Table 3
Hazard ratios of clinicopathologic characteristics for overall survival in PDAC.

Variables	Categories	Overall survival	
		HR (95% CI)	*p*
Univariate analysis			
aPKCλ/ι expression	high versus low	2.34 (1.46–3.74)	0.00036[a]
Age, years	<65 versus ≥65	1.07 (0.69–1.67)	0.77
Pathologic tumor status	pT1 + pT2 versus pT3	1.79 (0.73–4.44)	0.21
Pathologic node status	pN0 versus pN1	1.40 (0.79–2.45)	0.25
Pathologic metastasis status	pM0 versus pM1	2.61 (1.48–4.60)	0.00091[a]
Tumor Grade	Grade 1 + Grade 2 versus Grade 3	1.74 (0.93–3.25)	0.82
Tumor Size (cm)	<2.0 versus ≥2.0	1.78 (0.94–3.58)	0.077
Surgical margin	negative versus positive	1.81 (1.15–2.84)	0.011[a]
Chemoradiotherapy	received versus not received	0.67 (0.34–1.20)	0.18
Multivariate analysis			
aPKCλ/ι expression	high versus low	2.71 (1.68–4.40)	0.0000056[a]
Pathologic metastasis status	pM0 versus pM1	2.83 (1.59–5.03)	0.00041[a]
Tumor Grade	Grade 1 + Grade 2 versus Grade 3	2.01 (1.06–3.79)	0.032[a]
Chemoradiotherapy	received versus not received	0.54 (0.29–0.98)	0.041[a]

The result of the model chi-square was significant ($p < 0.001$).
Variables were selected by the forward selection method (likelihood ratio test).
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.
[a] Significant.

important effector of oncogenic K-ras, both *in vitro* and in *vivo* [21,56–61]. Therefore, one possibility is that of aPKCλ/ι playing a role as a critical downstream K-ras effector molecule.

The other possibility is involvement of IL-6 mediated signaling. IL-6, a cytokine involved in immune and hematopoietic activities, has been implicated in the progression of a variety of human cancers [62]. We demonstrated in an experiment conducted at our laboratory, that aPKCλ/ι activates transcription of the IL-6 gene and promotes the growth prostate cancer cells [22,63]. In fact, significantly enhanced IL-6 production has been shown in pancreatic cancer patients with cachexia [64,65]. Thus, there is a possibility that aPKCλ/ι induces tumor progression by stimulating IL-6 production in pancreatic cancer.

Table 4
Hazard ratios of clinicopathologic characteristics for overall survival in IPMN.

Variables	Categories	Overall survival	
		HR (95% CI)	*p*
Univariate analysis			
aPKCλ/ι expression	high versus low	5.25 (1.63–16.89)	0.0053[a]
Age, years	<65 versus ≥65	1.71 (0.52–5.60)	0.37
Histological grade	IPMN with low, intermediate and high grade dysplasia versus IPMN with an associated invasive carcinoma	12.61 (2.79–57.26)	0.0010[a]
Macroscopic type	Branch versus Mixed and Main	2.38 (0.66–8.67)	0.19
Morphological types	Gastric type versus others	4.22 (0.93–19.04)	0.61
Multivariate analysis			
aPKCλ/ι expression	high versus low	5.23 (1.47–18.55)	0.010[a]
Histological grade	IPMN with low, intermediate and high grade dysplasia versus IPMN with an associated invasive carcinoma	12.50 (2.71–57.70)	0.0012[a]

The result of the model chi-square was significant ($p < 0.001$).
Variables were selected by the forward selection method (likelihood ratio test).
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.
[a] Significant.

One of the limitations of our study was the small number of cases of IPMN. Furukawa et al. analyzed the prognostic relevance of the morphological types of IPMN using a sufficiently large number of cases ($n = 283$), and reported that morphological type was significantly associated with the patient prognosis, and that gastric type IPMN was associated with the best prognosis amongst the morphological types [66]. However, in our study, we could not demonstrate any superior prognosis of the gastric type IPMNs as compared to the other morphological types of IPMN (Table 4). This finding could suggest that our sample may not have been correctly representative of the population of IPMN. Thus, further analyses of a large number of cases are needed to confirm our results.

In conclusion, our data show the expression level of aPKCλ/ι as a promising prognostic marker common to different types of pancreatic neoplasms. Although further analysis is needed for clinical application, the accumulation of the knowledge of possible prognostic biomarkers that are independent of standard clinicopathological staging will help the decision of application of highly invasive treatment.

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References

[1] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277–300.
[2] Tanaka T, Ikeda M, Okusaka T, Ueno H, Morizane C, Hagihara A, et al. Prognostic factors in Japanese patients with advanced pancreatic cancer treated with single-agent Gemcitabine as first-line therapy. Jpn J Clin Oncol 2008;38:755–61.
[3] Hidalgo M. Pancreatic cancer. N Engl J Med 2010;362:1605–17.
[4] Yeo CJ, Cameron JL, Lillemoe KD, Sohn TA, Campbell KA, Sauter PK, et al. Pancreaticoduodenectomy with or without distal gastrectomy and extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma. Part 2. Randomized controlled trial evaluating survival, morbidity, and mortality. Ann Surg 2002;236:355–66.
[5] Miller RC, Iott MJ, Corsini MM. Review of adjuvant radiochemotherapy for resected pancreatic cancer and results from Mayo Clinic for the 5th JUCTS symposium. Int J Radiat Oncol Biol Phys 2009;75:364–8.

[6] Tonini G, Pantano F, Vincenzi B, Gabbrielli A, Coppola R, Santini D. Molecular prognostic factors in patients with pancreatic cancer. Expert Opin Ther Targets 2007;11:1553–69.
[7] Akimoto K, Mizuno K, Osada S, Hirai S, Tanuma S, Suzuki K, et al. A new member of the third class in the protein kinase C family, PKC lambda, expressed dominantly in an undifferentiated mouse embryonal carcinoma cell line and also in many tissues and cells. J Biol Chem 1994;269: 12677–83.
[8] Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 1995;9:484–96.
[9] Akimoto K, Takahashi R, Moriya S, Nishioka N, Takayanagi J, Kimura K, et al. EGF or PDGF receptors activate atypical PKClambda through phosphatidylinositol 3-kinase. EMBO J 1996;15:788–98.
[10] Akimoto K, Nakaya M, Yamanaka T, Tanaka J, Matsuda S, Weng QP, et al. Atypical protein kinase Clambda binds and regulates p70 S6 kinase. Biochem J 1998;335:417–24.
[11] Suzuki A, Akimoto K, Ohno S. Protein kinase Cλ/ι (PKCλ/ι): aPKC isotype essential for the development of multicellular organism. J Biochem 2003;133: 9–16.
[12] Ohno S. Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. Curr Opin Cell Biol 2001;13:641–8.
[13] Diaz-Meco MT, Berra E, Municio MM, Sanz L, Lozano J, Dominguez I, et al. A dominant negative protein kinase C zeta subspecies blocks NF-kappa B activation. Mol Cell Biol 1993;13:4770–5.
[14] Moscat J, Diaz-Meco MT, Albert A, Campuzano S. Cell signaling and function organized by PB1 domain interactions. Mol Cell 2006;23:631–40.
[15] Sanz L, Sanchez P, Lallena MJ, Diaz-Meco MT, Moscat J. The interaction of p62 with RIP links the atypical PKCs to NFκB activation. EMBO J 1999;18:3044–53.
[16] Regala RP, Weems C, Jamieson L, Khoor A, Edell ES, Lohse CM, et al. Atypical protein kinase C iota is an oncogene in human non-small cell lung cancer. Cancer Res 2005;65:8905–11.
[17] Regala RP, Weems C, Jamieson L, Copland JA, Thompson EA, Fields AP. Atypical protein kinase Ciota plays a critical role in human lung cancer cell growth and tumorigenicity. J Biol Chem 2005;280:31109–15.
[18] Eder AM, Sui X, Rosen DG, Nolden LK, Cheng KW, Lahad JP, et al. Atypical PKCiota contributes to poor prognosis through loss of apical-basal polarity and cyclin E overexpression in ovarian cancer. Proc Natl Acad Sci U S A 2005;102:12519–24.
[19] Zhang L, Huang J, Yang N, Liang S, Barchetti A, Giannakakis A, et al. Integrative genomic analysis of protein kinase C (PKC) family identifies PKCiota as a biomarker and potential oncogene in ovarian carcinoma. Cancer Res 2006;66: 4627–35.
[20] Kojima Y, Akimoto K, Nagashima Y, Ishiguro H, Shirai S, Chishima T, et al. The overexpression and altered localization of the atypical protein kinase C lambda/iota in breast cancer correlates with the pathologic type of these tumors. Hum Pathol 2008;39:824–31.
[21] Murray NR, Jamieson L, Yu W, Zhang J, Gökmen-Polar Y, Sier D, et al. Protein kinase Ciota is required for Ras transformation and colon carcinogenesis in vivo. J Cell Biol 2004;164:797–802.
[22] Ishiguro H, Akimoto K, Nagashima Y, Kojima Y, Sasaki T, Ishiguro-Imagawa Y, et al. aPKClambda/iota promotes growth of prostate cancer cells in an autocrine manner through transcriptional activation of interleukin-6. Proc Natl Acad Sci U S A 2009;106:16369–74.
[23] Takagawa R, Akimoto K, Ichikawa Y, Akiyama H, Kojima Y, Ishiguro H, et al. High expression of atypical protein kinase C lambda/iota in gastric cancer as a prognostic factor for recurrence. Ann Surg Oncol 2010;17:81–8.
[24] Scotti ML, Bamlet WR, Smyrk TC, Fields AP, Murray NR. Protein kinase Ciota is required for pancreatic cancer cell transformed growth and tumorigenesis. Cancer Res 2010;70:2064–74.
[25] Chari ST, Yadav D, Smyrk TC, DiMagno EP, Miller LJ, Raimondo M, et al. Study of recurrence after surgical resection of intraductal papillary mucinous neoplasm of the pancreas. Gastroenterology 2002;123(5):1500–7.
[26] Salvia R, Fernández-del Castillo C, Bassi C, Thayer SP, Falconi M, Mantovani W, et al. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. Ann Surg 2004;239(5):678–85 [discussion 685–7].
[27] Sohn TA, Yeo CJ, Cameron JL, Hruban RH, Fukushima N, Campbell KA, et al. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. Ann Surg 2004;239(6):788–97 [discussion 797–9].
[28] Ohashi K, Murakimi Y, Maruyama M, Takekoshi T, Ohta H, Ohashi I. Four cases of mucous secreting pancreatic cancer. Prog Dig Endosc 1982;20:348–51.
[29] Yamao K, Ohashi K, Nakamura T, Suzuki T, Shimizu Y, Nakamura Y, et al. The prognosis of intraductal papillary mucinous tumors of the pancreas. Hepatogastroenterology 2000;47:1129–34.
[30] Sohn TA, Yeo CJ, Cameron JL, Iacobuzio-Donahue CA, Hruban RH, Lillemoe KD. Intraductal papillary mucinous neoplasms of the pancreas: an increasingly recognized clinicopathologic entity. Ann Surg 2001;234:313–21.
[31] Raimondo M, Tachibana I, Urrutia R, Burgart LJ, DiMagno EP. Invasive cancer and survival of intraductal papillary mucinous tumors of the pancreas. Am J Gastroenterol 2002;97:2553–8.
[32] Maire F, Hammel P, Terris B, Paye F, Scoazec JY, Cellier C, et al. Prognosis of malignant intraductal papillary mucinous tumours of the pancreas after surgical resection. Comparison with pancreatic ductal adenocarcinoma. Gut 2002;51:717–22.
[33] Sohn TA, Yeo CJ, Cameron JL, Hruban RH, Fukushima N, Campbell KA, et al. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. Ann Surg 2004;239:788–97.
[34] Suzuki Y, Atomi Y, Sugiyama M, Isaji S, Inui K, Kimura W, et al. Cystic neoplasm of the pancreas: a Japanese multiinstitutional study of intraductal papillary mucinous tumor and mucinous cystic tumor. Pancreas 2004;28:241–6.
[35] Shimada K, Sakamoto Y, Sano T, Kosuge T, Hiraoka N. Invasive carcinoma originating in an intraductal papillary mucinous neoplasm of the pancreas: a clinicopathologic comparison with a common type of invasive ductal carcinoma. Pancreas 2006;32:281–7.
[36] Woo SM, Ryu JK, Lee SH, Yoo JW, Park JK, Kim YT, et al. Survival and prognosis of invasive intraductal papillary mucinous neoplasms of the pancreas: comparison with pancreatic ductal adenocarcinoma. Pancreas 2008;36:50–5.
[37] Murakami Y, Uemura K, Sudo T, Hayashidani Y, Hashimoto Y, Nakashima A, et al. Invasive intraductal papillary-mucinous neoplasm of the pancreas: comparison with pancreatic ductal adenocarcinoma. J Surg Oncol 2009;100:13–8.
[38] D'Angelica M, Brennan MF, Suriawinata AA, Klimstra D, Conlon KC. Intraductal papillary mucinous neoplasms of the pancreas: an analysis of clinicopathologic features and outcome. Ann Surg 2004;239:400–8.
[39] Raut CP, Cleary KR, Staerkel GA, Abbruzzese JL, Wolff RA, Lee JH. Intraductal papillary mucinous neoplasms of the pancreas: effect of invasion and pancreatic margin status on recurrence and survival. Ann Surg Oncol 2006;13: 582–94.
[40] Poultsides GA, Reddy S, Cameron JL, Hruban RH, Pawlik TM, Ahuja N, et al. Histopathologic basis for the favorable survival after resection of intraductal papillary mucinous neoplasm-associated invasive adenocarcinoma of the pancreas. Ann Surg 2010;251:470–6.
[41] Schnelldorfer T, Sarr MG, Nagorney DM, Zhang L, Smyrk TC, Qin R, et al. Experience with 208 resections for intraductal papillary mucinous neoplasm of the pancreas. Arch Surg 2008;143:639–46.
[42] Adsay NV, Fukushima N, Furukawa T, Hruban RH, Klimstra DS, Klöppel G, et al. Intraductal neoplasms of the pancreas. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system. 4th ed. Lyons, France: IARC Press; 2010. p. 304–13.
[43] Hruban RH, Boffetta P, Hiraoka N, Iacobuzio-Donahue C, Kato Y, Kern SE, et al. Ductal adenocarcinoma of the pancreas. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system. Lyons, France: IARC Press; 2010. p. 281–91.
[44] Hermanek P, Hutter RVP, Sobin LH, Wagner G, Wittekind Ch. TNM Atlas. 4th ed. Berlin: Springer-Verlag; 1997. p. 144–53.
[45] Japan Pancreas Society. Classification of pancreatic carcinoma (English ed.). Tokyo: Kanehara; 1998. p. 39.
[46] Tanaka M, Chari S, Adsay V, Fernandez-del Castillo C, Falconi M, Shimizu M, et al. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology 2006;6:17–32.
[47] Crippa S, Fernández-Del Castillo C, Salvia R, Finkelstein D, Bassi C, Domínguez I, et al. Mucin-producing neoplasms of the pancreas: an analysis of distinguishing clinical and epidemio logic characteristics. Clin Gastroenterol Hepatol 2010;8:213–9.
[48] Furukawa T, Klöppel G, Volkan Adsay N, Albores-Saavedra J, Fukushima N, Horii A, et al. Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study. Virchows Arch 2005;447:794–9.
[49] Basturk O, Khayyata S, Klimstra DS, Hruban RH, Zamboni G, Coban I, et al. Preferential expression of MUC6 in oncocytic and pancreatobiliary types of intraductal papillary neoplasms highlights a pyloropancreatic pathway, distinct from the intestinal pathway, in pancreatic carcinogenesis. Am J Surg Pathol 2010;34:364–70.
[50] Hruban RH, Pitman MB, Klimstra DS. Intraductal neoplasms. In: Hruban RH, Pitman MB, Klimstra DS, editors. Tumors of the Pancreas. Washington, DC: Amrican Registry of Pathology; 2007. p. 75–110.
[51] Hruban RH, Pitman MB, Klimstra DS. Adenocarcinoma variants. In: Hruban RH, Pitman MB, Klimstra DS, editors. Tumors of the pancreas. Washington, DC: American Registry of Pathology; 2007. p. 165–90.
[52] Li Q, Wang JM, Liu C, Xiao BL, Lu JX, Zou SQ. Correlation of aPKC-iota and E-cadherin expression with invasion and prognosis of cholangiocarcinoma. Hepatobiliary Pancreat Dis Int 2008;7:70–5.
[53] Maitra A, Fukushima N, Takaori K, Hruban RH. Precursors to invasive pancreatic cancer. Adv Anat Pathol 2005;12:81–91.
[54] Klimstra DS, Longnecker DS. K-ras mutations in pancreatic ductal proliferative lesions. Am J Pathol 1994;145:1547–50.
[55] Aoki K, Yoshida T, Matsumoto N, Ide H, Sugimura T, Terada M. Suppression of Ki-ras p21 levels leading to growth inhibition of pancreatic cancer cell lines with Ki-ras mutation but not those without Ki-ras mutation. Mol Carcinog 1997;20:251–8.
[56] Zhang J, Anastasiadis PZ, Liu Y, Thompson EA, Fields AP. Protein kinase C (PKC) βII induces cell invasion through a Ras/Mek-, PKCι/Rac 1-dependent signaling pathway. J Biol Chem 2004;279:22118–23.
[57] Bjorkoy G, Perander M, Overvatn A, Johansen T. Reversion of Rasand phosphatidylcholine-hydrolyzing phospholipase C-mediated transformation of NIH 3T3 cells by a dominant interfering mutant of protein kinase Cλ is accompanied by the loss of constitutive nuclear mitogen-activated protein kinase/extracellular signal-regulated kinase activity. J Biol Chem 1997;272: 11557–65.

[58] Kampfer S, Windegger M, Hochholdinger F, Schwaiger W, Pestell RG, Baier G, et al. Protein kinase C isoforms involved in the transcriptional activation of cyclin D1 by transforming Ha-Ras. J Biol Chem 2001;276:42834–42.
[59] Uberall F, Hellbert K, Kampfer S, Maly K, Villunger A, Spitaler M, et al. Evidence that atypical protein kinase C-λ and atypical protein kinase C-ζ participate in Ras-mediated reorganization of the F-actin cytoskeleton. J Cell Biol 1999;144:413–25.
[60] Coghlan MP, Chou MM, Carpenter CL. Atypical protein kinases Cλ and -ζ associate with the GTP-binding protein Cdc42 and mediate stress fiber loss. Mol Cell Biol 2000;20:2880–9.
[61] Regala RP, Davis RK, Kunz A, Khoor A, Leitges M, Fields AP. Atypical protein kinase Cι is required for bronchioalveolar stem cell expansion and lung tumorigenesis. Cancer Res 2009;69:7603–11.
[62] Hong DS, Angelo LS, Kurzrock R. Interleukin-6 and its receptor in cancer: implications for translational therapeutics. Cancer 2007;110:1911–28.
[63] Ishiguro H, Akimoto K, Nagashima Y, Kagawa E, Sasaki T, Sano JY, et al. Coexpression of aPKCλ/ι and IL-6 in prostate cancer tissue correlates with biochemical recurrence. Cancer Sci 2011;102:1576–81.
[64] Okada S, Okusaka T, Ishii H, Kyogoku A, Yoshimori M, Kajimura N, et al. Elevated serum interleukin-6 levels in patients with pancreatic cancer. Jpn J Clin Oncol 1998;28:12–5.
[65] Martignoni ME, Kunze P, Hildebrandt W, Künzli B, Berberat P, Giese T, et al. Role of mononuclear cells and inflammatory cytokines in pancreatic cancer-related cachexia. Clin Cancer Res 2005;11:5802–8.
[66] Furukawa T, Hatori T, Fujita I, Yamamoto M, Kobayashi M, Ohike N, et al. Prognostic relevance of morphological types of intraductal papillary mucinous neoplasms of the pancreas. Gut 2011;60:509–16.

Positioning of nasobiliary tube using magnet-loaded catheters

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Bibliography
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Background and study aims: In endoscopic nasobiliary drainage (ENBD), repositioning the catheter from the mouth to the nose is complicated. We devised a method using catheters with magnets and verified its utility and safety.

Patients and methods: We prospectively enrolled 20 patients undergoing ENBD at Yokohama City University Hospital.

Results: The procedures were successful in all 20 cases and no case required a change of operators to a senior doctor. The mean time for the procedure was 36.6 seconds. The emetic reflex was induced 0.5 times on average using the magnet method. The mean X-ray exposure time was 29.6 seconds. No complications occurred.

Conclusions: The magnet-loaded catheter method for positioning the ENBD catheter before finally leading it through the nose took little time and was performed successfully and safely. Therefore, the magnet method could become the first choice among techniques for ENBD catheter placement.

Introduction

Endoscopic nasobiliary drainage (ENBD) is used with endoscopic retrograde cholangiopancreatography (ERCP) and has been employed for over 30 years [1,2]. ENBD has recently been replaced by endoscopic retrograde biliary drainage (ERBD). Nevertheless, ENBD remains useful, for drainage in cholangitis; for cytological investigation, as it provides a continuous biliary sample [3,4]; in extracorporeal shock wave lithotripsy (ESWL) for biliary stones, to permit contrast injection and focusing on the stone [5]; and in treatment for perforation of the bile duct [6,7]. Also, the efficacy of preoperative ENBD for hilar and superior cholangiocarcinoma has recently been reported [8–10].

However, because leading the ENBD catheter from the mouth to the nose is painful for patients and complicated for operators, ENBD has been avoided. Currently, the finger method and the guide wire method [11] have been proposed as alternative placement techniques. Each method has some advantages, but also disadvantages. The finger method is speedy, but painful for patients and operators. The guide wire method is not painful, but is complicated for operators. We have devised a novel easier and quicker method using magnets, and have evaluated this technique for utility and safety.

Patients and methods

This was a prospective case series at a single center. Patients who underwent ENBD at Yokohama City University Hospital were included in the study, but patients were excluded if they had previously received a pacemaker. All patients gave written informed consent before the ENBD procedure and the study was performed in accordance with the ethical standards of the committees of the institute and the Declaration of Helsinki (Protocol B110901010, 1 October 2011), and was registered (ClinicalTrials.gov, ID UMIN000008118).

For sedation and analgesia, 5 mg of midazolam and 7.5 mg of pentazocine were administered for induction, and additional amounts given as needed. The ENBD tubes were placed under fluoroscopic guidance using a duodenal endoscope (JF-260V; Olympus, Tokyo, Japan). The ENBD tube was in all cases a 6-Fr reverse alpha type, and we used a Nelaton tube with two side holes. A trainee physician with 1–3 years' experience attempted placement of the ENBD tubes using the magnet method. If they failed to lead the ENBD tube to the mouth within 3 minutes, a senior gastroenterologist took over.

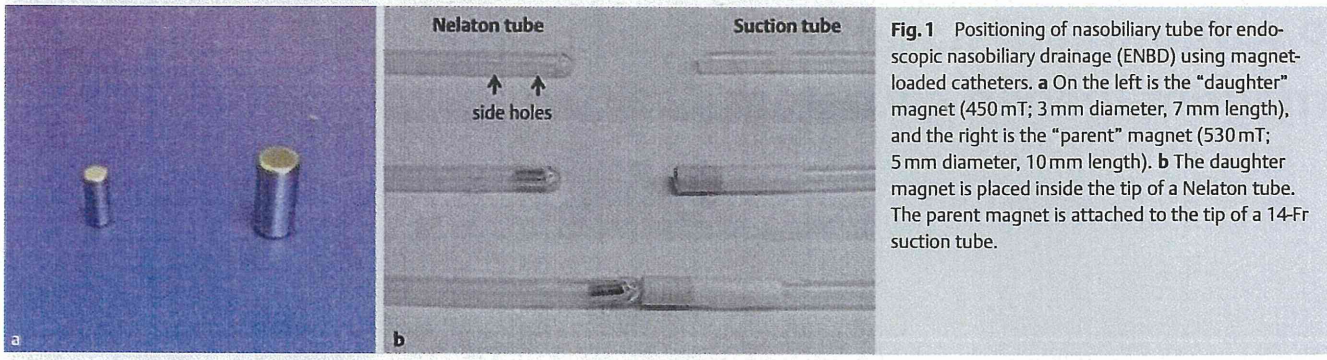

Fig. 1 Positioning of nasobiliary tube for endoscopic nasobiliary drainage (ENBD) using magnet-loaded catheters. **a** On the left is the "daughter" magnet (450 mT; 3 mm diameter, 7 mm length), and the right is the "parent" magnet (530 mT; 5 mm diameter, 10 mm length). **b** The daughter magnet is placed inside the tip of a Nelaton tube. The parent magnet is attached to the tip of a 14-Fr suction tube.

Table 1 Positioning of nasobiliary tube for endoscopic nasobiliary drainage (ENBD) using magnet-loaded catheters. Patient characteristics and results.

Patient no.	Gender; age; years	Diagnosis	Time, seconds	Emetic reflex, n	X-ray exposure, seconds
1	M; 75	Choledocholithiasis	79	0	52
2	M; 74	Cholangiocellular carcinoma	28	0	22
3	M; 80	Cholangitis	22	0	15
4	M; 76	Lymph node metastasis	30	0	25
5	M; 78	Gallbladder cancer	42	0	39
6	M; 41	Gallbladder cancer	34	0	30
7	F; 72	Gallbladder cancer	25	0	12
8	M; 49	Intrahepatic cholangiocarcinoma	40	3	40
9	M; 54	Intrahepatic cholangiocarcinoma	23	1	14
10	M; 73	Klatskin tumor	70	0	58
11	F; 55	Klatskin tumor	36	2	20
12	F; 55	Klatskin tumor	14	0	14
13	M; 53	Pancreatic cancer	37	0	32
14	M; 61	Pancreatic cancer	27	0	27
15	M; 75	Pancreatic cancer	31	0	31
16	M; 80	Pancreatic cancer	40	0	30
17	F; 72	Pancreatic cancer	40	0	40
18	M; 68	Pancreatic cancer	23	1	16
19	M; 69	Pancreatic cancer	18	0	9
20	F; 58	Post liver transplantation	73	2	66

Magnet technique

This method requires a "parent" magnet and a "daughter" magnet (**Fig. 1a**). The neodymium (Nd2Fe14B) rare-earth "daughter" magnet (450 mT; 3 mm diameter, 7 mm length) was placed inside the tip of the Nelaton tube through the side hole; the end hole was sealed to prevent the loss of the magnet. The "parent" magnet (neodymium rare-earth; 530 mT; 5 mm diameter, 10 mm length) was attached to the tip of a 14-Fr suction tube (**Fig. 1b**).

With the patient under sedation and having a mouthpiece fitted, the Nelaton tube with the daughter magnet was inserted through a nostril to the pharynx. Then the suction tube with the parent magnet was inserted through the mouthpiece. This attracted the daughter magnet in the Nelaton tube. The Nelaton tube was led out of the mouth (**Fig. 2**, **Video 1**).

Video 1

Placement of nasobiliary tube using magnet-loaded catheters. The Nelaton tube with a magnet is inserted through the nostril. The suction tube with a magnet is inserted through the mouth. The two magnets attract each other. Then the suction tube is led back out of the mouth with the Nelaton tube attached. The procedure was easy because of the good visibility of magnets under X-ray fluoroscopy. There was no emetic reflex movement during this procedure.

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Evaluation of the method

We evaluated the time for the procedure, measuring from the moment of insertion of the Nelaton tube into the nostril to when the Nelaton tube was led out of the mouth. We recorded the X-ray exposure, and the number of times the emetic reflex was induced. We also assessed complications.

Results

A total of 20 patients were consecutively enrolled between December 2011 and June 2012. The clinical features of all patients are presented in **Table 1**.

The results are also shown in **Table 1**. The procedures were successful in all cases and in no case was a change in operator to a senior doctor required. The mean time for the procedure was 36.6 seconds (range 14–79). The mean number of times the emetic reflex was induced was 0.5 (0–3). The mean X-ray exposure time was 29.6 seconds (9–66). Patients undergoing the magnet method reported no memory of the procedure and no discomfort during the procedure, nor was there any pharyngeal

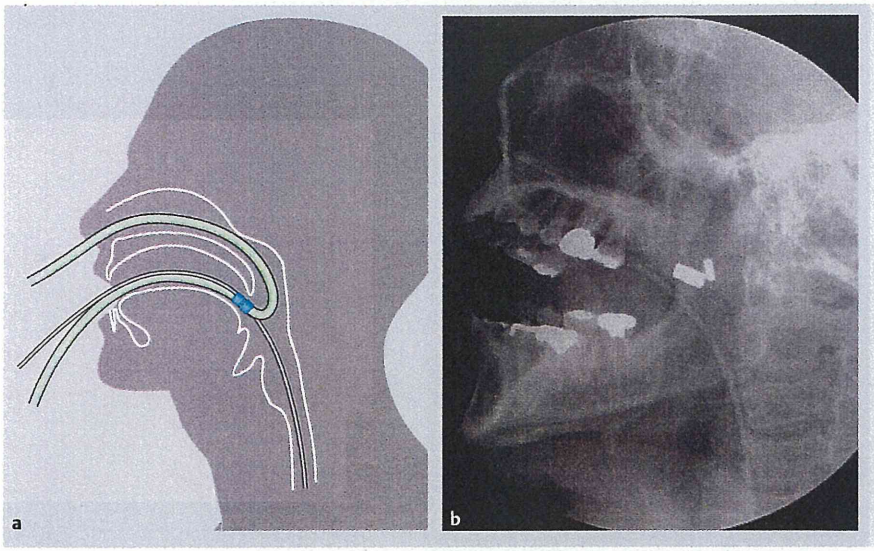

Fig. 2 Placement of nasobiliary tube using magnet-loaded catheters. **a** The Nelaton tube with the "daughter" magnet has been inserted through the nostril. The suction tube with the "parent" magnet has been inserted through the mouth. The two magnets attract each other. The suction tube is led back out of the mouth with the Nelaton tube attached. **b** X-ray image.

pain 2 hours later. There were no complications using the magnet method.

Discussion

In this prospective study, the magnet method was an excellent technique for repositioning the ENBD catheter through the nose. This method was superior in terms of feasibility, safety, and visibility, allowed placement in a shorter time and was less invasive than conventional methods. Even trainees were able to carry out this maneuver safely and reliably. The parts of the device are simple, available and cheap, and preparing the equipment is easy and takes only a few minutes. Thus, there is a definite possibility that the magnet method could become the first choice in ENBD.

The only concern was that the magnets could fall away from the tube. In the future, we need to develop a catheter in which the magnet is completely embedded.

Several techniques using magnets in the digestive system have been previously reported [12, 13]. These have mentioned a magnetic effect upon pacemakers and have stated that although the magnet is powerful, most of its effects can be ignored at a distance of 15 cm from the magnet [14]. We excluded pacemaker patients in this study as a precaution.

Our study had several limitations. First, it included a relatively small number of patients, and secondly, it was a single-center study. Consequently, further multicenter studies will be required to assess this new method.

In conclusion, the magnet method was found to be a superior method for leading the ENBD catheter to the nose, with regard to time required, success rate, and safety. Therefore, the magnet method is a better first-line choice than the conventional techniques. Additionally, although the methods for repositioning the ENBD tube are not directly involved in the treatment, the difficulty of this step has been one of the reasons that physicians and patients avoid ENBD. Taking into account the magnet method and, thus, the reduction in the complexity of ENBD and in the pain for patients, we hope that physicians will reconsider the use of ENBD.

Competing interests: None

References

1 *Nagai N, Toki F, Oi I* et al. Continuous endoscopic pancreatocholedochal catheterization. Gastrointest Endosc 1976; 23: 78–81
2 *Cotton PB, Burney PG, Mason RR.* Transnasal bile duct catheterization after endoscopic sphincterotomy. Gut 1979; 20: 285–287
3 *Yagioka H, Hirano K, Isayama H* et al. Clinical significance of bile cytology via an endoscopic nasobiliary drainage tube for pathological diagnosis of malignant biliary strictures. J Hepatobiliary Pancreat Sci 2011; 18: 211–215
4 *Uchida N, Kamada H, Ono M* et al. How many cytological examinations should be performed for the diagnosis of malignant biliary stricture via an endoscopic nasobiliary drainage tube? J Gastroenterol Hepatol 2008; 23: 1501–1504
5 *Sauerbruch T, Stern M.* Fragmentation of bile duct stones by extracorporeal shock waves. A new approach to biliary calculi after failure of routine endoscopic measures. Gastroenterology 1989; 96: 146–152
6 *Park SY, Park CH, Cho SB* et al. The safety and effectiveness of endoscopic biliary decompression by plastic stent placement in acute suppurative cholangitis compared with nasobiliary drainage. Gastrointest Endosc 2008; 68: 1076–1080
7 *Zhou PH, Yao LQ, Zhang YQ* et al. Endoscopic biliary drainage for biliary obstruction. Hepatobiliary Pancreat Dis Int 2003; 2: 598–601
8 *Maguchi H, Takahashi K, Katanuma A* et al. Preoperative biliary drainage for hilar cholangiocarcinoma. J Hepatobiliary Pancreat Surg 2007; 14: 441–446 Epub 2007 Sep 28
9 *Arakura N, Takayama M, Ozaki Y* et al. Efficacy of preoperative endoscopic nasobiliary drainage for hilar cholangiocarcinoma. J Hepatobiliary Pancreat Surg 2009; 16: 473–477
10 *Kawakami H, Kuwatani M, Onodera M* et al. Endoscopic nasobiliary drainage is the most suitable preoperative biliary drainage method in the management of patients with hilar cholangiocarcinoma. J Gastroenterol 2011; 46: 242–248
11 *Kunishi Y, Kaneko T, Sugimori K.* Introduction of roping techniques: a new induction method of ENBD tube to nostril using a guide wire [in Japanese]. Gastroenterol Endosc 2012; 54: 2046–2047
12 *Yamanouchi E, Kawagichi H, Endo I* et al. A new interventional method of anastomosis with magnets: magnetic compression anastomosis in five clinical cases. Radiology 1998; 209: 567
13 *Yamanouchi E, Sugiura T, Yasuda Y* et al. Review of 30 cases of magnetic compression anastomosis (MCA). Radiology 2000; 217: 584
14 *Yamanouchi E, Kumano R, Kobayashi K* et al. Treatment for bowel or biliary obstruction by magnetic compression anastomosis: development of Yamanouchi's method and its clinical evaluation [in Japanese]. J Nippon Med Sch 2002; 69: 471–475

LIPIDS IN HEALTH AND DISEASE

RESEARCH Open Access

Critical role of leukotriene B_4 receptor signaling in mouse 3T3-L1 preadipocyte differentiation

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Abstract

Background: Various inflammatory mediators related to obesity might be closely related to insulin resistance. Leukotrienes (LTs) are involved in inflammatory reactions. However, there are few reports regarding the role of LTs in adipocyte differentiation. Therefore, we investigated the role of leukotriene B_4 (LTB_4)-leukotriene receptor (BLT) signaling in mouse 3T3-L1 fibroblastic preadipocyte differentiation to mature adipocytes.

Methods: Mouse 3T3-L1 preadipocytes were treated with lipoxygenase (LOX) inhibitors, BLT antagonist, and small interfering RNA (siRNA) for BLT1 and BLT2 to block the LTB_4-BLT signaling pathway, then the adipocyte differentiation such as lipid accumulation and the increase in triglyceride was evaluated.

Results: Blockade of BLT signaling by treatment with a LOX inhibitor or a BLT antagonist suppressed preadipocyte differentiation into mature adipocytes. In addition, knockdown of BLT1 and BLT2 by siRNAs dramatically inhibited differentiation. These results indicate the LTB_4-BLT signaling pathway may positively regulate preadipocyte differentiation and be a rate-limiting system to control adipocyte differentiation.

Conclusions: The LTB_4-BLT signaling pathway provides a potent regulatory signal that accelerates the differentiation of mouse 3T3-L1 preadipocytes. Further investigations are necessary to confirm the exact role of LTB_4 and BLTs signaling pathways in preadipocyte differentiation.

Keywords: Leukotrienes, Preadipocyte differentiation, Mouse 3T3-L1 fibroblasts, BLT, siRNA

Background

Diabetes mellitus, hyperlipidemia, hypertension, and atherosclerosis have recently been defined as typical life style-related diseases. A common background of these diseases is obesity, which is thought to cause insulin resistance resulting in the onset of disease [1,2]. Recently, the incidence of obesity and associated metabolic syndrome has dramatically increased. Although high caloric western-style foods are believed to be the main cause of this dramatic increase, other possible risk factors could exist. The involvement of various inflammatory mediators such as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6) on obesity might be closely related to insulin resistance [1-4]. One of the most important organs in obesity and insulin resistance are the adipose tissues, as adipocytes generate adipocytokines that are important in the onset of metabolic syndrome [1,2,5].

Leukotrienes (LTs) such as leukotriene B_4, C_4, and D_4 (LTB_4, LTC_4, and LTD_4, respectively) are generated through lipoxygenase (LOX) pathways and induce inflammatory and allergic reactions such as leukocyte activation, capillary permeability, and bronchial contraction [6,7]. LTB_4 binds to specific receptors, BLT1 and BLT2, to activate signaling pathways [8,9]. LTs have been reported to be involved in the proliferation of epithelial, endothelial and mesangial cells [10,11]. In addition, we previously reported that LTB_4 controls immature neural stem cell proliferation and differentiation via the BLT signaling pathway [12]. Thus, LTB_4 and its signaling pathway might be involved in cell proliferation and differentiation. However, there have been few reports regarding the role of LTs in adipocyte differentiation.

In this study, we investigated the role of LTB_4 and BLTs signaling pathways in preadipocyte differentiation

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in a mouse fibroblastic 3T3-L1 cell line, a widely used cell line for research of preadipocyte differentiation [13]. We analyzed the effects of LOX inhibitors, a BLT antagonist and BLTs-specific siRNAs on the differentiation of 3T3-L1 to clarify the function of BLTs on preadipocyte differentiation. Our results suggest a potentially important and novel role for LTB_4 and BLT functions on preadipocyte differentiation.

Results

Effects of LOX inhibitors and BLT antagonist on mouse 3T3-L1 preadipocyte differentiation

Mouse 3T3-L1 cells can differentiate from fibroblastic cells into mature adipocytes in induction medium for differentiation containing insulin (INS), dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX) and rosiglitazone (ROSI), a specific ligand for peroxisome proliferator-activated receptor gamma (PPARγ) [13,14]. We used these induction conditions for the differentiation of mouse 3T3-L1 preadipocytes (Figure 1). Nordihydroguaiaretic acid (NDGA), a pan-LOX inhibitor, inhibited the accumulation of lipids, the decrease of triacylglycerol (TG) contents and the index of mouse 3T3-L1 preadipocyte differentiation into mature adipocytes (Figure 2A). No alterations in cell proliferation were observed under our experimental conditions (data not shown). AA-861, a 5-LOX inhibitor, also inhibited the differentiation of mouse 3T3-L1 preadipocytes into mature adipocytes (Figure 2B). These

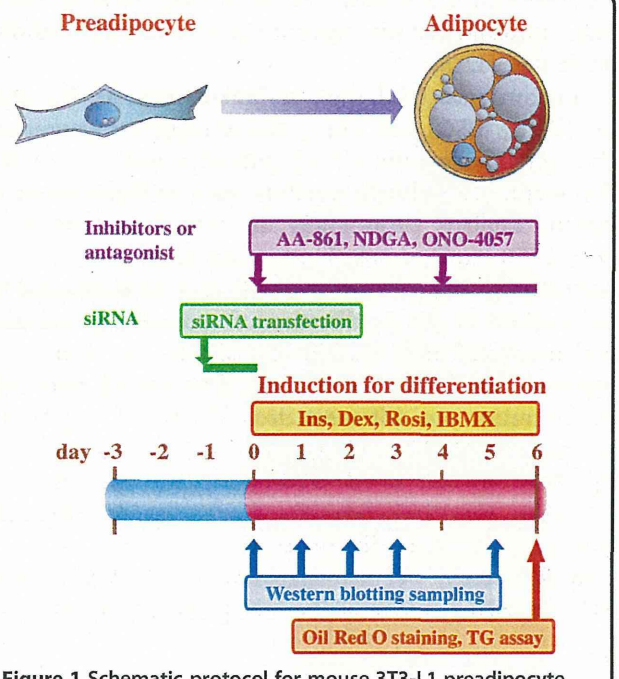

Figure 1 Schematic protocol for mouse 3T3-L1 preadipocyte differentiation.

suppressive effects occurred in a concentration-dependent manner. In addition, ONO-4057, a specific LTB_4 receptor antagonist, suppressed mouse 3T3-L1 preadipocyte differentiation (Figure 2C). These results suggest that the LTB_4-BLT signaling pathway may be involved in preadipocyte differentiation.

Effect of BLT1 and BLT2 knockdown by siRNA on mouse 3T3-L1 preadipocyte differentiation

To investigate whether BLTs are expressed on preadipocytes, we performed western blot analysis, which showed that both BLT1 and BLT2 were expressed in mouse 3T3-L1 preadipocytes from the start to late phases of differentiation (Figure 3). In addition, the level of LTB_4 secreted from preadipocytes into the culture medium was 31.8 ± 8.4 nmol/L (mean ± SEM, n=3). These results indicate that the BLT-signaling pathway induces the differentiation of preadipocytes to mature adipocytes.

Therefore, we designed siRNAs specific for BLT1 or BLT2 to knockdown receptor expression. siRNAs against BLT1 and BLT2 successfully suppressed the expression of BLT1 and BLT2 (Figure 4A). Indicators of preadipocyte differentiation such as lipid accumulation and TG contents were decreased by BLT1 siRNA (Figure 4B and C). Similar results were observed with BLT2 siRNA (Figure 4D and E). These results clearly indicated that the LTB_4-BLT signaling pathway accelerates mouse 3T3-L1 preadipocyte differentiation, and blockade or knockdown of BLTs leads to the suppression of preadipocyte differentiation.

Combination knockdown of BLT1 and BLT2 by siRNA on mouse 3T3-L1 preadipocyte differentiation

To clarify the role of each receptor, BLT1 and BLT2, on adipocyte differentiation, we performed combination treatment of BLT1-siRNA and BLT2-siRNA. The combined treatment of BLT1-siRNA (12.5 nM) and BLT2-siRNA (12.5 nM) remarkably decreased lipid accumulation and TG contents in comparison to single knockdown (Figure 5A and B) indicating that combination knockdown of BLT1 and BLT2 by specific siRNA efficiently suppressed preadipocyte differentiation.

Discussion

The mouse fibroblastic 3T3-L1 cell line established by Green is widely used for the investigation of adipocyte differentiation [13,15,16]. The involvement of various molecules for adipocyte differentiation has been investigated using this cell line. However, it is not fully understood whether inflammation-related lipid mediators, such as LTs and prostaglandins (PGs), promote or inhibit the onset of metabolic syndrome. Several previous reports indicated that PGD_2-derived 15-deoxy-$\Delta^{12,14}$-PGJ_2 promoted

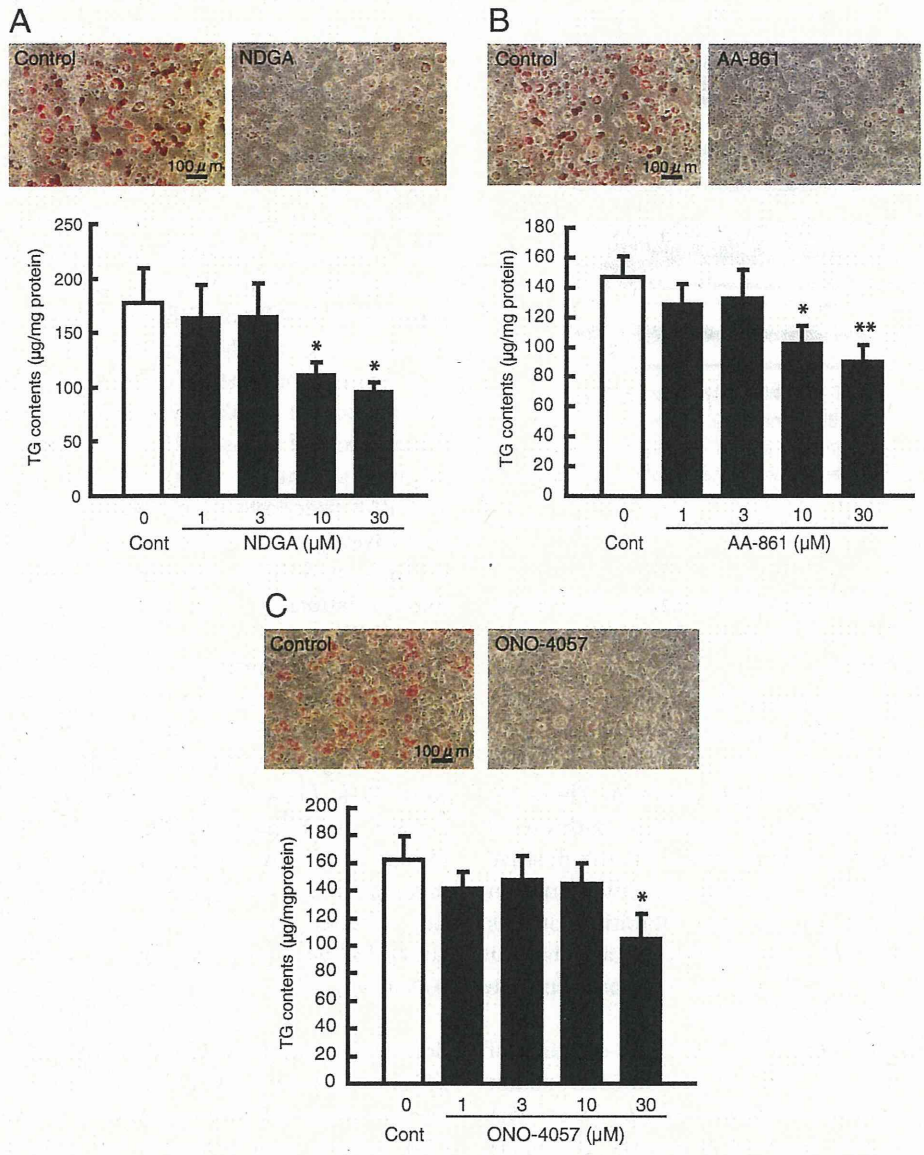

Figure 2 Effects of LOX inhibitors and the BLT antagonist on mouse 3T3-L1 preadipocyte differentiation. (A), (B) and **(C)**: Effects of NDGA **(**LOX inhibitor, **A)**, AA-861 **(**5-LOX inhibitor, **B)**, or ONO-4057 **(**a specific BLT antagonist, **C)** on lipid accumulation in mouse 3T3-L1 preadipocytes. Mouse 3T3-L1 preadipocytes were treated with NDGA, AA-861 or ONO-4057 for 6 days. Then, accumulation of triacylglycerol (TG), a marker of lipid accumulation (bottom panel), in matured adipocytes was measured and expressed as TG contents (μg/mg protein). Each column represents the mean ± SEM from 4-8 independent experiments. *$P<0.05$, **$P<0.01$ vs. vehicle control. Upper left panel shows representative photographs of differentiated mouse 3T3-L1 preadipocytes treated with vehicle for 6 days. Upper right panel shows representative photographs of undifferentiated mouse 3T3-L1 preadipocytes treated with NDGA, AA-861 or ONO-4057 for 6 days. Cells were stained with Oil Red O method to visualize lipid accumulation. Scale bar represents 100 μm.

adipocyte differentiation via activation of the PPARγ pathway [17,18]. Therefore, cyclooxygenase-related prostanoids are considered involved in the enhancement of adipocyte differentiation. In contrast, there have been few reports regarding the involvement of LOX-related metabolites in adipocyte differentiation.

In this study, 5-LOX inhibitors and a specific LTB_4 receptor antagonist inhibited the differentiation of mouse 3T3-L1 preadipocytes into mature adipocytes. Furthermore, BLT1 and BLT2 knockdown by siRNA suppressed mouse 3T3-L1 preadipocyte differentiation. In addition, combination knockdown of BLT1 and BLT2 by siRNA on mouse 3T3-L1 preadipocytes remarkably decreased lipid accumulation and TG contents in comparison to single knockdown alone. These results clearly indicate that the LTB_4-BLT signaling pathway is involved

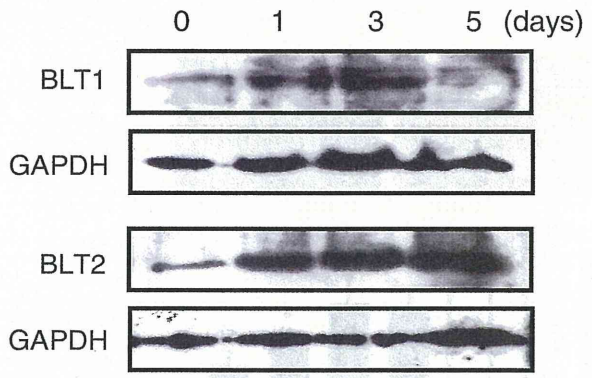

Figure 3 Expression of BLT1 and BLT2 during preadipocyte differentiation. Detection of BLT1 and BLT2 was performed by western blot analysis using anti-BLT1 or -BLT2 antibodies. GAPDH was used as an internal standard for confirmation of equally applied amounts of protein.

in mouse 3T3-L1 preadipocyte differentiation, and blockade or knockdown of BLTs leads to the suppression of preadipocyte differentiation.

Furthermore, we demonstrated that both LTB_4 receptors, BLT1 and BLT2, were expressed in mouse 3T3-L1 preadipocytes. We also confirmed the release of LTB_4 from preadipocytes into the culture medium. These results indicate that a paracrine or autocrine pathway of BLT-signaling operates in preadipocytes, for the positive regulation of mouse 3T3-L1 preadipocyte differentiation from adipocyte progenitors, because inhibition of this pathway with LOX inhibitors, a BLT antagonist, or siRNAs for BLTs induced the inhibition of preadipocyte differentiation.

Interestingly, a recent paper showed that deletion of BLT1 protected mice from high-fat diet-induced insulin resistance [19]. Such observations clearly show that BLT1 signaling is closely involved in insulin resistance. However, the results of a BLT1 knockout mouse study were considered to be due to systemic mechanisms. Therefore, the local action of BLT1 signaling on adipose tissues should be investigated. Our present data using 3T3-L1 adipocytes may partly support these previous observations. To clarify the issue, an adipocyte specific BLT1-conditional knockout mouse study is required. The involvement of BLT1 signaling may be important for adipocyte differentiation and related systemic disorders such as insulin resistance and obesity.

To investigate the potential mechanisms of BLT signaling-mediated acceleration of 3T3-L1 preadipocyte differentiation, we performed DNA microarray analysis to identify the molecules regulated by LTB_4-BLT signaling. Many molecules were significantly altered by treatment with a 5-LOX inhibitor, AA-861 or a specific BLT antagonist, ONO-4057 (unpublished data). Among them, we initially focused on the expressions of PPARγ and CCAAT-enhancer-binding protein, alpha (C/EBPα) which is known as important key transcriptional regulators to control adipocyte differentiation. However, both molecules did not show significant changes at the microarray analysis. Namely, increase in PPARγ expression was from 0.9 to 1.3-fold, that in C/EBPα expression was from 0.7 to 1.1-fold, respectively. Therefore, it is expected that LTB_4-BLT signaling pathway promoted 3T3-L1 preadipocyte differentiation via other molecules independent to PPARγ or C/EBPα. Further investigations will be required to clarify the molecules.

In conclusion, the LTB_4-BLT signaling pathway provides a potent regulatory signal that accelerates the differentiation of mouse 3T3-L1 preadipocytes. Our results imply a potentially important and novel role for LTB_4 and BLT functions on preadipocyte differentiation. Further investigations are necessary to confirm the exact role of LTB_4 and BLTs signaling pathways in preadipocyte differentiation.

Material and methods

Reagents and antibodies

INS, DEX and IBMX were purchased from Sigma Japan (Tokyo, Japan). ROSI was purchased from GlaxoSmith Kline K. K. (Tokyo, Japan). LT synthetase, LOX inhibitor, NDGA, and 5-LOX specific inhibitor, AA-861, were purchased from Sigma Japan. ONO-4057, a specific BLT antagonist, was a kind gift from ONO Pharmaceutical Co. Ltd. (Osaka, Japan). Anti-BLT1 and -BLT2 polyclonal antibodies were purchased from Cayman Chemicals (Ann Arbor, MI, USA).

Cell culture and induction of adipocyte differentiation

Mouse 3T3-L1 preadipocytes have been frequently used to study the differentiation of preadipocytes in vitro. Cell culture and induction of differentiation of preadipocytes were performed according to the previously described method (Figure 1) [14,20]. Briefly, mouse 3T3-L1 preadipocytes were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma Japan) supplemented with 10% fetal bovine serum, 1% MEM non-essential amino acids, 100 IU/mL penicillin and 0.1 mg/mL streptomycin. At 3 days after reaching confluency (day 0), the medium was replaced with induction medium for differentiation containing INS (150 nM), DEX (1 μM), IBMX (100 μM) and ROSI (PPARγ-ligand, 1 μM). The differentiation medium was changed every 4 days until analysis (day 6).

The LOX inhibitor (NDGA/AA-861) or specific BLT antagonist (ONO-4057) was prepared in dimethyl sulfoxide (DMSO, Sigma Japan) and added to the differentiation medium from day 0 to day 6 (Figure 1). The DMSO concentration was maintained up to 0.1% of