全エクソーム解析を用いた肝芽腫における網羅的ゲノム解析.（口頭）	星野論子，西村力，関正史，加藤元博，吉田健一，宮野悟，林泰秀，小川誠司，岡 明，滝田順子	第117回日本小児科学会	平成26年4月11日～13日	国内
次世代シークエンサーを用いた神経芽腫における11q領域の責任遺伝子探索.（口頭）	吉田美沙，関正史，星野論子，樋渡光輝，加藤元博，吉田健一，小川誠司，林泰秀，滝田順子，岡明.	第117回日本小児科学会	平成26年4月11日～13日	国内
神経芽腫120検体におけるゲノム異常と予後解析.（口頭）	瓜生久美子，関正史，加藤元博，星野論子，樋渡光輝，中川原章，林泰秀，小川誠司，滝田順子，岡明	第117回日本小児科学会	平成26年4月11日～13日	国内
当院でのテイコプラニン標準投与における血中濃度解析.（ポスター）	塩澤亮輔，渡邉健太郎，樋渡光輝，加藤元博，滝田順子，岡 明.	第117回日本小児科学会	平成26年4月11日～13日	国内
再発を繰り返すランゲルハンス組織球症の1例.（ポスター）	樋渡光輝，渡邉健太郎，塩澤亮輔，加藤元博，滝田順子，岡 明.	第117回日本小児科学会	平成26年4月11日～13日	国内
次世代シーケンサーによる小児胚細胞腫瘍の変異解析.（口頭）	塩澤亮輔，関正史，星野論子，吉田健一，吉田美沙，瓜生久美子，加藤元博，小川誠司，滝田順子，岡 明.	第73回日本癌学会学術総会，	平成26年9月25日～27日	国内
神経芽腫大規模検体におけるgenetic landscapeと予後解析.（口頭）	瓜生久美子，西村力，吉田健一，関正史，星野論子，吉田美沙，加藤元博，樋渡光輝，林泰秀，田尻達郎，中川原章，小川誠司，滝田順子.	第73回日本癌学会学術総会，	平成26年9月25日～27日	国内
神経芽腫における11q領域に関連した責任遺伝子探索.（ポスター）	吉田美沙，関正史，加藤元博，瓜生久美子，星野論子，西村力，樋渡光輝，吉田健一，岡 明，小川誠司，林泰秀，滝田順子.	第73回日本癌学会学術総会，	平成26年9月25日～27日	国内
エクソーム解析とトランスクリプトーム解析を用いた肝芽腫における統合解析.（口頭）	星野論子，関正史，吉田健一，加藤元博，白石友一，佐藤悠佑，千葉健一，宮野悟，林泰秀，岩中督，岡明，小川誠司，滝田順子.	第73回日本癌学会学術総会，	平成26年9月25日～27日	国内
新規ALK結合蛋白質であるFlotillin-1は、ALKの細胞膜結合の調整節を介してALKシグナルを制御する（口頭）	冨山新太，上北尚正，山口英樹，上野英明，滝田順子，佐々木一樹，中川原章，森健太郎，堺隆一.	第73回日本癌学会学術総会，	平成26年9月25日～27日	国内
胸膜肺芽腫におけるDICER 1 RNase IIIBドメイン変異のmiRNA産生への影響.（ポスター）	関正史，吉田健一，白石友一，佐藤悠佑，島村徹平，千葉健一，田中洋子，花田良二，岡 明，宮野悟，林泰秀，小川誠司，滝田順子.	第73回日本癌学会学術総会，	平成26年9月25日～27日	国内
Transcriptome profiling of neuroblastoma by RNA-Seq.（口頭）	Hiwatari M, Seki M, Shiozawa R, Kato M, Yoshida K, Ogawa S, Takita J.	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内
Integrated analysis of clonal evolution in hepatoblastoma with familial adenomatous polyposis.（口頭）	Hoshino N, Seki M, Yoshida K, Kato M, Sato Y, Kasahara M, Nakazawa A, Miyano S, Hayashi Y, Oka A, Iwanaka T, Takita J.	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内

Landscape of genomic alteration of pediatric germ cell tumors.（口頭）	Shiozawa R, Seki M, Hoshino N, Yoshida K, Yoshida M, Uryu K, Hiwatari M, Kato M, Ogawa S, Oka A, Takita J.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
Integrated genetic and epigenetic analysis defines novel molecular clusters in rhabdomyosarcoma.（口頭）	Seki M, Nishimura R, Yoshida K, Shimamura T, Shiraishi Y, Sato Y, Hoshino N, Nagae G, Okuno Y, Hosoi H, Tanaka Y, Okita H, Taguchi T, Hanada R, Oka A, Miyano S, Aburatani H, Hayashi Y, Ogawa S, Takita J.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
The role of epigenetic dysregulation in neuroblastoma.（口頭）	Hoshino N, Seki M, Yoshida K, Kato M, Nishimura R, Sato Y, Miyano S, Nagae G, Hayashi Y, Oka A, Aburatani H, Iwanaka T, Ogawa S, Takita J.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
中心静脈カテーテル関連血流感染(CRBSI)の予防に対する試みとその効果.（口頭）	渡邉健太郎，加藤元博，張田豊，関口昌央，塩澤亮輔，樋渡光輝，滝田順子，岡 明.	第56回日本小児血液・がん学会学術集会	平成26年11月28日～30日	国内
再発膵芽腫のマルチサンプリングによる腫瘍内不均一およびクローン進化の考察.（口頭）	磯部知弥，関正史，吉田健一，白石友一，千葉健一，田中洋子，佐藤悠佑，加藤元博，井口晶裕，濱麻人，田中祐吉，宮野悟，小川誠司，岡 明，滝田順子.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
重篤な慢性心不全を合併した21トリソミー児のAMKLに対する緩和的化学療法.（口頭）	関口昌央，加藤元博，渡邉健太郎，塩澤亮輔，樋渡光輝，林泰佑，平田陽一郎，滝田順子，岡 明.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
神経芽腫におけるターゲット遺伝子の深々度シークエンス.（口頭）	瓜生久美子，西村力，吉田健一，関正史，星野論子，吉田美沙，加藤元博，樋渡光輝，岡 明，林泰秀，田尻達郎，中川原章，滝田順子.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
神経芽腫におけるATM pathway関連遺伝子の異常.（口頭）	吉田美沙，関正史，加藤元博，瓜生久美子，星野論子，西村力，樋渡光輝，吉田健一，岡 明，小川誠司，林泰秀，滝田順子.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
神経芽腫再発2例に対する網羅的ゲノム解析.（口頭）	物井綾香，関正史，吉田健一，佐藤悠佑，加藤元博，樋渡光輝，星野論子，竹谷健，白石友一，千葉健一，田中洋子，宮野悟，岡 明，林泰秀，小川誠司，滝田順子.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
先天性高悪性脳腫瘍の1例.（口頭）	木本豪，加藤元博，関口昌央，渡邉健太郎，塩澤亮輔，樋渡光輝，阿部浩幸，田中麻理子，武笠晃丈，滝田順子，岡 明.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内
慢性活動性EBV感染症の1倍検例.（ポスター）	塩澤亮輔，樋渡光輝，加藤元博，田中淳，滝田順子，岡 明.	第56回日本小児血液・がん学会学術集会,	平成26年11月28日～30日	国内

ALK 陽性思春期神経芽腫に対するクリゾチニブの治療経験.（ポスター）	松野良介，大貫裕太，藤田祥央，花村麻衣子，塚田大樹，秋山康介，外山大輔，池田祐一，関正史，加藤元博，樋渡光輝，滝田順子，磯山恵一．	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内
小児がん化学療法に対する病棟薬剤師の取り組み.（ポスター）	清水啓道，本多秀俊，大野能之，長瀬幸恵，加藤元博，樋渡光輝，滝田順子，岡 明，鈴木洋史．	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内
未分化大細胞型リンパ腫における ALK 転座染色体の過剰を伴った急性転化.（ポスター）	星野顕宏，野村恵子，関正史，樋渡光輝，吉田健一，小川誠司，滝田順子，金兼弘和．	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内
腎静脈浸潤を認めた先天性間葉芽腎腫の一例.（ポスター）	藤代準，杉山正彦，新井真理，石丸哲也，吉田真理子，魚谷千都恵，宮川亨平，加藤元博，滝田順子，土田晋也，高橋尚人，岩中督．	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内
FGFR-1 増幅を伴う治療抵抗性未分化肉腫の1例.（ポスター）	杣津晋平，合井久美子，渡邊敦，犬飼岳史，蓮田憲夫，高野邦夫，近藤哲夫，中澤温子，宮地充，細井創，滝田順子，後藤裕明，杉田莞爾．	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内
Congenital and childhood plexiform (multinodular) cellar schwannoma の1乳児例.（ポスター）	宮川亨平，藤代準，高見尚平，加藤怜子，出家亨一，魚谷千都絵，吉田真理子，石丸哲也，新井真理，杉山正彦，岩中督，加藤元博，渡邉健太郎，滝田順子，柴原順二．	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内
小児造血細胞移植後フォローアップ外来の取り組みと課題.（口頭）	岩崎美和，割田陽子，滝田順子，加藤元博，樋渡光輝，大友英子，宮里由香里．	第56回日本小児血液・がん学会学術集会，	平成26年11月28日～30日	国内

2．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
日本小児外科学会悪性腫瘍委員会：小児の外科的悪性腫瘍，2012年登録症例の全国集計結果の報告	米倉竹夫、田尻達郎、伊勢一哉、小野　滋、大植孝治、佐藤智行、杉藤公信、菱木知郎、平井みさ子、文野誠久、本多昌平、風間理郎、杉山正彦、中田光政、仲谷健吾、脇坂宗親、近藤知史、上原秀一郎、鬼武美幸、木下義晶，	日小外会誌	2014	国内
Efficacy of preoperative transcatheter arterial chemoembolization combined with systemic chemotherapy for treatment of unresectable hepatoblastoma in children	M Hirakawa,A Nishie,Y Asayama,N Fujita,K Ishigami,T Tajiri,T Taguchi,H Honda.	Jpn J Radiol	2014	国外

Prenatal administration of neuropeptide bombesin promotes lung development in a rat model of nitrofen-induced congenital diaphragmatic hernia.	K Sakai, O Kimura, T Furukawa,S Fumino, K Higuchi,J Wakao,K Kimura,S Aoi,K Masumoto, T Tajiri	J Pediatr Surg	2014	国外
腹部腫瘍により発見された Herlyn-Werner-Wunderlich 症候群の１例	竹内雄毅、樋口恒司、坂井宏平、文野誠久、青井重善、古川泰三、木村修、田尻達郎	日本小児外科学雑誌	2014	国内
胸壁悪性軟部肉腫に対する肋骨合併切除・胸郭再建術	樋口恒司、木村修、古川泰三、文野誠久、青井重善、坂井宏平、土屋邦彦、家原知子、細井創、田尻達郎	小児外科	2014	国内
腸間膜リンパ管腫切除術	文野誠久、金聖和、坂井宏平、樋口恒司、青井重善、古川泰三、木村修、田尻達郎	小児外科	2014	国内
胆汁うっ滞　先天性胆道拡張症	文野誠久、加藤久尚、樋口恒司、出口英一、田尻達郎	周産期医学	2014	国内
膵・胆管合流異常の診断の最前線：膵・胆管合流異常における DIC-CT の診断意義	文野誠久、坂井宏平、東真弓、青井重善、古川泰三、田尻達郎	胆と膵	2014	国内
第 11 節　小児固形悪性腫瘍における遺伝子解析による悪性度診断と遺伝子治療　遺伝子治療・診断の最先端技術と新しい医薬品・診断薬の開発	田尻達郎	遺伝子治療・診断の最先端技術と新しい医薬品・診断薬の開発	2014	国内
QOL を重視した小児外科医療の進歩	田尻達郎	京都小児科医会会報	2014	国内
PAX3-NCOA2 fusion gene has a dual role in promoting the proliferation and inhibiting the myogenic differentiation of rhabdomyosarcoma cells.	Yoshida H, Miyachi M, Sakamoto K, Ouchi K, Yagyu S, Kikuchi K, Kuwahara Y, Tsuchiya K, Imamura T, Iehara T, Kakazu N, Hojo H, Hosoi H.	Oncogene.	2014 Dec 4	国外
Identification of COL3A1 and RAB2A as novel translocation partner genes of PLAG1 in lipoblastoma.	Yoshida H, Miyachi M, Ouchi K, Kuwahara Y, Tsuchiya K, Iehara T, Konishi E, Yanagisawa A, Hosoi H.	Genes Chromosomes Cancer.	2014 Apr 4	国外
A novel fusion partner of ALK in an inflammatory myofibroblastic tumor.	Ouchi K, Miyachi M, Tsuma Y, Tsuchiya K, Iehara T, Konishi E, Yanagisawa A, Hosoi H.	Pediatr Blood Cancer.	2015 Feb 14	国外
38. 神経芽腫	米田光宏	小児外科診療ハンドブック～実地診療に役立つ周術期管理と手術のポイント～	2014・2・10	国内

Two Cases of Neuroblastoma Comprising Two Distinct Clones.	Yamazaki F, Nakazawa A, Osumi T, Shimojima N, Tanaka T, Nakagawara A, Shimada H.	Pediatric Blood Cancer	2014	国外
RASSF1A methylation may have two biological roles in neuroblastoma tumorigenesis depending on the ploidy status and age of patients.	Haruta M, Kamijo T, Nakagawara A, Kaneko Y.	Cancer Letters.	2014	国外
Flotillin-1 regulates oncogenic signaling in neuroblastoma cells by regulating ALK membrane association.	Tomiyama A, Uekita T, Kamata R, Sasaki K, Takita J, Ohira M, Nakagawara A et al.	Cancer Res.	2014	国外
NCYM, a cis-antisense gene of MYCN, encodes a de novo evoluted protein that inhibits GSK3b resulting in the stabilization of MYCN in human neuroblastoma.	Suenaga Y, Islam SMR, Alagu J, Kaneko Y, Kato M, Tanaka Y, Kawana H, Hossain S, Matsumoto D, Yamamoto M, Shoji W, Itami M, Shibta T, Nakamura Y, Ohira M, Haraguchi S, Takatori A, Nakagawara A.	PLoS Genet.	2014	国外
RUNX3 interacts with MYCN and facilitates protein degradation in neuroblastoma.	Yu F, Gao W, Yokochi T, Suenaga Y, Ando K, Ohira M, Nakamura Y, Nakagawara A.	Oncogene	2014	国外
Identification of novel candidate compounds targeting TrkB to induce apoptosis in neuroblastoma.	Nakamura Y, Suganami A, Fukuda M, Hasan MK, Yokochi T, Takatori A, Sato S, Hoshino T, Tamura Y, Nakagawara A.	Cancer Med.	2014	国外
Two cases of neuroblastoma comprising two distinct clones. Pediatr.	Yamazaki F, Nakazawa A, Shimojima N, Tanaka T, Nakagawara A, Shimada H.	Blood Cancer.	2014	国外
Metastatic neuroblastoma confined to distant lymph nodes (stage 4N) predeicts outcome in patients with stage 4 disease: A study from the International Neuroblastoma Risk Group Database.	Morgenstern DA, London WA, Stephens D, Volchenboum S, Hero B, Cataldo AD, Nakagawara A, Shimada H, Ambros P, Matthay KK, Cohn SL, Peason ADJ, Irwin MS.	J. Clin. Oncol	2014	国外
Flotillin-1 regulates oncogenic signaling in neuroblastoma cells by regulating ALK membrane association.	Tomiyama A, Uekita T, Kamata R, Sasaki K, Takita J, Ohira M, Nakagawara A, Kitanaka C, Mori K, Yamaguchi H, Sakai R.	Cancer Res	2014	国外
Significance of clinical and biologic features in stage 3 neuroblastoma: A report from the International Neuroblastoma Risk Group project.	Meany HJ, London WB, Ambros PF, Matthay KK, Monclair T, Simon T, Garaventa A, Berthold F, Nakagawara A, Cohn SL, Peason ADJ, Park JR.	Pediatr. Blood Cancer .	2014	国外

Clinical, biological, and prognostic differences on the basis of primary tumor site in neuroblastoma: a report from the international neuroblastoma risk group project.	Vo KT, Matthay KK, Neuhaus J, London WB, Hero B, Ambros PF, Nakagawara A, Miniati D, Wheeler K, Pearson ADJ, Cohn SL, DuBois SG.	J. Clin. Oncol	2014	国外
Revised risk estimation and treatment stratification of low- and intermediate-risk neuroblastoma patients by integrating clinical and molecular prognostic markers.	Oberthuer A, Juraeva D, Hero B, Volland R, Carolina S, Schmidt R, Faldum A, Kahlert Y, Engesser A, Asgharzadeh S, Seeger RC, Ohira M, Nakagawara A, Scaruffi P, Tonini GP, Janoueix-Lerosey I, Delattre O, Schleiermacher G, Vandesompele J, Speleman F, Noguera R, Piqueras M, Benard J, Valent A, Avigad S, Yaniv I, Grundy RG, Ortmann M, Shao C, Schwab M, Eils R, Simon T, Theissen J, Berthold F, Westermann F, Brors B, Fischer M.	Clin. Cancer Res.	2014 [Epub ahead of print]	国外
Neuroblastoma in older children, adolescents and young adults. A report from the International Neuroblastoma Risk Group Project.	Mosse YP, Deyell RJ, Berthold F, Nakagawara A, Ambros PF, Monclair T, Cohn SL, Pearson AD, London WB, Matthay KK.	Pediatr. Blood Cancer.	2014	国外
Nr4a3, a possibile oncogenic factor for neuroblastoma associated with CpGi methylation within the third exon.	Uekusa S, Kawashima H, Sugito K, Yoshizawa S, Shinojima Y, Igarashi J, Ghosh S, Wang X, Fujiwara K, Ikeda T, Koshinaga T, Soma M, Nagase H.	Int J Oncol 44:1669-77.	2014	国外
Bilateral Wilms tumors treated according to the Japan Wilms Tumor Study Group protocol.	Oue T, Koshinaga T, Okita H, Kaneko Y, Hinotsu S, Fukuzawa M.	Pediatr Blood Cancer 61:1184-9. 2014.	2014	国外
Trim32 facilitates degradation of MYCN on spindle poles and induces asymmetric cell division in human neuroblastoma cells.	Izumi H, Kaneko Y.	Cancer Res 74:5620-5630, 2014	2014	国外
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Ⅴ．代表的論文

Identification of *COL3A1* and *RAB2A* as Novel Translocation Partner Genes of *PLAG1* in Lipoblastoma

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Lipoblastoma is a rapidly growing, benign neoplasm in children. Surgical excision is usually curative, with a recurrence rate of about 20%. Because the histology of lipoblastoma is heterogeneous and overlaps with other lipomatous tumors, some lipoblastoma cases have been difficult to diagnose. The detection of *PLAG1* gene rearrangement is useful for the diagnosis of lipoblastoma. Three fusion partner genes are known in relation to *PLAG1* in lipoblastoma *HAS2* at 8q24.1, *COL1A2* at 7q22, and *RAD51L1* at 14q24. Herein, we describe another two novel fusion genes in lipoblastoma tumor specimens. We checked six tumors for the presence of two known fusion genes, *HAS2-PLAG1* and *COL1A2-PLAG1*. Only *HAS2-PLAG1* was found in one of the cases. Next, we attempted to identify potential *PLAG1* fusion partners using 5'RACE. Sequence analysis revealed two novel fusion genes, *COL3A1-PLAG1* in three cases and *RAB2A-PLAG1* in one case, respectively. As a result of the translocations, the constitutively active promoter of the partner gene drives the ectopic expression of *PLAG1*. We also evaluated whether a high level of *PLAG1* expression can be used to help differentiate lipomatous tumors. *PLAG1* expression was evaluated by real-time PCR in five lipoblastoma tumor specimens. The expressions were 70–150 times higher in lipoblastomas than in human adipocytes. However, *PLAG1* expression was low in one case of lipoma. These results demonstrate that *PLAG1* overexpression is a potential marker of lipoblastoma. Our findings, in agreement with previous studies, show that lipoblastoma is a group of lipomatous tumors with *PLAG1* rearrangement and overexpression.

INTRODUCTION

Lipomatous tumors are relatively rare, accounting for only about 6% of all soft tissue neoplasms in childhood. One type of lipomatous tumor, lipoblastoma is a rapidly growing, benign neoplasm. It is a mesenchymal tumor of fetal white fat tissue that appears most commonly in children under three years of age and affects males three times more often than females (McVay et al., 2006). It can present anywhere in the body, but is most commonly seen in the trunk and extremities. Surgical excision is usually curative, with a recurrence rate of about 20% (Jimenez, 1986; Hicks et al., 2001). Histologically, lipoblastoma shows a characteristic lobular architecture, with lobules containing lipoblasts embedded in a myxoid matrix, whereas lipoma is a tumor composed of only mature fat without lobulation. The presence of lipoblasts helps to distinguish lipoblastoma from other lipomatous tumors. However, cytogenetic analysis is sometimes needed to reliably distinguish lipoblastoma from other lipomatous tumors (Weiss, 1996; Kuhnen et al., 2002; de Saint Aubain Somerhausen et al., 2008; Morerio et al., 2009). Thus, it is important to identify cytogenetic markers of lipoblastoma.

Lipoblastoma is associated with overexpression and other abnormalities of *PLAG1* (pleomorphic adenoma gene 1) located in chromosome band 8q12 (Astrom et al., 2000; Hibbard et al., 2000; Brandal et al., 2006). *PLAG1* is an oncogene that was first observed in pleomorphic adenoma of the salivary glands and encodes a zinc finger protein with two putative nuclear localization signals (Kas et al., 1997). The oncogenicity of *PLAG1* is partly becuase of activation of the insulin-like growth factor 2

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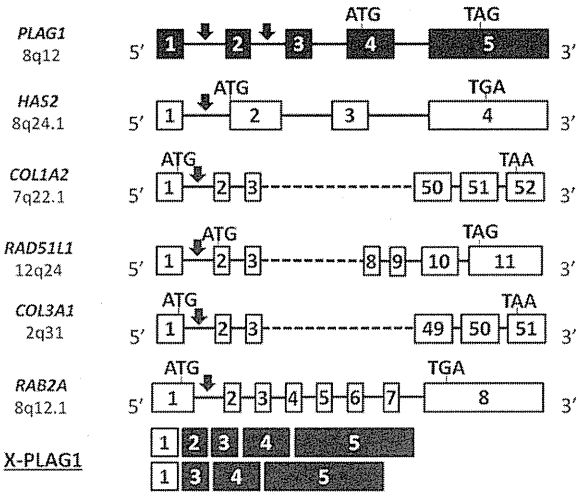

Figure 1. Schematic representation of fusion transcripts in human lipoblastoma. The chromosomal organization of the *PLAG1* gene (black box), and *HAS2, COL1A2, RAD51L1, COL3A1, and RAB2A* (white box) are given. Positions of translocation breakpoints (arrows), translation initiation sites (ATG), and stop codons (TAG, TGA and TAA) are indicated. Schematic compositions of two patterns of hybrid transcripts are presented in the lower part.

(IGF2) mitogenic pathway (Hensen et al., 2002), and *PLAG1* overexpression was shown to induce tumorigenesis in a mouse model (Declercq et al., 2005). The 8q12 rearrangement results in promoter-swapping, in which the *PLAG1* promoter element is replaced by promoter regions from another gene: hyaluronic acid synthase 2 (*HAS2*) at 8q24.1, collagen 1 α 2 (*COL1A2*) at 7q22 or *RAD51L1* at 14q24 (Fig. 1), at least the former two of which up-regulate *PLAG1* gene expression (Hibbard et al., 2000; Deen et al., 2013). Usually, exon 2 or 3 of *PLAG1* combines with exon 1 of the N-terminal side of the partner genes, resulting in accelerated transcription of the fusion gene with translation starting in at the *PLAG1* start codon in exon 4 (Van Dyck et al., 2007). In this way, overexpression of *PLAG1* arising from the fused gene is closely associated with the oncogenesis of lipoblastoma.

Herein, we describe two novel fusion genes related to *PLAG1* in lipoblastoma patients with *PLAG1* overexpression. In addition, we evaluated whether a high level of *PLAG1* expression can be used in the diagnosis of lipomatous tumors.

MATERIALS AND METHODS

Patients and Tumor Tissue Samples

Archival material from pediatric patients diagnosed with lipomatous tumors was retrieved from the specimen files at the Department of Pediatrics, Kyoto Prefectural University of Medicine (KPUM) from 1997 to 2013. The samples (one lipoma and five lipoblastomas) were collected from a total of six patients (four males and two females). The patients' characteristics are summarized in Table 1. All six of the lipomatous tumors occurred in children of age three years or under. Five of the cases presented with truncal lipomatous tumors (two in neck-mediastinum, two in shoulder, and one in chest wall), and one presented with a tumor in the right sole.

Tumor specimens were surgically resected before any therapy, **snap-frozen** in liquid nitrogen, and stored at −80°C until use. The patients were selected on the basis of histological examination of tumor specimens. We included one case of lipoma (case 1) to clarify whether *PLAG1* rearrangement and overexpression are limited to lipoblastoma. Written informed consent for using the samples for research was given by the parents according to the protocol approved by the institutional review board of KPUM in accordance with the Declaration of Helsinki.

Reverse Transcription-PCR

Total RNA was extracted from tumor specimens using the RNeasy mini kit (Qiagen, Hilden,

TABLE 1. Clinical cytogenetic, and molecular data for six lipomatous tumors

Case no.	Age	Sex	Tumor size (cm)	Localization	Karyotype	Gene fusion product	Histological Diagnosis
1	0y 7m	M	7.2 x 6.0	rt. shoulder	ND	-	lipoma
2	3y 2m	M	6.0 x 3.0	neck~mediastinum	ND	*HAS2-PLAG1*	lipoblastoma
3	0y 5m	M	8.0 x 4.0	neck~mediastinum	46,XY,add(2)(q21),add(7)(q11.2), add(8)(q24)[5]/46,XY[15]	*COL3A1-PLAG1*	lipoblastoma
4	0y 7m	M	3.0 x 2.3	lt. chest	ND	*RAB2A-PLAG1*	lipoblastoma
5	3y 8m	F	5.0 x 4.2	rt. sole	46,XX[20]	*COL3A1-PLAG1*	lipoblastoma
6	2y 1m	F	3.5 x 2.5	rt. shoulder	ND	*COL3A1-PLAG1*	lipoblastoma

ND: not done, rt.: right, lt.: left

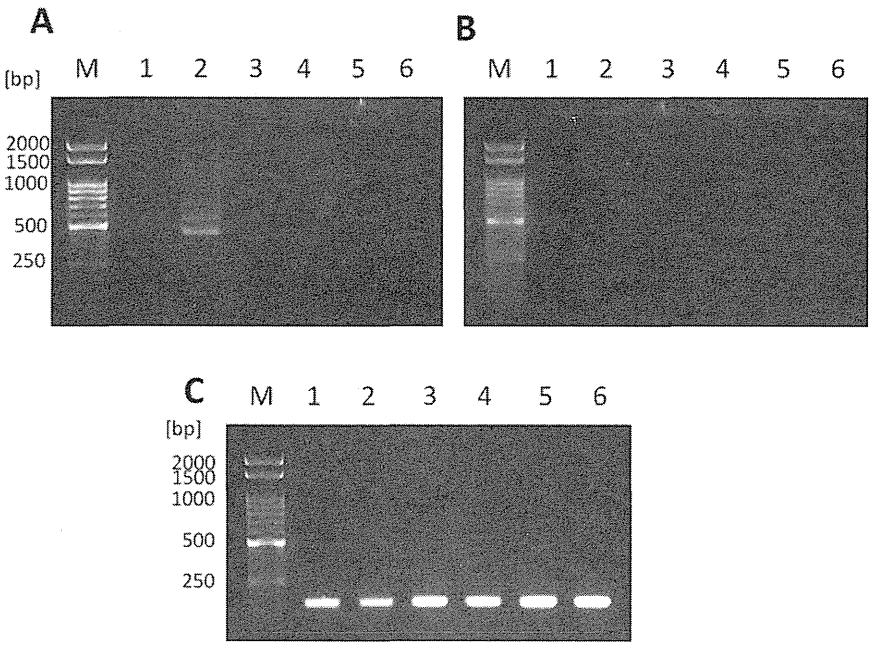

Figure 2. RT-PCR analysis of two known fusion genes related to *PLAG1* (*HAS2-PLAG1* and *COL1A2-PLAG1*) in cases 1–6. (A) *HAS2-PLAG1*. The sizes of the products were very close to their expected sizes of 521 and 406 bp (corresponding to fragments with and without exon 2 of *PLAG1*, respectively), and were found only in case 2. (B) *COL1A2-PLAG1*. None of the six cases were positive for this fusion gene (expected sizes 594 and 489 bp, corresponding to fragments with and without exon 2 of *PLAG1*, respectively). (C) *GAPDH* (expected size 142 bp).

Germany) according to the manufacturer's instructions. The complementary DNA (cDNA) for reverse transcriptase (RT)-PCR was synthesised using the SuperScript VILO cDNA Synthesis Kit (Invitrogen, Basel, Switzerland) according to the manufacturer's instructions. The *PLAG1* fusion transcript was detected by RT-PCR using *HAS2*, *COL1A2*, *COL3A1* or *RAB2A*, and *PLAG1* primers (Supporting information Table 1) and Ex Taq Hot Start Version (Takara Bio, Otsu, Japan) according to the manufacturer's instructions. PCR mixtures were denatured at 95°C for 10 sec, and 30 cycles of PCR (denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec) were performed in a thermal cycler followed by a final extension at 72°C for 7 min. The PCR products were analyzed by electrophoresis on a 1.0% agarose gel. As an internal control for the intactness of the RNA, cDNA of the *GAPDH* gene was also amplified. Gene Ladder 100 (Wako Pure Chemical Industries, Osaka, Japan) was used as a DNA size marker.

Rapid Amplification of cDNA Ends (5'-RACE) and Sequencing

One microgram of total RNA was reverse-transcribed to cDNA, followed by 5'-RACE, using the 5'-Full RACE Core set (Takara Bio) and the primer pairs listed in Supporting information Table 1. Amplified products after the second PCR were cloned with the TOPO TA Cloning Kit for Sequencing with One Shot TOP10 Chemically Competent *E. coli* (Invitrogen). The constructed plasmid DNA was sequenced with the use of the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA) and the ABI PRISM 3130 Sequence Detection System (Applied Biosystems).

Quantitative Real-Time RT-PCR

The cDNA was amplified by quantitative real-time PCR (qRT-PCR) using the 7300 Real-Time PCR System (Applied Biosystems) with SYBR Green I (Takara Bio) and the primer pairs shown in Supporting information Table 1. The PCR conditions were as follows: 10 sec at 95°C, followed by 40 cycles 95°C for 5 sec and 60°C for 31 sec. Relative target mRNA expression was determined using the comparative threshold (ΔC_T) method, in which the C_T value of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control and was subtracted from that of the target mRNA. Data are expressed as the ratio of target mRNA to GAPDH mRNA (calculated as $2^{\Delta CT}$). Standard curve analysis with stepwise dilution samples demonstrated that each

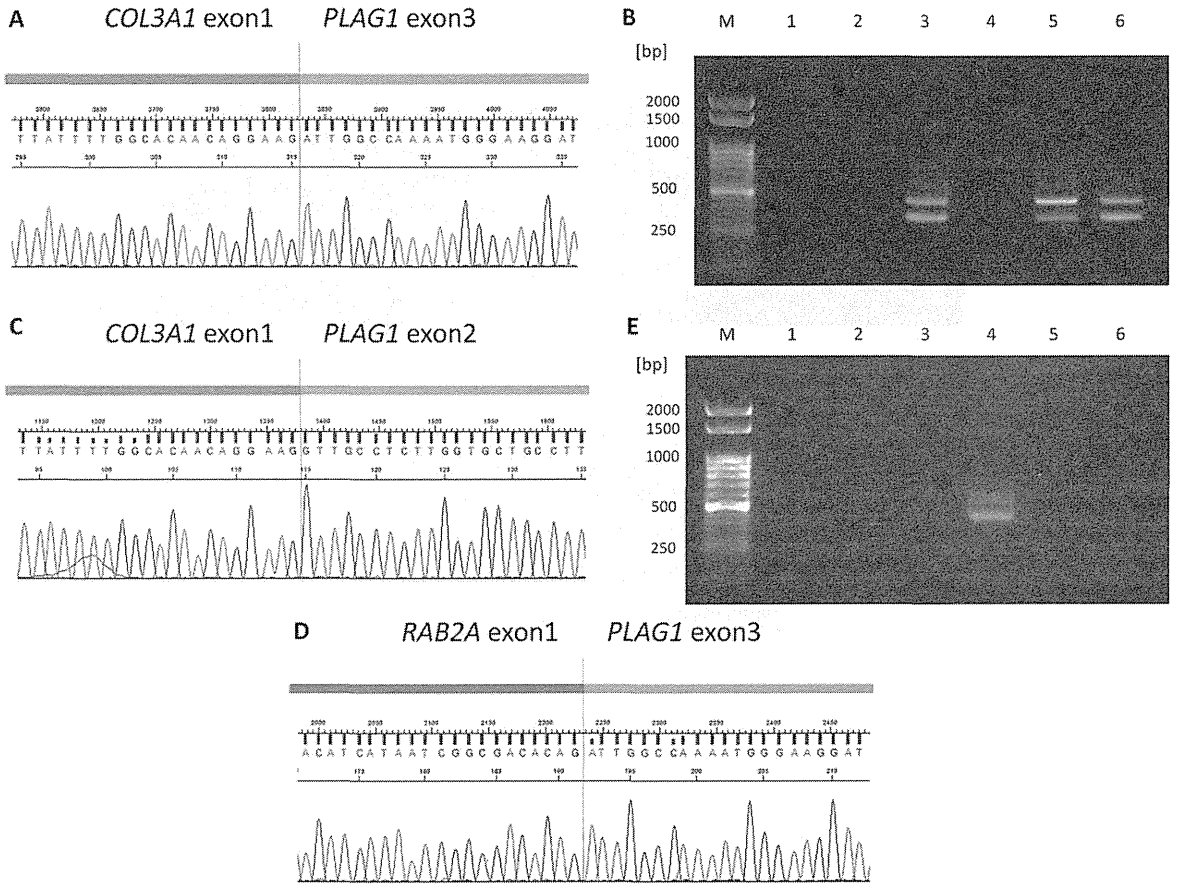

Figure 3. Novel fusion partners in lipoblastoma. (A) cDNA sequences showing the fusion of the *COL3A1* exon 1 with *PLAG1* exon 3. (B) RT-PCR analysis showing that the *COL3A1-PLAG1* fusion gene was present in cases 3, 5, and 6. The sizes of the bands were 450 and 345 bp, corresponding to fragments with and without exon 2 of *PLAG1*, respectively. (C) cDNA sequences showing the fusion of the *COL3A1* exon 1 with *PLAG1* exon 2. (D) Sequence analyses showing the fusion of the *RAB2A* exon 1 with *PLAG1* exon 3. (E) RT-PCR analysis showing that the *RAB2A-PLAG1* fusion gene was present in case 4. The sizes of the bands were 590 and 485 bp, corresponding to fragments with and without exon 2 of *PLAG1*, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

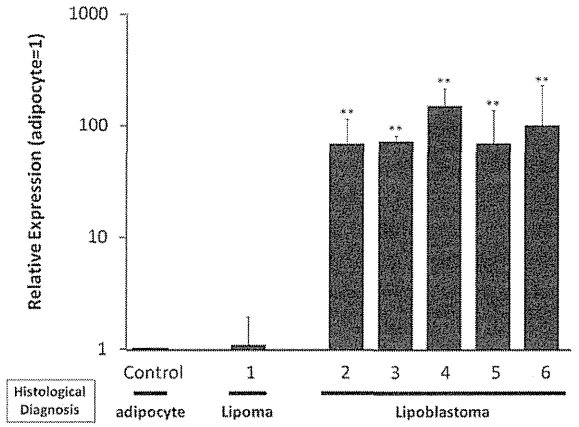

Figure 4. Relative expressions of *PLAG1* in six lipomatous tumor cases, as assessed by real-time PCR. Human adipocytes were used as control. The numbers under the bars corresponded to the case numbers shown in Table 1. Results represent the means ± SD of three independent experiments. $^{**}P < 0.01$ compared with adipocytes.

primer had similar amplification efficiencies. qRT-PCR reactions were performed in triplicate.

RESULTS

Identification of Two Novel *PLAG1* Fusion Partners in Lipoblastoma

First, we examined whether the lipoma and five lipoblastoma tumor specimens had two known fusion genes, *HAS-PLAG1* and *COL1A2-PLAG1*. Only one of these fusion genes, *HAS2-PLAG1*, was found in one of the six cases (case 2, a lipoblastoma) (Fig. 2). We used 5'RACE to identify other potential *PLAG1* fusion partners in the other cases. In case 3, PCR amplification of the 5' end of the *PLAG1* transcripts gave about 500 base pair (bp) products. Sequencing of this product revealed that exon 1 of the collagen type 3 alpha 1 gene (*COL3A1*) fused to exon 3 of *PLAG1* (Fig. 3A).

RT-PCR analysis showed that two other cases (cases 5 and 6) contained the *COL3A1-PLAG1* fusion gene (Fig. 3B), indicating that *COL3A1-PLAG1* is a recurrent chimeric gene in lipoblastoma. Because each case had two bands, we performed sequence analysis, showing that exon 1 of *COL3A1* was fused to either exon 2 or exon 3 of *PLAG1* (Fig. 3B and 3C).

None of the above fusion genes was detected in case 4 (Fig. 2 and 3B). However, 5'-RACE gave an about 650 bp product. Sequence analysis revealed another novel *PLAG1* fusion partner: the *RAB2A* gene. Exon 1 of *RAB2A* was fused to exon 3 of *PLAG1* (Fig. 3D). RT-PCR analysis indicated that only case 4 contained the *RAB2A-PLAG1* fusion gene and two alternative splicing transcripts were detected (Fig. 3E).

PLAG1 was overexpressed in all five lipoblastomas (cases 2–6). These expressions were from 70 to 150 times higher than the expression of *PLAG1* in human adipocytes (Fig. 4). *PLAG1* expression was low in the lipoma (case 1).

DISCUSSION

We found two novel fusion genes, *COL3A1-PLAG1, RAB2A-PLAG1*, in lipoblastomas, with the former being present in three of five cases. This suggests that *COL3A1-PLAG1* is a frequent fusion gene. *COL3A1* maps to chromosome band 2q31 and encodes the pro-alpha1 chains of type III collagen, a fibrillar collagen that is found in extensible connective tissues such as skin, lung, uterus, intestine, and the vascular system. Mutations in this gene have been associated with Ehlers-Danlos syndrome type IV, and with aortic and arterial aneurysms (Lee et al., 2008; Jeong et al., 2012). Another novel fusion partner, *RAB2A*, a member of the RAS oncogene family, maps to chromosome band 8q12.1. RAB proteins are small molecular weight guanosine triphosphatases (GTPases) that contain highly conserved domains involved in GTP binding and hydrolysis. RABs are membrane-bound proteins that are involved in vesicular fusion and trafficking (Ali et al., 2004; Mountjoy et al., 2008). It is also worth noting that RAB activity controls the connection between insulin signals and Glut4 trafficking in fat cells (Baldini et al., 1992; Baldini et al., 1995; Kaddai et al., 2008). Sequence analysis showed that the first exons of *COL3A1* or *RAB2A* fused to either exon 2 or exon 3 of *PLAG1* (Fig. 1). *PLAG1* has a genomic fusion breakpoint in intron 1 resulting in alternative splicing of exon 2 (Hibbard et al., 2000). The start codon of *PLAG1* is located in exon 4 (Fig. 1: "ATG" on *PLAG1*), and the coding sequences of *PLAG1* in both fusion genes (*HAS2-PLAG1* and *COL3A1-PLAG1*) are preserved. Our result is consistent with known splice variants of *PLAG1* (Hibbard et al., 2000), and supports the molecular mechanism of overexpression of *PLAG1* through promoter swapping; as a result of the translocation, the constitutively active promoter of the partner gene drives the ectopic expression of *PLAG1*. All five lipoblastoma cases examined in this study showed rearrangement of the *PLAG1* gene. Similarly, 82% of lipoblastomas examined in one study had a rearrangement of the 8q11–q13 region including *PLAG1* (Bartuma et al., 2008). Most lipoblastomas are characterized by cytogenetic rearrangement of the *PLAG1* region, and it is likely that *PLAG1* activation is a key tumorigenic event in lipoblastoma (Hicks et al., 2001). PLAG1 acts as a transcriptional activator of the *IGF2* gene (Voz et al., 2000). *IGF2* has four promoters. At the *IGF2* promoter 3 consensus binding site, *IGF2* is up-regulated by PLAG1 interactions, and IGF2 enhances proliferation of adipocyte progenitor cells (Butterwith and Goddard, 1991). This suggests that *IGF2* transcriptional up-regulation contributes to oncogenic proliferation in *PLAG1*-mutant lipoblastoma cells (Hibbard et al., 2000).

Of the five lipomatous tumor cases with *PLAG1* overexpression, each was found to express a fusion gene, although it appears that the start codons in the 5' partners of *PLAG1* were ignored so that translation started with the start codon of *PLAG1*. These results suggest that *PLAG1* overexpression is caused by formation of a fusion gene, in agreement with previous reports (Astrom et al., 2000; Hibbard et al., 2000). Because the histology of lipoblastoma is heterogeneous and overlaps with other lipomatous tumors, some lipoblastoma cases can be difficult to diagnose. Although *PLAG1* up-regulation is a hallmark of lipoblastoma, it is rarely used in clinical practice. Our findings raise the possibility that lipomatous tumors with *PLAG1* overexpression should be redefined as lipoblastomas cytogenetically and can be useful to distinguish lipoblastoma from other lipomatous tumor.

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ORIGINAL ARTICLE

PAX3-NCOA2 fusion gene has a dual role in promoting the proliferation and inhibiting the myogenic differentiation of rhabdomyosarcoma cells

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We analyzed a complex chromosomal translocation in a case of embryonal rhabdomyosarcoma (RMS) and showed that it generates the fusion gene *PAX3* (paired box 3)-*NCOA2* (nuclear receptor coactivator 2). To understand the role of this translocation in RMS tumorigenesis, we established two types of stable mouse myoblast C2C12 cell lines expressing PAX3-NCOA2 and PAX3-FOXO1A (forkhead box O1A), respectively. Compared with control cells, PAX3-NCOA2 cells grew faster, were more motile, were less anchorage dependent, progressed more quickly through the G1/S phase of cell cycle and showed greater transcriptional activation of the PAX3 consensus-binding site. However, PAX3-NCOA2 cells proliferated more slowly and differentiated more weakly than did PAX3-FOXO1A cells. Both PAX3-NCOA2 cells and PAX3-FOXO1A cells formed tumors in nude mice, although the PAX3-NCOA2-induced tumors grew more slowly. Our results may explain why *NCOA2* rearrangement is mainly found in embryonal rhabdomyosarcoma, which has a better prognosis than alveolar rhabdomyosarcoma, which expresses the *PAX3-FOXO1A* fusion gene. These results indicate that the *PAX3-NCOA2* fusion gene has a dual role in the tumorigenesis of RMS: promotion of the proliferation and inhibition of the myogenic differentiation of RMS cells.

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Keywords: rhabdomyosarcoma; PAX3-NCOA2; PAX3-FOXO1A; tumorigenesis; inhibiting differentiation

INTRODUCTION

Rhabdomyosarcoma (RMS) is the most common childhood soft tissue tumor. RMS can be divided into two main histopathological subgroups: embryonal rhabdomyosarcoma (ERMS) and alveolar rhabdomyosarcoma (ARMS). These subgroups differ considerably in their clinical phenotype and molecular features. The prognosis of ERMS is more favorable than that of ARMS. The majority of cases of the more aggressive ARMS are associated with one of two reciprocal translocations: t(2;13)(q35;q14), which generates an intronic fusion of *PAX3* (paired box 3) and *FOXOA1* (forkhead box O1A) that is also known as *FKHR* (forkhead in human rhabdomyosarcoma) and t(1;13)(p36;q14), which generates an intronic fusion of *PAX7* and *FOXOA1*.[1] The PAX3-FOXO1A translocation is associated with increased treatment failure and mortality rate.[2] PAX family members are transcription factors that regulate pattern formation during embryogenesis.[3] Several PAX genes, including *PAX3* and *PAX7*, contribute to tumorigenesis.[4] Pax3 is expressed in the developing nervous system and in somite compartments that give rise to skeletal muscle progenitors.[5,6] Exons 2, 3 and 4 of PAX3 encode the paired box, which is a distinctive feature of the PAX family, whereas the homeodomain is encoded by exons 5 and 6, and functions as a DNA-binding domain. Forkhead box (or *FOX*) genes have diverse roles including control of embryonic development and adult tissue-specific gene expression. FOXO1A has one transactivation domain (TAD) at the C-terminus,[7] and translocations between PAX3 and FOXO1A lead to an increase in transcriptional activation of the DNA-binding domains on PAX3. PAX3-FOXO1A induces cellular proliferation[8] and malignant transformation[9] and suppresses apoptosis.[10]

Recurrent *NCOA2* (nuclear receptor coactivator 2) gene rearrangements occur in ERMS.[11,12] Mosquera *et al.*[11] reported *NCOA2* gene rearrangements (*SRF-NCOA2* and *TEAD1-NCOA2*) in three cases of spindle cell RMS. Sumegi *et al.*[12] identified the fusion gene *PAX3-NCOA2* in each case of ERMS and ARMS that they investigated. We previously found a translocation involving chromosome band 2q35, which is the locus of the *PAX3* gene.[13] We later identified *NCOA2* as a candidate *PAX3* partner gene using fluorescence *in situ* hybridization (FISH). NCOA2 belongs to the p160 protein family, which also includes SRC-1 (steroid receptor coactivator), NCOA2/TIF2/ GRIP1/SRC-2 and pCIP/ACTR/AIB-1/RAC-3/TRAM-1/ SRC-3. NCOA2 has several functional domains including a PAS (sequence similarity with the Per Arndt-Sim motifs)/bHLH (sequence similarity with basic helix–loop–helix motifs) domain, a receptor interaction region, and two TADs.[14–17] NCOA2 is necessary for myogenic differentiation.[17] In nuclear receptor signaling, NCOA2 binds to nuclear receptors predominantly through its nuclear receptor-interacting domain (NID)[18,19] and recruits two transcriptional co-activators, CBP (CREB-binding protein)/p300 and CARM-1. CBP/p300 is recruited through its CBP interaction domain (CID/TAD1)[16,20] and CARM-1, an arginine

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