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分担研究報告書

難治性固形腫瘍に対する国際共同試験の必要性の検討

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研究要旨

国際共同臨床試験を通じて薬剤開発を行う必要がある小児固形腫瘍のうち、肝芽腫、横紋筋肉腫、ウイルムス腫瘍の計28例のこれまでの治療成績と、再発・難治性となった例の臨床経過と、再発後の予後因子について後方視的に検討した。横紋筋肉腫とウイルムス腫瘍の予後はよかったが、肝芽腫では難治例が認められ、12例中2例は化学療法が奏功しなかった。これらの例には、新規薬剤の開発が必要であり、希少小児がんである肝芽腫に対しては、国際共同臨床試験の実施が必須である。

A．研究目的

国際共同臨床試験を通じて薬剤開発を行う必要がある小児固形腫瘍のうち、肝芽腫、横紋筋肉腫、ウイルムス腫瘍のこれまでの治療成績と、再発・難治性となった例の臨床経過と、再発後の予後因子について後方視的に検討することで、どのような対象に対する薬剤開発が必要であるかを明らかにすることを目的とする。

B．研究方法

2000年1月～2012年12月に発症し、鹿児島大学病院で治療した肝芽腫12例、横紋筋肉腫6例、ウイルムス腫瘍10例の合計28例を対象とした。

（倫理面への配慮）

対象の28例は、診療録を後方視的に検討したのみで、倫理的な問題はない。

C．研究結果

肝芽腫12例の発症時年齢は、中央値2歳(0-13歳)、男8、女4で、stage II/III/IVがそれぞれ、1,6,5例であった。初期治療として、全例が、多剤併用化学療法＋原発切除±放射線治療を受けた。1例が再発、2例が初期治療中に増悪、1例が初期治療中に合併症により死亡した。再発・再燃後の治療は、イリノテカン、再切除、肝移植などであった。再発・増悪した1例を含む9例が生存しており、5年の全生存率は80.2%であった。再発・増悪した2例は、それぞれ9か月,49か月で原疾患の増悪によって死亡した。1例は、治療終了後に肺に局所再発し、肺病変を切除後、イリノテカン投与を投与し、再発後48か月時点で無病で生存している。

横紋筋肉腫6例の発症時年齢は、中央値9歳(1-19歳)、男3、女3で、stage I/II/IIIがそれぞれ、3,1,2例、グループ3が5例、4が1例であった。初期治療として、全例が、多剤併用化学療法＋原発切除または放射線治療を受け、2例は大量化学療法と自家末梢血幹細胞移植術も受けた。このうち1例が32か月で局所再発した。再発後の治療は、多剤併用化学療法＋放射線治療であった。再発例を含む全例が生存しており、5年の全生存率は100%であった。再発した例は、再発後16か月時点で、無病で生存している。

ウイルムス腫瘍の10例の発症時年齢は、中央値2歳(1-6歳)、男6、女4で、stage I/II/III/IVがそれぞれ、5,2,2,1例であった。初期治療として、全例が、多剤併用化学療法＋原発切除±放射線治療を受けた。このうち1例が18か月で局所再発した。再発後の治療は、切除＋多剤併用化学療法＋放射線治療であったが、局所に再々発し、再

度切除した。この再発例を含む全例が生存しており、5年の全生存率は100%であった。再発した例は、再発後13か月時点で、無病で生存している。

肝芽腫、横紋筋肉腫、ウイルムス腫瘍の予後は比較的よく、今回の検討では、全28例の5年生存率は91.9%、平均生存期間は137か月(95%信頼区間117 ～ 157か月)であった。このうち肝芽腫の5年生存率は他の疾患に比べて有意に悪かった($p = 0.037$)。再発した5例のうち3例が生存しており、再発後の平均生存期間は41か月(95%信頼区間21～61か月)であった。

D．考察

全28例中、死亡した3例は全例が肝芽腫であった。つまり、肝芽腫の中には難治性のものが含まれる。死亡例のうち1例は、化学療法が一時的に奏功したのみで、寛解に至らず14か月で再燃した。もう1例は、初期治療から化学療法が奏功しなかった。これらの極めて難治例には、これまでの薬剤と作用機序の異なる新規の薬剤が必要である。肝芽腫は、発症数の少ない希少な小児がんであり、このような小児がんに対する薬剤の開発のためには、国際共同臨床試験を行う必要がある。

E．結論

肝芽腫には、化学療法がまったく奏功しないか、奏功しても一時的で、寛解にさえ至らない極めて難治性のものがある。これらの希少でかつ難治性の疾患には、国際共同臨床試験を通じて新規薬剤の開発を行う必要がある。

F．研究発表

1. 論文発表

なし

2. 学会発表

岡本康裕，他．進行期神経芽腫の再発後の生存期間の検討．第28回鹿児島小児外科研究会，2012年3月31日，鹿児島市．

H．知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3.その他

なし

Ⅴ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tajiri T, Kimura O, Fumino S, Furukawa T, Iehara T, Souzaki R, Kinoshita Y, Koga Y, Suminoe A, Hara T, Kohashi K, Oda Y, Hishiki T, Hosoi H, Hiyama E, Taguchi T	Surgical strategies for unresectable hepatoblastomas	Journal of Pediatric Surgery	47(12)	2194-98	2012
Iehara T, Hiyama E, Tajiri T, Yoneda A, Hamazaki M, Fukuzawa M, Hosoi H, Sugimoto T, Sawada T	Is the prognosis of stage 4s neuroblastoma in patients 12 months of age and older really excellent?	European Journal of Cancer	48(11)	1707-1712	2012
Ogura T, Hiyama E, Kamei N, Kamimatsuse A, Ueda Y, Ogura K	Clinical feature of anaplastic lymphoma kinase-mutated neuroblastoma	ournal of Pediatric Surgery	47(10)	1789-96	2012
Uekusa S, Sugito K, Kawashima H, Yoshizawa S, Furuya T, Ohashi K, Ikeda T, Koshinaga T, Mugishima H	Successful treatment for hepatoblastoma in a 1-year-old boy with trisomy 18	Pediatrics International	54(3)	428–430	2012
吉澤信輔, 杉藤 公信, 蘇我晶子, 植草省太, 金田 英秀, 古屋武史, 大橋研介, 井上幹也, 池田太郎, 越永従道	超低出生体重児にWilson-Mikity症候群とBeckwith-Wiedemann症候群を伴った肝芽腫の1例	日本小児外科学会雑誌	48(6)	956-960	2012
Yoshida A, Shibata T, Wakai S, Ushiku T, Tsuta K, Fukayama M, Makimoto A, Furuta K, Tsuda H	Anaplastic lymphoma kinase status in rhabdomyosarcomas	Modern Pathology	2013	1-10	2013
Kojima Y, Hashimoto K, Ando M, Yonemori K, Yamamoto H, Kodaira M, Yunokawa M, Shimizu C, Tamura K, Hosono A, Makimoto A, Fujiwara Y	Comparison of dose intensity of vincristine, dactinomycin, and cyclophosphamide chemotherapy for child and adult rhabdomyosarcoma: a retrospective analysis	Cancer Chemotherapy and Pharmacology	70(3)	391-397	2012
Yamamoto Y, Makimoto A	A case of stage IV neuroblastoma treated with aggressive surgery following intensive neoadjuvant chemotherapy with autologous stem cell transplantation	Japanese Journal of Clinical Oncology	42(4)	359	2012
Kojima Y, Hashimoto K, Ando M, Yonemori K, Hirakawa A, Kodaira M, Yunokawa M, Shimizu C, Tamura K, Katsumata N, Hosono A, Makimoto A, Fujiwara Y	Clinical outcomes of adult and childhood rhabdomyosarcoma treated with vincristine, dactinomycin, and cyclophosphamide chemotherapy	Journal of Cancer Research and Clinical Oncology	138(7)	1249-1257	2012
Kobayashi S, Ito M, Sano H, Mochizuki K, Akaihata M, Waragai T, Hosoya M, Kikuta A	Clinical analysis of combination therapy for febrile neutropenic patients in childhood cancer	Pediatrics International	55(1)	65-71	2013

Akaihata M, Kikuta A, Mochizuki K, Nemoto K, Ito M, Sano H, Kobayashi S, Ohto H, Hosoya M	Maintenance of surface antigens and the absence of an apoptotic marker are observed during storage of granulocyte concentrates collected by bag separation method	Transfusion and Apheresis Science	47(1)	43-47	2012
Kimura H, Ito Y, Kawabe S, Gotoh K, Takahashi Y, Kojima S, Naoe T, Esaki S, Kikuta A, Sawada A, Kawa K, Ohshima K, Nakamura S	EBV-associated T/NK-cell lymphoproliferativedisease in nonimmunocompromised hosts: prospective analysis of 108 cases	Blood	119(3)	673-686	2012
López-Terrada D, Alaggio R, Dávila MTD, Czauderna P. Hiyama E, Katzenstein H, Leuschner I, Malogolowkin M, Meyers R, Ranganathan S, Tanaka Y, Tomlinson G, Fabrè M, Zimmermann A, Finegold MJ, Towards an International Pediatric Liver Tumor Consensus Classification	Proceedings of the Los Angeles COG International Liver Tumors Symposium	Modern Pathology		In press	

VI. 研究成果の刊行物・別刷

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Surgical strategies for unresectable hepatoblastomas ☆

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Abstract

Background: The aim of this study was to assess the surgical strategies for unresectable hepatoblastomas at the initial diagnosis based on the experience of two institutions.

Methods: The PRETEXT (Pretreatment evaluation of tumor extent) and POST-TEXT (Post treatment extent of disease) staging, surgical treatments, and clinical outcomes were retrospectively analyzed for 12 cases with PRETEXT III or IV and M(−) of 29 hepatoblastomas treated based on the JPLT-2 (The Japanese Study Group for Pediatric Liver Tumor-2) protocol at two institutions between 1998 and 2011.

Results: Two of the 9 cases with PRETEXT III status were downstaged to POST-TEXT II. One of the 3 cases with PRETEXT IV showed downstaging to POST-TEXT III. Four of the 7 cases with P2 or V3 (indicated for liver transplantation) in the PRETEXT staging system showed P2 or V3 in POST-TEXT staging after 2 cycles of CITA (JPLT-2 standard regimen), and one case showed P2 or V3 in POST-TEXT staging at the initial operation and underwent primary liver transplantation. The initial surgical treatments were 1 lobectomy, 2 segmentectomies, 6 trisegmentectomies, 2 mesohepatectomies, and 1 primary liver transplantation. Both patients who underwent mesohepatectomies had bile leakage, and 1 of 5 trisegmentectomies had an acute obstruction of the right hepatic vein. Two patients underwent rescue living donor liver transplantation. Both of these patients showed P2 or V3 positive findings in POST-TEXT staging after 2 cycles of CITA.

Conclusions: POST-TEXT staging and P and V factors should be evaluated after 2 cycles of CITA for unresectable hepatoblastomas detected at the initial diagnosis. The patients should be referred to the transplantation center if the POST-TEXT IV, P2, or V3 is positive at that time. Liver resection by

☆ The authors declare that they have no conflicts of interest.

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trisegmentectomy is recommended in view of the incidence of surgical complications. Careful treatment, such as back-up transplantation, should thus be considered for liver resection in the cases with POST-TEXT IV, P2, or V3 status after initial 2 cycles of CITA.

Hepatoblastoma is the most common childhood liver tumor. Multicenter trials have made it clear that pre- and/or postoperative chemotherapy is necessary to improve the outcome of the treatment of hepatoblastoma [1,2]. The Japanese Study Group for Pediatric Liver Tumor (JPLT) established JPLT-1, the first nationwide protocol for liver tumors in childhood, to improve the outcome in children with liver tumors in Japan [3].

Furthermore, a refined version of the protocol, designated JPLT-2, was opened to participating institutions in 1998. The PRETEXT (Pretreatment evaluation of tumor extent) staging system was adopted from SIOPEL (International Society of Pediatric Oncology Liver Tumor Study Group) as an internationally approved staging system for tumor localization. All patients other than non-metastatic PRETEXT I patients are subjected to preoperative chemotherapy. A second-line regimen including ifosfamide is applied for tumors showing chemoresistance. A protocol including high-dose chemotherapy with autologous stem cell transplantation is used for patients with metastatic disease. The JPLT2 protocol resulted in an improvement in the 5-year overall survival rate (OS) in non-metastatic cases to 100% for PRETEXT I, 87.1% for PRETEXT II, 89.7% for PRETEXT III, and 71.2%, for PRETEXT IV. The 5-year OS in metastatic cases was 43.9%. The outcome in unresectable and metastatic tumors remained unsatisfactory [4].

Liver transplantation is the only option for those children with disease contained to the liver that is deemed unresectable after a predetermined course of chemotherapy. Long-term survival of 80% to 90% has been reported in children with unresectable hepatoblastoma who undergo primary transplantation [5,6]. The excellent results of primary liver transplantation for unresectable hepatoblastoma, in contrast to the poor outcomes reported in children requiring rescue transplant for recurrence after a primary resection, have led some surgeons to advocate for the expanded use of primary transplantation [7].

There are questions associated with the surgical strategies for PRETEXT III or IV hepatoblastomas such as the following: Extensive liver resection or liver transplantation? Which patients truly need total hepatectomy with liver transplantation? When should this be decided? The indications for liver transplantation of hepatoblastomas depend on POST-TEXT (Post treatment extent of disease). The consensus of indication is POST-TEXT IV or P2 (Involvement of the main portal vein) or V3 (Involvement of all three hepatic veins and/or the IVC) in POSTTEXT [8]. P2 means the involvement of the main portal vein. V3 means the involvement of all three hepatic veins and/or the IVC [9].

The aim of this study was to assess the surgical strategies for unresectable hepatoblastomas at the initial diagnosis. The PRETEXT and POST-TEXT staging, surgical treatments, and clinical outcomes were retrospectively analyzed for PRETEXT III or IV and M (−) hepatoblastomas treated based on the JPLT2 protocol at two institutions between 1998 and 2011.

1. Patients and methods

All 29 patients treated for hepatoblastoma at the two institutions (Kyushu University Hospital and Kyoto Prefectural University of Medicine Hospital) from 1998 through 2011 were retrospectively reviewed. This study was performed according to the Ethical Guidelines for Clinical Research published by the Ministry of Health, Labor, and Welfare of Japan on July 30, 2003 (revised 2008) and complies with the Helsinki Declaration of 1964 (revised 2008).

The tumors were evaluated using state-of-the-art imaging, including computed tomography; clinical staging was based on PRETEXT (pretreatment extent of tumor) as applied in SIOPEL [9,10]. M (metastatic), E (extrahepatic invasion), P (main portal invasion), V (invasion of all three hepatic veins or the vena cava), and R (rupture at diagnosis) were added as annotations to PRETEXT staging.

All 29 patients with hepatoblastomas were treated based on the JPLT2 protocol in principle [4]. Briefly, PRETEXT I tumors were primarily resected, and PRETEXT II–IV cases were treated with preoperative chemotherapy. At least two courses of a combination of 80 mg/m^2 cisplatin on day 1, followed by 30 mg/m^2 pirarubicin on days 2 and 3, which was designated CITA, were repeated preoperatively. CITA was allowed to be substituted with transarterial chemoembolization using 30 mg/m^2 pirarubicin and 200 mg/m^2 carboplatin (CATA-L) at the discretion of the physician. CITA was repeated until surgical resection became feasible. A combination of 3 g/m^2 ifosfamide on days 1 and 2, 400 mg/m^2 carboplatin on day 3, 30 mg/m^2 pirarubicin on days 4 and 5, and 100 mg/m^2 etoposide on days 1–5 (ITEC) was given until the tumor became resectable if CITA (CATA-L) failed to induce PR (as defined below). Postoperative chemotherapy was given to all cases. PRETEXT I and II tumors were treated with four courses of half-dose CITA (low-CITA). PRETEXT III, IV, and metastatic cases were treated with two courses of CITA. Patients who required salvage with ITEC preoperatively were treated with two courses of ITEC.

The PRETEXT and POST-TEXT staging, surgical treatments, and clinical outcomes were retrospectively analyzed for 12 cases with PRETEXT III or IV and M (−) of 29 hepatoblastomas.

2. Results

Table 1 shows a summary of the 12 cases with PRETEXT III or IV and M (−) hepatoblastomas treated based on the JPLT-2 regimen. Ten cases were PRETEXT III, and 2 cases were PRETEXT IV. Three cases underwent liver transplantation. One case had a primary liver transplantation due to POST-TEXT IV at surgery, while the other 2 cases underwent rescue liver transplantations. Two of the 10 cases with PRETEXT III showed a downstaging to POST-TEXT II at the initial operation. Five cases underwent trisegmentectomy, and 1 case underwent mesohepatectomy. One of 2 cases with PRETEXT IV showed downstaging to POST-TEXT III at the initial operation. One case with POST-TEXT III underwent mesohepatectomy, and another case with POST-TEXT IV underwent primary liver transplantation. A summary of the 12 cases with PRETEXT III or IV showed that 3 cases (25%) showed downstaging in POST-TEXT at the time of the operation. The initial surgical treatments were 1 lobectomy, 2 segmentectomies, 6 trisegmentectomies, 2 mesohepatectomies, and 1 primary liver transplantation.

The P and V factors in PRETEXT staging were retrospectively evaluated, POST-TEXT staging after 2 cycles of CITA, and POST-TEXT staging at surgery, respectively. Two of 5 cases with P2 or V3 in PRETEXT III showed P2 or V3 in POST-TEXT staging after 2 cycles of CITA, and no cases showed P2 or V3 in POST-TEXT staging at initial operation. Both two cases with P2 or V3 in PRETEXT IV showed also P2 or V3 in POST-TEXT staging after 2 cycles of CITA, and one case showed neither P2 nor V3, and another case showed P2 or V3 in POST-TEXT staging at the initial operation, and then underwent primary liver transplantation.

Two of the cases in the current series underwent rescue liver transplantation. One patient (Case No. 3) who underwent rescue liver transplantation was a 3 month old female with PRETEXT IV, and P2 and V0. The POST-TEXT staging after 2 cycles of CITA was III, and P2 and V0. POST-TEXT staging at the initial operation was III, and P1 and V0 after an additional 5 cycles of CITA. The patient underwent mesohepatectomy. The medical insurance would not cover transplantation for hepatoblastoma in Japan at that time. Multiple recurrence occurred in the posterior area 2 months after mesohepatectomy, and then a right lobectomy was performed. Multiple recurrence of the residual liver and liver dysfunction occurred 5 months after right lobectomy, and then living donor related liver transplantation was performed. However, the patient died due to graft failure 4 days after the operation. The other patient who underwent rescue liver transplantation was a 10 month-old male with a

Table 1 Summary of characteristics for 12 hepatoblastomas with PRETEXT III or IV and M (−).

	Patient age at diagnosis	PRETEXT	P	V	POSTTEXT after 2 cycles of CITA	P	V	neoadjuvant chemotherapy	POSTTEXT at surgery	P	V	operation	adjuvant chemotherapy	outcome
1	1 Y	III	0	1	II	0	0	CITA 4 cycles	II	0	0	right lobectomy	ITEC 3 cycles	Alive, NED
2	11 M	III	0	0	III	0	0	CITA 3 cycles	III	0	0	segmentectomy (S4+S5)	CITA 2 cycles	Alive, NED
3	3 M	IV	2	0	III	2	0	CITA 7 cycles	III	1	0	mesohepatectomy → right lobectomy → transplantation	ITEC → CPT11 4 cycles	transplantation related death
4	10 M	III	2	2	III	1	2	CITA 4 cycles	III	1	2	trisegmentectomy (left)	CITA 2 cycles	Alive, NED
5	1 Y	III	2	0	III	2	0	CITA 4 cycles	III	1	0	trisegmentectomy (left)	CITA 2 cycles	Alive, NED
6	7 Y	III	2	2	III	0	0	CITA 5 cycles	III	0	0	mesohepatectomy	CITA 2 cycles	Alive, NED
7	3 Y	III	2	2	III	1	1	CITA 4 cycles	III	1	1	trisegmentectomy (right)	CITA 2 cycles	Alive, NED
8	10 M	III	2	3	III	1	3	CITA 4 cycles	III	1	2	trisegmentectomy (left) → transplantation	(−)	Alive, NED
9	1 Y	IV	1	3	IV	1	3	CITA 4 cycles+ITEC 2 cycles	IV	1	3	transplantation	CPT-11 2 cycles	Alive, NED
10	1 Y	III	1	2	III	1	2	CITA 5 cycles	II	1	2	trisegmentectomy (right)	CITA 1 cycle	Alive, NED
11	1 Y	III	0	0	III	0	0	CITA (4)	III	0	0	S2+3 segmentectomy+partial resection	CITA 4 cycles	Alive, NED
12	9 M	III	0	0	III	0	0	CITA (3)	III	0	0	trisegmentectomy (left)	CITA 2 cycles	Alive, NED

PRETEXT III disease, with P2 and V3 (Case No. 8). The POST-TEXT staging after 2 cycles of CITA was III, and P1 and V3. POST-TEXT staging at the time of initial operation was III, and P1 and V2 after an additional 2 cycles of CITA, and the patient underwent trisegmentectomy. Acute liver dysfunction due to obstruction of the right hepatic vein occurred after left trisegmentectomy. Living donor related liver transplantation was performed 7 days after left trisegmentectomy. Both of the 2 cases that underwent rescue liver transplantation showed P2 or V3 positive in POST-TEXT staging after 2 cycles of CITA.

There were some surgical complications among the 11 cases that underwent liver resections. Both of the 2 cases with mesohepatectomies had bile leak complications, and only one of 6 trisegmentectomies had acute obstruction of right hepatic vein. There were 3 liver transplantations (one primary transplantation and two rescue transplantations). One of the 2 rescue transplantations had acute graft failure. Therefore, 11 cases were alive with evidence of disease, while 1 case died due to transplantation related complications (graft failure after 4 days of transplantation).

3. Discussion

The current Children's Oncology Group (COG) hepatoblastoma protocol (AHEP0731), determines tumor extent from computed tomography (CT) imaging using the PRETEXT system [10], which is now referred to as POST-TEXT for studies obtained after treatment with neoadjuvant chemotherapy. Children with POST-TEXT III or IV tumors are referred to the pediatric liver transplant center and extreme liver resection at diagnosis if possible and no later than just after the second cycle of chemotherapy. This strategy is designed to prevent protracted courses of chemotherapy and ensure expeditious access to transplant for truly unresectable tumors. Meanwhile, Lautz et al. advocated that an excellent oncologic cure, comparable with that reported for primary transplantation, is possible with nontransplant resection in appropriately selected children with POST-TEXT III or IV hepatoblastomas [8].

The present study retrospectively analyzed the PRETEXT and POST-TEXT staging, surgical treatments, and clinical outcomes for 12 hepatoblastomas with PRETEXT III or IV and M (−). Two of 12 cases with PRETEXT III or IV, showed down staging in POST-TEXT after 2 cycles of CITA, and 1 case showed downstaging at the initial operation. Three cases (25%) showed downstaging in POST-TEXT at the time of the operation. Four of 7 cases with P2 or V3 in PRETEXT staging showed P2 or V3 in POST-TEXT staging after 2 cycles of CITA, and one case showed P2 or V3 in POST-TEXT staging at the initial operation, and then underwent primary liver transplantation. Six cases (86%) showed down staging in P or V factors at the initial operation. These 6 patients underwent liver resection at the initial operation. However, both cases that underwent rescue liver transplantation showed P2 or V3 positive in POSTTEXT staging after 2 cycles of CITA. In the retrospective view of one patient (Case No. 3) who underwent rescue liver transplantation, if medical insurance was appropriate for transplantation for hepatoblastoma at right lobectomy (second operation), liver transplantation should have been performed. Another patient (Case No. 8) underwent rescue liver transplantation because the drainage area of RHV was extremely small, though the involvement of the RHV (right hepatic vein) was not found at POST-TEXT staging at the initial operation. Primary transplantation should therefore have been performed in this case as well. Regarding surgical complications, both mesohepatectomies had bile leak complications.

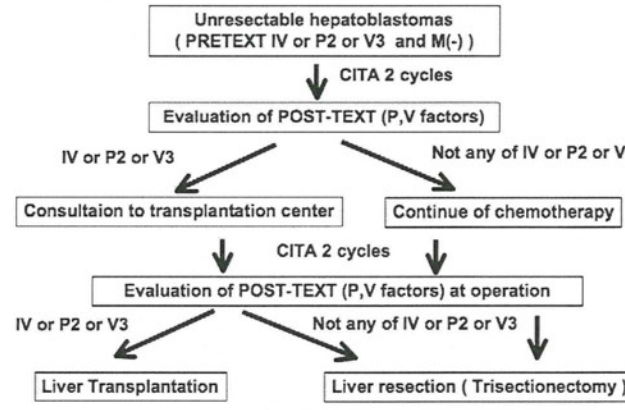

Fig. 1 Plan of surgical strategies for unresectable hepatoblastomas.

Preliminary plan of a surgical strategy for unresectable hepatoblastomas can be recommended from these results (Fig. 1). POST-TEXT staging and P, V factors should be evaluated after 2 cycles of CITA. The patients should be referred to the transplantation center if the POST-TEXT IV, or P2 or V3 is positive at that time. The final decision to perform either resection or transplantation should be determined based on POST-TEXT staging and P, V factors after an additional 2 cycles of CITA. Trisegmentectomy is recommended, because mesohepatectomies have a high rate of surgical complications, such as bile leaks. Careful treatment, such as backup transplantation should therefore be considered for cases presenting with POST-TEXT IV or P2 or V3 after the initial 2 cycles of CITA.

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References

[1] Suita S, Tajiri T, Takamatsu H, et al. Improved survival outcome for hepatoblastoma based on an optimal chemotherapeutic regimen. A report from the study group for pediatric solid malignant tumors in the Kyushu area, Japan. J Pediatr Surg 2004;39:195-8.

[2] Ortega JA, Douglass EC, Feusner JH, et al. Randomized comparison of cisplatin/vincristine/fluorouracil and cisplatin/ continuous infusion doxorubicin for treatment of pediatric hepatoblastoma: a report from the Children's Cancer Group and the Pediatric Oncology Group. J Clin Oncol 2000;18:2665-75.
[3] Sasaki F, Matsunaga T, Iwafuchi M, et al. Outcome of hepatoblastoma treated with the JPLT-1 (Japanese Study Group for Pediatric Liver Tumor) Protocol-1: a report from the Japanese Study Group for Pediatric Liver Tumor. J Pediatr Surg 2002;37:851-6.
[4] Hishiki T, Mtasunaga T, Sasaki F, et al. Outcome of hepatoblastomas treated using the Japanese Study Group for Pediatric Liver Tumor (JPLT) protocol-2: report from JPLT. Pediatr Surg Int 2010;27:1-8.
[5] Browne M, Sher D, Grant D, et al. Survival after liver transplantation for hepatoblastoma: a 2-center experience. J Pediatr Surg 2008;43: 1973-81.
[6] Faraj W, Dar F, Marangoni G, et al. Liver transplantation for hepatoblastoma. Liver Transpl 2008;14:1614-9.
[7] Otte JB, de Ville de Goyet J, Reding R. Liver transplantation for hepatoblastoma: indications and contraindications in the modern era. Pediatr Transplant 2005;9:557-65.
[8] Lautz TB, Ben-Ami T, Tantemsapya N, et al. Successful nontransplant resection of POST-TEXT III and IV hepatoblastoma. Cancer 2011; 117:1976-83.
[9] Roebuck DJ, Aronson D, Clapuyt P, et al. 2005 PRETEXT: a revised staging system for primary malignant liver tumours of childhood developed by the SIOPEL group. Pediatr Radiol 2007;37:123-32.
[10] Brown J, Perilongo G, Shafford E, et al. Pretreatment prognostic factors for children with hepatoblatoma—results from the International Society of Paediatric Oncology (SIOP) Study SIOPEL 1. Eur J Cancer 2000;36:1418-25.

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Is the prognosis of stage 4s neuroblastoma in patients 12 months of age and older really excellent?

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KEYWORDS
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Abstract *Purpose:* In the International Neuroblastoma Risk Group (INRG) classification system, stage 4s was changed into stage MS in children less than 18 months of age. Stage MS is defined as a metastatic disease with skin, liver and bone marrow, similar to INSS stage 4s. To evaluate the outcome of stage 4s cases in patients 12 months of age and over and to determine the appropriate treatment strategy.

Method: We performed a retrospective review of 3834 patients registered with the Japanese Society of Pediatric Oncology and Japanese Society of Pediatric Surgeons between 1980 and 1998.

Results: The rates of stage 4s patients were 10.7%, 6.3% and 3.3% in patients of $\leqslant$11 months of age, from $\geqslant$12 to $\leqslant$17 months of age, $\geqslant$18 months of age, respectively. The 5 year event-free survival rates were 89.4%, 100% and 53.1%, respectively. The rates of *MYCN* amplification and unfavourable histology were smaller in stage 4s groups than stage 4 groups in all ages.

Conclusion: In the children 12 months of age and older, stage 4s cases are markedly different from stage 4 cases in regard to the clinical features and prognosis. The prognosis of stage 4s cases from $\geqslant$12 to $\leqslant$17 months of age is excellent. The concept of stage MS appears to be appropriate.

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1. Introduction

Neuroblastoma is the most common extra cranial solid tumour in childhood. The prognosis of the disease is largely dependent on the age of the child and the extension of the tumour at diagnosis. In general, the prognosis for neuroblastoma in infants is much more favourable than in older children. Stage 4s neuroblastoma, first described by Evans, is a special metastatic disease for patients <12-months-old associated with a favourable prognosis.[1,2] Although these patients have wide metastatic disease, they have a favourable prognosis and also have high rates of a spontaneous regression. The stage 4s neuroblastoma is defined as an infant <12-months-old with metastases restricted to the liver, skin, and/or bone marrow, in which the primary tumour is localised (stage 1 or 2).

Recently, the International Neuroblastoma Risk Group (INRG) classification system was developed in order to establish a consensus approach for pre-treatment risk stratification. The new International Neuroblastoma Risk Group Staging System (INRGSS) was developed for the INRG.[3,4] To classify neuroblastoma patients by INRG classification system, we used the criteria of INRG stage, age, histological category, grade of tumour differentiation, *MYCN* status, 11q aberrations and tumour cell ploidy. In this INRG system, stage 4s changes to stage MS in children <18 months old. Stage MS is defined as a metastatic disease with special features, similar to INSS stage 4s, although there is no restriction regarding the size of the primary tumour. The metastases are restricted to the skin, liver and bone marrow. Age is not a component of the definition of stage MS.[3,4] Therefore, stage MS includes children aged from more than 12 months to less than 18 months of age. Conventionally, an age of $\geqslant$12 months has been the reference point for decisions for stage 4 neuroblastoma. Recently, an age cutoff of 18 months was proposed in a large-scale research study.[5]

The present study was undertaken to clarify how high the frequency of the stage 4s cases of $\geqslant$12 months and to clarify whether the prognosis is excellent or not for a decision-appropriate treatment strategy. This is the first report of stage 4s neuroblastoma in patients $\geqslant$12 months of age.

2. Patients and method

A retrospective review of 3834 patients with neuroblastoma was performed. The patients were registered with the Committee of Neuroblastoma in the Japanese Society of Pediatric Oncology and Japanese Society of Pediatric Surgeons between 1980 and 1998.

The patients were divided into three groups: $\leqslant$11-months of age, $\geqslant$12 to $\leqslant$17-months of age and $\geqslant$18-months of age.

From these three groups, we extracted the cases suited for stage 4s. The cases of metastasis were limited to the skin, liver or bone marrow. The primary tumours were observed within the tumour capsule (C_1) or outside the tumour capsule but not beyond the midline (C_2) and without contra lateral regional lymph node, in other words, the tumour of stage 3 was omitted. The maximum diameter of the primary tumours is less than 10 cm. In infancy, the stage 4s definition excluded bone marrow metastasis with more than 10% tumour cell infiltration. However, in this study, the ratio of the infiltration tumour cells is not considered in the stage 4s cases >12 months old. Thereafter, we examined the frequency of these extracted cases and compared the clinical feature and prognosis of stage 4 with those of 4s cases.

The stage 4s cases <12 months old were given either six cycles of the low-dose regimen, consisted of a low-dose of cyclophosphamide and vincristine over a 2-week period to shrink the tumour, followed by surgical resection. Stage 4 cases were treated with intensive chemotherapy consisting of cyclophosphamide and pirarubicin, cisplatin, vincristine or etoposide. Infants less than 12-months old were treated with reduced dosages. After 1992, many cases, especially cases with *MYCN* amplification, received high-dose chemotherapy with stem cell transplantation.

Amplification of the *MYCN* had been studied in children with those tumours since 1990 in JAPAN.

The histology of the primary tumour was mandatory to allow diagnosis of the neuroblastoma according to the International Neuroblastoma Pathology Classification, with the central review system by the Committee of Japanese Pediatric Tumor Pathology since 1994.

2.1. Statistical analysis

The Kaplan and Meier product limit methods were used to estimate the event-free survival (EFS) and the over-all survival (OS). The EFS calculated from diagnosis to the first event; relapse, progression or death (exception of other reason death). OS is calculated from diagnosis to death, excluded other reason death. Because the number of the events of each group was very small, we omitted the other reason death not to make bias. The Cox proportional hazards model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CI).The exact test from the permutation of the log-rank statistic was used to compare the EFS or OS probabilities between subgroups of patients. Differences between the two groups in categorical data were analysed by means of Fisher's exact probability test or the chi-square test. Two-sided *P*-values under 0.05 were considered as significant.

3. Results

The rates of stage 4s patients were 10.7%, 6.3% and 3.3% in patients $\leqslant$11-months of age, 12–17 months of age, and $\geqslant$18 months of age, respectively.

Stage 4s patients frequently present even at 12 months of age and older, although their frequency decreases with age. Since 1985, the high numbers of patients under 11 months of age is the reason why cases detected by screening are included. However, there is no difference in the frequency of patients detected by screening between stage 4 and 4s groups (Table 1).

The rates of *MYCN* amplified stage 4s patients were 3.7%, 0% and 0% in those ⩾11 months of age, 12–17-months of age and ⩾18 months of age, respectively. The rates of *MYCN* amplified patients were smaller in the stage 4s groups than in the stage 4 groups in each group ($P < 0.001$, $P = 0.04$ and $P < 0.001$, respectively). Similarly, the rates of patients with unfavourable histology were smaller in the stage 4s groups than in the stage 4 groups. However, the difference in the frequency of patients with unfavourable histology between the two stage groups was not significant in those 12–17-months of age, because of the small number of patients. The stage 4s patients displayed a lower mean serum LDH value than the stage 4 patients in each group (Table 1).

In stage 4s patients ⩽11-months old, observation and surgery alone were 6.2% and 6.9%, respectively. Infants less than 12-months old were treated with a different protocol between stage 4s and 4 group. Those stage 4s patients received less dose chemotherapy than stage 4. Patients >12-months of age with stage 4s and 4 tumour received the same induction chemotherapy and most of them received surgical resection. The other hand, the number of patients who received high-dose chemotherapy with stem cell transplantation were smaller in the stage 4s groups than in the stage 4 groups in each age category ($P = 0.002$ and $P = 0.017$, respectively), for patients ⩽11 months of age and 12–17-months of age. All patients ⩽11-months of age who received high dose chemotherapy have tumours with *MYCN* amplification. There are no patients who received surgical resection only or

Table 1
Characteristics of patients with INSS stage 4 or stage 4s neuroblastoma.

		⩽11 m			12–17 m			⩽18 m		
		No.	%	*P*	No.	%	*P*	No.	%	*P*
Patients	Total	2579			252			1003		
	Stage 4s	275	10.7		16	6.3		33	3.3	
	Stage 4	294	11.4		73	29.0		523	52.1	
Screening	Stage 4s	174	63.2							
	Stage 4	154	52.4	0.173						
MNA/no-MNA	Stage 4s	8/206	3.7		0/9	0		0/32	0	
	Stage 4	30/156	16.1	<0.001	18/36	25	0.04	68/186	26.8	<0.001
UFH/FH	Stage 4s	4/95	5.3		0/5	0		4/23	14.8	
	Stage 4	11/78	12.4	0.014	8/16	33.3	0.129	78/97	44.6	0.003
LDH(U/L) (mean level)	Stage 4s	672.4			441.4			675.5		
	Stage 4	1483.8		<0.001	4755.6		<0.001	2316.4		0.019
Therapy										
Observation	Stage 4s	17	6.2		0	0		0	0	
Surgery alone		19	6.9		1	6.2		0	0	
Chemo+surgery		239	86.9		15	93.8		33	100	
Radiation		13	4.7		4	25		13	39.3	
HDT with SCT		4	1.5		0	0		5	15.2	
Observation	Stage 4	0	0		0	0		0	0	
Surgery alone		8	2.7		0	0		0	0	
Chemo+surgery		286	97.3	0.002	73	100	0.017	512	97.9	0.130
Radiation		90	30.6		23	31.5		162	31	
HDT with SCT		19	6.4		20	27.4		142	27.2	
Outcome										
Alive	Stage 4s	258	93.8		15	93.8		17	51.5	
Dead of disease		9	3.3		0	0		12	36.4	
Therapic death		5	1.8		0	0		2	6.1	
Other reason death		1	0.4		0	0		0	0	
Unknown		2	0.7		1	6.2		2	6.1	
Alive	Stage 4	215	73.1		19	26		112	21.4	
Dead of disease		39	13.3		44	60.3		351	67.1	
Therapic death		15	5.1		9	12.3		39	7.5	
Other reason death		3	1		0	0		6	1.1	
Unknown		22	7.5		1	1.4		15	2.9	

Abbreviations: MNA, MYCN amplification; UFH, unfavorable histology; FH, favorable histology; HDT, high dose therapy; SCT, stem cell transplantation.

observation in stage 4 and 4s patients aged ⩾18-months. In other words, stage 4s patients aged <18-months received less intensive treatment than stage 4 patients.

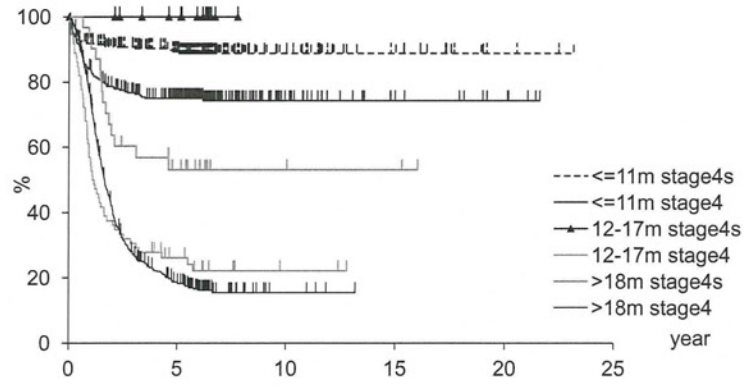
The details of prognosis of each groups were described in Table 1.The prognoses of stage 4s patients were good. Especially, the 5-year overall and event-free survival rates of the cases ⩽11 months of age, 12–17-months of age were excellent (91.2/89.4% and 100/100%, respectively) (Figs. 1 and 2). Comparing stage 4s with stage 4 in the same age, it was found that groups of stage 4s had a significantly better prognosis than the stage 4 groups (Figs. 1 and 2). For example, the *P*-values of the event-free survival rates were 0.004, 0.006 and <0.001, in patients ⩾11 months of age, 12–17-months of age and ⩾18 months of age, respectively.

4. Discussion

After Evans and DAngio reported on the uniqueness of stage 4s tumours concerning their spontaneous regression, most stage 4s tumours have been considered to be low risk tumours with an excellent prognosis.[1,2] Although there has been one report that stage 4s neuroblastoma patients do not have a poor prognosis even with *MYCN* amplification,[6] other reports have reported a poor prognosis in patients with stage 4s tumours with unfavourable prognostic factors such as *MYCN* amplification.[7–9] However, the tumours with poor prognostic factors are few in stage 4s neuroblastoma; the Children's Cancer Group Study reported that *MYCN* amplified tumour represented 0% of 80 stage 4s tumours,[10] and *MYCN* amplified cases constituted only 6% in 94 cases with stage 4s from the French Society of Pediatric Oncology.[11] In addition, it was reported that only 3.8% of all stage 4s tumours show an unfavourable histology.[10] From our results, only eight cases (3.7%) with stage 4s tumours showed *MYCN* amplification, and a few cases showed an unfavourable histology.

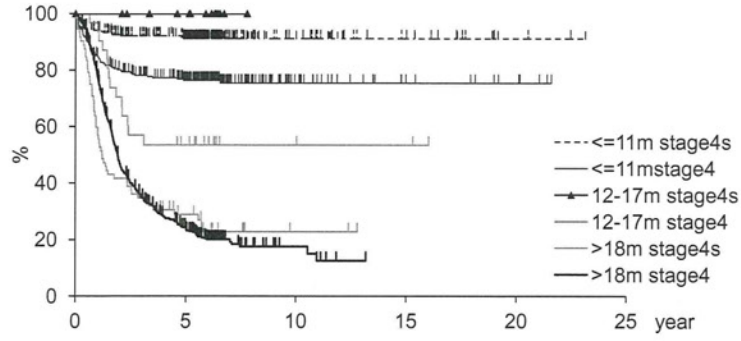
Presently, a few cases with unfavourable prognostic factors were evident in stage 4s cases involving infants both ⩽11 months of age and ⩾12 months of age. In the stage 4s patients, the serum LDH level was lower than the stage 4 patients in each group. These mean that the stage 4s neuroblastoma cases were less aggressive than the stage 4 cases at all ages were. These result that stage 4s patients who are ⩾12 months of age should have a better prognosis than stage 4 patients have, and these stage 4s cases should be different from stage 4 cases. Recently, some studies were conducted to clarify the biological difference between stage 4s and stage 4 using microarray analyses.[12,13] Although these studies did not discriminate between these stages in terms of genomic abnormalities, the possibility of a relationship between some partial chromosomal aberration, such as 17q, and clinical behaviour has been suggested.[13]

According to our research, the ratio of the stage 4s cases decreased and those of stage 4 increased with increasing age. The following two hypotheses can be suggested to the reason why the incidence of stage 4s cases changes with age. Firstly, stage 4 tumours, which are different from stage 4s tumours, developed with advancing age. The different biological characteristics of stage 4s and stage 4 tumours support this view. The number of cases detected might clinically decrease, because stage 4s tumours show spontaneous regression.[14–16] The second hypothesis assumes that stage 4s



	N	5y-EFS	
< = 11m stage 4s	273	89.4%	HRs;1.17
< = 11m stage 4	272	75.0%	95%CI;1.65-3.14, *P*=0.004
12-17m stage 4s	15	100%	HRs;3.57
12-17m stage 4	72	26.1%	95%CI;2.81-4.52, *P*=0.006
> = 18m stage 4s	31	53.1%	HRs;2.71
> = 18m stage 4	508	18.7%	95%CI;1.59-4.62, *P*<0.001

Fig. 1. Comparison of event-free survival rates of between stage 4s and stage 4 in patients <11 months old, ⩾12 to ⩽17 months, and ⩾18 months old. The event-free survival rates; The groups of stage 4s had better prognosis than the groups of stage 4 in patients <11 months old, ⩾12 to ⩽17 months, and ⩾18 months old (hazard ratios; 1.17, 3.57 and 2.71, $P = 0.001$, $P = 0.006$ and $P < 0.001$, respectively).



	N	5y-OS	
< = 11m stage 4s	273	91.2%	HRs;1.06 95%CI;1.79-4.64, *P*<0.001
< = 11m stage 4	272	76.3%	
12-17m stage 4s	15	100%	HRs;3.56 95%CI;2.67-4.63, *P*=0.007
12-17m stage 4	72	28.9%	
> = 18m stage 4s	31	53.5%	HRs;2.38 95%CI;1.40-4.06, *P*=0.001
> = 18m stage 4	508	24.4%	

Fig. 2. Comparison of overall survival rates of between stage 4s and stage 4 in patients <11 months old, ⩾12 to ⩽17 months, and ⩾18 months old. The overall survival rates; The groups of stage 4s had the better prognosis than the groups of stage 4 in patients <11 months old, ⩾12 to ⩽17 months, and ⩾18 months old (hazard ratios; 1.06, 3.56 and 2.38, $P < 0.001$, $P = 0.007$ and $P = 0.001$, respectively).

tumour change into stage 4 tumours, thus acquiring malignancy and thereby inducing clonal evolution.

However, it has been reported in a small number of cases that stage 4s tumours that progress to stage 4 tumours ultimately die.[17–19]

Presently, the cases with stage 4s tumours displayed a better prognosis than those cases with stage 4 tumour in infants aged ⩾12 months. Especially, the stage 4s cases aged 12–17 months had a good prognosis (100% 5 year event-free survival rate). According to the report of other countries, cases involving infants ⩾12 months of age with metastatic disease are now classified into stage 4 and receive intensive treatment.[20–22] In our study, cases ⩾12-months of age with stage 4s and 4 tumours were treated with the same induction chemotherapy consisting of cyclophosphamide and pirarubicin, cisplatin, vincristine or etoposide. The number of patients who received high-dose chemotherapy with stem cell transplantation was smaller in the stage 4s groups than in the stage 4 groups in aged 12–17-months category. As these stage 4s cases from 12–17-months of age were previously defined high risk group, they should now receive less intensive chemotherapy. It has been reported that the patients from 12–18 months of age with stage 4 non-amplified *MYCN* neuroblastoma have a better prognosis than older children.[20,21] This group may include stage 4s cases from 12 to 17 months of age. These cases are appropriate as a low risk group as well as the cases aged ⩽11 months. Therefore, the concept of stage MS of INRG of patients <18 months of age is proper.

On the other hand, the 5-year overall and event-free survival rates of stage 4s patients aged ⩾18-months were not so good (namely, 53.1% and 53.5%, respectively). This group should therefore be classified as belonging to a high risk group, and the initial intensive treatment should not be reduced.

Conflict of interest statement

None declared.

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References

1. Evans AE, DAngio GJ, Randolph J. A proposed staging for children with neuroblastoma. *Cancer* 1971;**27**:374–8.
2. DAngio GJ, Evans AE, Koop CE. Special pattern of widespread neuroblastoma with a favorable prognosis. *Lancet* 1971;**1**:1046–9.
3. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system. *J Clin Oncol* 2009;**27**(2):289–97.
4. Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. *J Clin Oncol* 2009;**27**(2):298–303.
5. London WB, Boni L, Simon T, et al. The role of age in neuroblastoma risk stratification: the German, Italian, and children's oncology group perspectives. *Cancer Lett* 2005;**228**(1–2): 257–66.
6. Tonini GP, Boni L, Pession A, et al. MYCN oncogene amplification in neuroblastoma is associated with worse prognosis, except in stage 4s: the Italian experience with 295 children. *J Clin Oncol* 1997;**15**(1):85–93.

7. van Noesel MM, Hählen K, Hakvoort-Cammel FG, et al. Neuroblastoma 4S: a heterogeneous disease with variable risk factors and treatment strategies. *Cancer* 1997;**80**(5):834–43.
8. Bourhis J, Dominici C, McDowell H, et al. N-myc genomic content and DNA ploidy in stage IVS neuroblastoma. *J Clin Oncol* 1991;**9**(8):1371–5.
9. Schneiderman J, London WB, Brodeur GM, et al. Clinical significance of MYCN amplification and ploidy in favorable-stage neuroblastoma: a report from the Children's Oncology Group. *J Clin Oncol* 2008;**26**(6):913–8.
10. Nickerson HJ, Matthay KK, Seeger RC, et al. Favorable biology and outcome of stage IV-S neuroblastoma with supportive care or minimal therapy: a Children's Cancer Group study. *J Clin Oncol* 2000;**18**(3):477–86.
11. Schleiermacher G, Rubie H, Hartmann O. Et al; Neuroblastoma Study Group of the French Society of Paediatric Oncology. Treatment of stage 4s neuroblastoma – report of 10 years' experience of the French Society of Paediatric Oncology (SFOP). *Br J Cancer* 2003;**89**(3):470–6.
12. Bénard J, Raguénez G, Kauffmann A, et al. MYCN-non-amplified metastatic neuroblastoma with good prognosis and spontaneous regression: a molecular portrait of stage 4S. *Mol Oncol* 2008;**2**(3):261–71.
13. Lavarino C, Cheung NK, Garcia I, et al. Specific gene expression profiles and chromosomal abnormalities are associated with infant disseminated neuroblastoma. *BMC Cancer* 2009;**9**:44.
14. De Bernardi B, Gerrard M, Boni L, et al. Excellent outcome with reduced treatment for infants with disseminated neuroblastoma without MYCN gene amplification. *J Clin Oncol* 2009;**27**(7):1034–40.
15. Pritchard J, Hickman JA. Why does stage 4s neuroblastoma regress spontaneously? *Lancet* 1994;**344**(8926):869–70.
16. Matthay KK. Stage 4S neuroblastoma: what makes it special? *J Clin Oncol* 1998;**16**(6):2003–6.
17. Bourhis J, Dominici C, McDowell H, et al. N-myc genomic content and DNA ploidy in stage IVS neuroblastoma. *J Clin Oncol* 1991;**9**:1371–5.
18. Wilson PC, Coppes MJ, Solh H, et al. Neuroblastoma stage IV-S: a heterogeneous disease. *Med Pediatr Oncol* 1991;**19**(6):467–72.
19. Heij HA, Verschuur AC, Kaspers GJ, et al. Is aggressive local treatment necessary for diffuse liver involvement in patients with progression of stage 4s neuroblastoma to stage 4? *J Pediatr Surg* 2008;**43**(9):1630–5.
20. George RE, London WB, Cohn SL, et al. Hyperdiploidy plus non-amplified MYCN confers a favorable prognosis in children 12 to 18 months old with disseminated neuroblastoma: a Pediatric Oncology Group study. *J Clin Oncol* 2005;**23**(27):6466–73.
21. Schmidt ML, Lal A, Seeger RC, et al. Favorable prognosis for patients 12 to 18 months of age with stage 4 non-amplified MYCN neuroblastoma: a Children's Cancer Group Study. *J Clin Oncol* 2005;**23**(27):6474–80.
22. Valteau-Couanet D, Michon J, Boneu A, et al. Results of induction chemotherapy in children older than 1 year with a stage 4 neuroblastoma treated with the NB 97 French Society of Pediatric Oncology (SFOP) protocol. *J Clin Oncol* 2005;**23**(3):532–40.

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Original Articles

Clinical feature of anaplastic lymphoma kinase–mutated neuroblastoma

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Prognosis

Abstract

Purpose: Anaplastic lymphoma kinase (*ALK*) has recently been identified as a gene conferring a predisposition for neuroblastoma. We have analyzed tyrosine kinase domain mutations and amplification/expression of the *ALK* gene and focused on clinical features of neuroblastoma cases with *ALK* aberrations.

Methods: The frequency of *ALK* mutations, copy number gain, and expression were analyzed in 538 neuroblastoma tumors derived from 361 cases, including 161 cases detected by mass screening. These cases were analyzed according to clinicopathologic features including the International Neuroblastoma Staging System and patient outcomes.

Results: Three cases (0.8%) had *ALK* amplification, and 16 cases (5.2%) had missense mutations at positions F1174, F1245, D1249, and R1275. Among them, 7 cases were diagnosed at more than 14 months of age, and 11 cases were infants, including 9 cases detected by mass screening and 1 multiple neuroblastoma with a germline mutation. Of the 11 infants, 3 cases relapsed, and 1 case died of disease. Among cases detected by screening, activated *ALK* cases showed significantly worse prognosis ($P = .002$). Of 7 older cases, 5 had *MYC* amplifications, and 5 died of disease. The expression levels of *ALK* were up-regulated in cases with unfavorable outcomes. In cases with activated ALK neuroblastoma, survival rates of patients detected by screening were significantly better than those in the clinically detected group ($P = .025$).

Conclusions: The results of the present study support the hypothesis that activated *ALK* tumors represent a specific subset of neuroblastomas. These tumors usually develop in infants and may have a high capacity for recurrence.

Neuroblastoma, derived from neuroblasts in the neural crest, is the most common malignant solid tumor in children. The incidence of neuroblastoma is about 1 case per 7000 babies born per year [1]. More than 90% of neuroblastomas are diagnosed within the first 5 years of age, and they exhibit 3 distinct patterns of clinical behavior:

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life-threatening progression, spontaneous regression, or maturation to ganglioneuroma. Many patients who are diagnosed at more than 1 year of age have advanced neuroblastomas with metastasis and usually show poor outcome despite multimodal therapies. Because more than 80% of neuroblastomas produce catecholamines, their metabolites (vanillylmandelic acid [VMA] and homovanillic acid [HVA]) are detectable in the urine. Consequently, mass screening projects to detect earlier-stage neuroblastomas have been carried out in several countries [2-5]. In Japan, large-scale mass screening of infants increased the annual incidence of the disease more than 2-fold. In contrast, both the incidence of advanced neuroblastoma in older children and the cumulative mortality rate of neuroblastoma were reduced significantly [2]. These findings indicate that a large number of neuroblastomas occur in infants and spontaneously regress or mature silently without clinical detection, whereas some of them may progress into malignant phenotypes [2]. These screening projects also provided us with a large number of neuroblastoma samples for investigation of the biologic characteristics of neuroblastoma. Transforming some tumors from a favorable type to unfavorable one has been suggested [6], but the precise molecular mechanism of malignant transformation of these tumors remains to be elucidated.

Clinically, it is essential to distinguish progressive neuroblastomas from favorable tumors because appropriate multimodal therapies are necessary to improve the prognosis of patients with progressive tumors. In contrast, aggressive therapy should be avoided in patients with favorable tumors to minimize side effects, late complications, and medical expenses. To more clearly distinguish the molecular and biologic heterogeneity of neuroblastomas, numerous analyses have been performed, and several distinct differences have been found including *MYCN* amplification, DNA ploidy, chromosomal loss or gain, expression of *NTRK1*, telomerase activation, and others [7].

ALK, a receptor tyrosine kinase, was initially identified through the analysis of a specific translocation in a subtype of non-Hodgkin lymphoma [8]. Recently, *ALK* was identified as a gene conferring a predisposition to neuroblastoma [9-12]. It harbors mutations in the tyrosine kinase domain (TKD) leading to aberrant activation of the gene and the downstream pathway [9]. Germline mutations of *ALK* were shown to be responsible for some hereditary neuroblastomas, and *ALK* has also been found to be mutated in some sporadic neuroblastomas [10-12]. Two hotspot mutations (F1174 and R1275) were identified as activating mutations [13], and several other mutations in TKD have been identified in sporadic neuroblastomas [14]. In these tumors, activating mutations and *ALK* amplification are the underlying mechanisms for *ALK*-dependent tumorigenesis and might be associated with a specific clinical phenotype in this disease [13,14].

In this article, we searched for acquired and germline *ALK* mutations in more than 300 Japanese neuroblastomas, including cases detected by mass screening. We investigated the characteristics of the neuroblastoma cases with *ALK* mutations, especially focusing on the treatment and the clinical outcomes of neuroblastoma cases with aberrant *ALK* genes. This study is the first to examine clinical features and investigate both mutations and copy number alterations of *ALK* in both a large set of sporadic neuroblastoma cases and cases detected by mass screening.

1. Materials and methods

1.1. Samples

Over the past 2 decades, more than 500 neuroblastomas derived from more than 400 cases, including approximately 180 detected by mass screening, have been stored at our university hospital. In some cases, we were consulted for molecular analysis by other hospitals in Japan. In the present study, genomic DNA was extracted from 361 neuroblastoma samples including 161 detected by mass screening. The tumor stages at surgery and tumor histology were classified according to the International Neuroblastoma Staging System (INSS) [15] and the International Neuroblastoma Pathological Classification (INPC) [16], respectively. Between 1991 and 2010, all patients were diagnosed at our hospital or affiliated hospitals as having neuroblastoma. Most patients were treated according to Japanese neuroblastoma protocols for infants or advanced stage neuroblastoma (A1, new A1, or A3) [17]. The follow-up period for all patients was more than 1 year. This research was approved by the ethical committee of our university (ID no. Hiro-Rin-20). Written informed consent for this research was obtained from parents of all patients. None of the patients had prior therapy before surgery or biopsy to obtain tumor specimens. Venous blood (5-7 mL) was taken from patients before surgery. Tumor DNA and constitutive DNA from each patient were extracted and purified using standard methods.

1.2. Affymetrix platform

Array experiments were done according to standard protocols for Affymetrix GeneChip Mapping 100K arrays (Affymetrix, Inc, Santa Clara, CA) [18]. The 100K single nucleotide polymorphisms (SNPs) or SNP 6.0 arrays were scanned with the Affymetrix GeneChip Scanner 3000 using GeneChip Operating System 1.2 (Affymetrix). Genotype calls and intensity of the SNPs were processed by GeneChip DNA Analysis Software. Individual SNP copy numbers and chromosomal regions with gains or losses were evaluated with the Affymetrix GeneChip Chromosome Number Tool 2.0.

1.3. *ALK* mutation analysis

Polymerase chain reaction (PCR) primers were designed for exons 23, 24, and 25 of the *ALK* gene, according to previous reports [9-12], and each exon was amplified from

genomic and constitutional DNA using exon-specific primers. The sequencing products were purified using Centri-Sep Spin Columns (Princeton Separations Inc., Adelphia, NJ) and then prepared for running on the ABI 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

1.4. Reverse transcription–PCR analysis of the expression of *ALK and MYCN* genes

Total RNA extracted from each tumor sample was reverse-transcribed using a high-capacity complementary archive kit (Applied Biosystems). An aliquot of the complementary DNA (equivalent to 20 ng total RNA) was subjected to real-time reverse transcription–PCR using the TaqMan Gene Expression Assay (Applied Biosystems) for *ALK*, and Pre-Developed TaqMan Assay Reagents (Applied Biosystems) for *GAPDH* as an internal control. More than 3 independent measurements were averaged, and relative gene expression levels were calculated as ratios to *GAPDH* expression for each sample.

1.5. Statistical analysis

Tests of association between aberrations and *ALK* expression levels were performed with the use of Wilcoxon *t* test. A log-rank test was used to evaluate survival rates. A Cox proportional hazards model was applied to compare the survival rates of each group.

All probabilities were 2-tailed, with a P value less than .05 regarded as statistically significant.

2. Results

2.1. Single nucleotide polymorphism analysis data

Among the 361 tumors analyzed by SNP array, 3 tumors harbored amplifications of the *ALK* gene, and 94 tumors showed chromosome 2 gains. Among the cases with chromosome 2p gains, 82 included the *ALK* gene, and 27 had *MYCN* amplification. Two cases had *ALK* amplification with *MYCN* amplification, and no cases had *ALK* amplification without *MYCN* amplification.

2.2. DNA sequencing

In exons 23 to 25 of the *ALK* gene, missense mutations were detected in 16 of 361 investigated cases (4.4%) (Tables 1 and 2). In those 16 cases with *ALK* missense mutations, changes of amino acids were as follows: F1174 (n = 6), R1275 (n = 6), F1245 (n = 3), and D1249 (n = 1) (Fig. 1). Among these cases, we detected 1 constitutional mutation (F1174L, 3522C>A) in the case with multifocal tumors (case 13 in Table 2). At base 3528 in exon 23 and base 3657 in exon 25, C>T substitutions were detected in other 2 cases, but these were silent mutations.

2.3. Expression analysis

We used reverse transcription–PCR to analyze gene expression of *ALK* in neuroblastoma tumor samples with *ALK* mutations or amplification (Fig. 2). The highest expression was detected in the 2 unfavorable tumors with *ALK* amplification. The expression levels of the tumors with *ALK* missense mutations were significantly higher than other tumors without mutations ($P < .05$). In the 2 tumors with silent mutations, *ALK* expression levels were similar to those without mutations. In the cases with *ALK* aberrations, *ALK* expression levels in deceased patients were relatively high but not significantly so.

2.4. Clinical and biologic features of cases with *ALK* mutations or amplifications

For patients with *ALK* mutated or amplified tumors, the clinical features of clinically detected cases are shown in Table 1, whereas mass-screening detected cases are presented in Table 2. In the 3 cases with *ALK* amplifications, one INSS 4S case (case 19) was detected by mass screening and had a *MYCN* amplification. The other 2 cases (cases 9 and 10) included an INSS 3 patient who was diagnosed at 14 months of age and an INSS 4 patient who was diagnosed at 22 months of age. All these cases were aggressive and showed poor outcomes.

Among the 16 cases with missense mutations of *ALK*, half were clinically detected, and half were detected by large-scale screening. In the 8 clinically detected cases (Table 1), 2 hotspot mutations were detected in 7 cases. Among them, 5 were detected as INSS4 tumors at more than 18 months of age and were unfavorable according to the INPC. In these 5 cases, serum levels of neuron specific enolase (NSE) were more than 100 ng/mL and urine levels of VMA and HVA were elevated, except for case 3.The remaining 3 cases were infants with INSS1, 3, or 4 tumors. Of these infants, 2 have survived disease-free, but one case with 2p gain has survived with remnants of the tumor. Review of the treatments provided to the 8 clinically detected cases revealed that primary tumor resection was feasible in 3 of the infants and an older INSS3 case, but in 4 older INSS 4 cases, the primary tumor was resected after preoperative chemotherapy, followed by postoperative chemotherapy including megachemotherapy with stem cell transplantation. All 3 infant cases have survived, but 4 of the 5 older cases died of disease.

In 8 cases detected by mass screening (Table 2), 6 were hot spot mutations, none had a *MYCN* amplification, and 4 had 2p gains. All cases were localized tumors (INSS 1-3) except for 1 patient (case 12) with favorable histology and 1 patient (case 11) died of disease. In the constitutional