The joinpoint regression model was used to describe changes in trends [6]. We allowed for up to four joinpoints for each model. The computation of mortality rates and their standard errors was implemented in SAS 9.0. Joinpoint analyses were carried out using Joinpoint software 3.3.1 from the Surveillance Research Program of the US National Cancer Institute. Time trends were assessed for all childhood cancer combined and for six major categories, including leukemia, lymphoma, malignant brain tumor, malignant kidney tumor and malignant bone tumor.

The standardized mortality ratio (SMR) by sex was calculated for 47 prefectures in Japan by taking the ratio of the observed to expected deaths. The z-value was computed for each SMR, on the basis of the assumption that observed deaths follow a Poisson distribution. The maps were developed using adjusted SMR by gender.

results

mortality

Tables 1 and 2 give age-adjusted mortality rates in Japan and five other developed countries for all malignant tumors and for

Table 2. Childhood cancer mortality rate (per 100 000) in Japan and other selected countries (girls)

Period of death	Japan	Canada	United States	Italy	UK	New Zealand
Total malignant tumors						
1970–1974	5.10	6.12	5.13	6.90	5.55	6.85
1975–1979	4.61	4.83	4.07	5.90	4.69	6.35
1980–1984	3.88	4.24	3.59	5.48	4.27	4.39
1985–1989	3.30	3.43	3.06	4.36	3.81	5.27
1990–1994	2.75	2.80	2.69	4.19	3.01	3.81
1995–1999	2.23	2.73	2.39	3.29	2.65	3.54
2000–	1.89	2.06	2.28	2.86	2.47	3.06
Leukemia						
1970–1974	2.86	2.80	2.26	3.28	2.43	3.08
1975–1979	2.50	2.34	1.70	2.53	1.82	1.86
1980–1984	1.79	1.71	1.30	2.17	1.59	1.66
1985–1989	1.50	1.37	1.09	1.51	1.26	1.84
1990–1994	1.20	0.89	0.91	1.47	0.89	1.04
1995–1999	0.88	0.87	0.78	1.07	0.91	1.34
2000–	0.68	0.46	0.69	0.82	0.76	0.90
Lymphomas						
1970–1974	0.33	0.39	–	0.54	0.31	0.27
1975–1979	0.35	0.18	–	0.39	0.27	0.41
1980–1984	0.31	0.23	0.16	0.26	0.22	0.21
1985–1989	0.28	0.22	0.13	0.28	0.14	0.25
1990–1994	0.25	0.12	0.09	0.16	0.09	0.10
1995–1999	0.10	0.09	0.08	0.17	0.09	0.20
2000–	0.06	0.39	0.06	0.18	0.09	0.05
Central nervous system tumors						
1980–1984	0.39	1.01	0.84	1.13	0.93	1.43
1985–1989	0.38	0.88	0.77	0.99	0.98	1.37
1990–1994	0.44	0.75	0.77	0.90	0.88	1.26
1995–1999	0.47	0.84	0.71	0.72	0.74	0.88
2000–	0.42	0.69	0.69	0.78	0.71	1.00
Malignant kidney tumors						
1970–1974	0.20	0.32	0.25	0.44	0.37	0.38
1975–1979	0.11	0.23	0.19	0.33	0.26	0.36
1980–1984	0.12	0.13	0.15	0.27	0.18	0.00
1985–1989	0.07	0.11	0.13	0.18	0.18	0.10
1990–1994	0.07	0.10	0.09	0.18	0.15	0.27
1995–1999	0.05	0.14	0.11	0.11	0.12	0.21
2000–	0.06	0.11	0.10	0.10	0.12	0.11
Malignant bone tumors						
1980–1984	0.17	0.20	0.16	0.26	0.29	0.13
1985–1989	0.16	0.14	0.12	0.27	0.26	0.31
1990–1994	0.12	0.13	0.13	0.23	0.14	0.05
1995–1999	0.14	0.12	0.11	0.16	0.13	0.18
2000–	0.11	0.16	0.11	0.12	0.20	0.25

the main types of childhood cancer. A total of 33 059 childhood cancer deaths were reported in Japan during 1970–2006, of which 353 cancer deaths occurred in 2006. For all cancers combined, the mortality rate during 2000–2006 was 2.20 per 100 000 population for boys and 1.89 for girls. Leukemia was the most common diagnosis. Death rates from leukemia were 0.84 for boys and 0.68 for girls. Mortality from childhood CNS tumors was 0.43 for boys and 0.42 for girls. Geographic variations were observed. The rates of childhood CNS tumor and malignant kidney tumor were lower for both genders in Japan than in other countries.

temporal changes in mortality

Trends of age-standardized mortality from childhood cancer are shown in Figures 1 and 2 and Tables 3 and 4. Mortality for all cancers combined decreased since 1970s in Japan. For boys, a declining trend of 1.58% per year ($P < 0.05$) was observed during 1970–1979, followed by an accelerated decline of 3.78% per year ($P < 0.05$) during 1979–2006. For girls, mortality was high in the 1970s and remained stable in 1996–2006 at a low level, after two significant periods of decline (1972–1995 and 1995–1999). The average annual per cent change (AAPC) in recent 10 years was −3.8% ($P < 0.05$%) for boys and −1.9% ($P < 0.05$) for girls. In recent 5 years, declining trend only occurred in boys. The average annual per cent change during 2002–2006 was −3.8% ($P < 0.05$%) for boys, and for girls a nonsignificant decline was observed from 2002 (AAPC = −0.6%, $P > 0.05$) for girls.

The mortality rate from leukemia in boys remained stable during 1970–1976 (APC = −1.10, $P > 0.05$) and then declined by 4.77% per year ($P < 0.05$) during 1976–2006. For girls, mortality decreased by 4.53% per year ($P < 0.05$) throughout the whole period. The average annual change in recent 10 years was −4.8% ($P < 0.05$%) for boys and −4.5% ($P < 0.05$%) for girls. Similar decline trends were also observed in Canada, the United States, Italy, UK (girls) and New Zealand.

In contrast with the dramatic decline in mortality for childhood leukemia, mortality rates from childhood CNS tumor in Japan remained stable at a low level for both genders during 1980–2006. The average annual change in recent 10 years was 0.5% ($P > 0.05$) for boys and 0.0% ($P > 0.05$) for girls. On the contrary, Canada, the United States, UK and New Zealand (girls) showed significant declining trends in the whole period.

With reference to the pattern of mortality for lymphomas, death rates for boys were stable during 1970–1985 and declined significant thereafter by 8.56% per year. The trend for girls leveled off during 1970–1991 and showed a declining trend of 11.85% per year during 1991–2006; however, except for New Zealand females, the death rates in other countries for both genders significantly declined throughout the whole period.

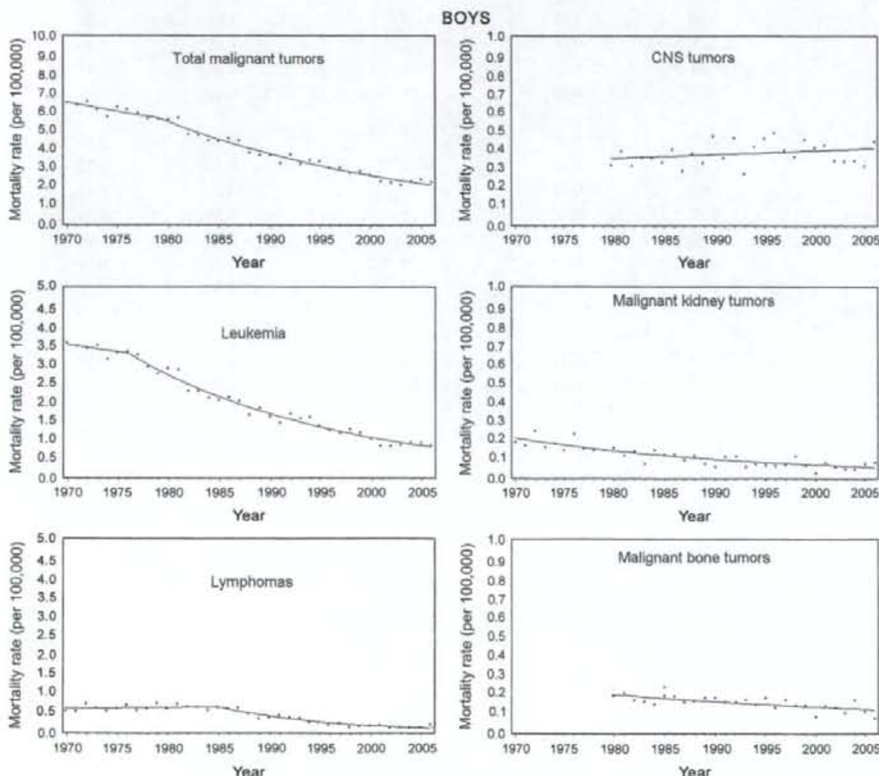

Figure 1. Mortality rates of childhood cancer deaths, boys, Japan, 1970–2006.

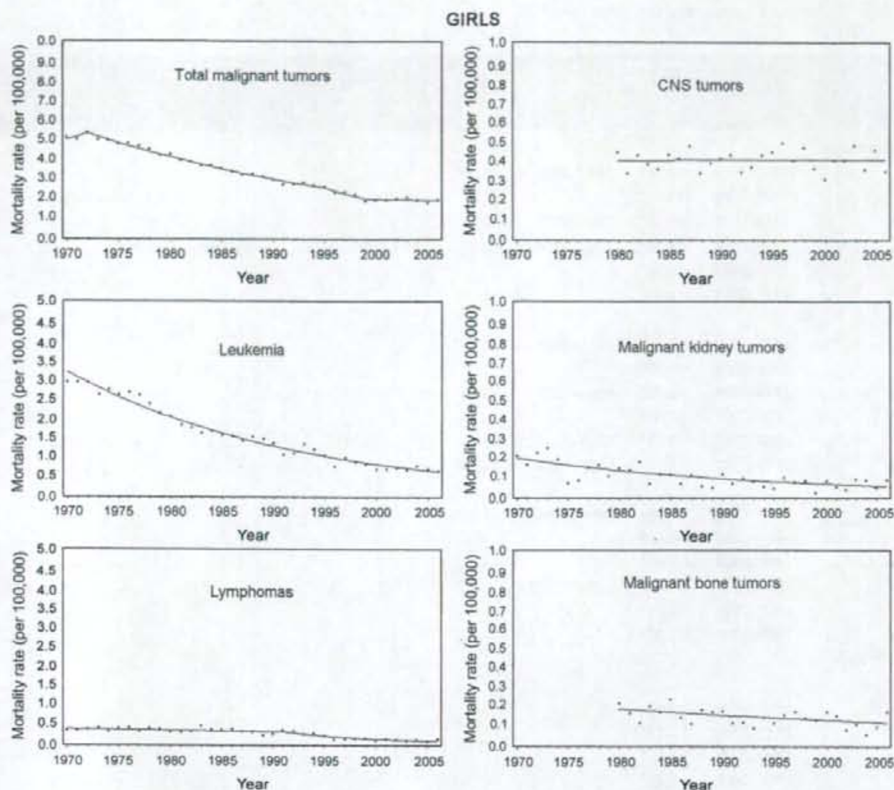

Figure 2. Mortality rates of childhood cancer deaths, girls, Japan, 1970–2006.

Regression analysis also revealed that the death rate for malignant kidney tumors declined by 4.12% per year for boys and 3.98% per year for girls during 1970–2006. Similar trends were observed for malignant bone tumor. Mortality decreased by 2.03% per year for boys and 1.79% per year for girls throughout the whole period.

Mortality rates varied from prefecture to prefecture in Japan. A map of SMR by gender is shown in Figure 3. The SMR was significantly highest among children in Kochi prefecture for boys and Tokushima and Kagoshima prefectures for girls.

discussion

In this study, we quantified the childhood cancer burden in Japan, focusing on mortality, and compared these figures with other developed countries. The results indicated that mortality from childhood cancer in Japan is substantial, while the number of deaths is small. There were 33 059 cases of childhood cancer death over the period 1970–2006 in Japan. Approximately 400 deaths each year were attributed to cancer in children aged 0–14 years. Mortality from all cancers combined in Japan is comparable to that in the European, North American and Oceanic countries included in this study for contrast.

The joinpoint regression method was used in our research to evaluate the trend in childhood cancer deaths. This method has allowed a detailed and accurate description of the pattern of childhood cancer mortality since it identifies the calendar years in which statistically significant changes in trends occurred. This offers a clearer picture of actual trends in mortality over long periods of time rather than using only one trend statistics. We also reported the average annual percentage change in this study. The AAPC can be used to characterize a short segment based on a joinpoint model fit over a much longer series. This is especially advantageous for situations when the data are sparse (e.g. a rare cancer or data from a small geographic area) [7]. Our results showed a declining cancer mortality rate for boys in the whole period and a stable trend for girls in recent 5 years. It is unlikely that the observed time trends in the mortality rate are due to variations in the completeness and accuracy of the population data because the analyzed data were provided by official sources and based on the population census. The significant time trend observed for most tumor types is congruent with improvements in diagnosis, therapy and supportive care.

The dramatic decrease in mortality observed for childhood leukemia, which accounts for ~50% of all childhood cancer

Table 3. The APC of childhood cancer mortality rates (boys)

Country	Trend 1		Trend 2		Trend 3		Trend 4		AAPC	
	Years	APC	Years	APC	Years	APC	Years	APC	Last 10 observations	Last 5 observations
Total malignant tumors										
Japan	1970–1979	−1.58*	1979–2006	−3.78*					−3.8*	−3.8*
Canada	1970–2004	−3.64*							−3.6*	−3.6*
United States	1970–1998	−3.22*	1998–2005	−0.26					−0.3	−0.9
Italy	1970–1985	−2.32*	1985–1989	−8.69	1989–1993	6.96	1993–2003	−5.89*	−5.9*	−5.9*
UK	1970–2005	−2.93*							−2.9*	−2.9*
New Zealand	1970–2004	−2.50*							−2.5*	−2.5*
Leukemia										
Japan	1970–1976	−1.10	1976–2006	−4.77*					−4.8*	−4.8*
Canada	1970–2004	−5.00*							−5.0*	−5.0*
United States	1970–1984	−4.95*	1984–2005	−3.39*					−3.4*	−3.4*
Italy	1970–2003	−3.69*							−3.7*	−3.7*
UK	1970–2005	−3.74*	2003–2005	−27.41					−9.6	−16.4
New Zealand	1970–1997	−2.12*	1997–2004	−18.03*					−14.7*	−18.0*
Lymphomas										
Japan	1970–1985	0.39	1985–2006	−8.56*					−8.6*	−8.6*
Canada	1970–2004	−6.10*							−6.1*	−6.1*
United States	1980–2005	−5.63*							−5.6*	−5.6*
Italy	1970–2003	−4.46*							−4.5*	−4.5*
UK	1970–2005	−4.56*							−4.6*	−4.6*
New Zealand	1970–2004	−2.57*							−2.6*	−2.6*
Central nervous system tumors										
Japan	1980–2006	0.48							0.5	0.5
Canada	1980–2004	−2.13*							−2.1*	−2.1*
United States	1980–2005	−1.07*							−1.1*	−1.1*
Italy	1980–2003	−2.19*							−2.2*	−2.2*
UK	1980–2005	−1.25*							−1.2*	−1.2*
New Zealand	1980–2004	−0.86							−0.9	−0.9
Malignant kidney tumors										
Japan	1970–2006	−4.12*							−4.1*	−4.1*
Canada	1970–1996	−7.91*	1996–2004	17.70*					14.5*	17.7*
United States	1970–1987	−5.46*	1987–2005	−1.73*					−1.7*	−1.7*
Italy	1970–2003	−4.91*							−4.9*	−4.9*
UK	1970–2005	−3.64*							−3.6*	−3.6*
New Zealand	1970–2004	−1.99							−2.0*	−2.0*
Malignant bone tumors										
Japan	1980–2006	−2.03*							−2.0*	−2.0*
Canada	1980–2004	−2.32*							−2.3*	−2.3*
United States	1980–1990	−4.41*	1990–2005	1.31					1.3	1.3
Italy	1980–2003	−4.43*							−4.4*	−4.4*
UK	1980–2005	−2.93*							−2.9*	−2.9*
New Zealand	1980–2004	−0.23							−0.2	−0.2

*$P < 0.05$.

APC is the annual per cent change; AAPC is average annual per cent change.

deaths, is consistent with improvements in survival, particularly for patients with acute lymphoblastic leukemia. This increase in survival is due to more effective antileukemic therapy, such as multidrug chemotherapy protocols, with a reduction in the number of relapses and resistant disease, but also due to improvements in supportive care, such as antibiotics, antifungal treatment, blood banking, transplant procedures and pediatric intensive care. In fact, the 5-year survival rate of acute lymphoblastic leukemia increased from 20% to 30% in the 1960s to 60% to 75% in the 1980s in developed countries. Current survival rates are ~80% for acute lymphoblastic leukemia (ALL) [8] and 50%–70% for acute myelogenous leukemia. In Japan, a population-based study in Osaka prefecture indicated that the 5-year survival rate of childhood leukemia increased from 32.4% in 1975–1984 to 60.4 in 1985–1994 [1]. National incidence trends could not be

Table 4. The APC of childhood cancer mortality rates (girls)

Country	Trend 1		Trend 2		Trend 3		Trend 4		AAPC	
	Years	APC	Years	APC	Years	APC	Years	APC	Last 10 observations	Last 5 observations
Total malignant tumors										
Japan	1970–1972	3.24	1972–1995	−3.21*	1995–1999	−6.46*	1999–2006	−0.57	−1.9*	−0.6
Canada	1970–2004	−3.42*							−3.4*	−3.4*
United States	1970–1977	−4.46*	1977–1995	−2.72*	1995–2005	−1.07*			−1.1*	−1.1*
Italy	1970–2003	−2.80*							−2.8*	−2.8*
UK	1970–2005	−2.73*							−2.7*	−2.7*
New Zealand	1970–2004	−2.57*							−2.6*	−2.6*
Leukemia										
Japan	1970–2006	−4.53*							−4.5*	−4.5*
Canada	1970–2004	−5.28*							−5.3*	−5.3*
United States	1970–1980	−6.09*	1980–2005	−3.14*					−3.1*	−3.1*
Italy	1970–2003	−4.33*							−4.3*	−4.3*
UK	1970–2005	−3.88*							−3.9*	−3.9*
New Zealand	1970–2004	−3.17*							−3.2*	−3.2*
Lymphomas										
Japan	1970–1991	−1.13	1991–2006	−11.85*					−11.8*	−11.8**
Canada	1970–2004	−4.55*							−4.6*	−4.6*
United States	1980–2005	−4.39*							−4.4*	−4.4*
Italy	1970–2003	−3.93*							−3.9*	−3.9*
UK	1970–2005	−4.56*							−4.6*	−4.6*
New Zealand	1970–2004	−0.35							−0.4	−0.4
Central nervous system tumors										
Japan	1980–2006	0.03							0.0	0.0
Canada	1980–2004	−1.50*							−1.5*	−1.5*
United States	1980–2005	−0.87*							−0.9*	−0.9*
Italy	1980–2003	−2.28*							−2.3*	−2.3*
UK	1980–2005	−1.68*							−1.7*	−1.7*
New Zealand	1980–2004	−2.32*							−2.3*	−2.3*
Malignant kidney tumors										
Japan	1976–2006	−3.98*							−4.0*	−4.0*
Canada	1970–2004	−2.90*							−2.9*	−2.9*
United States	1970–1991	−4.60*	1991–2005	0.16					0.2	0.2
Italy	1970–2003	−4.62*							−4.6*	−4.6*
UK	1970–2005	−3.49*							−3.5*	−3.5*
New Zealand	1970–2004	−2.91*							−2.9*	−2.9*
Malignant bone tumors										
Japan	1980–2006	−1.79*							−1.8*	−1.8*
Canada	1980–2004	−0.24							−0.2	−0.2
United States	1980–2005	−1.59*							−1.6*	−1.6*
Italy	1980–2003	−3.52*							−3.5*	−3.5*
UK	1980–2005	−2.22*							−2.2*	−2.2*
New Zealand	1980–2004	1.52							1.5	1.5

*$P < 0.05$.

APC is the annual per cent change; AAPC is average annual per cent change.

obtained in the current study. Research in Great Britain [9, 10], Italy [11] and Sweden [12] showed increased trends in childhood leukemia. A report from Britain indicated that small peaks in the incidence of ALL in 1976 and 1990 coincided with the years immediately following influenza epidemics [13]. Other explanations of the increased trend were characteristics of the environment, such as population mixing, although the etiology of cancer remains complicated and largely unknown.

The stable trend in mortality for childhood CNS tumor implied a modest increase trend in the incidence rate in Japan because of the survival improvement reported in childhood CNS tumors in developed countries in recent decades, while progress in therapy for brain tumors has not been as great as for leukemia. For CNS tumors, computed tomography, which was introduced in the 1970s, and magnetic resonance imaging, which has been used widely since the 1980s, has become

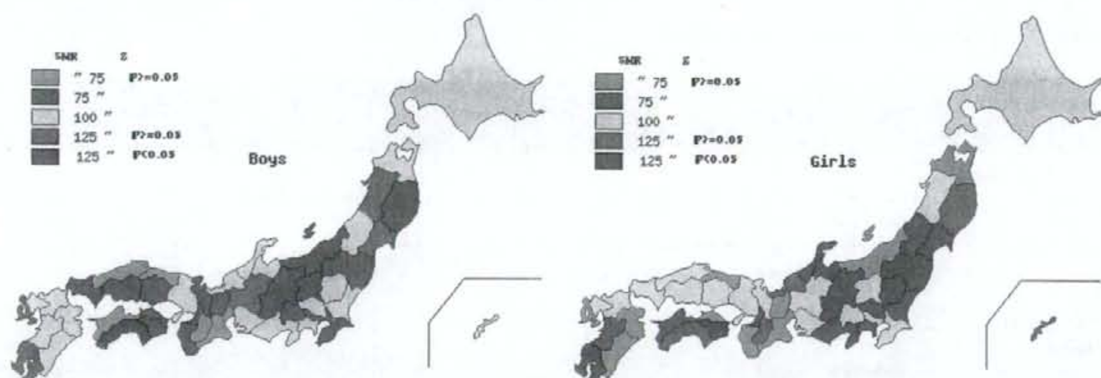

Figure 3. Standardized mortality ratios for childhood cancer in Japan, 2000–2006 by prefecture.

a standard tool for CNS tumor diagnosis and evaluation [14]. Furthermore, improvements in neurosurgical techniques have occurred during the past two decades, including stereotactic surgery, Cavitron Ultrasonic Surgical Aspirator and so on. Childhood cancer survival research from Osaka prefecture in Japan reported a slight increase in 5-year survival [1]. Incidence trends were not evaluated in this study. Data from the population-based cancer registry of Hokkaido prefecture in Japan indicated that the incidence of childhood brain tumors has been increasing, though the cause is unknown [15]. Other studies conducted in developed countries reported a significant increase in childhood CNS tumor incidence [10, 12, 16–21]. This has been explained by changes in detection and/or reports of childhood CNS tumors [22]. Because magnetic resonance imaging became ubiquitous at tertiary pediatric centers in the mid-1980s, it is likely to have increased the rate of detection; however, in the current study, the mortality rate of childhood CNS tumors in Japan was low and constant since the 1980s, and no significant increase in the number of deaths occurred in the middle of the 1980s to support the suggestion that the incidence increase was due to improved diagnostic techniques, if this increase really exists in Japan, and it seems unlikely to explain the long-term continued leveling off of mortality. The etiology of childhood CNS tumors remains largely unknown. Environmental factors are suggested to have a relationship with brain tumors. Further investigation in this field is needed to identify the incidence trends and reasonable explanations for these trends in Japan.

A previous childhood cancer mortality study in Japan presented data up to 1998. Furthermore, trend analysis was according to the correlation coefficient between the mortality rate and death year. Our analysis provides an updated mortality rate and reliable time trend analysis. In general, the mortality trends observed in other developed countries were compatible with Japan, although some differences were apparent. For example, a decrease in mortality during 0–14 years was observed in leukemia in the United States, Canada, Italy, New Zealand and Japan; however, the mortality rate from CNS tumors has decreased in the United States, Canada, UK and Italy in recent two decades. No evidence of decline appeared during 1980-2006 in Japan. For lymphoma, the decline occurred relatively late in Japan, compared with a significant decline without a leveling off period in the United States, Canada, Italy and UK. There is no simple explanation for these trend disparities. It is possible that the distribution of the histology pattern is markedly different among different countries, even in the same diagnostic group. The possible causes for these disparities in the childhood cancer death rate (e.g. late diagnosis, poor treatment quality, lack of health insurance and difficulty in accessing health care) need to be studied further.

A high mortality rate was observed in Kochi prefecture in boys and Tokushima and Kagoshima prefectures in girls. As mentioned above, the geographic disparity might be due to differences in cancer incidence and survival in different regions. Studies of the relationship between social class and childhood cancer have not been consistent. Research from Brazil suggested that higher decreases in the mortality rate were observed in more developed regions, possibly reflecting better health care [23]. We did not perform a similar ecologic study here, because of the small number of death, and we could not even calculate mortality by subtype by prefecture. Further detailed individual-level study is needed to identify a more reasonable explanation for the mortality disparities in childhood cancer.

A few points should be borne in mind when interpreting these findings. Some stable trends in the present study, such as mortality in lymphoma, and malignant bone tumors in New Zealand are more difficult to explain because of the small absolute number and substantial random variation. Other limitations included the wide time span and changes in diagnostic capabilities during the study period, and we were not able to collect any information on social status, employment of individuals and other genetic, environmental factors that would have allowed us to analysis etiological hypotheses.

Despite these limitations, when considering the absence of a national cancer registry system in Japan, estimates of incidence may have their own limitations (for example, they may be significantly influenced by errors in diagnosis and classification); evaluation of death may be an alternate effective method to identify more population-based point estimates of

mortality from childhood cancer under these circumstances. Furthermore, the results presented here are based on 100% national coverage and provide an important baseline for monitoring the further progress against childhood cancer in Japan. Analysis of trends in national mortality rates over several decades may provide additional insight into the burden and impact of childhood cancer and suggest more targeted avenues for interventions that further delineate and ultimately reduce mortality from childhood cancer.

conclusions

The present study provides updated figures and trends in childhood cancer mortality in Japan and other developed countries. This will help to estimate care needs and to plan interventions and the quantity of appropriate childhood cancer treatment. Comprehensive efforts designed to identify risk factors for childhood cancer, promote early detection and reduce morbidity and mortality are warranted.

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Human Osteoblasts Support Hematopoietic Cell Development in vitro

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Key Words

Cell adhesion molecule · Cell signaling · Cytokines · Hematopoiesis · Osteoblasts · Stem cell factor

Abstract

Background/Aim: Although osteoblasts are thought to be the major component of the hematopoietic stem cell niche in the bone marrow microenvironment, the role of osteoblasts in hematopoiesis is still unclear. The ability of human osteoblasts to support early hematopoiesis was investigated. ***Methods and Results:*** Human CD34+ bone marrow cells cultured on human osteoblasts were capable of surviving without addition of cytokines and differentiated into myeloid cells with slight proliferation. The results of immunohistochemical experiments suggested activation of FAK and AKT in hematopoietic cells attached to osteoblasts. When stem cell factor, Flt3-L, and IL-3 were added to the coculture system, each cytokine distinctively enhanced proliferation and differentiation of CD34+ bone marrow cells. ***Conclusion:*** The results suggest that human osteoblasts have the ability to support hematopoietic cell development in vitro.

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Introduction

Bone marrow stromal cells, including fibroblasts, adipocytes, macrophages, endothelial cells, and osteoblasts, have been ascribed an important role in hematopoiesis. In addition to the effect of cytokines secreted by stromal cells, direct contact between hematopoietic and stromal cells or extracellular matrix synthesized by bone marrow stromal cells has been implicated in the formation of various hematopoietic cells [1, 2]. Having the ability to self-renew in this environment, hematopoietic stem cells (HSCs) proliferate and differentiate into hematopoietic cells of various lineages, e.g. lymphocytes and myeloid cells.

HSCs are thought to be located in a specific stromal niche in the bone marrow for cell maintenance, proliferation and differentiation. Recent publications have highlighted that osteoblasts are the major component of the HSC niche in the bone marrow microenvironment [3–5]. Zhang et al. [3] presented osteoblasts as key components of the niche that supports HSC development, being functionally controlled via the bone morphogenetic protein signaling pathway. They also suggested that N-cadherin and β-catenin are responsible for the interaction between osteoblasts and long-term HSCs. Calvi et al. [4] demonstrated that osteoblasts are a regulatory com-

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ponent of the HSC niche and control stem cell function through the Notch 1-Jagged 1 pathway. They further stated that parathyroid hormone affects the ability of osteoblasts to expand HSCs. Arai et al. [5] reported that the tight adhesion between HSCs and osteoblasts induced through the Tie2/Ang-1 signaling pathway plays an important role in the maintenance of HSCs in a quiescent state in the bone marrow niche. Despite these findings, however, the role of osteoblasts in hematopoiesis is still unclear.

In this study, we investigated the effect of a primary human osteoblast culture on proliferation and differentiation of human CD34+ bone marrow cells and found that human osteoblasts have the ability to maintain and support hematopoietic cell development in vitro. Primary human osteoblast cultures may provide an in vitro model for the hematopoietic microenvironment.

Materials and Methods

Recombinant human stem cell factor (SCF), human fms-like tyrosine kinase 3 ligand (Flt3-L), and human interleukin (IL)-3 were purchased from Pepro Tec (London, UK). All cytokines were dissolved in phosphate-buffered saline and diluted with culture medium to the concentration indicated. The following mouse monoclonal antibodies (mAbs) against human antigens were used: carboxyfluorescein succinimidylester (CFS)-conjugated anti-CD184 (CXCR4) from Dako Cytomation (Glostrup, Denmark), phycoerythrin- and allophycocyanin-conjugated anti-CD33, phycoerythrin-cyanine (PC) 5-conjugated anti-CD19, PC7-conjugated anti-CD34, allophycocyanin-conjugated anti-CD45, purified anti-CD29 (β_1-integrin), anti-CD49d (α_4-integrin), anti-CD106 (VCAM-1), and anti-fibronectin from Beckman/Coulter (Westbrook, Mass., USA), purified anti-CD166 (activated leukocyte cell adhesion molecule), anti-β-catenin, anti-N-cadherin, and anti-paxillin from Becton Dickinson Biosciences (San Diego, Calif., USA), anti-vinculin from Chemicon International (Temecula, Calif., USA), and anti-osteocalcin from Takara Bio (Shiga, Japan). Purified mAbs were labeled with the Zenon™ Alexa Fluor® 488 and 546 mouse IgG1 labeling kit according to the manufacturer's protocol (Invitrogen, Carlsbad, Calif., USA). The rabbit polyclonal Abs against phospho-specific AKT from Cell Signaling Technology (Beverly, Mass., USA) and phospho-specific FAK from Affinity BioReagents (Golden, Colo., USA) were also used. Unless otherwise indicated, all other chemical reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Human CD34+ bone marrow cells and a primary culture of human osteoblasts purchased from Cambrex Bio Science Walkersville (Walkersville, Md., USA) were used. The cells had been isolated from human tissue after obtaining informed consent from the donor and were maintained in RPMI 1640 medium (Sigma-Aldrich, St. Louis, Mo., USA) supplemented with 10% (v/v) fetal calf serum (Sigma-Aldrich) at 37°C under a humidified 5% CO_2 atmosphere. The primary culture of human osteoblasts was isolated from the spongy section of the bone head via an explant using the methods established by the provider. The provider confirmed that the majority of the cells were positive for alkaline phosphatase and bone mineralization (von Kossa stain), representing osteoblast phenotypes.

Cells from the primary culture of human osteoblasts were plated and grown to approximately three-fourth confluence on a 12-well tissue plate (Asahi Techno Glass, Chiba, Japan). The CD34+ cells were plated at a concentration of 4×10^4 cells/well/2 ml on the human osteoblasts in RPMI 1640 medium supplemented with 10% fetal calf serum, with and without cytokines. Each cytokine was added at a concentration of 100 ng/ml. After cultivation for the periods indicated, the cells were harvested with 0.25% trypsin plus 0.02% ethylenediamine tetraacetic acid (Immuno-Biological Laboratories, Gunma, Japan), and the number of cells per well was counted. All experiments were performed in triplicate, and cell numbers are reported as means ± SD. For the histological studies, cells were cultured on type-I collagen-coated coverslips (Asahi Techno Glass). At the end of the culture period, the cells on the coverslips were examined after either May-Grünwald-Giemsa staining or immunohistochemical staining.

RT-PCR was performed as described previously [6]. The primer sets used in this study are listed in table 1. The PCR products were separated on 1.5% agarose gel.

For immunostaining, coverslips were fixed with ice-cold acetone for 15 min, incubated with fluorescence-labeled mouse mAbs at room temperature for 30 min, and washed in phosphate-buffered saline. In case of the rabbit polyclonal Abs, secondary goat anti-rabbit IgG Ab conjugated with Alexa Fluor 546 (1:300 dilution, Invitrogen) was used for labeling. The cells were then stained with 4′,6-diamidino-2-phenylindole (200 ng/ml, Sigma-Aldrich) and Alexa Fluor 633-conjugated phalloidin (Invitrogen).

Confocal laser scanning was performed with a FV500 confocal laser scanning microscope (Olympus, Tokyo, Japan). A water immersion objective (×40, NA1.7) was used, and simultaneous multifluorescence acquisitions were performed with the 351- and 488-, 546-, and 633-nm laser lines to excite 4′,6-diamidino-2-phenylindole, Alexa Fluor 488, 546, and 633, respectively. Fluorescence images were selected using appropriate multifluorescence dichroic mirrors and band pass filters using the sequential acquisition mode.

A multicolor immunofluorescence study was performed using a combination of carboxyfluorescein succinimidylester, phycoerythrin, PC-5, and PC-7. Cells were stained with fluorescence-labeled mAbs and analyzed by flow cytometry (EPICS-XL, Beckman/Coulter), as described previously [7]. The gates were set to exclude dead cells and osteoblasts, and 1×10^4 gated cells were analyzed. Data are displayed as histograms of log-fluorescence intensity versus log-fluorescence intensity.

Results

As described in Materials and Methods, the primary culture of human osteoblasts used in this study was qualified and confirmed by the provider, i.e. that the majority of the cells represent osteoblast phenotypes. Using im-

Table 1. Primer sequences

Primer		Sequence	Product size, bp
IL-1α	Sense	5′-TGCTGCATTACATAATCTGG-3′	738
	Anti-sense	5′-TCATGAAATCCTAGGTCTGT-3′	
IL-1β	Sense	5′-CTGGAGAGTGTGATCCCAA-3′	212
	Anti-sense	5′-GACACAAATTGCATGGTGAA-3′	
IL-3	Sense	5′-ACCTTCGAAGGCCAAACCTG-3′	271
	Anti-sense	5′-AGAGAACGAGCTGGACGTTG-3′	
IL-6	Sense	5′-GAGTACAAAAGTCCTGATCC -3′	587
	Anti-sense	5′-TATTTGAGGTAAGCCTACAC-3′	
IL-7	Sense	5′-AAAGTTTCAGAAGGCACAAC-3′	314
	Anti-sense	5′-CTAGGAAGCATTCCACTCTG-3′	
IL-9	Sense	5′-TCTCAGATGACCAATACCAC-3′	216
	Anti-sense	5′-TCATATCTTGCCTCTCATCC-3′	
G-CSF	Sense	5′-ATAGCGGCCTTTTCCTCTAC-3′	863
	Anti-sense	5′-TGATGTTCGGGAGTCAAACC-3′	
M-CSF	Sense	5′-GCAACTGCCTGTACCCCAAA-3′	614
	Anti-sense	5′-CTGAGCAGGGCAGATGGATG-3′	
GM-CSF	Sense	5′-AGCATGTGAATGCCATCCAG-3′	434
	Anti-sense	5′-TTGGTCCCTCCAAGATGACC-3′	
Flt3-L	Sense	5′-TGGATGGAGCGGCTCAAGAC-3′	136
	Anti-sense	5′-TGTTGGTCTGGACGAAGCGA-3′	
LIF	Sense	5′-GCTGTTGGTTCTGCACTGGA-3′	621
	Anti-sense	5′-ACTCCTGAGATCCCTCGGTT-3′	
OPG	Sense	5′-AGATCCTGAAGCTGCTCAGT-3′	319
	Anti-sense	5′-AAAGCCTCAAGTGCCTGAGA-3′	
SCF	Sense	5′-ACAGCTTGACTGATCTTCTG-3′	711
	Anti-sense	5′-TTGTAAGACTTGGCTGTCTC-3′	
SDF-1α	Sense	5′-ATTCAGGAGTACCTGGAGAA-3′	522
	Anti-sense	5′-CAGTGTCTGAAGAAAGGACA-3′	
VEGF	Sense	5′-CTACCTCCACCATGCCAAGT-3′	577
	Anti-sense	5′-AGATCTGGTTCCCGAAACCC-3′	
CD166	Sense	5′-GTATTCCAGAACACGATGAG-3′	272
	Anti-sense	5′-TATCTCTGGACAACTAGGAC-3′	
Fibronectin	Sense	5′-GGATGACTCGTGCTTTGACC-3′	321
	Anti-sense	5′-TGCCACTGTTCTCCTACGTG-3′	
N-cadherin	Sense	5′-GTGCTGATGTTTGTGGTATG-3′	514
	Anti-sense	5′-CTGAAGTTCAGTCATCACCT-3′	
Jagged-1	Sense	5′-CAGGACCTGGTTAACGGATT-3′	936
	Anti-sense	5′-CGTTTCTACAAGGGTTGCTC-3′	
GAPDH	Sense	5′-CCACCCATGGCAAATTCCATGGCA-3′	598
	Anti-sense	5′-TCTAGACGGCAGGTCAGGTCCACC-3′	

munohistochemical staining, the majority of cells were positive for osteocalcin (fig. 1a), an osteoblast-specific protein, indicating that the cells indeed possess the characteristics of osteoblast.

First, we investigated the expression of adhesion-related molecules in the osteoblasts by immunohistostaining. As shown in figure 1b, filamentous distribution of CD106 was detected by confocal microscopy. Fibronectin was also expressed in the form of filaments around the cells, but occasionally it was distributed in the form of coarse bundles (arrowhead). Expression of the protein N-cadherin in the form of a fine mesh was also detected by immunohistostaining (fig. 1b). We also detected mRNA expression of adhesion-related molecules CD16

fibronectin, N-cadherin, and Jagged-1 in the osteoblasts (fig. 1c).

Next, we characterized cytokine expression of cells in the primary culture of human osteoblasts. RT-PCR revealed mRNA expression of cytokines, including IL-1β, IL-6, Flt3-L, leukemia inhibitory factor (LIF), osteoprotegrin (OPG), stromal cell-derived factor (SDF-1α), and vascular endothelial growth factor (VEGF), in osteoblasts (fig. 1c). No expression of other cytokines, i.e. IL-1α, IL-3, IL-7, IL-9, granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), or SCF, was detected under the same conditions (fig. 1c).

To investigate the effect of human osteoblasts on hematopoiesis in vitro, we cultured human CD34+ bone marrow cells on cells from the primary culture of the human osteoblasts. When human CD34+ bone marrow cells were cultured alone in the absence of cytokine addition, they almost all died (fig. 2). By contrast, the human CD34+ bone marrow cells were maintained and proliferated slightly on human osteoblasts in the absence of cytokine supplementation. As shown in figure 2, part of the hematopoietic cells adhered to osteoblasts, forming a small colony, whereas another part of hematopoietic cells floated (data not shown). The culture initially contained 40,000 human CD34+ bone marrow cells, 2 and 4 weeks later 40,000–160,000 and 120,000–270,000 hematopoietic cells were collected, respectively.

We analyzed the subsets of the hematopoietic cells that had survived on the osteoblasts by flow cytometry. When examined after 2 weeks of coculture, the majority of hematopoietic cells were CD33+ myeloid cells (fig. 3). In addition, the human osteoblasts had the ability to maintain a few human CD34+ bone marrow cells (fig. 3), and a small amount of hematopoietic cells still remained to express CD34 after 2 weeks of coculture.

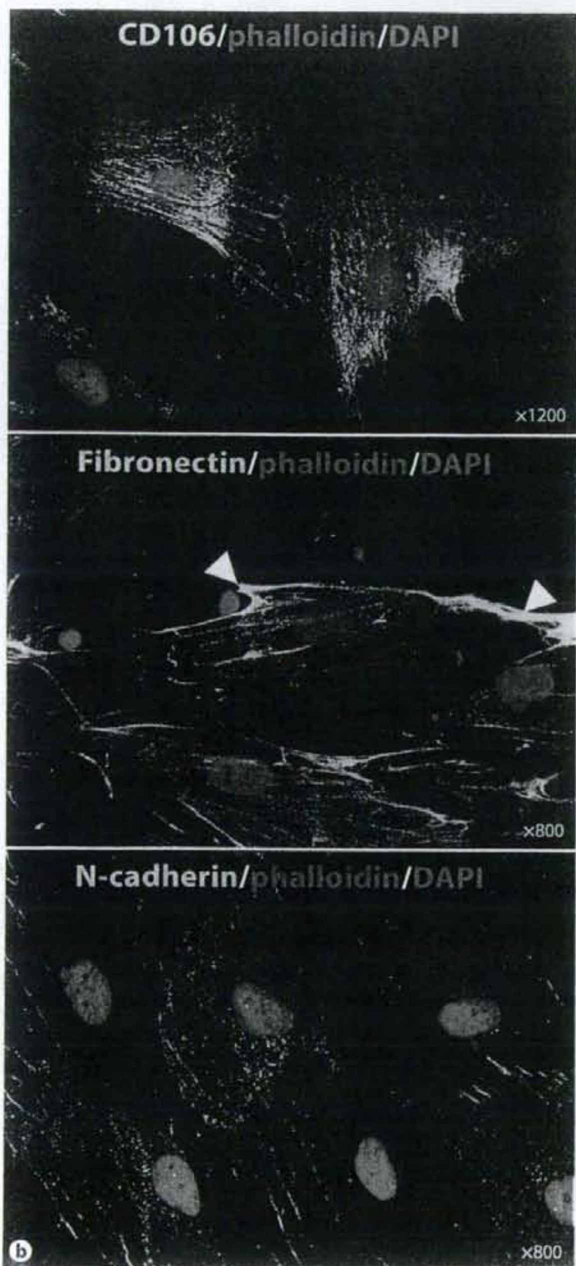
It is noteworthy that occasionally proliferation of CD19+ B-lineage cells on osteoblasts was observed, and one experiment revealed that 46.2 and 13.0% of the hematopoietic cells were B-lineage cells after 2 and 4 weeks of coculture, for example (data not shown). However, the proliferation of B-lineage cells seems to be dependent on the lot of human CD34+ bone marrow cells. Since CD34+ bone marrow cells contained a sizable population of CD19+CD34+ cells in those cases (data not shown), it is most likely that osteoblasts can maintain the B-lineage-committed cells rather than promote B-cell differentiation.

Fig. 1. Expression of osteocalcin and cell adhesion molecules in a primary culture of osteoblasts detected by immunohistochemistry. **a** Osteoblast primary culture grown on coverslips was stained with fluorescein-labeled antibodies to the osteocalcin and examined by confocal microscopy.

Next, we investigated the effect of addition of cytokines, including SCF, Flt3-L and IL-3, on the growth and differentiation of human CD34+ bone marrow cells cultured on human osteoblasts. In the absence of osteoblasts, SCF and IL-3 differently supported the proliferation of hematopoietic cells and 180,000 ± 42,426 and 1,295,000 ± 49,497 cells had grown after a 2-week cocultivation from an initial 40,000 CD34+ bone marrow cells, respectively (fig. 3b). By contrast, Flt3-L induced no significant proliferation of hematopoietic cells (fig. 3b).

In the presence of osteoblasts, however, the subsequent hematopoietic cell number was significantly increased compared with hematopoietic cells cultured with cytokine alone. When 55,000 ± 21,213 hematopoietic cells had grown from an initial 40,000 CD34+ bone marrow cells after a 2-week cocultivation on human normal osteoblasts, cytokines SCF, Flt3-L, and IL-3 markedly increased hematopoietic cell recovery to 990,000 ± 183,848, 355,000 ± 77,782, and 2,370,000 ± 367,696, respectively (fig. 3c). In each case, the majority of cells were CD33+ (fig. 3c). An especially remarkable increase in the CD34+ cell number was achieved with the combination of osteoblasts and IL-3 (fig. 3d).

We further investigated the effect of other factors which may affect either osteoblasts or CD34+ bone marrow cells, including parathyroid hormone, vitamin D, MIP-1α, BMP-2, IL-7, and LIF, whereas no significant ef-



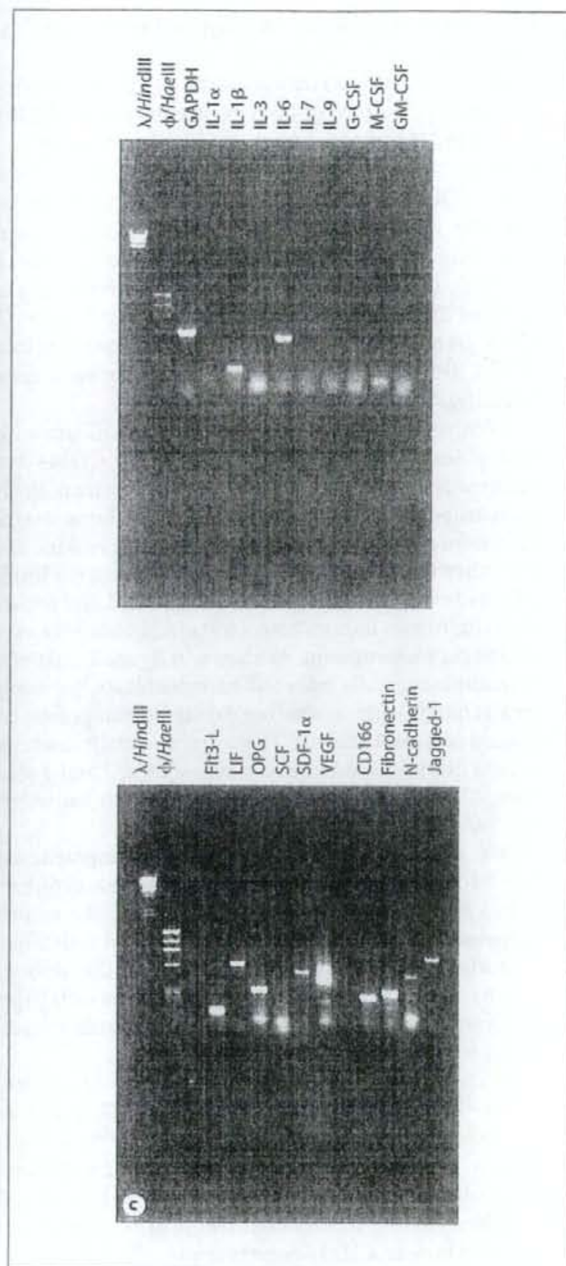


Fig. 1. b The same samples were stained with fluorescein-labeled antibodies to the cell adhesion molecules indicated and examined by confocal microscopy. **c** Expression of cytokines and cell adhesion molecules in osteoblasts detected by RT-PCR. To investigate mRNA expression of the cytokines and cell adhesion molecules indicated, total RNAs were extracted from a primary culture of human osteoblasts and RT-PCR analysis was performed. The PCR products obtained were subjected to electrophoresis with molecular weight markers ϕ/X174/*Hae*III and λ/*Hind*III.

Fig. 2. Human CD34+ bone marrow cells grown on osteoblast primary cultures. **a** Human CD34+ bone marrow cells were cultured for 2 weeks on osteoblast primary cultures grown on coverslips and examined by phase-contrast microscopy (magnification ×100). **b** After May-Grünwald-Giemsa staining, cells were examined by light microscopy. The small nuclear cells gathered at the center are human CD34+ bone marrow cells (arrow), and the large nuclear cells present at the circumference are human osteoblasts (magnification ×400).

fect on the growth and differentiation of hematopoietic cells cultured on osteoblasts was observed (data not shown).

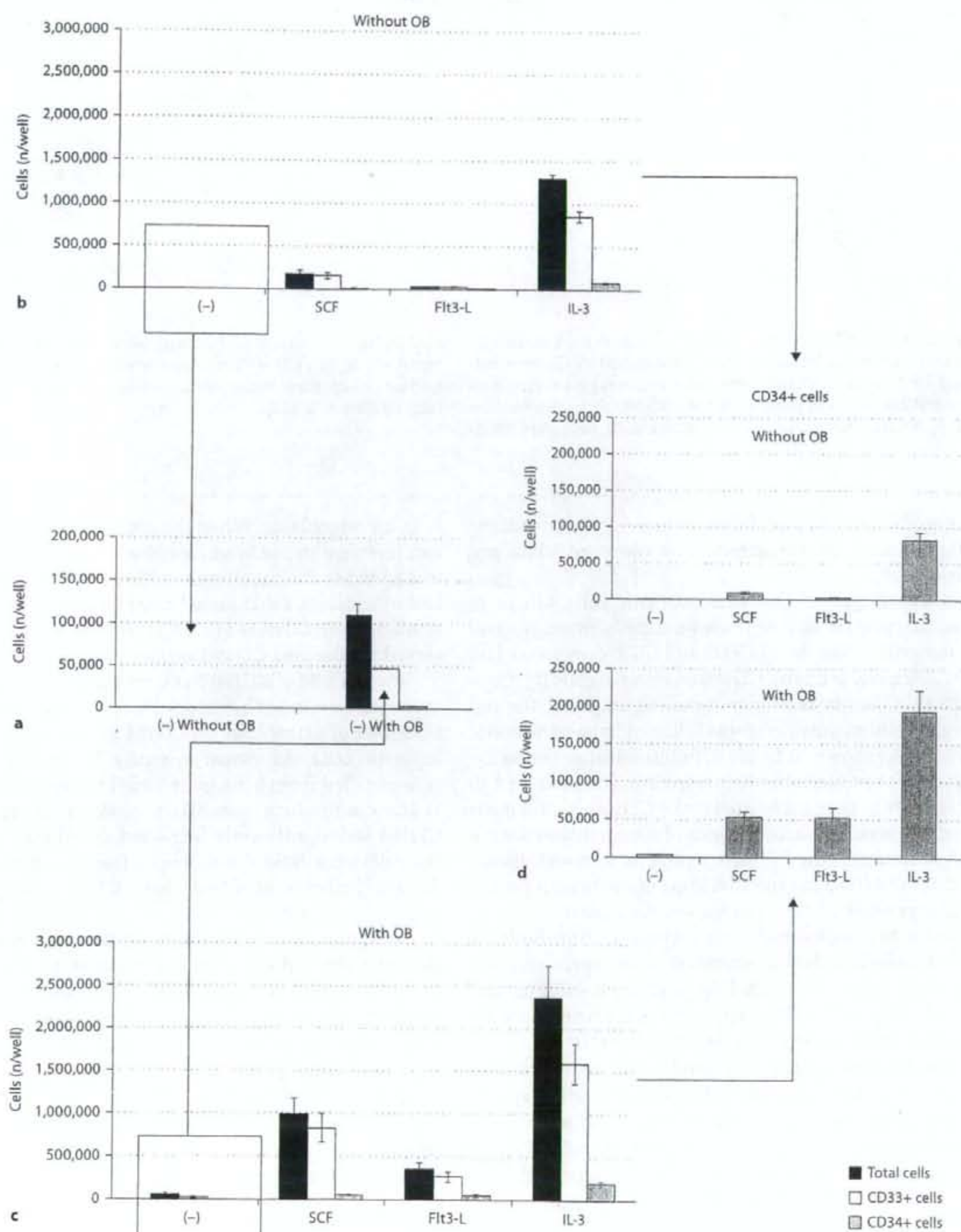
It was suggested that hematopoietic cells adhere to bone marrow stromal cells via binding between β_1- and α_4-integrin, a complex of CD29 and CD49d, expressed on hematopoietic cells and CD106 on stromal cells [8]. Consequently, the intracellular signaling required for cell survival and maintenance was initiated in hematopoietic cells [9]. As shown in figure 4, immunohistostaining experiments revealed that hematopoietic cells adhered to osteoblasts as they got twisted to the CD106 that filamentously expressed on osteoblasts. Immunohistostaining also showed that the CD166 staining pattern was similar to that of fibronectin and that some of the hematopoietic cells expressed CD166, and those cells looked like those adhering to osteoblasts that also express CD166. Both the hematopoietic cells and osteoblasts also expressed other signaling molecules, including paxillin, β-catenin, and vinculin (fig. 4). Paxillin expression in the hematopoietic cells was much greater than in osteoblasts (fig. 4).

We also investigated the phosphorylation of signaling molecules in hematopoietic cells using activation-specific antibodies. As shown in figure 5, confocal microscopic analysis showed phosphorylation of FAK and AKT, and there was clear colocalization of phosphorylated FAK and AKT with CD49d and CD29, respectively (fig. 5, bottom panels).

Thus, we tested the effect of cell adhesion mediated by CD29 and CD49d on the maintenance of hematopoietic cells on osteoblasts. When the anti-CD29 antibody that can interfere in the binding between CD29 and CD106 was added to the coculture of CD34+ bone marrow cells and osteoblasts, subsequent hematopoietic cell proliferation was clearly reduced (fig. 6). Identical results were observed for the anti-CD49d antibody (fig. 6).

Since CD184 (CXCR4) has been suggested to contribute to the homing of hematopoietic cells, we investigated the effect of osteoblasts on CD184 expression by hematopoietic cells. As shown in figure 7a, human CD34+ cells purified from bone marrow did not express CD184. When cultured on osteoblasts, however, expression of CD184 was significantly increased and the hematopoietic cells were divided into several fractions based on the diverse patterns of CD184 and CD33 co-expression (fig. 7).

In the absence of osteoblasts, on the other hand, each cytokine also induced CD184 expression by hematopoietic cells in a distinct manner (fig. 7). When CD34+ bone marrow cells were cultured in the presence of osteoblasts, addition of cytokines further enhanced CD184 expression (fig. 7). We also examined the effect of SDF-1, a ligand for CD184, and anti-SDF-1 antibody on the proliferation and differentiation of CD34+ bone marrow cells cultured on osteoblasts in the presence of each cytokine, but no significant effect was observed (data not shown).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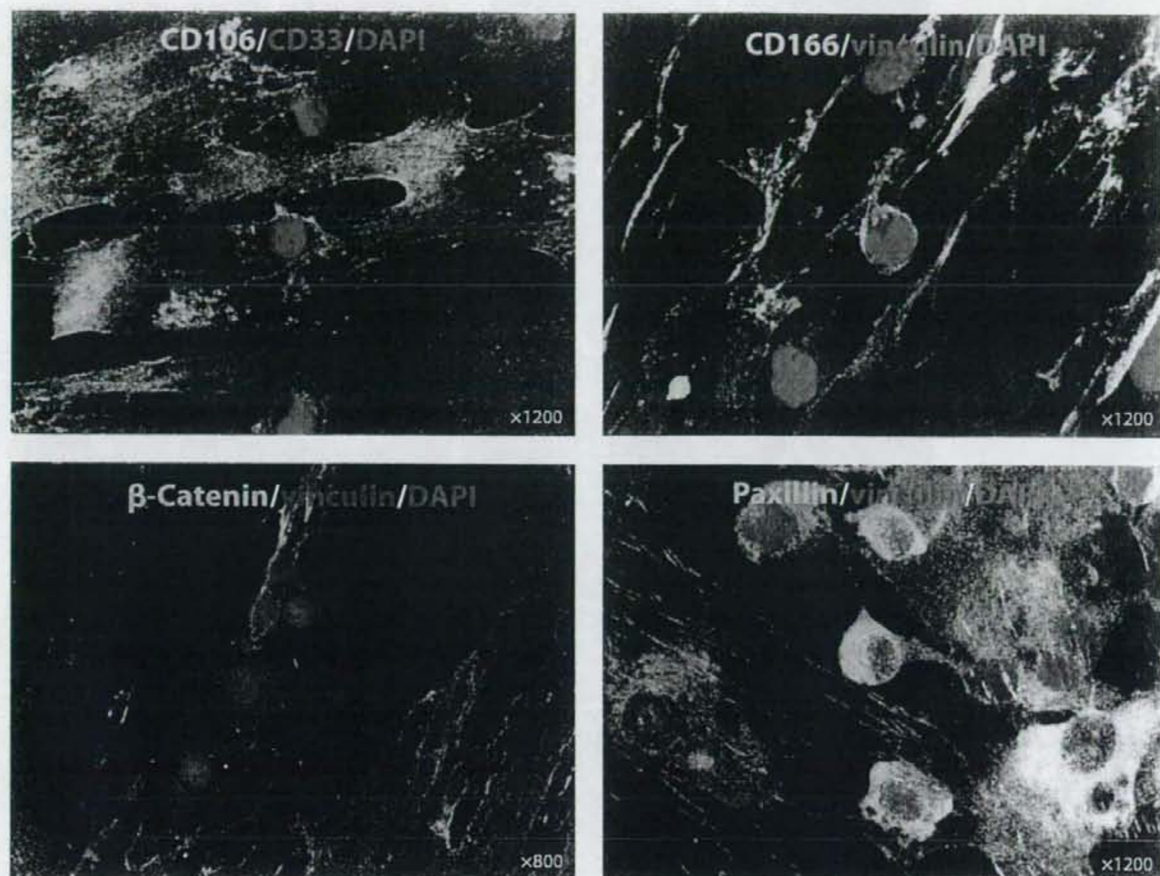
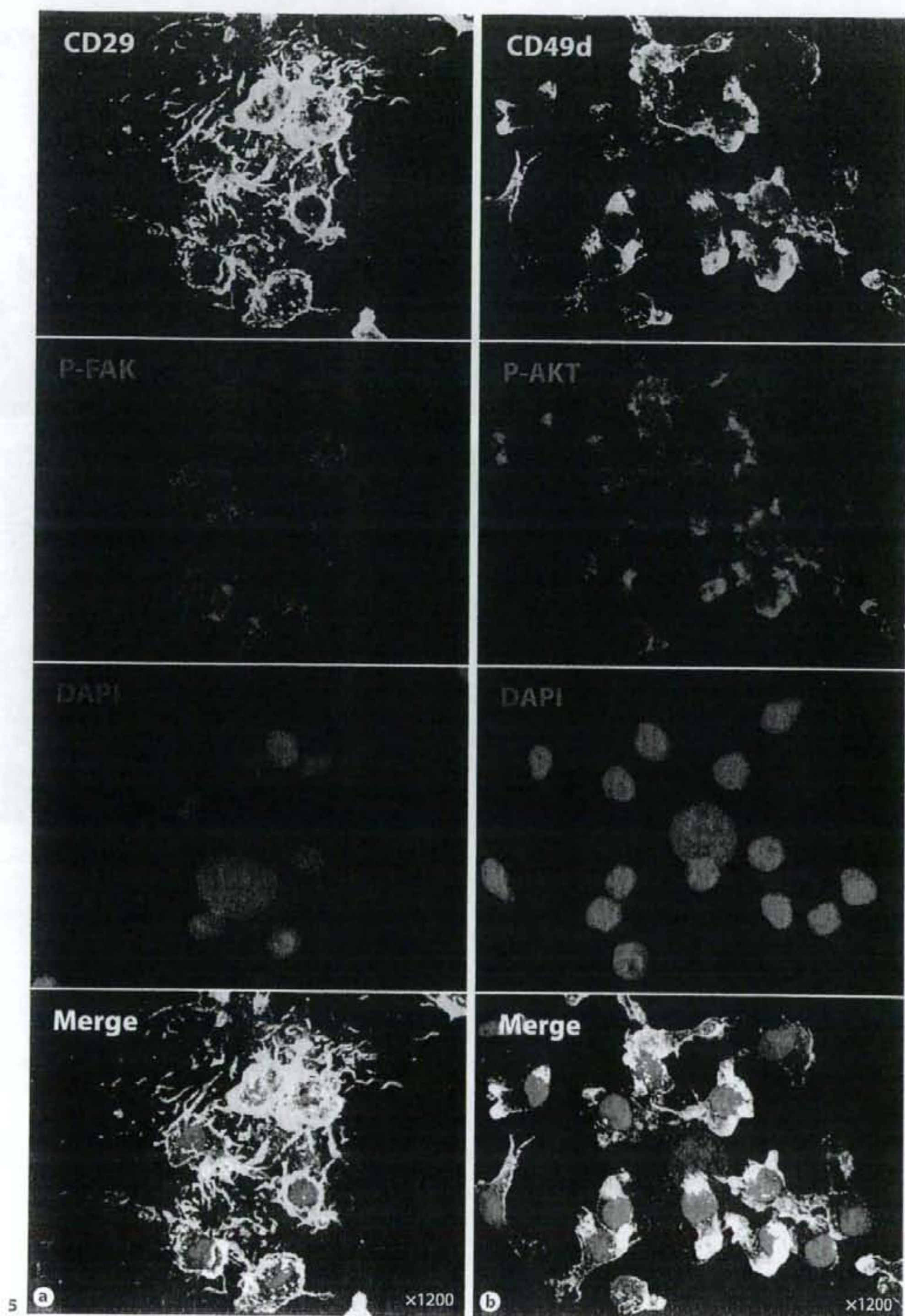


Fig. 4. Immunohistochemical analysis of the interaction between osteoblasts and human CD34+ bone marrow cells. Human CD34+ bone marrow cells were grown on a primary culture of osteoblasts for 2 weeks and examined as in figure 1b.

Fig. 3. Effect of human osteoblasts and cytokines on the growth and differentiation of human CD34+ bone marrow cells. Human CD34+ bone marrow cells were cultured with or without human osteoblasts (OB) for 2 weeks in the presence or absence of the cytokines indicated. After cultivation, the ensuing hematopoietic cells were collected, counted, and positivity for CD33 and CD34 was determined by flow cytometry. The actual total cell numbers and the numbers of cells in each subpopulation are represented by bar graphs. **a** With or without OB (in the absence of cytokines); **b** without OB (in the presence of cytokines); **c** with OB (in the presence of cytokines); **d** CD34+ cells extracted from **b** and **c**.

Discussion

As reported in this paper, when cultured on human osteoblasts, human CD34+ bone marrow cells were able to survive without the addition of any cytokine, and differentiated into myeloid cells with slight proliferation, suggesting that human osteoblasts possess the ability to support the survival and differentiation of hematopoietic cells in vitro. Analysis by confocal microscopy suggested that cell adhesion molecules, including CD29/49d, CD106, and CD166, are involved in cell-to-cell interaction between hematopoietic cells and osteoblasts. Since we observed that FAK and AKT were colocalized with CD29/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49d and phosphorylated in hematopoietic cells adhering to osteoblasts, we suspect that cell-to-cell interaction induces activation of integrin-bound kinases, leading to cell survival signals in hematopoietic cells in which AKT is involved. Although CD34+ bone marrow cells were cultured in the presence of 30% of the cultured supernatant of osteoblasts, most cells died over a 4-week culture period (data not shown), suggesting that the soluble factors derived from osteoblasts are not sufficient to support the survival of human CD34+ bone marrow cells, and adhesion to osteoblasts must be important for the survival of hematopoietic cells.

Human osteoblasts have been reported to produce several hematopoietic cytokines, including IL-1β, IL-6, IL-7, G-CSF, M-CSF, GM-CSF, tumor necrosis factor-α, LIF, OPG, receptor activator of NF-κB ligand, SDF-1, VEGF, and osteoclast differentiation factor [1, 2, 10–12], and not to produce IL-1α, IL-3, or SCF [10]. However, in our experiment, human osteoblasts did not produce IL-7, G-CSF, M-CSF, or GM-CSF. Although the precise reason for the discrepancy is not clear, it may be attributable to differences in cell culture conditions or donor age. Alternatively, different subsets or differentiation states related to differential cytokine production may be present among the osteoblasts.

Several cytokines have been shown to contribute to the maintenance, proliferation, and differentiation of HSCs. For example, Flt3-L and SCF play an important role in the early stage of hematopoiesis [13]. An in vivo study has demonstrated that SCF and IL-3 prevent unirradiated hematopoietic progenitors from undergoing apoptosis, and Flt3-L has been demonstrated to induce survival and proliferation of CD34+CD38– cells [14], suggesting the effects of these cytokines on hematopoiesis in vivo to some extent [15], but their effects in vitro, whether alone or in combination, are still a matter of controversy [2]. The results of this study demonstrate that SCF and IL-3, but not Flt3-L, induce proliferation of CD34+ bone marrow cells to some extent in our culture condition. When added to the coculture system of human osteoblasts and human CD34+ bone marrow cells, however, each of them significantly promoted the proliferation of hematopoietic cells. SCF and Flt3-L induced in particular significant growth of hematopoietic cells cultured on osteoblasts. Since our RT-PCR experiments revealed no expression of SCF and IL-3 mRNA in osteoblasts, the major role of osteoblasts in hematopoiesis could be to maintain HSCs as HSCs and therefore the lack of proliferation-inducing cytokines is appropriate for this role. In the context of the microenvironment, other cells should supply these factors to the niche. Alternatively, it is also possible that disaggregated osteoblasts do not produce these factors when they are grown in monocultures but do so in the niche when in the appropriate context.

CD184, a receptor for CXC subfamily chemokines, was originally identified as an orphan receptor [16]. It was suggested that CD184 and its sole ligand SDF-1 play an important role in hematopoiesis and are required for homing of stem cells and progenitor cells from the liver to the bone marrow [2, 16–18], but their role at the molecular level remains unknown. Tokoyoda et al. [18] stated that contact between the earliest HSCs and SDF-1-expressing cells is necessary for B lymphopoiesis. In our study, the CD184 expression pattern was dramatically altered by cytokines and the presence of osteoblasts. Although the exact mechanism of action remains to be elucidated, the different expression pattern of CD184 may be related to the different function of hematopoietic cells,

Fig. 5. Phosphorylation of cell signaling molecules in hematopoietic cells cultured on osteoblasts detected by immunohistochemistry. Human CD34+ bone marrow cells were grown on a primary culture of osteoblasts for 2 weeks and stained with the combination of phospho-specific antibodies and anti-cell adhesion molecule antibodies and examined as in figure 4. **a** CD29 versus phosphorylated FAK; **b** CD49d versus phosphorylated AKT.

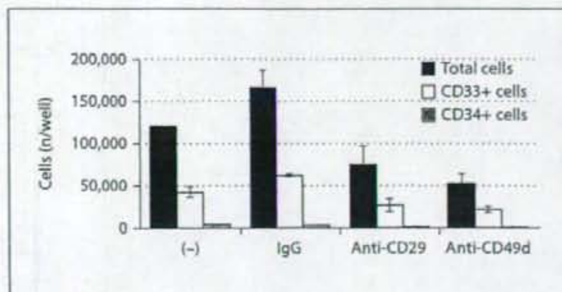
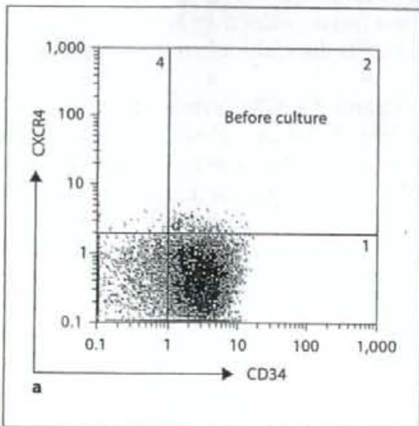


Fig. 6. Effect of anti-integrin antibodies on growth of CD34+ bone marrow cells on human osteoblasts. Human CD34+ bone marrow cells were cultured on osteoblasts for 2 weeks in the presence or absence of antibodies against CD29 or CD49d at a concentration of 5 μg/ml. Following cultivation, hematopoietic cells were collected, counted, and positivity for CD33 and CD34 was determined by flow cytometry (see fig. 3). Purified mouse IgG served as a negative control.

Fig. 7. Expression of CD184 in hematopoietic cells grown on human osteoblasts. Human CD34+ bone marrow cells were cultured for 2 weeks (see fig. 3). Hematopoietic cells were collected and examined by flow cytometry. Two-parameter histograms for CD184 versus CD34 (**a**) or CD33 (**b**) are shown.



e.g. homing. Further investigation to identify the role of CD184 expression in hematopoiesis is now underway.

In conclusion, human osteoblasts have the ability to support the survival and differentiation of human CD34+ bone marrow cells. Addition of cytokines to this culture system stimulates human CD34+ bone marrow cells to differentiate into various blood cells. Osteoblasts provide a useful in vitro model of the hematopoietic microenvironment, and further studies are required to elucidate the role of the microenvironment in early hematopoiesis.

Acknowledgments

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CURRENT TOPICS

小児腫瘍のグループスタディーと病理

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はじめに

今から約6年前に各種難治性疾患に関わるエビデンス創出の基盤づくりのための公的研究費投入が開始された．それを受けて，小児がんについても標準的治療法開発を目指した取り組みが開始された．現在，我が国では小児がんの病型ごとの臨床研究グループがつくられ，統一治療プロトコールに基づく臨床試験などが実施されている．各研究グループには数十～200程度の医療施設が参加している．それらの組織体制は種々であるが，運営委員会や幹事会等の運営母体，プロトコール作成や研究立案を行う各種委員会，研究事務局，データセンター，中央診断システム等から構成されている．

上記の組織体制の中で，病理医が関与する場は主として中央診断システムの部分である．その他，臨床研究グループにおける中央診断に関わる業務は様々で，病理診断以外に，遺伝子診断(増幅，欠失，変異，キメラ遺伝子発現等)，染色体診断，骨髄スメア等の形態診断，CT・MRI等画像診断，などが存在する．中央診断システムの役割は標準化された診断による試験参加適格性判定や治療層別化判定に関連する情報提供が基本であるが，一部では治療層別化に有用な新規マーカー開発研究や病態解明に結びつく基礎研究も行われる場合がある．病理中央診断については日本病理学会小児腫瘍組織分類委員会の委員の多くが関わってきた．

小児がんは稀少であり，年間の発生数が1,500～2,000程度と予想されることから，これらの症例を効率よく収集するシステムとしても臨床研究グループによる症例のリクルート，その中での中央診断システムは貴重である．また，診断後の余剰検体や研究用検体を保存し，基礎研究の推進に活用する仕組みも確立中である．また，小児がんの年間発生数把握に関する取り組みも始まっている．我が国におけるがん登録に対する取り組みは甚だしく遅れており，小児がん登録についても言わずもがなの状況である．しかしながら上記の臨床研究の推進ならびに予後の著明な改善に伴い，小児がん登録の重要性が増している．

本稿ではこれらの取り組みの現状を紹介する．

I．小児がん臨床研究グループの活動

小児がんを扱う我が国の臨床研究グループとして専門家の間で認知されているものは**表1**に示した7つである．この中で日本小児白血病・リンパ腫研究グループ(JPLSG)が最も規模が大きい．小児血液腫瘍のプロトコールスタディを実施していた既存の4つの臨床研究グループ(CCLSG，JACLS，KYCCSGおよびTCCSG)がインターグループとして結集して形成されたもので，我が国の主たる小児がん治療施設のほとんどが参加している．小児血液腫瘍および血液系関連疾患に対する臨床試験11件を現在実施している(**表2**)．JPLSGのホームページでの組織図によると，代議員会と運営委員会が運営の中心となっており，その周囲に各種委員会，データセンター，検体センター等が配置されている．血液腫瘍以外の小児固形腫瘍については，基本的には病型ごとに研究グループが形成されている．小児の代表的な固形腫瘍である神経芽腫，横紋筋肉腫，Ewing肉腫，Wilms腫，肝芽腫については，それぞれ，JNBSG，JRSG，JESS，JWiTS，JPLTのグループが形成されている．なお，小児脳腫瘍についてはJPBTCというNPOとして活動している．また，多くの研究グループは何らかの形で公的研究費の支援を受けながら活動している．治療介入型の臨床試験を実施しているグループが大半だが，ガイドライン治療を実施し基盤となる情報収集を目的とした観察研究に近い形の研究もある．

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