*n* (%)	*n* (%)	Gender, *n* (%)		Age, *n* (%)					Residential areas at diagnosis, *n* (%)						
				Male	Female	0	1–4	5–9	10–14	15–19	Hokkaido	Tohoku	Kanto-Koshinetsu	Tokai-Hokuriku	Kinki	Chugoku-Shikoku	Kyushu-Okinawa
	Other		46 (7.3)	25	21	2	7	10	23	4	2	4	20	6	7	1	6
Hodgkin lymphoma		107 (2.0)		61 (57.0)	46 (43.0)	0 (0.0)	6 (5.6)	28 (26.2)	55 (51.4)	18 (16.8)	4 (3.7)	9 (8.4)	37 (34.6)	19 (17.8)	15 (14.0)	7 (6.5)	16 (15.0)
Histiocytosis		624 (11.8)		329 (52.7)	295 (47.3)	113 (18.1)	259 (41.5)	139 (22.3)	95 (15.2)	18 (2.9)	27 (4.3)	36 (5.8)	213 (34.1)	96 (15.4)	125 (20.0)	55 (8.8)	72 (11.5)
	Langerhans cell histiocytosis		345 (55.3)	199	146	66	143	81	50	5	20	20	110	50	66	39	40
	Hemophagocytic lymphohistiocytosis		265 (42.5)	123	142	44	108	56	44	13	6	15	97	44	55	16	32
	Other		14 (2.2)	7	7	3	8	2	1	0	1	1	6	2	4	0	0
Transient abnormal myelopoiesis associated with Down syndrome		184 (3.5)		100 (54.4)	84 (45.7)	182 (98.9)	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.2)	3 (1.6)	86 (46.7)	23 (12.5)	28 (15.2)	13 (7.1)	27 (14.7)
Other	lymphoproliferative disorders		51 (1.0)		24 (47.1)	27 (52.9)	1 (2.0)	9 (17.7)	19 (37.3)	18 (35.3)	4 (7.8)	0 (0.0)	4 (7.8)	13 (25.5)	8 (15.7)	9 (17.7)	11 (21.6)
6 (11.8)																	
Other hematologic malignancies		6 (0.1)		2 (33.3)	4 (66.7)	5 (83.3)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	2 (33.3)	0 (0.0)	2 (33.3)	1 (16.7)	0 (0.0)	0 (0.0)	1 (16.7)
Hematological malignancies	Total	5,287 (100.0)		3,019 (57.1)	2,268 (42.9)	572 (10.8)	1,803 (34.1)	1,375 (26.0)	1,253 (23.7)	284 (5.4)	215 (4.1)	370 (7.0)	2,029 (38.4)	766 (14.5)	880 (16.6)	449 (8.5)	578 (10.9)

Among NHL patients, those with Burkitt lymphoma (BL, 24.5 %), precursor T-lymphoblastic lymphoma (21.7 %), or diffuse large B cell lymphoma (DLBCL, 19.3 %), respectively, accounted for more than 20 %. When combined with those with anaplastic large cell lymphoma (ALCL, 15.9 %) and those with precursor B-lymphoblastic lymphoma (11.3 %), patients with these types of lymphoma accounted for 92.7 % of all NHL patients. Three subtypes, i.e., nodular sclerosis (37 cases), nodular lymphocyte predominance (29 cases), and mixed cellularity (31 cases), accounted for 90.7 % of all HL patients. While NHL patients were predominantly male, accounting for 2.5 times the number of female patients, no significant gender difference was observed for HL, with only 1.3 times male predominance. Peak incidences of both NHL and HL occurred at 5 years of age or older. While the incidence of BL peaked between ages 5 and 9, the incidence of precursor T-lymphoblastic lymphoma, DLBCL, and ALCL increased with age and peaked between ages 10 and 14.

The majority of histiocytosis included LCH, at 345 cases (55.3 %), followed by HLH at 265 cases (42.5 %). Incidences of both HLH and LCH mainly occurred in children aged four and under.

Among patients with MDS and/or MPN, those with MDS made up the majority (136 cases, 45.9 %), followed by MPN (111 cases, 37.5 %), including chronic myeloid leukemia (CML), and MDS/MPN (49 cases, 16.6 %), including juvenile myelomonocytic leukemia (JMML) and chronic myelomonocytic leukemia (CMML). The breakdown of registered cases of MDS by the JSPH guideline for diagnosis showed that refractory anemia (33 cases) account for 22 %, refractory cytopenia with multi-lineage dysplasia (25 cases) for 18 %, refractory anemia with excess blasts (RAEB)-1 (22 cases) for 16 %, and RAEB2 (19 cases) for 14 %, respectively.

Down-TAM accounted for 3.5 % of all cases, with the incidence rate being slightly higher in males (1.2 times higher than females).

No significant regional difference was observed when looking at age-specific incidences (data not shown).

Survival

Table 3 and Figs. 1, 2, 3, 4, 5 and 6 show disease-specific prognostic information for 5,287 cases of patients who were diagnosed with pediatric hematological malignancies between 2006 and 2010. The median observation period (range) was 1.7 (0.0–5.3) years. The point estimate (95 %CI) of 2y-OS for all pediatric patients with hematological malignancies was 91.6 (90.7–92.5) %. No difference was observed in survival rates in terms of gender (2y-OS 91.5 % for male, 91.9 % for female; log-rank test p value = 0.76) or residential areas at diagnosis (2y-OS 88.0 % for Hokkaido, 93.3 % for Tohoku, 91.5 % for Kanto-Koshinetsu, 91.7 % for Tokai-Hokuriku, 94.2 % for Kinki, 92.2 % for Chugoku-Shikoku, and 88.1 % for Kyushu-Okinawa; p value = 0.11). Survival rates in different age categories showed that children aged 5–9 years had the best prognosis (94.4 %). This was followed by children aged 1–4 years (93.8 %) and those aged 10–14 years (90.8 %), indicating a 2y-OS of more than 90 %. On the other hand, the 2y-OS for patients aged 15–19 years was about 10 % points lower at 80.5 %. The 2y-OS for infants less than 1 year old did not reach 90 % either at 85.6 % (log-rank test p value < 0.0001).

The comparison of 2y-OS among different diseases indicates that patients with HL (95.2 %) had the best prognosis.

Table 3 Survival for Japanese children and adolescents diagnosed with hematological malignancies

Disease	n	1 year		2 year		3 year		4 year		5 year	
		1-yr OS	95 % CI	2-yr OS	95 % CI	3-yr OS	95 % CI	4-yr OS	95 % CI	5-yr OS	95 % CI
Acute lymphoblastic leukemia	2,464	97.3	96.5–97.9	94.2	93.0–95.2	91.1	89.4–92.5	89.1	87.0–90.9	88.7	86.4–90.6
B-precursor	2,110	98.0	97.3–98.6	96.2	95.1–97.0	93.6	92.0–94.9	91.8	89.7–93.5	91.3	88.9–93.2
Mature B	60	95.6	83.4–98.9	84.7	68.8–92.9						
T cell	269	92.4	87.9–95.2	81.3	74.3–86.5	71.0	61.8–78.3	66.9	56.3–75.4		
Unknown	25	90.5	67.0–97.5	75.4	33.3–93.0						
Acute myeloid leukemia	891	91.2	88.9–93.0	83.3	80.1–86.1	77.4	73.3–80.9	76.3	71.9–80.1	75.2	70.3–79.4
M0	33	81.2	60.5–91.8	71.8	49.3–85.7	59.9	30.0–80.3				
M1	73	87.7	75.8–94.0	80.5	66.3–89.2	76.8	61.0–86.9				
M2	218	93.5	88.8–96.3	85.9	79.2–90.6	78.9	70.2–85.3	76.4	66.4–83.8	72.2	58.8–81.9
M3, M3v	70	95.6	87.0–98.6	95.6	87.0–98.6						
M4, M4Eo	112	91.7	84.0–95.8	79.0	68.1–86.6	72.4	59.5–81.7				

Table 3 continued

Disease	*n*	1 year		2 year		3 year		4 year		5 year	
		1-yr OS	95 % CI	2-yr OS	95 % CI	3-yr OS	95 % CI	4-yr OS	95 % CI	5-yr OS	95 % CI
M5a, M5b	124	83.7	74.9–89.6	76.7	66.4–84.2	70.5	58.3–79.7	67.4	54.1–77.6		
M6a, M6b	14	90.9	50.8–98.7	90.9	50.8–98.7						
M7	212	94.0	89.1–96.8	84.8	77.0–90.1	80.2	70.8–86.9				
Unknown	35	90.7	73.9–96.9	82.9	63.2–92.6	56.8	21.9–81.0				
Rare leukemia	36	90.6	73.5–96.9	75.2	51.5–88.5	68.9	43.9–84.5				
Myelodysplastic syndrome (MDS) and/or myeloproliferative neoplasms (MPN)	296	96.2	93.1–98.0	92.8	88.4–95.6	87.9	81.8–92.1	85.3	76.6–91.0		
MPN	111	100.0	–	98.6	90.2–99.8	96.7	87.4–99.2				
MDS/MPN	49	90.6	76.5–96.4	86.6	69.9–94.4	80.0	57.2–91.4				
MDS	136	95.0	89.2–97.7	90.0	81.9–94.6	82.6	71.3–89.7	76.2	57.8–87.4		
Non-Hodgkin lymphoma	628	94.6	92.3–96.2	92.1	89.3–94.2	90.5	87.0–93.1				
Lymphoblastic-T-precursor	136	96.4	90.6–98.6	90.4	82.2–95.0	86.9	77.0–92.7	83.1	69.8–90.9		
Lymphoblastic-B-precursor	71	98.4	89.3–99.8	96.2	85.3–99.1						
Burkitt	154	93.3	87.4–96.4	91.1	84.4–95.0						
Diffuse large B cell	121	97.1	91.3–99.1	97.1	91.3–99.1						
Anaplastic large cell	100	91.0	82.7–95.4	91.0	82.7–95.4						
Other	46	90.7	76.9–96.4	83.8	66.7–92.6						
Hodgkin lymphoma	107	98.7	91.2–99.8	95.2	85.5–98.4						
Histiocytosis	624	94.9	92.8–96.5	93.9	91.4–95.6						
Langerhans cell histiocytosis	345	99.3	97.3–99.8	98.7	95.8–99.6						
Hemophagocytic lymphohistiocytosis	265	88.9	84.3–92.3	87.7	82.8–91.4						
Other	14	100.0	–	90.0	47.3–98.5						
Transient abnormal myelopoiesis associated with Down syndrome	184	89.8	84.3–93.4	89.8	84.3–93.4						
Other lymphoproliferative disorders	51	89.5	76.6–95.5	86.3	71.6–93.7						
Other hematologic malignancies	6	83.3	27.3–97.5	83.3	27.3–97.5						
Hematological malignancies, Total	5,281	95.2	94.6–95.8	91.6	90.7–92.5	88.8	87.6–89.8	87.5	86.2–88.8	87.0	85.4–88.3
Gender											
Female	2,268	95.3	94.2–96.1	91.9	90.5–93.1	89.3	87.4–90.8	87.6	85.5–89.5	86.8	84.3–88.9
Male	3,019	95.2	94.3–96.0	91.5	90.2–92.6	88.4	86.8–89.8	87.5	85.7–89.0	87.2	85.3–88.8
Age											
0	572	88.0	84.8–90.5	85.6	82.0–88.5	83.7	79.7–87.0				
1–4	1,803	96.7	95.7–97.5	93.8	92.4–95.0	91.4	89.5–93.0	90.9	88.8–92.6		
5–9	1,375	97.1	96.0–97.9	94.4	92.7–95.7	91.0	88.7–92.9	89.5	86.7–91.7		
10–14	1,253	95.4	93.9–96.5	90.8	88.6–92.5	88.1	85.5–90.3	85.9	82.7–88.6	84.0	79.6–87.5
15–19	284	90.6	86.2–93.7	80.5	74.2–85.5	73.7	65.8–80.1			69.1	56.6–78.6
Residential areas											
Hokkaido	215	92.8	87.9–95.8	88.0	81.7–92.3	85.9	78.8–90.8			82.6	72.1–89.4
Tohoku	370	96.6	93.9–98.1	93.3	89.4–95.8	89.1	83.7–92.7	86.9	80.5–91.3		
Kanto-Koshinetsu	2,029	95.0	93.9–96.0	91.5	89.9–92.8	87.8	85.6–89.6	86.5	84.0–88.6		
Tokai-Hokuriku	766	95.5	93.6–96.8	91.7	89.1–93.7	90.8	88.0–93.0	90.3	87.3–92.7	89.0	84.7–92.2
Kinki	880	97.1	95.6–98.0	94.2	92.1–95.7	90.7	87.7–93.0	89.0	85.3–91.8	88.0	83.7–91.2
Chugoku-Shikoku	449	94.0	91.1–96.0	92.2	88.8–94.6	88.1	83.4–91.6				
Kyushu-Okinawa	578	93.6	91.1–95.4	88.1	84.6–90.9	87.3	83.7–90.9	87.3	83.7–90.2	85.1	80.5–88.7

yr year, *OS* overall survival

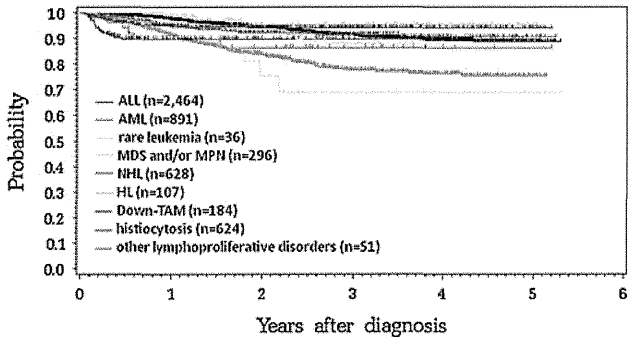

Fig. 1 Overall survival for patients diagnosed with hematological malignancies (n = 5,287). *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *MDS and/or MPN* myelodysplastic syndrome and/or myeloproliferative neoplasms, *NHL* non-Hodgkin lymphoma, *HL* Hodgkin lymphoma, *Down-TAM* transient abnormal myelopoiesis associated with Down syndrome

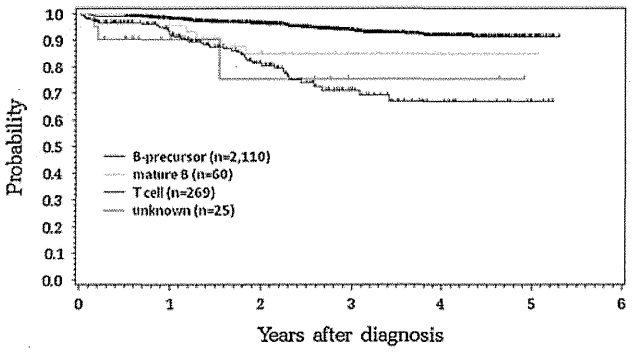

Fig. 2 Overall survival for patients diagnosed with acute lymphoblastic leukemia

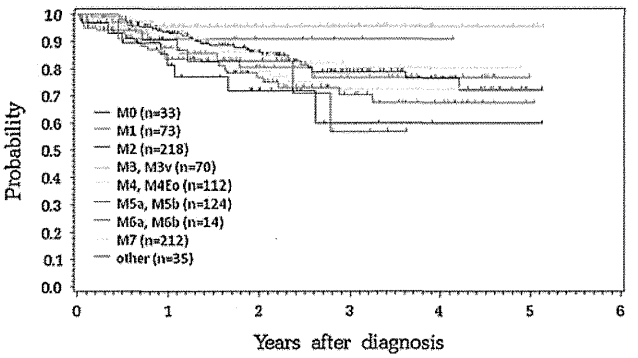

Fig. 3 Overall survival for patients diagnosed with acute myeloid leukemia

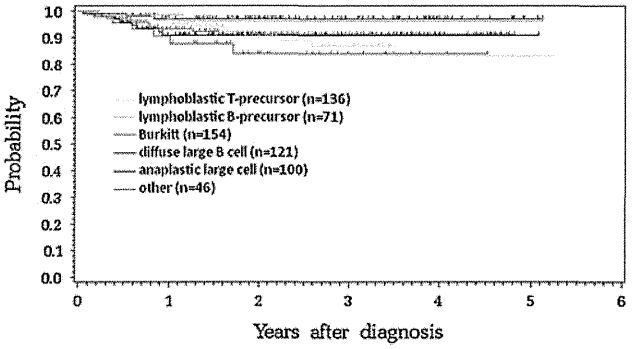

Fig. 4 Overall survival for patients diagnosed with non-Hodgkin lymphoma

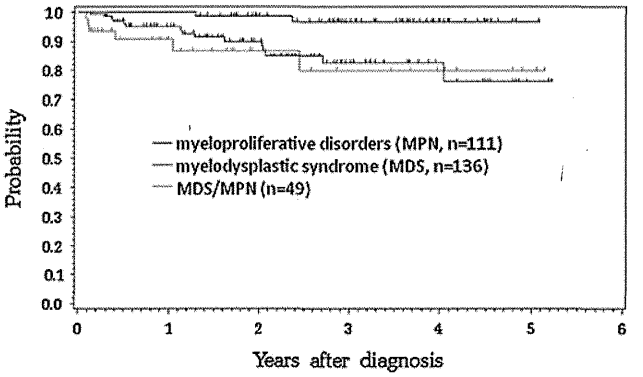

Fig. 5 Overall survival for patients diagnosed with myelodysplastic syndrome and/or myeloproliferative. *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasms

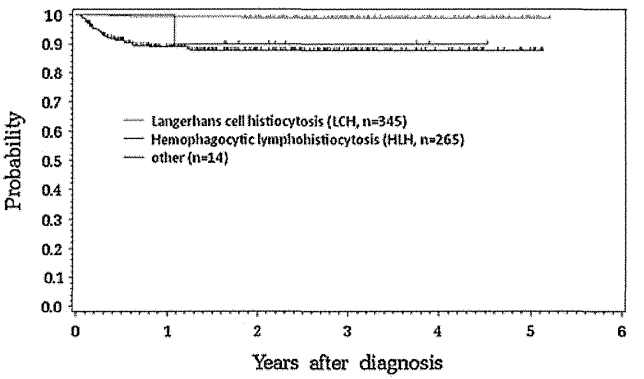

Fig. 6 Overall survival for patients diagnosed with histiocytosis

This was followed by patients with ALL (94.2 %), histiocytosis (93.9 %), MDS/MPN (92.4 %), and NHL (92.1 %). All patients had a survival rate of 90 % or more within 2 years after their disease was diagnosed. The 2y-OS for patients with Down-TAM (89.8 %) and those with other LPD (86.3 %) was estimated more than 85 %, while that for AML (83.3 %) and rare leukemia (75.2 %) was inferior to it.

Examination based on the immunophenotypic classification of ALL shows that patients with B-precursor ALL had the highest 2y-OS at 96.2 %, surpassing the 2y-OS for patients with mature B cell ALL (84.7 %) or T cell ALL (81.3 %).

Examination based on the FAB classification of AML indicates that M3 had the best prognosis (2y-OS 95.6 %),

followed by M6 (90.6 %). The 2y-OS accounted for more than 80 % with the exception of M0 (71.8 %), M5 (76.7 %), and M4 (79.0 %) whose survival rates did not reach 80 %.

Any type of NHL, without being based on the immunological classification, indicated a survival rate of more than 90 % within 2 years after diagnosis; especially, DLBCL (97.1 %) and precursor B-lymphoblastic lymphoma (96.2 %) had excellent prognoses.

Among histiocytosis, LCH indicated an extremely excellent 2y-OS of 98.7 % while that of HLH was 87.7 %. As for MDS and/or MPN, the 2y-OS of MPN was 98.6 % and the best, followed by MDS (90.0 %) and MDS/MPN (86.6 %).

Discussion

This study, which includes the largest childhood cohorts of hematological malignancies ever reported in Japan, documented progressive improvements in survival for children enrolled onto the disease registry project of the JSPH between 2007 and 2011. Considering the number of participating institutions, we estimated that our patient sample collected through the system represented about 80 % of all the cases of hematological malignancies in Japan. As cases newly diagnosed during the 5 years from January 1, 2006 to December 31, 2010, it has reported 5,287 cases of hematological malignancies from 187 institutions (diagnosis and treatment departments) across 47 prefectures nationwide; this result is equivalent to the prevalence of 4.5 cases per 100,000 people per year. The number of registered cases for age 15–19 years is much lower than those at age 14 or younger, which may be reflecting the fact that patients over the age of 16 usually visit internists rather than pediatrician. In order to figure out the exact trends in disease incidence for this age category in Japan, it is necessary to establish a registration system that can be accessed both by internists and pediatricians.

Regarding the incidence by disease group, ALL accounted for approximately half of hematological malignancies and more than 80 % when combined with AML, NHL and histiocytosis, which accounted for 10–15 %, respectively. The incidence by disease was nearly constant regardless of the diagnosis year. The reason why reports of Down-TAM almost doubled in 2010 is inferred that such increase accompanied wider recognition and utilization of the TAM central diagnosis system realized through a nationwide clinical observational study, JPLSG TAM-10, started in the same year (UMIN # 000005418).

According to the immunophenotypic classification counting of ALL, although the percentage of B-precursor ALL in Japan is higher than that in the US (85.6 vs. 63 %), T-ALL and mature B-ALL showed almost the same ratios [18]. It was also consistent with the findings in the US that the age of peak incidence was under 4 years old and that the incidence of ALL is slightly higher among male children than female children [19]. It is reported that introduction of risk-stratified treatment and improvement in supportive care have helped to achieve better treatment results of ALL with its 5y-OS higher than 85 % [14]. Our data showed that the 2y-OS of ALL was 94.2 % while its 5y-OS also exceeded 80 %. This indicates an improvement compared to the results of the European disease registry data during the second half of the twentieth century (1978–1997) [20], suggesting that prognoses as good as those in the results of recent foreign clinical studies have been achieved nationwide [14].

A dramatic improvement in the success rate of the treatment of AML has been seen [21], from about 20 % during the 1970s to 55 % in the decade following 2000. Although there are differences in thinking among different groups studying the treatment, such as those with regard to chemotherapy as well as hematopoietic stem cell transplantation depending on the disease risk, the overall survival in clinical trial has also been improved to have reached 42 $\sim$ 62 % [22–24]. In the AML99 clinical trials (2000–2002) [25–28] conducted in Japan, good results of a 5y-OS of over 76 % were obtained. In the present study also, it was found that by and large a good 2y-OS of higher than 80 % has been obtained even when M3 (2y-OS: 95.6 %), with the best prognosis, is excluded.

The incidence of HL in our data was very low compared to that from other countries. There are reports, both domestic and from overseas, that the incidences of both NHL and HL are relatively high in adolescents, and that the ratio of male to female children is high [29–33]. In our data, NHL is uncommon in infant, and the incidence of NHL increases throughout life. Although NHL is more common than HL in children younger than 15 years, the relative incidence of HL increases in children older than 10 years, making the incidence of HL in children aged between 15 and 19, almost twice that of NHL. In addition, higher incidence of NHL in male children was observed, while there was a slight male predominance in the incidence of HL, with an incidence ratio of 1.3 in male and female children. For NHL, favorable outcomes of treatment were obtained in more than 90 % of the cases within 2 years after diagnosis regardless of the immunological classification, which were similar to reports of clinical trials from inside and outside of the country [34–37].

Similarly, there was a high incidence of LCH, which accounts for the majority of cases of histiocytosis, in the age group of 1–4 years as in the overseas reports [38], and the prognosis was also good [39]. About half of the patients (42.5 %) with histiocytosis were diagnosed as having

HLH. In accordance with the previous literature in Japan, our data showed that the incidence of HLH cases per year was about 50 (mean 53, range 43–64) [40]. And clinical outcomes of HLH were considerably improved compared to the results of HLH-94 study [41].

With regard to MDS and/or MPN, hematopoietic stem cell transplantation, rather than conventional chemotherapy, has come to be a good indication [42] in cases in which there is an HLA-matched sibling donor. Children with low-risk MDS, including refractory anemia and refractory anemia with ring sideroblasts, were not candidates for hematopoietic stem cell transplantation [43]. Although the 5y-OS of children under the age of 16 was, respectively, 50–67 % for MDS and 51–75 % for MPN, depending on the type of transplant, in the national survey results (2011 report) from 1991 to 2010 by the Japan Society for Hematopoietic Cell Transplantation (JSHCT), our data showed that there was an improvement to 86.6 % for MDS/MPN including JMML, for which the prognoses are the worst [44], although the follow-up period was not sufficient.

In treatments for hematological malignancies during childhood and adolescents, long-term toxicity, including treatment-related deaths and secondary neoplasms, still remain as important issues. Therefore, we will continuously evaluate the trends in the national levels of diagnosis and treatment through the JSPH disease registry project and will show data concerning trends in disease incidence and deaths accompanied by prognostic information. Continuous activity to monitor the level of medical care is considered quite important in aiming at the development of more effective treatments which maintain long-term safety.

Acknowledgments The survey on hematological malignancies incidence in Japan was conducted with contributions from the 187 institutions, described in Appendix 1. The authors thank deeply the members, especially Kaori Nagai, Kazumi Takeuchi, Maki Nishimura, and Midori Otomo of the Data Management Department of the NPO-OSCR, for their support in the management of the electronic or paper-based survey system and in the cleaning and tabulation of the registered data.

Conflict of interest The authors have no financial relationship to declare.

Appendix

See appendix Table 4.

Table 4 Institutions with registered cases of hematological malignancies

S. no.	District	Institutions
1	Hokkaido	Oji General Hospital
2	Hokkaido	Sapporo Medical University Hospital
3	Hokkaido	Hokkaido Medical Center for Child Health and Rehabilitation
4	Hokkaido	Sapporo Hokuyu Hospital
5	Hokkaido	Hokkaido University Hospital
6	Hokkaido	KKR Sapporo Medical Center
7	Hokkaido	Asahikawa Medical University Hospital
8	Hokkaido	Hospital Hakodate Hokkaido
9	Hokkaido	Kushiro city General Hospital
10	Hokkaido	National Hospital Organization Hokkaido Cancer Center
11	Tohoku	Hirosaki University school of Medicine and Hospital
12	Tohoku	Nakadori General Hospital
13	Tohoku	Akita University Hospital
14	Tohoku	Iwate Medical University Hospital
15	Tohoku	Iwate Prefectural Chubu Hospital
16	Tohoku	Iwaki Kyoritsu Hospital
17	Tohoku	Fukushima Medical University Hospital
18	Tohoku	Tohoku University Hospital
19	Tohoku	Miyagi Children's Hospital
20	Tohoku	Yamagata University Hospital
21	Tohoku	Sendai City Hospital
22	Kanto and Koshinetsu	Ibaraki Children's Hospital
23	Kanto and Koshinetsu	Tsukuba University Hospital

Table 4 continued

S. no.	District	Institutions
24	Kanto and Koshinetsu	Yokohama City University Hospital
25	Kanto and Koshinetsu	Saiseikai Yokohama City Nanbu Hospital
26	Kanto and Koshinetsu	Kitasato University Hospital
27	Kanto and Koshinetsu	Tokai University Hospital
28	Kanto and Koshinetsu	Showa University Fujigaoka Hospital
29	Kanto and Koshinetsu	Kanagawa Children's Medical Center
30	Kanto and Koshinetsu	St. Marianna University School of Medicine Hospital
31	Kanto and Koshinetsu	Gunma Children's Medical Center
32	Kanto and Koshinetsu	Gunma University Hospital
33	Kanto and Koshinetsu	Saitama Medical Center
34	Kanto and Koshinetsu	Saitama Children's Medical Center
35	Kanto and Koshinetsu	National Defense Medical College Hospital
36	Kanto and Koshinetsu	Teikyo University Chiba Medical Center
37	Kanto and Koshinetsu	Kameda Medical Center
38	Kanto and Koshinetsu	Nippon Medical School Chiba Hokusoh Hospital
39	Kanto and Koshinetsu	Kokuho Asahi General Hospital
40	Kanto and Koshinetsu	Japanese Red Cross Narita Hospital
41	Kanto and Koshinetsu	Chiba University Hospital
42	Kanto and Koshinetsu	Chiba Children's Hospital
43	Kanto and Koshinetsu	Matsudo City Hospital
44	Kanto and Koshinetsu	National Center for Global Health and Medicine
45	Kanto and Koshinetsu	Nihon University Itabashi Hospital
46	Kanto and Koshinetsu	Japanese Red Cross Musashino Hospital
47	Kanto and Koshinetsu	Teikyo University Hospital
48	Kanto and Koshinetsu	Tokyo Medical And Dental University Hospital Faculty of Medicine
49	Kanto and Koshinetsu	The Jikei University Daisan Hospital
50	Kanto and Koshinetsu	Tokyo Metropolitan Children's Medical Center
51	Kanto and Koshinetsu	The Jikei University Hospital
52	Kanto and Koshinetsu	Nippon medical School Hospital
53	Kanto and Koshinetsu	Tokyo Women's Medical University Medical Center East
54	Kanto and Koshinetsu	The University of Tokyo Hospital
55	Kanto and Koshinetsu	Keio University Hospital
56	Kanto and Koshinetsu	Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital
57	Kanto and Koshinetsu	Toho University Omori Medical Center
58	Kanto and Koshinetsu	Showa University Hospital
59	Kanto and Koshinetsu	Juntendo University Hospital
60	Kanto and Koshinetsu	National Center for Child Health and Development
61	Kanto and Koshinetsu	St. Luke's International Hospital
62	Kanto and Koshinetsu	Kyorin University Hospital
63	Kanto and Koshinetsu	Tokyo Dental College Ichikawa General Hospital
64	Kanto and Koshinetsu	Dokkyo Medical University Hospital
65	Kanto and Koshinetsu	Jichi Medical University Hospital
66	Kanto and Koshinetsu	Shinshu University Hospital
67	Kanto and Koshinetsu	Nagano Children's Hospital
68	Kanto and Koshinetsu	Niigata University Medical and Dental Hospital
69	Kanto and Koshinetsu	Niigata Cancer Center Hospital
70	Kanto and Koshinetsu	University of Yamanashi Hospital
71	Kanto and Koshinetsu	Japanese Red Cross Maebashi Hospital

Table 4 continued

S. no.	District	Institutions
72	Kanto and Koshinetsu	Saitama Medical University International Medical Center
73	Kanto and Koshinetsu	Yokosuka Kyosai Hospital
74	Kanto and Koshinetsu	Kofu Municipal Hospital
75	Kanto and Koshinetsu	Teikyo University School of medicine University Hospital, Mizonokuchi
76	Tokai and Hokuriku	Fujita Health University
77	Tokai and Hokuriku	Aichi Medical University Hospital
78	Tokai and Hokuriku	Komaki City Hospital
79	Tokai and Hokuriku	National Hospital Organization Nagoya Medical Center
80	Tokai and Hokuriku	Nagoya Daini Red Cross Hospital
81	Tokai and Hokuriku	Anjo Kosei Hospital
82	Tokai and Hokuriku	Japanese Red Cross Nagoya Daiichi Hospital
83	Tokai and Hokuriku	Nagoya University Hospital
84	Tokai and Hokuriku	Kasugai Municipal Hospital
85	Tokai and Hokuriku	Nagoya City University Hospital
86	Tokai and Hokuriku	Toyohashi Municipal Hospital
87	Tokai and Hokuriku	Ichinomiya Municipal Hospital
88	Tokai and Hokuriku	Okazaki City Hospital
89	Tokai and Hokuriku	Kanazawa University Hospital
90	Tokai and Hokuriku	Ishikawa Prefectural Central Hospital
91	Tokai and Hokuriku	Kanazawa Medical University Hospital
92	Tokai and Hokuriku	Gifu Municipal Hospital
93	Tokai and Hokuriku	Toki Municipal General Hospital
94	Tokai and Hokuriku	Gifu University Hospital
95	Tokai and Hokuriku	Hamamatsu Medical Center
96	Tokai and Hokuriku	Hamamatsu University School of Medicine, University Hospital
97	Tokai and Hokuriku	Shizuoka Children's Hospital
98	Tokai and Hokuriku	Iwata City Hospital
99	Tokai and Hokuriku	Seirei Hamamatsu General Hospital
100	Tokai and Hokuriku	Toyama University Hospital
101	Tokai and Hokuriku	Fukui Red Cross Hospital
102	Tokai and Hokuriku	University of Fukui Hospital
103	Tokai and Hokuriku	Mie University Hospital
104	Tokai and Hokuriku	National Mie Hospital
105	Tokai and Hokuriku	Nagoya City East Medical Center
106	Kinki	National Hospital Organization Osaka National Hospital
107	Kinki	Osaka City University Hospital
108	Kinki	Kinki University Hospital
109	Kinki	Yao Municipal Hospital
110	Kinki	Matsushita Memorial Hospital
111	Kinki	Osaka Medical Center and Research Institute for Maternal and Child Health
112	Kinki	Toyonaka Municipal Hospital
113	Kinki	Osaka University Hospital
114	Kinki	Sakai Hospital Kinki University Faculty of Medicine
115	Kinki	Osaka Medical College Hospital
116	Kinki	Kansai Medical University Hirakata Hospital
117	Kinki	Kitano Hospital, The Tazuke Kofukai Medical Research Institute
118	Kinki	Osaka City General Hospital
119	Kinki	Osaka Red Cross Hospital

Table 4 continued

S. no.	District	Institutions
120	Kinki	Osaka General Medical Center
121	Kinki	Nakano Children's Hospital
122	Kinki	Kishiwada City Hospital
123	Kinki	Japanese Red Cross Kyoto Daiichi Hospital
124	Kinki	Kyoto-Katsura Hospital
125	Kinki	Kyoto University Hospital
126	Kinki	Kyoto City Hospital
127	Kinki	National Hospital Organization Maizuru Medical Center
128	Kinki	University Hospital, Kyoto Prefectural University of Medicine
129	Kinki	Takashima General Hospital
130	Kinki	Shiga University of Medical Science Hospital
131	Kinki	Shiga Medical Center for Children
132	Kinki	Otsu Red Cross Hospital
133	Kinki	Tenri Hospital
134	Kinki	Nara Medical University Hospital
135	Kinki	Kobe University Hospital
136	Kinki	Kobe City Medical Center General Hospital
137	Kinki	Japanese Red Cross Society Himeji Hospital
138	Kinki	Akashi Municipal Hospital
139	Kinki	Hyogo Prefectural Kobe Children's Hospital
140	Kinki	Hyogo College of Medicine Hospital
141	Kinki	Nishi-Kobe Medical Center
142	Kinki	Japanese Red Cross Society Wakayama Medical Center
143	Kinki	Wakayama Medical University Hospital
144	Chugoku and Shikoku	Ehime Prefectural Central Hospital
145	Chugoku and Shikoku	Ehime University Hospital
146	Chugoku and Shikoku	National Hospital Organization Okayama Medical Center
147	Chugoku and Shikoku	Okayama University Hospital
148	Chugoku and Shikoku	Okayama Saiseikai General Hospital
149	Chugoku and Shikoku	Kawasaki Medical School Hospital
150	Chugoku and Shikoku	Kurashiki Central Hospital
151	Chugoku and Shikoku	National Hospital Organization Kagawa Children's Hospital
152	Chugoku and Shikoku	Kagawa University Hospital
153	Chugoku and Shikoku	National Hospital Organization Kochi Medical Center
154	Chugoku and Shikoku	Japanese Red Cross Kochi Hospital
155	Chugoku and Shikoku	Kochi Medical School Hospital
156	Chugoku and Shikoku	Shimane University Hospital
157	Chugoku and Shikoku	Shimane Prefectural Central Hospital
158	Chugoku and Shikoku	Tokushima University Hospital
159	Chugoku and Shikoku	Tottori University Hospital
160	Chugoku and Shikoku	Tottori Prefectural Chuou Hospital
161	Chugoku and Shikoku	Hiroshima University Hospital
162	Chugoku and Shikoku	Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital
163	Chugoku and Shikoku	Yamaguchi University Hospital
164	Chugoku and Shikoku	Tokushima Red Cross Hospital
165	Chugoku and Shikoku	Matsue Red Cross Hospital
166	Kyushu and Okinawa	National Hospital Organization Beppu Medical Center
167	Kyushu and Okinawa	Oita Prefectural Hospital