Suzuki A, Iwamura C, Shinoda K, Tumes DJ, Y Kimura M, Hosokawa H, Endo Y, Horiuchi S, Tokoyoda K, Koseki H, Yamashita M, Nakayama T.	Polycomb group gene product Ring1B regulates Th2-driven airway inflammation through the inhibition of Bim-mediated apoptosis of effector Th2 cells in the lung.	J. Immunol.	184	4510-4520	2010
Majewski IJ, Ritchie ME, Phipson B, Corbin J, Pakusch M, Ebert A, Busslinger M, Koseki H, Hu Y, Smyth GK, Alexander WS, Hilton DJ, Blewitt ME.	Opposing roles of polycomb repressive complexes in hematopoietic stem and progenitor cells.	Blood	116	731-739	2010
Watarai H, Fujii S, Yamada D, Rybouchkin A, Sakata S, Nagata Y, Iida-Kobayashi M, Sekine-Kondo E, Shimizu K, Shozaki Y, Sharif J,Matsuda M, MochidukiS, Hasegawa T, Kitahara G, Endo TA, ToyodaT, Ohara O, Harigaya K, Koseki H, Taniguchi M.	Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells.	J. Clin. Invest.	120	2610-2618	2010
Pereira CF, Piccolo FM, Tsubouchi T, Sauer S, Ryan NK, Bruno L,Landeira D, Santos J, Banito A, Gil J, KosekiH, Merkenschlager M, Fisher AG.	ESCs require PRC2 to direct the successful reprogramming of differentiated cells toward pluripotency.	Cell Stem Cell	6	547-556	2010
Oka A, Mita A, Takada Y, Koseki H, Shiroishi T.	Reproductive isolation in hybrid mice due to spermatogenesis defects at three meiotic stages. Spermatogenic Disruptions at Three Meiotic Stages in Hybrid Males Between House Mouse Subspecies or Species.	Genetics	186	339-351	2010

Wada T, Ishiwata K, Koseki H, Ishikura T, Ugajin T, OhnumaN, Obata K, Ishikawa R, Yoshikawa S, Mukai K, Kawano Y, Minegishi Y,Yokozeki H, Watanabe N, Karasuyama H.	Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks.	J. Clin. Invest.	120	2867-2875	2010
Negishi M, Saraya A, Mochizuki S, Helin K, Koseki H, Iwama A.	A novel zinc finger protein Zfp277 mediates transcriptional repression of the Ink4a/arf locus through polycomb repressive complex 1.	PLoS One	5	e12373	2010
Koseki H.	Epigenetics in development: decorating the genome to show or hide?	Dev. Growth Differ.	52	481	2010
Sharif J, Endo TA, Toyoda T, Koseki H.	Divergence of CpG island promoters: a consequence or cause of evolution?	Dev. Growth Differ.	52	545-554	2010
Chiba T, Seki A, Aoki R, Ichikawa H, Negishi M, Miyagi S, Oguro H, Saraya A, Kamiya A, Nakauchi H, Yokosuka O, and Iwama A.	The polycomb-groupgene Bmi1 promoteshepatic stem cell expansion and tumorigenicity in both Ink4a/Arf-dependent and independent manners.	Hepatology	52	1111-1123	2010
Yamashita Y, Yuan J, Suetake I, Suzuki H, Ishikawa Y, Choi YL, Ueno T, Soda M, Hamada T, Haruta H, Takada S, Miyazaki Y, Kiyoi H, Ito E, Naoe T, Tomonaga M, Toyota M,Tajima S, Iwama A, and Mano H.	Array-based genomic resequencing of human leukemia.	Oncogene	24	3723-3731	2010
Sugawara T, Oguro H, Negishi M, Morita Y, Ichikawa H, Iseki T, Yokosuka O, Nakauchi H,Iwama A.	FET family proto-oncogene Fus contributes to self-renewal ofhematopoietic stem cells.	Exp. Hematol.	38	696-706	2010

Koizumi T, Negishi M, Nakamura S, Oguro H, Satoh K, Ichinose M, and Iwama A.	Depletion of Dnmt1-associated protein 1 triggers DNA damage and compromises the proliferative capacity of hematopoieticstem cells.	Int. J. Hematol.	91	611	2010
Aoki R, Chiba T, Miyagi S, Negishi M, Konuma T, Taniguchi H, Ogawa M, Yokosuka O, and Iwama A.	The polycomb-group gene product Ezh2 regulates proliferation and differentiation of murine hepatic stem/progenitor cells.	J. Hepatology	52	854-863	2010
Oguro H, Yuan J, Ichikawa H, Ikawa T, Yamazaki S, Kawamoto H,Nakauchi H, and Iwama A.	Poised lineage specification in multipotent hematopoietic stem and progenitor cells by the polycomb protein Bmi1.	Cell Stem Cell	6	279-286	2010
Tanaka H, Takeuchi M ,Takeda Y, Sakai S, Oda K, Abe D, Ohwada C, Ozawa S, Sakaida E, Shimizu N, Saito Y, Miyagi S, Iwama A, and Nakaseko C.	Identification of a novel TEL-Lyn fusion gene in primary myelofibrosis.	Leukemia	24	197	2010
Takenobu H, Shimozatoo, Nakamura T, OchiaiH, Yamaguchi Y, OhiraM, Nakagawara A, Kamijo T.	CD133 suppresses neuroblastoma cell differentiation via signal pathway modification.	Oncogene	30	97-105	2011.
Zhang L, Haraguchi S, Koda T, Hashimoto K, Nakagawara A.	Muscle atrophy and motor neuron degeneration in human NEDL1 transgenic mice.	J. Biomed. Biotechnol.	2011	831092	2011
Iwama E, Tsuchimoto D, Iyama T, Sakumi K, Nakagawara A, Takayama K, Nakanishi Y, Nakabeppu Y.	Cancer-related PRUNE2 protein is associated with nucleotides and is highly expressed in mature nerve tissues.	J. Mol. Neurosci.	44	103-114	2011
Ryu M, Hamano M, Nakagawara A, Shinoda M, Shimizu H, Miura T, Yoshida I, Nemoto A, Yoshikawa A.	The benchmark analysis of gastric, colorectal and rectal cancer pathways: toward establishing standardized clinical pathway in the cancer care.	Jpn. J. Clin. Oncol.	41	2-9	2011
Ozaki T, Nakagawara A.	p53: the attractive tumor suppressor in the cancer research field.	J. Biomed. Biotechnol.	2011	603925	2011

Okoshi R, Kubo N, Nakashima K, Shimozato O, Nakagawara A, Ozaki T.	CREB represses p53-dependent transactivation of MDM2 through the complex formation with p53 and contributes to p53-mediated apoptosis in response to glucose deprivation.	Biochem. Biophys. Res. Commun.	406	79-84	2011
Kimura M, Takenobu H, Akita N, Nakazawa A, Ochiai H, Shimozato O, Fujimura YI, Koseki H, Yoshino I, Kimura H, Nakagawara A, Kamijo T.	Bmi1 regulates cell fate via tumor suppressor WWOX repression in small cell lung cancer cells.	Cancer Sci.	102	983-990	2011
Kawahara N, Sugimura H, Nakagawara A, Masui T, Miyake J, Akiyama M, Wahid IA, Hao X, Akaza H.	The 6th Asia Cancer Forum: What Should We Do to Place Cancer on the Global Health Agenda? Sharing Information Leads to Human Security.	Jpn. J. Clin. Oncol.	1	723-729	2011
Ozaki T, Nakagawara A.	Role of p53 in cell death and human cancers.	Cancers	3	994-1013	2011
Takahashi A, Tokita H, Takahashi K, Takeoka T, Murayama K, Tomotsune D, Ohira M, Iwamatsu A, Ohara K, Yazaki K, Koda T, Nakagawara A, Tani K,	A novel potent tumour promoter aberrantly overexpressed in most human cancers.	Scientific Reports	1	15	2011
Isogai E, Ohira M, Ozaki T, Oba S, Nakamura Y, Nakagawara A.	Oncogenic LMO3 collaborates with HEN2 to enhance neuroblastoma cell growth through transactivation of Mash1.	PLoS ONE	6	e19297	2011
London WB, Castel V, Monclair T, Ambros PF, Pearson AD, Cohn SL, Berthold F, Nakagawara A, Ladenstein RL, Iehara T, Matthay KK.	Clinical and Biologic Features Predictive of Survival After Relapse of Neuroblastoma: A Report From the International Neuroblastoma Risk Group Project.	J. Clin. Oncol.	29	3286-3292	2011

Nakajima T, Yasufuku K, Nakagawara A, Kimura H, Yoshino I.	Multi-gene mutation analysis of metastatic lymph nodes in non-small cell lung cancer diagnosed by EBUS-TBNA.	Chest.	140	1319-1324	2011
Kawahara N, Roh JK, Akaza H, Inoue H, Shibuya K, Iwasaki M, Tsuji T, Nishiyama M, Nakagawara A, Watanabe K, Nozaki S, Inoue M,Sugimura H, Miyake J,Li F.	The 7th Asia Cancer Forum: from the perspective of human security, how can we collaborate as Asians in order to place cancer on the global health agenda? How can we fill in the gaps that exist among us?	Jpn. J. Clin. Oncol.	41	825-831	2011
Shih YY, Lee H, Nakagawara A, Juan HF, Jeng YM, Tsay YG, Lin DT, Hsieh FJ, Pan CY ,Hsu WM, Liao YF.	Nuclear GRP75 Binds Retinoic Acid Receptors to Promote Neuronal Differentiation of Neuroblastoma.	PLoS One.	6	e26236	2011
Taggart DR, London WB, Schmidt ML, Dubois SG, Monclair TF, Nakagawara A, De Bernardi B, Ambros PF, Pearson AD, Cohn SL, Matthay KK.	Prognostic Value of the Stage 4S Metastatic Pattern and Tumor Biology in Patients With Metastatic Neuroblastoma Diagnosed Between Birth and 18 Months of Age.	J. Clin. Oncol.	29	4358-4364	2011
Ozaki T, Yamada C, Nakagawara A.	A novel role of RUNX3 in the regulation of p53-mediated apoptosis in response to DNA damage.	Seikagaku	83	751-754	2011
Akter J, Takatori A, Hossain S, Ozaki T, Nakazawa A, Ohira M, Suenaga Y, Nakagawara A.	Expression of NLRR3 orphan receptor gene is negatively regulated by MYCN and Miz-1, and its down-regulation is associated with unfavorable outcome in neuroblastoma.	Clin. Cancer Res.	17	6681-6692	2011
Shih YY, Nakagawara A, Lee H, Juan HF, Jeng YM, Lin DT, Yang YL, Tsay YG, Huang MC, Pan CY, Hsu WM, Liao YF.	Calreticulin Mediates Nerve Growth Factor-Induced Neuronal Differentiation.	J. Mol. Neurosci.	47	571-581	2011

Li X, Isono K, Yamada D, Endo TA, Endoh M, Shinga J, Koseki YM, Otte AP, Casanova M, Kitamura M, Kamijo T, Sharif J, Ohara O, Toyada T, Bernstein BE, Brockdorff N and Koseki H.	Mammalian Polycomb like Pcl2/Mtf2 is a novel regulatory component of PRC2 that can differentially modulate Polycomb activity at both the Hox gene cluster and at Cdkn2a genes.	Mol. Cell Biol.	31	351-364	2011
Yanagisawa R, MatsudaK, Sakashita K, Nakazawa Y, Tanaka M, Saito S, Yoshikawa K, Kamijo T, Shiohara M, Koike K	Disappearance of Minimal Residual Disease After the Early Withdrawal of Immunosuppressants in a Patient With Juvenile Myelomonocytic Leukemia.	Pediatr. Blood Cancer	56	501-502	2011
Sasaki M, Kawahara K, Nishio M, Mimori K, Kogo R, Hamada K, Itoh B, Wang J, Komatsu Y, Yang YR, Hikasa H, Horie Y, Yamashita T, Kamijo T, Zhang Y ,Zhu Y, Prives C, Nakano T, Mak TW, SasakiT, Tomohiko MaehamaT, Mori M, and SzukiA.	Regulation of the MDM2-P53 Pathway and Tumor Growth by PICT1/GLTSCR2 via Nucleolar RPL11.	Nat. Med.	31	944-951	2011
Kimura W, Machii M, Xue X, Sultana N, Hikosaka K, Sharkar MT, Uezato T, Matsuda M, Koseki H, Miura N.	Irxl1 mutant mice show reduced tendon differentiation and no patterning defects in musculoskeletal system development.	Genesis	49	2-9	2011
Oshima M, Endoh M, Endo TA, Toyoda T, Nakajima-Takagi Y, Sugiyama F, Koseki H, Kyba M, Iwama A, Osawa M.	Genome-wide analysis of target genes regulated by HoxB4 in hematopoietic stem and progenitor cells developing from embryonic stem cells.	Blood	117	e142-150	2011
Casanova M, Preissner T, Cerase A, Poot R, Yamada D, Li X, Appanah R, Bezstarosti K, Demmers J, Koseki H, Brockdorff N.	Polycomblike 2 facilitates the recruitment of PRC2 Polycomb group complexes to the inactive X chromosome and to target loci in embryonic stem cells.	Development	138	1471-1482	2011

Hojyo S, Fukada T, Shimoda S, Ohashi W, Bin BH, Koseki H, Hirano T.	The inc transporter SLC39A14/ZIP14 controls G-protein coupled receptor-mediated signaling required for systemic growth.	PLoS One.	6	e18059	2011
Nishida K, Yamasaki S, Hasegawa A, Iwamatsu A, Koseki H, Hirano T.	Gab2, via PI-3K, regulates ARF1 in FcεRI-mediated granule translocation and mast cell degranulation.	J. Immunol.	187	932-941	2011
Mishima Y, Miyagi S, Saraya A, Negishi M, Endoh M, Endo TA, Toyoda T, Shinga J, Katsumoto T, Chiba T, Yamaguchi N, Kitabayashil, Koseki H, Iwama A.	The Hbo1-Brd1/Brpf2 complex is responsible for global acetylation of H3K14 and required for fetal liver erythropoiesis.	Blood	118	2443-2453	2011
Takada Y, Naruse C, Costa Y, Shirakawa T, Tachibana M, Sharif J, Kezuka-Shiotani F, Kakiuchi D, Masumoto H,Shinkai Y, Ohbo K, Peters AH, Turner JM, Asano M, Koseki H.	HP1γ links histone methylation marks to meiotic synapsis in mice.	Development	138	4207-4217	2011
Mochizuki-Kashio M, Mishima Y, Miyagi S, Negishi M, Saraya A, Konuma T, Shinga J, Koseki H, Iwama A.	Dependency on the polycomb gene Ezh2 distinguishes fetal from adult hematopoietic stem cells.	Blood	118	6553-6561	2011
Zhang J, Gao Q, Li P, Liu X, Jia Y, Wu W, Li J, Dong S, Koseki H, Wong J.	S phase-dependent interaction with DNMT1 dictates the role of UHRF1 but not UHRF2 in DNA methylation maintenance.	Cell Res.	21	1723-1739	2011
Tan J, Jones M, Koseki H, Nakayama M, Muntean AG, Maillard I, Hess JL.	CBX8, a polycomb group protein, is essential for MLL-AF9-induced leukemogenesis.	Cancer Cell	20	563-575	2011
Sharif J, Endoh M, Koseki H.	Epigenetic memory meets G2/M: to remember or to forget?	Dev. Cell.	18;20	5-6	2011
Sharif J, Koseki H.	Recruitment of Dnmt1 roles of the SRA protein Np95 (Uhrf1) and other factors.	Prog. Mol. Biol. Transl. Sci.	101	289-310	2011

Yamazaki S, Ema H, Karlsson G, Yamaguchi T, Miyoshi H, Shioda S, Taketo MM, Karlsson S, Iwama A, and Nakauchi H.	Non-myelinating Schwann cells in the mouse bone marrow niche maintain haematopoietic stem cell hibernation through TGF-β signaling.	Cell	147	1146-1158	2011
Chiba T, Suzuki E, Negishi M, Saraya A, Miyagi S, Konuma T,Tanaka S, Tada M, Kanai F, Imazeki F, Iwama A,and YokosukaO.	3-deazaneplanocin isa promising therapeutic agent for the eradication of tumor-initiating hepatocellular carcinoma cells.	Int. J. Cancer	130	2557-2567	2011
Yuan J, Takeuchi M, Negishi M, Oguro H, Ichikawa H, and Iwama A.	Bmi1 is essential for leukemic reprogramming of myeloid progenitor cells.	Leukemia	25	1335-1343	2011
Konuma T, Nakamura S, Miyagi S, Negishi M, Chiba T, Oguro H, Yuan J, Mochizuki-Kashio M, Ichikawa H, Miyoshi H, Vidal M and Iwama A.	Forced expression of the histone demethylase Fbxl10 maintains self-renewing hematopoietic stem cells.	Exp. Hematol.	39	697-709	2011
Takeda Y, Nakaseko C ,Tanaka H, Takeuchi M,Yui M, Saraya A, Miyagi S, Wang C, TanakaS, Ohwada C, SakaidaE, Yamaguchi N, Yokote K, Hennighausen L,and Iwama A.	Direct activation of STAT5 by ETV6-Lyn fusion protein promotes induction of myeloproliferative neoplasm with myelofibrosis.	Br. J. Haematol.	153	589-598	2011
Kimura H, Nakajima T ,Takeuchi K, Soda M, Mano H, Iizasa T, Matsui Y, Yoshino M, Shingyoji M, Itakura M, Itami M, Ikebe D, Yokoi S, Kageyama H, Ohira M, Nakagawara A.	ALK fusion gene positive lung cancer and 3 cases treated with an inhibitor for ALK kinase activity.	Lung Cancer	75	66-72	2012
Yoshihara Y, Wu D, Kubo N, Sang M, Nakagawara A, Ozaki T.	Inhibitory role of E2F-1 in the regulation of tumor suppressor p53 during DNA damage response.	Biochem. Biophys. Res. Commun.	2421	57-63	2012
Kamijo T, Nakagawara A.	Molecular and genetic bases of neuroblastoma.	Int. J. Clin. Oncol.	17	190-195	2012

Hossain S, Takatori A, Nakamura Y, Suenaga Y, Kamijo T, Nakagawara A.	NLRR1 Enhances EGF-Mediated MYCN Induction in Neuroblastoma and Accelerates Tumor Growth In Vivo.	Cancer Res.	72	4587-4596	2012
Tonini GP, Nakagawara A, Berthold F.	Towards a turning point of neuroblastoma therapy.	Cancer Lett.	326	128-134	2012
Schleiermacher G, Mosseri V, London WB, Maris JM, Brodeur GM, Attiyeh E, Haber M, Khan J, Nakagawara A, Speleman F, Noguera R, Tonini GP, Fischer M, Ambros I, MonclairT, Matthay KK, Ambros P, Cohn SL, PearsonAD.	Segmental chromosomal alterations have prognostic impact in neuroblastoma: a reportf rom the INRG project.	Br. J. Cancer.	107	1418-1422	2012
Shum CK, Lau ST, Tsoi LL, Chan LK, Yam JW, Ohira M, Nakagawara A, Tam PK, Ngan ES.	Krüppel-like factor 4 (KLF4) suppresses neuroblastoma cell growth and determines non-tumorigenic lineage differentiation.	Oncogene		Epub ahead of print	2012
Kamijo T.	Role of stemness-related molecules in neuroblastoma.	Pediatric Res.	71	511-515	2012
Oonishi K, Cui X, Hirakawa H, Fujimori A, Kamijo T, Yamada S, Yokosuka O, Kamada T.	Different effects of carbon ion beams and X-rays on clonogenics urvival and DNA repair in human pancreatic cancer stem-like cells.	Radiother. Oncol.	105	258-265	2012
Kamijo T.	Neuroblastoma: Role of MYCN/Bmi1 Pathway in Neuroblastoma.	Pediatric Cancer			2012
Hashizume O, Shimizu A, Yokota M, Sugiyama A, Nakada K, Miyoshi H, Itami M, Ohira M,Nagase H, Takenaga K,Hayashi J.	Specific mitochondrial DNA mutation in mice regulates diabetes and lymphoma development.	Proc. Natl. Acad. Sci.	109	10528-10533	2012

Hisada K, Sanchez C, Endo TA, Endoh M, Roman-Trufero M, Sharif J, Koseki H, Vidal M.	RYBP represses endogenous retroviruses and preimplantation- and germ line-specific genes in mouse embryonic stem cells.	Mol. Cell Biol.	32	1139-1149	2012
T Takagi S, Saito Y, Hijikata A, Tanaka S, Watanabe T, Hasegawa T, Mochizuki S, Kunisawa J, Kiyono H, Koseki H, Ohara O, Saito T, Taniguchi S, Shultz LD, Ishikawa F.	Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation.	Blood	119	2768-2777	2012
Watarai H, Sekine-Kondo E, Shigeura T, Motomura Y, Yasuda T, Satoh R, Yoshida H, Kubo M, Kawamoto H, Koseki H, Taniguchi M.	Development and function of invariant natural killer T cells producing t(h)2- and t(h)17-cytokines.	PLoS Biol.	10	e1001255	2012
Oguro H, Yuan J, Tanaka S, Miyagi S, Mochizuki-Kashio M, Ichikawa H, Yamazaki S, Koseki H, Nakauchi H, Iwama A.	Lethal myelofibrosis induced by Bmi1-deficient hematopoietic cells unveils a tumor suppressor function of the polycomb group genes.	J. Exp. Med.	209	445-454	2012
Shinoda K, Tokoyoda K, Hanazawa A, Hayashizaki K, Zehentmeier S, Hosokawa H, Iwamura C, Koseki H, Tumes DJ, Radbruch A, Nakayama T.	Type II membrane protein CD69 regulates the formation of resting T-helper memory.	Proc. Natl. Acad. Sci.	109	7409-7414	2012
Lapthanasupkul P, Feng J, Mantesso A, Takada-Horisawa Y, Vidal M, Koseki H, Wang L, AnZ, Miletich I, Sharpe PT.	Ring1a/b polycomb proteins regulate the mesenchymal stem cell niche in continuously growing incisors.	Dev. Biol.	367	140-153	2012
Watarai H, Yamada D, Fujii S, Taniguchi M, Koseki H.	Induced pluripotency as a potential path towards iNKT cell-mediated cancer immunotherapy.	Int. J. Hematol.	95	624-631	2012

Nakamura S, Oshima M, Yuan J, Saraya A, Miyagi S, Konuma T, Yamazaki S, Osawa M ,Nakauchi H, Koseki H ,Iwama A.	Bmi1 confers resistance to oxidative stress on hematopoietic stem cells.	PLoS One.	7	e36209	2012
Tanaka S, Miyagi S, Sashida G, Chiba T, Yuan J, Mochizuki-Kashio M, Suzuki Y, Sugano S, Nakaseko C,Yokote K, Koseki H, Iwama A.	Ezh2 augments leukemogenecity by reinforcing differentiation blockage in acute myeloid leukemia.	Blood	120	1107-1117	2012
Visconte V, Rogers HJ ,Singh J, Barnard J, Bupathi M, Traina F, McMahon J, Makishima H, Szpurka H, Jankowska A, Jerez A, Sekeres MA, Sauntharajah Y ,Advani AS, Copelan E ,Koseki H, Isono K, Padgett RA, Osman S, Koide K, O'Keefe C, Maciejewski JP, Tiu RV.	SF3B1 haploinsufficiency leads to formation of ring sideroblastsin myelodysplastic syndromes.	Blood	120	3173-3186	2012
Endoh M, Endo TA, Endoh T, Isono K, Sharif J, Ohara O, Toyoda T, Ito T, Eskeland R, Bickmore WA, Vidal M, Bernstein BE, Koseki H.	Histone H2A Mono-Ubiquitination Is a Crucial Step to Mediate PRC1-Dependent Repression of Developmental Genes to Maintain ES Cell Identity.	PLoS Genet.	8	e1002774	2012
Onoguchi M, Hirabayashi Y, Koseki H, Gotoh Y.	A noncoding RNA regulates the neurogenin1 gene locus during mouse neocortical development.	Proc. Natl. Acad. Sci.	109	16939-16944	2012
Ku M, Jaffe JD, Koche RP, Rheinbay E, Endoh M, Koseki H, CarrSA, Bernstein BE.	H2A.Z landscapes and dual modifications in pluripotent and multipotent stem cells underlie complex genome regulatory functions.	Genome Biol.	13	R85	2012

Nakajima-Takagi Y, Osawa M, Oshima M, Takagi H, Miyagi S, Endoh M, Endo TA, Takayama N, Eto K, Toyoda T, Koseki H, Nakauchi H, Iwama A.	Role of SOX17 in hematopoietic development from human embryonic stem cells.	Blood	121	447-458	2012
Farcas AM, Blackledge NP, Sudbery I, Long HK, McGouran JF, RoseNR, Lee S, Sims D, Cerase A, Sheahan T W, Koseki H, Brockdorff N, Ponting CP, KesslerBM, Klose RJ.	KDM2B links the Polycomb Repressive Complex 1 (PRC1) to recognition of CpG islands.	Elife.	1	e00205	2012
Sakai S, Nakaseko C,Takeuchi M, Ohwada C, Shimizu N, Tsukamoto S, Kawaguchi T,Jiang M, Sato Y, Ebinuma H, Yokote K, Iwama A, Fukamachi I, Schneider WJ, SaitoY, and Bujo H.	Circulating soluble LR11/SorLA levels are highly increased and ameliorated by chemotherapy in acute leukemias.	Clinica. Chemica. Acta.	413	1542-1548	2012
Koseki H.	An interview with Haruhiko Koseki.	Development	139	3469-3470	2012
Shide K, Kameda T, Shimoda H, Yamaji T,Abe H, Kamiunten A,Sekine M, Hidaka T, Katayose K, Kubuki Y, Yamamoto S, Miike T, Iwakiri H, Hasuike S, Nagata K, Iwama A, Matsuda T, Kitanaka A and ShimodaK.	TET2 is essential forsurvival and hematopoietic stem cell homeostasis.	Leukemia	26	2216-2223	2012
Suzuki E, Chiba T, Zen Y, Miyagi S, TadaM ,Kanai F, Imazeki F, Miyazaki M, Iwama A, and Yokosuka O.	Aldehyde dehydrogenase 1 is associated with recurrence-free survival but not stem cell-like propertiesin hepatocellular carcinoma.	Hepatol. Res.	42	1100-1111	2012
Ashinuma H, Takiguchi Y, Kitazono S, Kitazono-Saitoh M, Kitamura A, Chiba T, Tada Y, Kurosu K, Sakaida E, Sekine I, Tanabe N, Iwama A, Yokosuka O, Tatsumi K.	Anti-proliferative action of metformin in human lung cancer cell lines.	Oncology Reports	28	8-14	2012

Chand D, Yamazaki Y, Ruuth K, Schönherr C, Martinsson T, Kogner P, Attiyeh EF, Maris J, Morozova O, Marra M A, Ohira M, Nakagawara A, Sandström PE, Palmer R, Hallberg B.	Cell and Drosophila model systems define three classes of ALK mutations in neuroblastoma.	Dis. Model Mech.		Epub ahead of print	2013
Wu D, Ozaki T, Yoshihara Y, Kubo N, Nakagawara A.	Runt-related Transcription Factor 1 (RUNX1) Stimulates Tumor Suppressor p53 Protein in Response to DNA Damage through Complex Formation and Acetylation.	J. Biol. Chem.	288	1353-1364	2013
Nozato M, Kaneko S, Nakagawara A, Komuro H.	Epithelial-mesenchymal transition-related gene expression as a new prognostic marker for neuroblastoma.	Int. J. Oncol.	42	134-140	2013
Kubo N, Wu D, Yoshihara Y, Sang M, Nakagawara A, Ozaki T.	Co-chaperon DnaJC7/TPR2 enhances p53 stability and activity through blocking the complex formation between p53 and MDM2.	Biochem. Biophys. Res. Commun.	430	1034-1039	2013
Sugimoto T, Gotoh T, Yagyu S, Kuroda H, Iehara T, Hosoi H, OhtaS, Ohira M, Nakagawara A.	A MYCN-amplified cell line derived from a long-term event-free survivor among our sixteen established neuroblastoma cell lines.	Cancer Lett.		Epub ahead of print	2013
Takagi D, Tatsumi Y, Yokochi T, Takatori A, Ohira M, Kamijo T, Kondo S, Fujii Y, Nakagawara A.	Shf, a novel adaptor protein, interacts with ALK receptor and negatively regulates its downstream signals in neuroblastoma.	Cancer Sci.		Epub ahead of print	2013
Yamaki T, Suenaga Y, Iuchi T, Alagu J, Takatori A, Itami M, Araki A, Ohira M, Inoue M,Kageyama H, Yokoi S, Saeki N, Nakagawara A.	Temozolomide suppresses MYC via activation of TAp63 to inhibit progression of human glioblastoma.	Sci Rep.		Epub ahead of print	2013

Nishimura T, Kaneko S, Kawana-Tachikawa A,Tajima Y, Goto H, ZhuD, Nakayama-Hosoya K, Iriguchi S, Uemura Y, Shimizu T, Takayama N, Yamada D, Nishimura K, Ohtaka M, Watanabe N, Takahashi S, Iwamoto A, Koseki H, Nakanishi M, Eto K, Nakauchi H.	Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation.	Cell Stem Cell	12	114-126	2013
Vizcardo R, Masuda K ,Yamada D, Ikawa T, Shimizu K, Fujii S, Koseki H, Kawamoto H.	Regeneration of Human Tumor Antigen-Specific T Cells from iPSCs Derived from Mature CD8(+) T Cells.	Cell Stem Cell	12	31-36	2013
Sharif J, Shinkai Y, Koseki H.	Is there a role for endogenous retrovirusesto mediate long-term adaptive phenotypic response upon environmental inputs?	Philos Trans R Soc Lond B Biol Sci.	368	20110340	2013

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ORIGINAL ARTICLE

CD133 suppresses neuroblastoma cell differentiation via signal pathway modification

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CD133 (prominin-1) is a transmembrane glycoprotein expressed on the surface of normal and cancer stem cells (tumor-initiating cells), progenitor cells, rod photoreceptor cells and a variety of epithelial cells. Although CD133 is widely used as a marker of various somatic and putative cancer stem cells, its contribution to the fundamental properties of cancer cells, such as tumorigenesis and differentiation, remains to be elucidated. In the present report, we found that CD133 was expressed in several neuroblastoma (NB) cell lines/tumor samples. Intriguingly, CD133 repressed NB cell differentiation, for example neurite extension and the expression of differentiation marker proteins, and was decreased by several differentiation stimuli, but accelerated cell proliferation, anchorage-independent colony formation and *in vivo* tumor formation of NB cells. NB cell line and primary tumor-sphere experiments indicated that the molecular mechanism of CD133-related differentiation suppression in NB was in part dependent on neurotrophic receptor RET tyrosine kinase regulation. RET transcription was suppressed by CD133 in NB cells and glial cell line-derived neurotrophic factor treatment failed to induce RET in CD133-expressing cells; RET overexpression rescued CD133-related inhibition of neurite elongation. Of note, CD133-related NB cell differentiation and RET repression were mainly dependent on p38MAPK and PI3K/Akt pathways. Furthermore, CD133 has a function in growth and RET expression in NB cell line- and primary tumor cell-derived tumor spheres. To the best of our knowledge, this is the first report of the function of CD133 in cancer cells and our findings may be applied to improve differentiation induction therapy for NB patients.
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Introduction

CD133 (AC133; human prominin-1) belongs to a family of cell-surface glycoproteins harboring five transmembrane domains (Corbeil *et al.*, 2001) and was originally found as a hematopoietic stem cell marker (Yin *et al.*, 1997). CD133 was subsequently shown to be expressed by a number of progenitor cells, including those of the epithelium, where it is expressed on the apical surface (Corbeil *et al.*, 2000). Previously, it was found that CD133-expressing cells in brain tumors have the capacity for unlimited self-renewal, as well as the ability, in small numbers, to initiate tumor formation and progression in immuno-deficient mice (Singh *et al.*, 2004), suggesting that CD133-expressing cells satisfy the important criteria required for tumor-initiating cells (TICs) (Reya *et al.*, 2001; Jordan *et al.*, 2006). Using similar methods, CD133 has recently been designated as a marker associated with TICs in the colon (O'Brien *et al.*, 2007; Ricci-Vitiani *et al.*, 2007), pancreatic (Olempska *et al.*, 2007), liver (Yin *et al.*, 2007), skin (Monzani *et al.*, 2007) and prostate (Collins *et al.*, 2005; Miki *et al.*, 2007) cancers. Maw *et al.* (2000) reported homozygosity for a 1-bp deletion (1878delG) in exon 16 of the CD133 gene predicted to cause a frameshift at codon 614 and a prematurely truncated protein lacking about half of the second extracellular loop, the final membrane-spanning segment and the cytoplasmic-C-terminal domain; this missense mutation caused retinal degeneration in four affected members of a consanguineous Indian family. This finding was further confirmed by an article describing that loss of Prom-1 in genetically modified mouse results in the progressive degeneration of mature photoreceptors with complete loss of vision (Zacchigna *et al.*, 2009); however, to the best of our knowledge, no reports have studied the function of CD133 in tumorigenesis.

Neuroblastoma (NB) is the most common pediatric solid malignant tumor derived from the sympathetic nervous system. Unlike the many childhood malignancies for which survival has been improved by recent therapies, high-risk NB is still one of the most difficult tumors to cure, with only 30% long-term survival despite intensive multimodal therapy (Maris *et al.*,

2007). The clinical presentation and treatment response of advanced NB, which results in relapse and a refractory state after a good responsive to the initial chemotherapy, suggest that TICs likely exist in NB tumors. A previous report indicated the isolation and characterization of putative TICs using primary-sphere formation with tumors and bone marrow metastases from NB patients, although CD133 expression was not detected in a bone marrow-derived high-risk NB tumor-sphere sample (Hansford *et al.*, 2007). On the other hand, it was reported that sub-cloned NB cells (designated 'intermediate type'), which have a significantly more malignant phenotype, with four- to fivefold greater plating efficiencies in soft agar and sixfold higher tumorigenicity in athymic mice, expressed high amounts of CD133 mRNA compared with less malignant subclones (Walton *et al.*, 2004); therefore, the function of CD133 in NB tumorigenesis and aggressiveness remains unresolved.

Previous reports about CD133 expression in NB and its function as a stem cell marker in several tumors prompted us to study the function of CD133 in NB cells (Walton *et al.*, 2004; Hansford *et al.*, 2007). Our results clearly indicated that CD133 also seems to regulate cell proliferation and tumorigenesis in NB cells. Importantly, CD133 represses NB cell differentiation and is decreased by several differentiation stimulators. We studied the molecular mechanism of CD133-related differentiation inhibition in NB cells and found that it was in part dependent on RET tyrosine kinase receptor regulation via signal pathway modification. Furthermore, CD133 is expressed in NB cell spheres and has a function in sphere growth and RET regulation.

In specific malignancies, for example NB and acute promyelocytic leukemia, differentiation induction therapy using retinoic acid is clearly effective. *In vitro* experiments indicated that all-*trans*-retinoic acid (ATRA) treatment induced morphological and biochemical differentiation in these cancer cells, suggesting that the induced differentiation seems to repress the tumorigenic activity of cancer cells (Brodeur *et al.*, 2000; Weinberg, 2006). Together, CD133 may regulate NB tumorigenesis and proliferation by preventing differentiation.

Results

CD133 has a function in NB cell proliferation

First, we checked the expression of CD133 in NB cell lines and found its expression in 7 out of 20 (53%) cell lines (Figure 3d and Supplementary Figure 1S). A high level of cell-surface expression of *CD133* was detected in TGW and SK-N-DZ cells, and modest expression was found in IMR32 (Figure 1a; Supplementary Figure 1Sa). Next, we knocked down *CD133* in highly expressing NB cells and analyzed the knockdown-induced phenotype. Figure 1b shows that infection of shRNA-reduced CD133 mRNA and protein and *CD133* knockdown in TGW cells effectively resulted in significant growth retardation. Inhibition of cell proliferation by CD133 small-interference RNA was also observed in SK-N-DZ cells (Supplementary Figure S1). Furthermore, stable knockdown of *CD133* in TGW cells suppressed cell proliferation under anchorage-independent conditions (Figure 1c). To test tumorigenicity *in vivo*, *CD133*-silenced TGW cells were injected subcutaneously into nude mice. Mock shRNA lentivirus-infected cells formed large tumors within 9 days post-injection; CD133 shRNA lentivirus-infected cells formed very small tumors (Figure 1d). Next, we examined the effect of CD133 on NB cell proliferation (Supplementary Figure 2S). CD133 was successfully expressed in SH-SY5Y cells by lentivirus. The proliferation rate of CD133-expressing SH-SY5Y cells was 2–2.5-fold greater than mock cells. Moreover, a soft agar colony formation assay showed that CD133-expressing cells formed more and bigger colonies than mock-control cells.

CD133 knockdown induces NB differentiation

In NB cells, differentiation into a neuronal phenotype is induced when cells are treated with several stimulations. Glial cell line-derived neurotrophic factor (GDNF) induced neurite outgrowth in TGW cells (Figure 2a, center). In CD133 knocked-down TGW cells, neurite formation was observed even under normal culture conditions (Figure 2a, KD). We scored cells with neurite length longer than the cell body diameter as neurite positive (Figure 2b). CD133 knocked-down cells showed intensified neurite extensions when compared with mock cells. Mock-infected and CD133 knocked-down cells were collected at the end of the experiment, and mRNA was extracted and subjected to RT–PCR (Figure 2c). With *GAP43/neurofilament (NF) 68* as neuronal differentiation markers, these expressions were constitutively upregulated in CD133 knocked-down cells. Along with differentiation induced by treatment with ATRA or phorbol-12-myristate-13-acetate (TPA) in parental TGW cells, CD133 expression was suppressed at both protein and mRNA levels (Supplementary Figure 3S). These results indicated that CD133 may suppress the differentiation of NB cells.

CD133 regulates RET expression in NB cells

To identify the mechanism of CD133-related cellular differentiation, we studied the expression of several neurotrophic receptors and RET receptors because they are the important signal transduction pathway molecules, which have important functions in sympathetic nerve and NB cell differentiation (Kaplan *et al.*, 1993; Klein, 1994; D'Alessio *et al.*, 1995; Enomoto *et al.*, 2001). We introduced *CD133* cDNA into several NB cell lines (Figure 3a), and checked the effect of CD133 overexpression on *RET* expression using a primer pair recognizing all *RET* isoforms, *RET51*, *RET9* and *RET43*, formed by alternative splicing of C-terminal exon cassettes (Myers *et al.*, 1995; Enomoto *et al.*, 2000). Intriguingly, in *RET* and all *RET* isoforms, transcriptions were suppressed in CD133-overexpressing NB cells (*RET* reduction was 1.3–3.8-fold by qPCR); however,

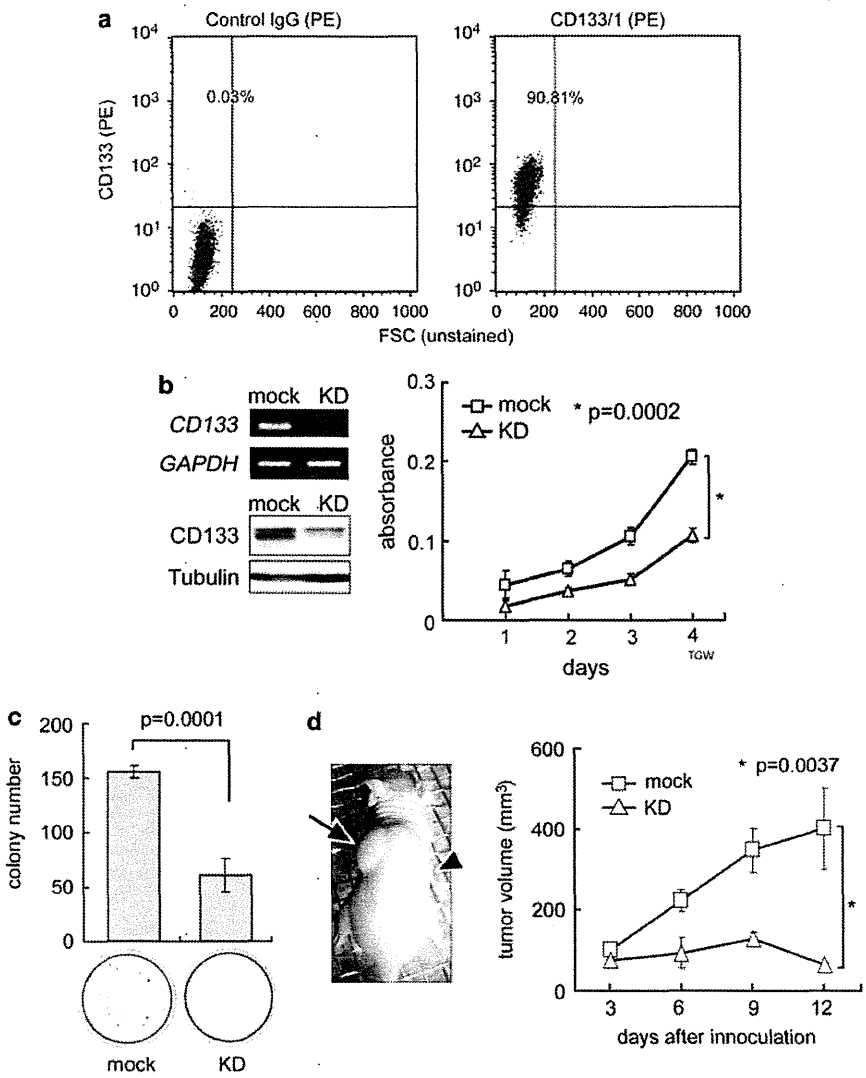

Figure 1 *CD133* knockdown inhibits the growth of human neuroblastoma (NB) cells. (**a**) Flow cytometric analysis of CD133 expression profiles in TGW cells. CD133 fluorescence is depicted on the *y* axis, and the percentage of CD133-positive cells is shown in the left upper corner of each plot. (**b**) Stable knockdown of *CD133* by lentivirus-mediated shRNA was performed as described in Materials and methods. *CD133* expression was detected by semi-quantitative RT–PCR and western blotting analysis in TGW cells. Growth curves were obtained by WST-8 assay. Anchorage-independent colony formation (**c**) and *in vivo* tumorigenic assay (**d**). TGW cells were stably transduced with shRNA against mock or CD133 (KD). (**c**) Colonies were stained with MTT dye and directly counted under a phase contrast microscope. (**d**) Tumor development in BALB/c AJcl *nu/nu* mice on injection of TGW cells stably infected with shRNA against mock (arrow) and CD133 (KD, arrowhead) cells. Tumor volume was measured every 3 days. Data are presented as the mean ± s.d. of tumors in four mice.

the effects of CD133 on *TrkA/B/C, p75NGFR* and *GDNF* expressions did not show a specific tendency. CD133 knockdown clearly increased *RET* mRNA (*RET* induction was 2.5–3.0-fold by qPCR). CD133-mediated RET downregulation was also observed at the protein level (Figure 3b). Furthermore, CD133 expression in primary NB spheres resulted in transcriptional suppression of *RET* (Figure 3c). These results suggest that CD133 suppresses *RET* gene transcription in NB cells.

To study the expression pattern of *CD133* and *RET* mRNA in human NBs, we performed semi-quantitative RT–PCR. *CD133* was expressed in 7 of 20 NB cell lines tested (Figure 3d), and only 1 NB cell line was *RET* positive in the 7 cell lines. We further studied *CD133* and *RET* expression in unfavorable patient-derived tumors (stages 3 and 4, TrkA(−), *MYCN* amplified). Again, *RET* expression was profoundly repressed in CD133-expressing NB tumors (Figure 3e). Finally, we studied the transcriptional activity of *RET* promoter in CD133-expressing cells. *RET* promoter reporter-derived luciferase activity was significantly suppressed in CD133-expressing cells (Figure 3e).

CD133 regulates NB cell differentiation in a RET-dependent manner

We investigated the biological effects of CD133 overexpression on *RET* downregulation in SH-SY5Y cells.

Significant neurite outgrowth was observed when mock-infected cells were stimulated with GDNF (Figure 4a). At the same time, no obvious difference was observed between mock- and GDNF-treated CD133-expressing cells. These results implied that CD133 overexpression inhibited NB cell differentiation.

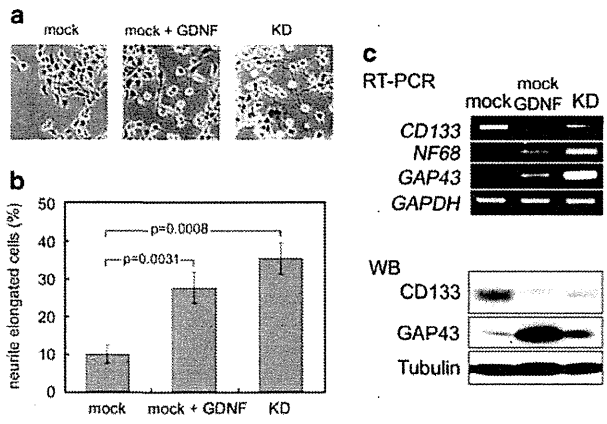

Figure 2 CD133 silencing induces differentiation in TGW cells. TGW cells were infected with lentivirus vectors encoding shRNA against *CD133* (right) or a mock (left) as a negative control. Ten days after infection, cells were treated with buffer (mock and KD) or GDNF (10 ng/ml, middle). Cells were scored for the presence of neurites longer than one cell diameter 72 h after treatment (photo: (**a**), bar graphs: (**b**)). Data are presented as the mean ± s.d. from at least three independent experiments. Statistical analysis was performed by Student's *t*-test. (**c**) NB differentiation-related molecule *neurofilament 68 (NF68)* and *GAP43* expressions in RT–PCR and WB. NF68 protein was not detected by WB in TGW cells.

We examined the effect of the co-expression of CD133 and RET (RET9) on SH-SY5Y cells. RET-expressing lentivirus was co-infected into stably CD133-expressing SH-SY5Y cells. Ten days after infection, ectopic RET and CD133 expressions were observed both at protein

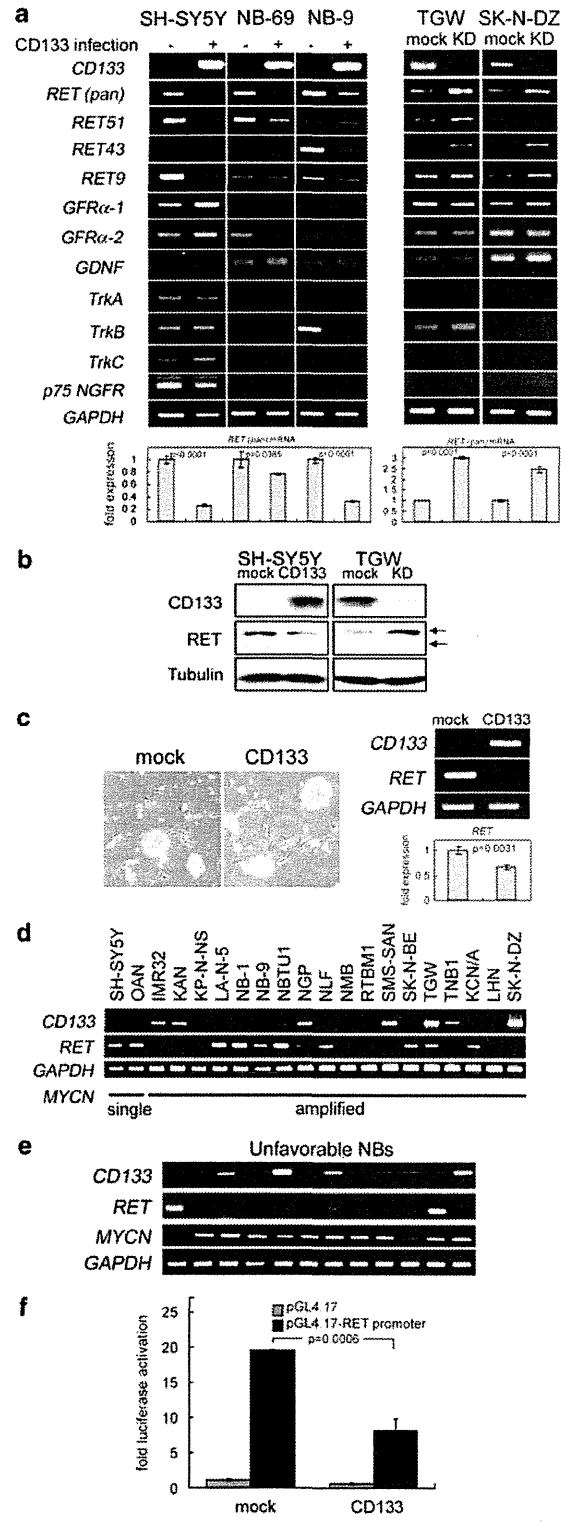

Figure 3 CD133 inhibits RET expression in NB cells. (**a**) SH-SY5Y, NB-69 and NB-9 cells were infected with mock or CD133-expressing lentivirus, and TGW and SK-N-DZ cells were stably infected with shRNA against mock or CD133 (KD) lentivirus. Semi-quantitative RT–PCR analyses were performed with CD133-modified NBs using specific primers against each *RET* isoform, *Trk* families, *GFRα-1/2* and *GDNF*. *GAPDH* was used as a loading control. Expression level of *RET* (pan) was analyzed by qPCR. In qPCR, relative *RET* values were normalized by *GAPDH*. Data are representative results of at least three independent experiments. (**b**) CD133-expressing SH-SY5Y or CD133 knocked-down TGW cell lysates were subjected to western blotting for CD133 and pan-RET expression. Pan-RET antibody detected two bands corresponding to RET isoforms (arrows). (**c**) Primary sphere from a stage 4 NB patient was infected with mock or CD133-expressing lentivirus. Five days after infection, RNA was extracted for semi-quantitative RT–PCR of *CD133*/*RET* and qPCR of *RET*. GAPDH was used as an internal control. Data are representative of three tumor samples. (**d**) Expression of *CD133* and *RET* mRNA in NB cell lines. In all, 18 NB cell lines with amplified *MYCN* and 2 cell lines with a single copy of *MYCN* were used for semi-quantitative RT–PCR analysis. (**e**) Semi-quantitative RT–PCR analysis in unfavorable primary NBs. The results of 12 NBs are shown. Unfavorable NBs: International NB Staging System (INSS) stage 3 or 4, *TrkA* (−), with *MYCN* amplified. (**f**) Effects of CD133 on *RET* promoter (0.8 kb) activity in SH-SY5Y cells. pGL4.17-*RET* promoter-driven luciferase activities were normalized to pRL-SV40 early enhancer/promoter-driven *Renilla* luciferase activities as the transfection control and expressed as relative values.

and mRNA levels (Figure 4b and data not shown). As seen in Figure 4a, GDNF significantly induced neurite outgrowth of CD133/RET co-expressing SH-SY5Y cells. CD133 single-infected cells did not respond to GDNF, suggesting that the response was dependent on RET receptor expression. However, the expression of neuronal cell differentiation markers induced by GDNF was not recovered by *RET* in CD133-expressing cells (Figure 4b). These findings indicated that CD133 inhibits GDNF-promoted neuronal differentiation via not only by *RET* but also by the other signal pathways.

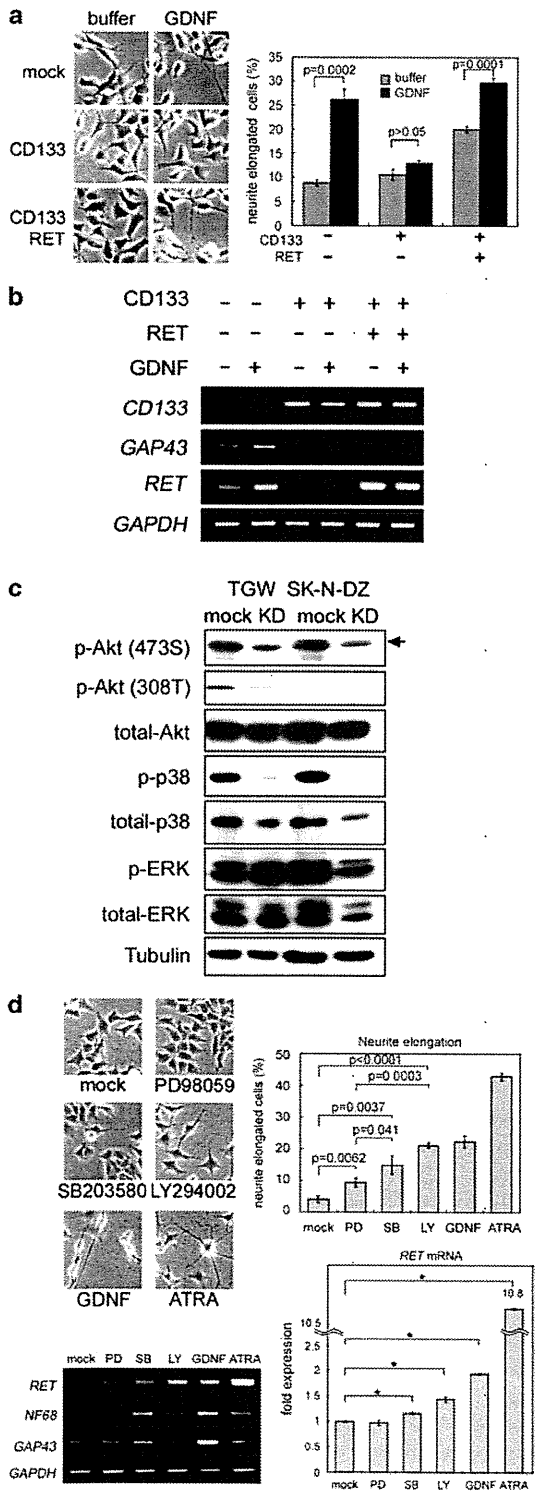

Figure 4 NB cell differentiation was regulated by CD133-dependent RET suppression via signal pathway modification. (**a**) Mock, CD133 and/or RET9 co-expressing SH-SY5Y cells were treated with GDNF (50 ng/ml) for 72 h. Cells were scored for the presence of neurites longer than one cell diameter after GDNF treatment. (**b**) CD133 and/or RET9 co-infected SH-SY5Y cells were cultured with or without GDNF treatment. Semi-quantitative RT–PCR analyses of *CD133*, *GAP43*, *RET* and *GAPDH* were performed. (**c**) The levels of phospho-Akt (p-Akt(473S) and p-Akt(308 T)), total-Akt, phospho-p38MAPK (p-p38), total-p38MAPK, phospho-ERK (p-ERK), total-ERK and tubulin were analyzed by western blot analysis. (**d**) TGW cells were cultured with DMSO (mock, 0.1%), PD98059 (PD, 5 μM), SB203580 (SB, 5 μM), LY294002 (LY, 5 μM), GDNF (50 ng/ml) or ATRA (5 μM) for 96 h. Cells were scored for the presence of neurite longer than one cell diameter after treatments. Semi-quantitative RT–PCR analysis of *RET*/*NF68*/*GAP43*/*GAPDH*, and qPCR of *RET* were performed.

CD133 regulates RET expression and NB cell differentiation by modification of signaling pathways

To identify the mechanism of *RET* downregulation in CD133-expressing cells, we studied the signaling molecule status in CD133 knocked-down cells (Figure 4c) and found a strong suppression of Akt (473S, 308T) and p38MAPK phosphorylation, but not ERK1/2 in both TGW and SK-N-DZ cells. To confirm the Akt and p38MAPK phosphorylation status caused by CD133 downregulation, we treated TGW cells with kinase inhibitors. MEK1 inhibitor (PD98059, PD), p38MAPK inhibitor (SB203580, SB) and PI3K inhibitor (LY294002, LY) induced neurite elongation in NB cells, and SB and LY were more effective for neurite elongation than PD. *RET* induction by kinase inhibitors was correlated with neurite elongation; however, differentiation markers *NF68* and *GAP43* were significantly induced by SB treatment. These results suggest that downregulation of p38MAPK and PI3K/Akt pathways has a function in CD133-related neurite elongation and differentiation marker expression is affected mainly by the p38MAPK pathway.

CD133 has a function in tumor-sphere growth and cell survival

It was previously reported that NB TICs were accumulated in NB spheres in serum-free media (SFM) (Hansford *et al.*, 2007). These observations prompted us to study the function of CD133 in tumor-sphere formation of NB cells. In IMR32 cells, only a small fraction of cells expressed CD133 (Supplementary Figure 1Sa). IMR32 cells were cultured in SFM with epidermal growth factor and fibroblast growth factor for a week, and sphere formation, upregulation of *CD133* (11.8-fold induction) and suppression of *RET* (2.8-fold reduction) were observed (Figure 5a). In primary NB cells from bone marrow metastasis,