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G. 知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

先天性角化不全症の効果的診断方法の確立と治療ガイドラインの作成に関する研究

Dyskeratosis congenita における免疫異常に関する研究

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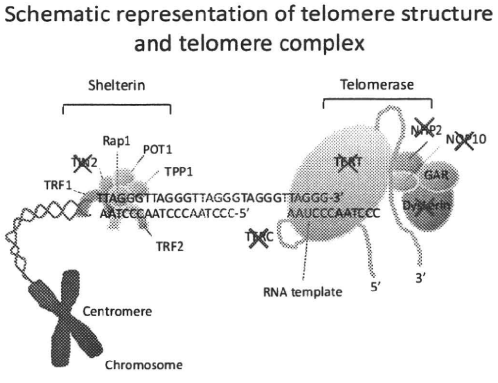
研究要旨： Dyskeratosis congenital (DKC)は先天性骨髄不全症のひとつであり、爪の異形成、白斑症、皮膚の脱色素を3徴とし、高発がん性である。DKCの原因としては17-36%が*DKC1*変異によるX連鎖DKCであるが、常染色体性DKCの原因遺伝子として*TERC, TERT, TINF2, NHP2, NOP10*が知られている。Hoyeraal-Hreidarsson症候群（HHS）は再生不良性貧血、免疫不全症、小頭症、小脳低形成などを特徴とするX連鎖免疫不全症であるが、その原因遺伝子も*DKC1*である。DKCとHHSがオーバーラップした症例も存在し、*DKC1*変異以外にも*TERC, TERT, TINF2*変異によるHHSも報告されている。DKCとHHSは連続した症候群と考えられるため、DKCにおける免疫異常について研究を行う。

A. 研究目的

Dyskeratosis congenita(DKC)は爪の異形成、白斑症、皮膚の脱色素といった皮膚粘膜異常を特徴とする先天性骨髄不全症であり、高率に白血病などに進展する予後不良な疾患である。その原因遺伝子はX連鎖DKCの*DKC1*の他に常染色体性DKCの*TERC, TERT, TINF2, NHP2, NOP10*が報告されている。これらはすべてテロメアを構成する成分であり、テロメアの異常によってDKCが生ずると考えられる(図1)。



Hoyeraal-Hreidarsson症候群（HHS）は再生不良性貧血、免疫不全症、小頭症、小脳低形成、発達障害を特徴とするX連鎖免疫不全症の一つであり、その原因遺伝子はX連鎖DKCと同じく、*DKC1*である。X連鎖DKCとHHSがオーバーラップする患者も報告されており、また*DKC1*のみならず、*TERT, TERC, TINF2*の異常によってもHHSを発症することが明らかとなりつつあり、DKCとHHSは連続した症候群と考えられる。よってDKCにおいてもHHSと同様に何らかの免疫学的異常が存在している可能性が考えられ、わが国のDKC患者における免疫学的異常についての解析を行うことを本研究の目的とする。

B. 研究方法

DKCの患者家族から同意が得られたら、ヘパリン加静脈血を採取し、当教室まで送っても

らう。単核球に分離後、テロメア長の測定、リンパ球サブセットにてT/B/NK細胞のみならずナイーブ・メモリーT細胞、B細胞分画についてフローサイトメトリーを用いて調べる。さらにTREC/KRECの定量も行う。また主治医から臨床症状、リンパ球数、血清免疫グロブリン値のデータを供与してもらう。

C. 研究結果

本年度はまずわが国におけるDKC患者を把握する。以前にわれわれは4例のX連鎖DKC患者を同定しており（Brit J Haematol 2005）、そのうちの1例では精神発達遅滞、低身長が認められ、HHSとのオーバーラップと考えられたが、免疫学的検査は十分に解析されていない。

D. 考察

HHSでは進行性の複合免疫不全症として低ガンマグロブリン血症、リンパ球減少、B細胞減少、T細胞機能異常を認めることがあり、DKCとHHSは連続した症候群と考えられ、テロメア長の欠損を伴う場合にはHHSを生じうることが予想される。

E. 結論

次年度はわが国のDKC患者における免疫学的異常についてX連鎖DKCを中心に解析を行う予定である。

F. 研究発表

1. 論文発表

なし

2. 学会発表

なし

G. 知的財産権の出願・登録状況

なし

Ⅲ. 研究成果の刊行に関する一覧

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Ⅳ. 研究成果の刊行物・別刷り

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Reduced-intensity conditioning for alternative donor hematopoietic stem cell transplantation in patients with dyskeratosis congenita

Nishio N, Takahashi Y, Ohashi H, Doisaki S, Muramatsu H, Hama A, Shimada A, Yagasaki H, Kojima S. Reduced-intensity conditioning for alternative donor hematopoietic stem cell transplantation in patients with dyskeratosis congenita.
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Abstract: DC is an inherited bone marrow failure syndrome mainly characterized by nail dystrophy, abnormal skin pigmentation, and oral leukoplakia. Bone marrow failure is the most common cause of death in patients with DC. Because previous results of HSCT with a myeloablative regimen were disappointing, we used a reduced-intensity conditioning regimen for two patients with classic DC, and one patient with cryptic DC who harbored the *TERT* mutation. Graft sources included two mismatched-related bone marrow (BM) donors and one unrelated BM donor. Successful engraftment was achieved with few regimen-related toxicities in all patients. They were alive 10, 66, and 72 months after transplantation, respectively. Long-term follow-up is crucial to determine the late effects of our conditioning regimen.

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Key words: dyskeratosis congenita – non-myeloablative hematopoietic stem cell transplantation

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DC is an inherited multisystem bone marrow failure syndrome characterized by nail dystrophy, abnormal skin pigmentation, oral leukoplakia, and cancer predisposition. Patients with DC have very short germ-line telomeres compared with normal individuals because of a defect of telomere maintenance. Until now, mutations in six genes (*DKC1*, *TERC*, *TERT*, *NOP10*, *NHP2*, and *TINF2*) involved in telomere maintenance have been identified in patients with DC (1).

Abbreviations: ATG, anti-thymocyte globulin; CMV, cytomegalovirus; DC, dyskeratosis congenita; EBV, Epstein–Barr virus; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; IST, immunosuppressive therapy; RIST, reduced-intensity stem cell transplantation; TBI, total body irradiation.

Bone marrow failure develops in 80–90% of patients with DC and is the most common cause of death, up to 60–70% (2, 3). Although androgen has been used to improve cytopenia since the 1960s, allogeneic HSCT is the only curative treatment for bone marrow failure in patients with DC. However, the outcome in previous reports has been disappointing because of unacceptable transplant-related toxicities such as severe pulmonary/liver complications especially in transplant using a myeloablative conditioning regimen or transplants from an alternative donor (3, 4).

To avoid transplant-related complications, RIST using a non-myeloablative conditioning regimen has been recently used in patients with DC, and encouraging short-term survival has been achieved. Reducing the intensity of

conditioning results in less tissue damage and decreased inflammatory cytokine release compared with myeloablative transplantation (5). However, until now, there have been only a few reports of non-myeloablative transplants, especially from an alternative donor. Here, we report our encouraging results of RIST from an alternative donor using a fludarabine-based conditioning regimen and *in vivo* T-cell depletion by ATG in three patients with DC.

Patients and methods

Case 1

Patient 1 was a 21-yr-old man with classical DC with nail dystrophy, abnormal skin pigmentation, oral leukoplakia, and bone marrow failure. He had no family history of physical or hematologic abnormalities. Nail changes began to develop in early childhood. He suffered from cytopenia and was diagnosed with aplastic anemia at the age of 9. At age 18, he was referred to our hospital and was diagnosed as having DC. He had very short telomeres – i.e., less than the first percentile for his age – although mutation analysis did not identify any mutations in *DKC1*, *TERC*, *TERT*, *NOP10*, or *TINF2*. As pancytopenia progressed, we planned HSCT from a sister who was mismatched at HLA DRB1 allele. He did not undergo HSCT before. Conditioning regimen included cyclophosphamide 750 mg/m^2/day, fludarabine 25 mg/m^2/day, and rabbit-ATG (Thymoglobulin; Genzyme, Cambridge, MA, USA) 2.5 mg/kg/day, all from days −5 to −2, and total lymphoid irradiation 3 Gy (1 fraction) on day −1. GVHD prophylaxis comprised tacrolimus (intravenous infusion of 0.02 mg/kg/day starting on day −1, with dose adjustments to maintain blood levels of 5–15 ng/dL) and short-term methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11). The administration route of tacrolimus was switched to oral after patients recovered from gastrointestinal toxicity.

Case 2

Patient 2 was a nine-yr-old girl with aplastic anemia without any physical abnormalities. At first, she was diagnosed with acquired aplastic anemia of unknown cause. However, she was identified as having a heterozygous *TERT* mutation (T726M) and very short telomeres in our retrospective study of mutation screening for telomere-related genes. She was born to healthy non-consanguineous parents and had no family history of physical or hematologic abnormalities. Subsequent screening of her family members revealed that her father had the same heterozygous *TERT* mutation (6). She was diagnosed with very severe aplastic anemia at the age of 8. She received IST with horse-ATG (Lymphoglobulin; Genzyme) 15 mg/kg/day intravenously for five days and cyclosporine because she had no HLA-matched family donor. However, the response to IST was poor, and she was still transfusion-dependent for six months after treatment. At first, she underwent HSCT from an HLA DRB1 one-allele-mismatched unrelated donor. The first conditioning regimen included cyclophosphamide 50 mg/kg/day for four days, TBI 5 Gy (two fractions), and rabbit-ATG (Thymoglobulin; Genzyme) 2.5 mg/kg for four days. Patient failed to engraft and had no autologous recovery of her bone marrow. She underwent a second transplant from an HLA B and DRB1 alleles-mismatched mother, 48 days post-transplant as salvage therapy. Conditioning regimen included fludarabine 30 mg/m^2/day and ATG 2.5 mg/kg/day from days −5 to −2, and melphalan 60 mg/m^2/day on days −2 and −1. GVHD prophylaxis was the same as for case 1.

Case 3

Patient 3 was an 18-yr-old man with classical DC with nail dystrophy, abnormal skin pigmentation, oral leukoplakia, and bone marrow failure. He had very short telomeres, and mutation analysis showed *DKC1* mutation. Nail changes began in early childhood, and pancytopenia was noted at age 13. Because pancytopenia progressed, we planned HSCT from an HLA 6/6 alleles-matched unrelated donor. He did not undergo HSCT before. Conditioning regimen included cyclophosphamide 750 mg/m^2/day, fludarabine 25 mg/m^2/day, and rabbit-ATG 2.5 mg/kg/day, all from days −5 to −2, and TBI 3 Gy (one fraction) on day −1. GVHD prophylaxis was the same as for cases 1 and 2.

Table 1 shows patient and disease characteristics. Pre-transplant cardiac, lung, or liver dysfunction was not observed in any patient except for slight elevation of liver transaminase levels in patient 2. Bone marrow examination

Table 1. Patient and disease characteristics

Patient no.	Sex	Age at diagnosis of DC	Mutation	Clinical triad	Other symptoms	Pre-transplant hematological data			Number of pre-transplant transfusions		Cytogenetics
						ANC (×10^9/L)	Hb (g/dL)	PLT (×10^9/L)	RBC	PLT	
1	Male	18	Not detected	Nail, skin, oral	Cerebellar hypoplasia, growth retardation	0.9	5.7	16	25	2	46, XY
2	Female	9	*TERT*	None	None	0.3	6	0.9	40	90	46, XX
3	Male	15	*DKC1*	Nail, skin, oral	None	0.84	7.7	19	0	2	46, XY

ANC, absolute neutrophil count; Hb, hemoglobin; PLT, platelet; RBC, red blood cell.

Table 2. Pre-transplant characteristics of donors and patients

Patient no.	Age at transplant	Donor	Donor sex	Donor age	ABO incompatibility	Source	HLA match	Mismatch locus
1	21	Sister	Female	24	Compatible	BM	5/6	DR
2	9	Mother	Female	36	Major	BM + PBSC	4/6	B, DR
3	18	UD	Female	37	Compatible	BM	6/6	–

UD, unrelated donor; BM, bone marrow; PBSC, peripheral blood stem cell.

revealed severe hypocellularity and normal karyotypes in all three patients. Table 2 shows pretransplant characteristics of donors and patients.

Supportive care

All patients received trimethoprim-sulfamethoxazole orally or inhaled pentamidine as prophylaxis against *Pneumocystis jiroveci*. Patients received standard doses of oral amphotericin B and acyclovir as fungal and viral prophylaxis. Patients received pre-emptive therapy with ganciclovir when CMV antigenemia became positive. Weekly viral studies for CMV, EBV and human herpesvirus 6 were obtained until day 90 post-transplant (7). Granulocyte colony-stimulating factor was started from day 5 to neutrophil engraftment. Acute and chronic GVHD was diagnosed and graded according to established criteria (8, 9).

Results

Transplant outcomes are shown in Table 3. Engraftment day was defined as the first of three consecutive days in which the patient had an absolute neutrophil count greater than 0.5×10^9/L. Neutrophil engraftment was achieved in all patients, although the days of platelet recovery were delayed. Analysis of short tandem repeats or fluorescent *in situ* hybridization of sex chromosomes revealed that all patients achieved >95% donor chimerism by day 100.

Engraftment syndrome developed in patient 3 and responded well to steroid therapy (10, 11). Acute GVHD did not occur in any patient, while chronic GVHD of the skin occurred in patient 3 and responded to tacrolimus therapy. Patients 1 and 2 discontinued their treatment with immunosuppressive drugs at 18 and 14 months, respectively, following transplant.

Increases in the EBV genome load were observed in patients 1 and 3. The dose of tacrolimus was decreased in patient 1, and one course of rituximab was administered in patient 3. As a result, EBV genome load decreased in both patients. Positive CMV antigenemia was seen only in patient 3. Preemptive therapy with ganciclovir was administered until the test for CMV antigenemia became negative. He did not progress to CMV disease.

To date, all three patients are alive with a follow-up of 10, 66, and 72 months, respectively. No patients have developed pulmonary or liver complications or malignancies.

Discussion

In our case reports, we report the outcome of two patients with classical DC and one patient with aplastic anemia harboring the *TERT* mutation to assess the feasibility and efficacy of a fludarabine-based non-myeloablative regimen. Our regimens are promising, as all three patients achieved complete chimerism and hematologic recovery without severe transplant-related toxicities.

Previously, results of HSCT using a myeloablative regimen for patients with DC were disappointing mainly because of pulmonary/liver complications and GVHD (12–19). Until recently, there were no survivors who received unrelated sources of stem cells (3). A high transplant-related mortality rate is considered

Table 3. Outcomes of transplantation

	Cell dose		Engraftment		GVHD				
Patient no.	NCC ($\times 10^8$)	CD34 ($\times 10^6$)	ANC (>500 μL)	PLT (>20 $\times 10^9$/L)	Acute	Chronic	Complication	Follow-up	Outcome
1	3.45	1.73	16	31	No	No	EBV reactivation	5 yr 6 months	Alive
2	9.6	2.6	23	123	No	No	DM, enteritis	6 yr	Alive
3	0.81	N.E	19	111	No	Skin	Sepsis, engraftment syndrome, CMV antigenemia, EBV reactivation	10 months	Alive

NCC, nuclear cell count; ANC, absolute neutrophil count; PLT, platelet; N.E, not evaluated.

to be associated with impaired restorative ability of tissue damage because of defective telomere maintenance. To avoid these complications, reduced-intensity regimens have been recently used and have achieved engraftment with fewer complications in both related and unrelated settings (16, 20–24). Most recently, Dietz et al. reported encouraging results of six patients with DC who underwent HSCT using fludarabine-based non-myeloablative regimens (26). Their non-myeloablative regimen consisted of cyclophosphamide 50 mg/kg for one day, fludarabine 40 mg/m^2 for five days, and TBI 2 Gy and alemtuzumab 0.2 mg/kg for five days. Engraftment was achieved in five of six patients. Four patients are alive, three of whom were recipients of unrelated grafts. Our regimen is similar to theirs, including cyclophosphamide, fludarabine, low-dose irradiation, and ATG instead of alemtuzumab. The results of HSCT from an alternative donor for DC are shown in Table 4.

It is still unclear whether HSCT can prolong the overall survival of patients with DC. Dietz et al. combined 18 cases who had undergone RIST in the literature with their six cases and calculated an overall survival rate of 65%, which was similar to another historical cohort that included both myeloablative and non-myeloablative transplants reported by Alter et al. (4). However, the follow-up periods in non-myeloablative transplants seem to be shorter than in myeloablative transplants. Although bone marrow failure is the most common cause of death in patients with DC, pulmonary fibrosis is another common cause of death (27). Alter et al. reviewed 65 patients who had received HSCT until 2008 (4). According to the review, nine of 30 deaths after HSCT were because of pulmonary fibrosis, suggesting that the high rate of this lung complication might originate from the natural history of DC. A prospective long-term follow-up study is necessary to clarify whether HSCT procedures, including conditioning agents and allogeneic immune responses to recipient's organ such as the lungs and liver, affect the natural course of DC.

Fludarabine is a potent immunosuppressive and less myeloablative agent, which has been used successfully in RIST for aplastic anemia (28) and other bone marrow failure syndromes

Table 4. Summary of HSCT from an alternative donor for dyskeratosis congenita

Patient	Age/sex	Donor source	HLA	Conditioning regimen	Outcome	Complication	References
1	23/M	MUD BM	6/6	CY 120 mg/kg and TBI 12 Gy	Death	Disseminated candidasis	Langston et al. (16)
2	20/M	MUD BM	6/6	CY 120 mg/kg and TBI 12 Gy	Death	Disseminated candidasis	Langston et al. (16)
3	29/M	MUD BM	6/6	CY 200 mg/kg and TBI 6 Gy	Death	Rejection Died of respiratory failure after 2nd BMT	Dokal et al. (17)
4	3/M	MUD	6/6	CY 120 mg/kg, Flu 180 mg/m^2 and ATG 160 mg/kg	Alive >15 months		Dror et al. (24)
5	8/F	MUD	6/6	CY 120 mg/kg, Flu 180 mg/m^2 and ATG 160 mg/kg	Alive >16 months	EBV reactivation	Dror et al. (24)
6	15/M	MUD	6/6	CY 120 mg/kg, Flu 180 mg/m^2 and ATG 160 mg/kg	Death	Cardio-respiratory arrest on day 0 Diffuse capillaritis	Brazzola et al. (25)
7	24/M	MMUD dUCB	4/6 4/6	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Death	Sepsis outside of hospital	Dietz et al. (26)
8	5/F	MUD BM	6/6	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Alive >40 months		Dietz et al. (26)
9	2/M	MUD BM	8/8	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Death	Adenoviral sepsis	Dietz et al. (26)
10	18/F	MMUD dUCB	4/6 5/6	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Alive >12 months	Acute GVHD grade IV (gut)	Dietz et al. (26)
11	25/M	MMUD dUCB	4/6 5/6	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Alive >12 months		Dietz et al. (26)
12	21/M	MMRD BM	5/6	CY 3 g/m^2, Flu 100 mg/m^2, TLI 3 Gy and ATG 10 mg/kg	Alive >5 yr	EBV reactivation	This report
13	9/F	MMRD BM+PBSC	4/6	MEL 120 mg/m^2, Flu 120 mg/m^2 and ATG 10 mg/kg	Alive >6 yr	DM, enteritis	This report
14	18/M	MUD	6/6	CY 3 g/m^2, Flu 100 mg/m^2, TBI 3 Gy and ATG 10 mg/kg	Alive >10 months	Sepsis, engraftment syndrome, EBV reactivation	This report

MUD, matched unrelated donor; MMUD, mismatched unrelated donor; MMRD, mismatched related donor; BM, bone marrow; PBSC, peripheral blood stem cell; dUCB, double unrelated cord blood; CY, cyclophosphamide; Flu, fludarabine; Alem, alemtuzumab; MEL, melphalan; BMT, bone marrow transplantation; DM, diabetes mellitus.

such as Fanconi anemia (29), Shwachman-Diamond syndrome (30), and Diamond-Blackfan anemia (31). In this study, fludarabine seemed to be well tolerated in patients with DC who achieved engraftment even after transplant from an alternative donor.

Reduction in the dose of cyclophosphamide may contribute to a decrease in transplant-related toxicity. We administered cyclophosphamide at a total dose of 3000 mg/m^2, which was a tolerable dose for our patients. In several reports, the total dose was reduced to 40–50 mg/kg with durable engraftment. However, one patient who received 50 mg/kg cyclophosphamide and an unrelated double-cord graft (one set of 4/6 and 4/6 HLA match) developed primary graft failure (26). The appropriate dose of cyclophosphamide remains undetermined.

The dose of irradiation is another important issue to achieve engraftment without increasing toxicities. Because patients with DC possess chromosomal instability, they are suspected to show increased radiosensitivity. In fact, a full-dose TBI regimen resulted in unacceptable toxicities in previous reports (16). From our experience as well as other reports, inclusion of low-dose TBI may contribute to achieve durable engraftment without undesirable complications.

Dietz et al. tried to provide a natural pulmonary compensation by delivering irradiation side-to-side, instead of anterior-to-posterior, with the patient in a seated position and the arms resting at the side of the thoracic cage (26). In our institute, patients are in a supine position with the arms at the side of the thoracic cage during TBI, which is delivered side-to-side. Our method also can provide for pulmonary compensation. In addition to the dose of irradiation, the method of irradiation may be important to assess the true effects on lungs in patients with DC.

GVHD prophylaxis is another important issue for successful transplant from an alternative donor. *In vivo* T-cell depletion can reduce the risk of GVHD in HSCT for bone marrow failure syndrome (32). Our conditioning regimen included rabbit-ATG for the purpose of *in vivo* T-cell depletion to prevent severe acute GVHD. Acute GVHD did not occur in any patient in our series, even in the patient who received both bone marrow and peripheral blood from an HLA haploidentical donor. Finke et al. reported the outcome of patients with hematologic malignancies who underwent HSCT from unrelated donors using a regimen containing rabbit-ATG (33). The cumulative incidence of grade II-IV acute GVHD and chronic GVHD for HLA-mismatched transplantation was 20% and 44%, respectively, which were equal to 21% and 43% for HLA-matched transplants. The authors concluded that a single-antigen mismatch might not compromise the outcome after HSCT from an unrelated donor when ATG is used in addition to standard GVHD prophylaxis.

In conclusion, our study indicated that RIST can provide successful engraftment with few complications in patients with DC, even in transplants from an alternative donor. Long-term follow-up is crucial to monitor the late effects of conditioning agents and allogeneic immune responses to the recipient's organs, such as the lungs and liver. Given these encouraging results, we believe that RIST should be explored further.

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Autoimmune lymphoproliferative syndrome-like disease with somatic KRAS mutation

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Autoimmune Lymphoproliferative Syndrome Like Disease With Somatic *KRAS* Mutation

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Abstract

Autoimmune lymphoproliferative syndrome (ALPS) is classically defined as a disease with defective FAS-mediated apoptosis (Type I–III). Germline *NRAS* mutation was recently identified in Type IV ALPS. We report two cases with ALPS like disease with somatic *KRAS* mutation. Both of the cases were characterized by prominent autoimmune cytopenia and lymphoadenopathy/splenomegaly. These patients did not satisfy the diagnostic criteria for ALPS or juvenile myelomonocytic leukemia (JMML), and are likely to be defined as a new disease entity of RAS associated ALPS like disease (RALD).

Introduction

ALPS is a disease characterized by dysfunction of the FAS-mediated apoptotic pathway[1,2], currently categorized as Type Ia, germline *TNFRSF6/FAS* mutation; Type Ib, germline *FAS ligand* mutation; Type Is, somatic *TNFRSF6/FAS* mutation; and Type II, germline *Caspase 10* mutation. Patients exhibit lymphadenopathy, hepatosplenomegaly, and autoimmune diseases such as immune cytopenia and hyper-γ-globulinemia. An additional subclassification has been proposed that includes Types III and IV, whereby Type III has been defined as that with no known mutation but with a defect in FAS-mediated apoptosis, and Type IV as one showing germline *NRAS* mutation[3]. Type IV is considered exceptional because the FAS-dependent apoptosis pathway is not involved in the pathogenesis, and this subclass is characterized by a resistance to IL-2 depletion-dependent apoptosis. Recent updated criteria and classification of ALPS suggested type IV ALPS as a RAS associated leukoproliferative disease [4].

JMML is a chronic leukemia in children. Patients show lymphadenopathy, hepatosplenomegaly, leukocytosis associated with monocytosis, anemia, thrombocytopenia, and occasional autoimmune phenotypes. About 80% of patients with JMML have been shown to have a genetic abnormality in their leukemia cells including mutations of *NF1*, *RAS* family[5], *CBL*, or *PTPN11*. The hallmarks of the laboratory findings of JMML include spontaneous colony formation in bone marrow (BM) or peripheral blood (PB) mononuclear cells (MNC) and hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) of CD34 positive BM-MNC[6].

Germline RAS pathway mutations cause Costello (*HRAS*), Noonan (*PTPN11*, *KRAS*, and *SOS1*), and cardio-facio-cutaneous (CFC) syndromes (*KRAS*, *BRAF*, *MEK1*, and *MEK2*). Patients with Costello and Noonan syndromes have an increased propensity to develop solid and hematopoietic tumors, respectively[7],

among these tumors the incidence of JMML in patients with germline mutation of *NF1* or *PTPN11* is well known.

We present two cases with autoimmune cytopenia and remarkable lymphadenopathy and hepatosplenomegaly, both of which were identified as having a somatic KRAS G13D mutation without any clinical features of germline *RAS* mutation such as CFC or Noonan syndrome.

Patients and Methods

All studies were approved by the ethical board of Tokyo Medical and Dental University.

Case 1

A 9-month-old boy had enormous bilateral cervical lymphadenopathy and hepatosplenomegaly (Supplemental data 1 Fig. 1a, b). Blood test revealed presence of hemolytic anemia and autoimmune thrombocytopenia. hyper-γ-globulinemia with various auto-antibodies was also noted. ALPS and JMML were nominated as the diseases to be differentially diagnosed. Detailed clinical history and laboratory data are provided as Supplemental data 1. The patient did not satisfy the criteria for the diagnosis of ALPS or JMML as discussed in results and discussion section.

Case 2

A 5-month-old girl had a fever, massive hepatosplenomegaly (Supplemental data 1 Fig. 1d). She was initially diagnosed with Evans syndrome based on the presence of hemolytic anemia and autoimmune thrombocytopenia with hyper-γ-globulinemia and auto-antibodies. Spontaneous colony formation assay and GM-CSF hypersensitivity of BM-MNC showed positivity. Then, tentative diagnosis of JMML was given, even though she showed no massive