Mukaiyama Y, Suzuki M, Morikawa T, Mori Y, Takeshima Y, Fujimura T, Fukuhara H, Nakagawa T, Nishimatsu H, Kume H, Homma Y	Multiple primary malignant neoplasms of the glottis, renal pelvis, urinary bladder, oral floor, prostate, and esophagus in a Japanese male patient: a case report.	World J Surg Oncol	12	294	2014
Aizawa N, Ito H, Sugiyama R, Fujimura T, Suzuki M, Fukuhara H, Homma Y, Igawa Y	Selective inhibitory effect of imidafenacin and 5-hydroxymethyl tolterodine on capsaicin-sensitive C-fibers of the primary bladder mechanosensitive afferent nerves in the rat.	J Urol		in press	2014
Sugihara T, Yasunaga H, Horiguchi H, Matsui H, Fujimura T, Nishimatsu H, Fukuhara H, Kume H, Yu C, Kattan MW, Kiyohide F, Homma Y	Robot-assisted versus other types of radical prostatectomy: population-based safety and cost comparison in Japan, 2012-2013.	Cancer Sci	105(11)	1421-1426	2014
Nishimatsu H, Kitamura Y, Yamada D, Nomiya A, Niimi A, Suzuki M, Fujimura T, Fukuhara H, Nakagawa T, Enomoto Y, Kume H, Igawa Y, Homma Y	Improvement of symptoms of aging in males by a preparation LEOPIN ROYAL containing aged garlic extract and other five of natural medicines - comparison with traditional herbal medicines (Kampo).	Aging Male	17(2)	112-116	2014
Sato YT, Fukuhara H, Suzuki M, Fujimura T, Nakagawa T, Nishimatsu H, Kume H, Morikawa T, Fukayama M, Homma Y	Long-term results of radical prostatectomy with immediate adjuvant androgen deprivation therapy for pT3N0 prostate cancer.	BMC Urol	14	13-18	2014
Kamei J, Nishimatsu H, Nakagawa T, Suzuki M, Fujimura T, Fukuhara H, Igawa Y, Kume H, Homma Y	Risk factors for septic shock in acute obstructive pyelonephritis requiring emergency drainage of the upper urinary tract.		46(3)	493-497	2014
Kadowaki N	Guest editorial: immunotherapy for hematological malignancies: the quest to overcome tolerogenic drive.	Int J Hematol	99(2)	105-106	2014

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Koyama-Nasu R, Nasu-Nishimura Y, Todo T, Ino Y, Saito N, Aburatani H, Funato K, Echizen K, Sugano H, Haruta R, Matsui M, Takahashi R, Manabe E, Oda T, Akiyama T	The critical role of cyclin D2 in cell cycle progression and tumorigenicity of glioblastoma stem cells.	Oncogene	32(33)	3840-3845	2013
Tsuji T, Nakamori M, Iwahashi M, Nakamura M, Ojima T, Iida T, katsuda M, Hayata K, Ino Y, Todo T, Yamaue H	An armed oncolytic herpes simplex virus expressing thrombospondin-1 has an enhanced in vivo antitumor effect against human gastric cancer.	Int J Cancer	132(2)	485-494	2013
Taguchi S, Nakagawa T, Hattori M, Niimi A, Nagata M, Kawai T, Fukuhara H, Nishimatsu H, Ishikawa A, Kume H, Homma Y	Prognostic Factors for Metastatic Urothelial Carcinoma Undergoing Cisplatin-based Salvage Chemotherapy.	Jpn J Clin Oncol	43(9)	923-928	2013
Nishimatsu H, Suzuki E, Nomiya A, Niimi A, Suzuki M, Fujimura T, Fukuhara H, Homma Y	Adrenomedullin and Angiopoietin-1 Additively Restore Erectile Function in Diabetic Rats: Comparison with the Combination Therapy of Vascular Endothelial Growth Factor and Angiopoietin-1.	J Sex Med	10(7)	1707-1719	2013
Yamada D, Nishimatsu H, Kumano S, Hirano Y, Suzuki M, Fujimura T, Fukuhara H, Enomoto Y, Kume H, Homma Y	Reduction of prostate cancer incidence by naftopidil, an α1 -adrenoceptor antagonist and transforming growth factor-β signaling inhibitor.	Int J Urol.	20(12)	1220-1227	2013

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Miyazaki H, Kume H, Suzuki M, Fujimura T, Fukuhara H, Enomoto Y, Nishimatsu H, Homma Y	Leukopenia as a risk factor for osteonecrosis of the jaw in metastatic prostate cancer receiving zoledronic acid and docetaxel.	BJU International		E520-525	2012
Nakamura M, Fujimura T, Nagata M,Hosoda C, Suzuki M, Fukuhara H, Enomoto Y, Nishimatsu H, Kume H, Igawa Y, Homma Y.	Association between lower urinary tract symptoms and sexual dysfunction assessed using the core lower urinary tract symptom score and International index of erectile function-5 questionnaires.	Aging Male	15	111-114	2012

Sugihara T, Yasunaga H, Horiguchi H, Nishimatsu H, Fukuhara H, Enomoto Y, Kume H, Ohe K, Matsuda S, Homma Y.	Management trends, angioembolization performance and multiorgan injury indicators of renal trauma from Japanese administrative claims database.	International Journal of Urologyl	19	559-563	2012
Fujimura T, Kume H, Nishimatsu H, Sugihara T, Nomiya A, Tsurumaki Y, Miyazaki H, Suzuki M, Fukuhara H, Enomoto Y, Homma Y.	Assessment of lower urinary tract symptoms in men by international prostate symptom score and core lower urinary tract symptom score.	BJU International	109	1512-1516	2012
Nagata M, Sakurai-Yageta M, Yamada D, Goto A, Ito A, Fukuhara H, Kume H, Morikawa T, Fukayama M, Homma Y, Murakami Y.	Aberrations of a cell adhesion molecule CADM4 in renal clear cell carcinoma.	InternationalJournal of Cancer	130	1329-1337	2012
Itoh Y, Nakahara H, Itoh R, Ito A, Satou T.	Osteoplastic ameloblastoma: a case report and literature review	Oral Surg Oral Med Oral Pathol Oral Radiol	113	23-28	2012
Enomoto A, Uchihashi T, Izumoto T, Nakahara H, Hamada S	Suppurative arthritis of the temporomandibular joint associated with bisphosphonate:	J Oral Maxillofacial. Surg.	70	1376-1379	2012
Morimoto Y, Hamada S, Ogura T, Nakahara H, Enomoto A, Uchihashi T, Hiraoka S, Kogo M.	A case of malignant melanoma discovered as a result of metastatic disease of the temporomandibular joint.	Journal of Oral and Maxillofacial Surgery,Medicine,and Pathology.	25	74-78	2012
内橋俊大、中原寛和、榎本明史、内橋隆行、栗本聖之、泉本貴子、濱田傑	下顎骨に発生した良性歯原性腫瘍および悪性腫瘍の鑑別	近大医学誌	37	87-91	2012
泉本貴子、中原寛和、榎本明史、松井裕一、内橋俊大、栗本聖之、内橋隆行、上田貴史、濱田傑	周術期院内紹介患者の歯性感染症治療	近大医学誌	38(1,2)	69-72	2013
Tajiri T, Kimura O, Fumino S, Furukawa T, Iehara T, Souzaki R, Kinoshita Y, Koga Y, Suminoe A, Hara T, Kohashi K, Oda Y, Hishiki T, Hosoi H, Hiyama E, Taguchi T.	Surgical strategies for unresectable hepatoblastomas.	J Pediatr Surg.	47	2194-2198	2012
Shiohama T, Fujii K, Hayashi M, Hishiki T, Suyama M, Mizuochi H, Uchikawa H, Yoshida S, Yoshida H, Kohno Y.	Phrenic nerve palsy associated with birth trauma - Case reports and a literature review.	Brain Dev.	35	363-366	2013

Inoue Y, Ochiai H, Hishiki T, Shimojo N, Yoshida H, Kohno Y.	Food allergy after cord blood stem cell transplantation with tacrolimus therapy in two patients who developed veno-occlusive disease.	Allergol Int.	61	497-499	2012

MRI of the Salivary Grand

唾液腺疾患のMRI

編集
柏木伸夫
近畿大学医学部放射線医学教室講師

64
MEDICAL VIEW

執筆者一覧

◆ 編 集

柏木伸夫 近畿大学医学部放射線医学教室講師

◆ 執筆者(掲載順)

中原寛和 近畿大学医学部歯科口腔外科准教授

渡口真史 近畿大学医学部放射線医学教室

柏木伸夫 近畿大学医学部放射線医学教室講師

原田博史 生長会府中病院病理診断科副部長

寺尾恭一 近畿大学医学部耳鼻咽喉科准教授

土井勝美 近畿大学医学部耳鼻咽喉科主任教授

北野睦三 近畿大学医学部耳鼻咽喉科講師

酒井美緒 大阪府立成人病センター放射線診断科副部長

池田耕士 関西医科大学放射線科学講師

臨床解剖

中原寛和

唾液腺は唾液の産生，分泌を担う外分泌腺であり，左右に一対ずつある3つの大唾液腺（耳下腺，顎下腺，舌下腺）と小唾液腺に分かれている。小唾液腺は口腔内，中咽頭に多数，広汎に分布している[1]（図1）。

耳下腺

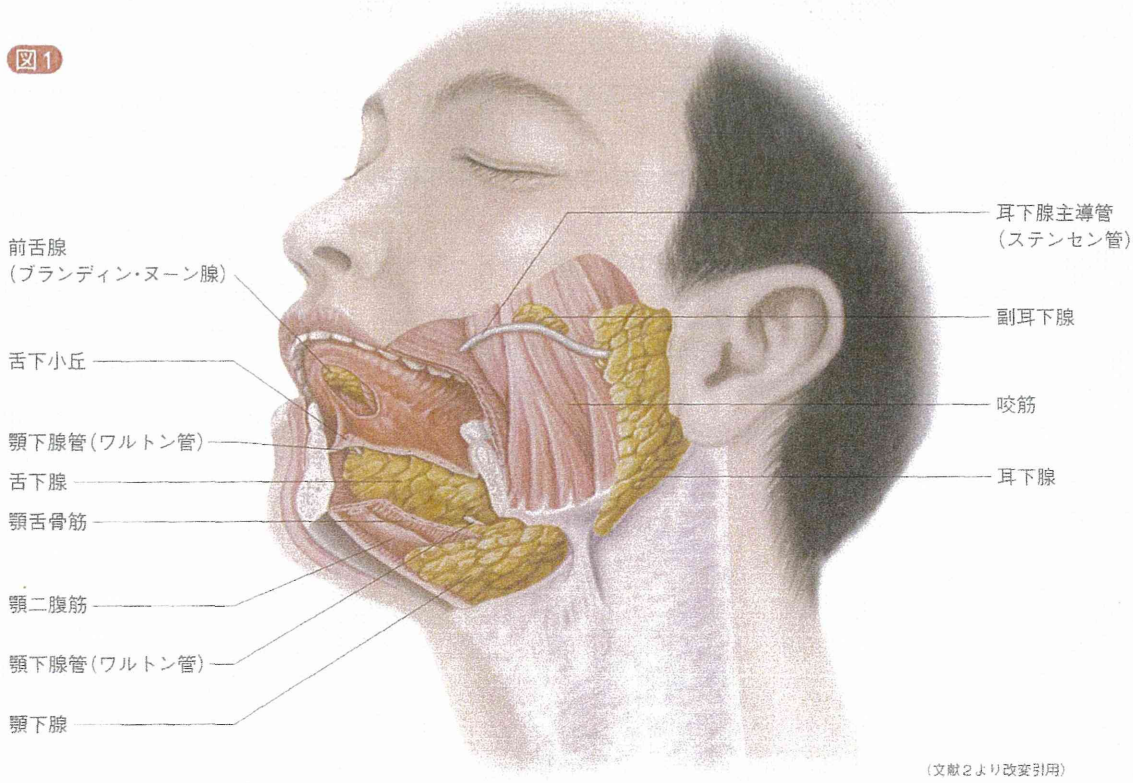
◆ 位置

耳下腺は最大の大唾液腺で，外耳孔前下方の皮膚直下に拡がっており，浅葉と深葉に分かれている。浅葉は外耳道，頬骨弓の高さから下顎角外側を越えて胸鎖乳突筋前縁の皮膚直下に存在している。深葉は，浅葉の深部に広がり内面は咬筋から下顎枝後縁，さらには内側翼突筋，傍咽頭隙に接している（図1，2）。

◆ 耳下腺管

耳下腺の主導管（ステンセン管，Stensen's duct）は腺の前上部から出て，頬骨弓下の咬筋直上を横走し，その前縁で内側に曲がり，頬筋，頬粘膜を貫き，上顎第二大臼歯付近の頬粘膜の耳下腺乳頭に開口する（図1～3）。副耳下腺が，主導管の周囲に存在することがある（図1）。

図1



（文献2より改変引用）

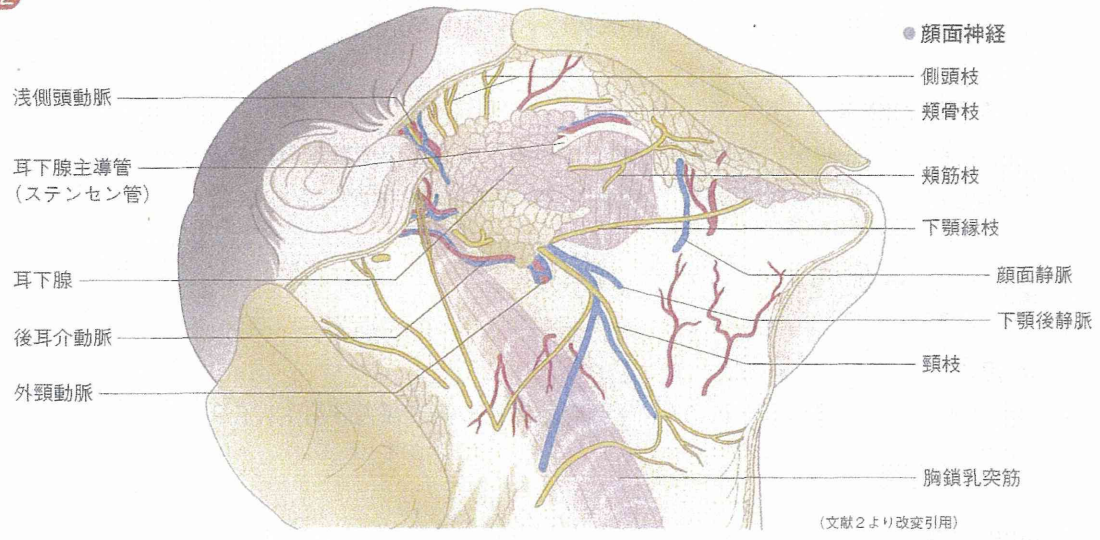
◆ 顔面神経との位置関係

顔面神経主幹は茎乳突孔から出て，耳下腺の皮膜に覆われ腺内に入る(図2，4，5)。顔面神経は腺内で分枝・吻合し，耳下腺神経叢を形成しており，耳下腺神経叢の外側の浅葉，内側の深葉に分けられている(図2，5)。耳下腺神経叢より末梢は耳下腺の上縁から側頭枝，頬骨枝が，前縁から頬筋枝と下顎縁枝が，下縁付近より頸枝が分枝している(図2，5)。

◆ 耳下腺の脈管

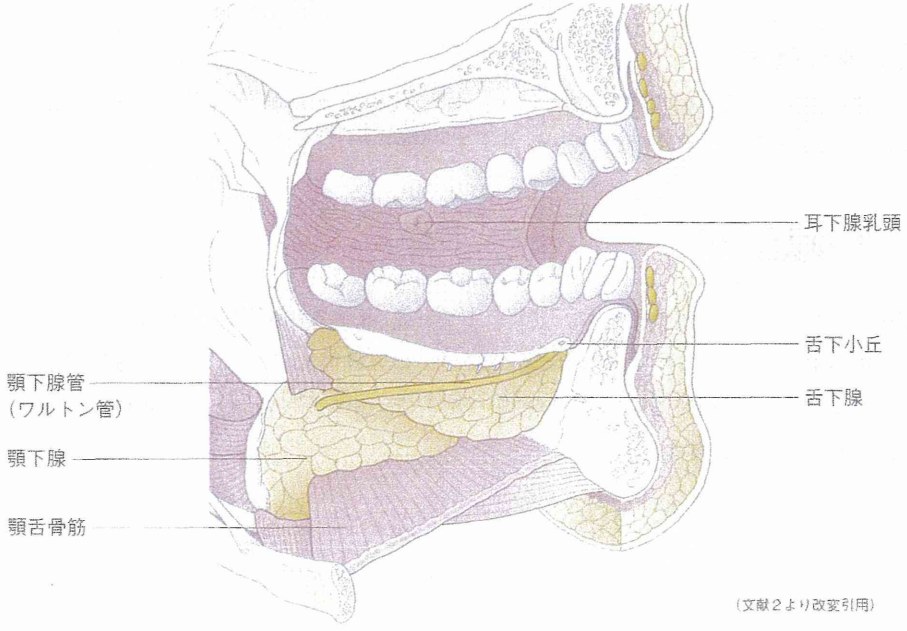
耳下腺の栄養は外頸動脈の枝が担っている。腺深葉内側を走る外頸動脈は，まず後耳介動脈を分枝し，次に腺内で顎動脈と浅側頭動脈の2終枝に分かれる。これらの血管から耳下腺枝が分布している。耳下腺の静脈血は下顎後静脈に集められ，顔面静脈と吻合する(図2)。

図2



(文献2より改変引用)

図3



(文献2より改変引用)

◆ 耳下腺のリンパ節

耳下腺表面および腺内にリンパ節は豊富に存在する。耳介前リンパ節は浅葉の上方で，耳介の前方にあり，耳下腺リンパ節は胸鎖乳突筋前縁の耳下腺下極にある。腺内にも浅リンパ節および深リンパ節がある。

◉— 顎下腺

◆ 位置

顎下腺は顎二腹筋の前腹と後腹および下顎骨下縁でつくられる顎下三角に位置しており，表面は広頸筋，内面は顎舌骨筋に接している(図1, 3)。

◆ 顎下腺管

顎下腺管(ワルトン管，Wharton's duct)は腺中層より出て顎舌骨筋の後縁を回って口腔底を前方に走り，舌下小丘に開口する(図1, 3, 6)。

◆ 顎下腺の脈管

顎下線には顔面動脈の直接枝が分布している。さらに顔面動脈の枝であるオトガイ下動脈や上行口蓋動脈の枝，舌動脈の枝が分布している場合もある。

◉— 舌下腺

◆ 位置

舌下腺は舌下粘膜の直下にあり，下顎骨と舌筋の間で顎舌骨筋上に位置している(図1, 3, 6)。

◆ 舌下腺管

導管は顎下腺管と合流して舌下小丘に開口する(図1, 3, 6)。

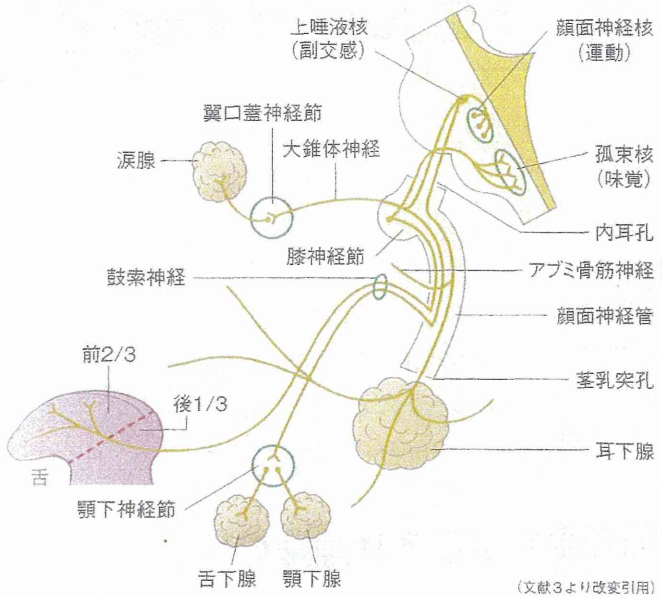
◆ 舌下腺の脈管

舌下腺は主に舌動脈の直接枝および舌動脈の枝の舌下動脈と舌深動脈の枝，顔面動脈の枝のオトガイ下動脈の枝が分布する(図6)。

◉— 小唾液腺

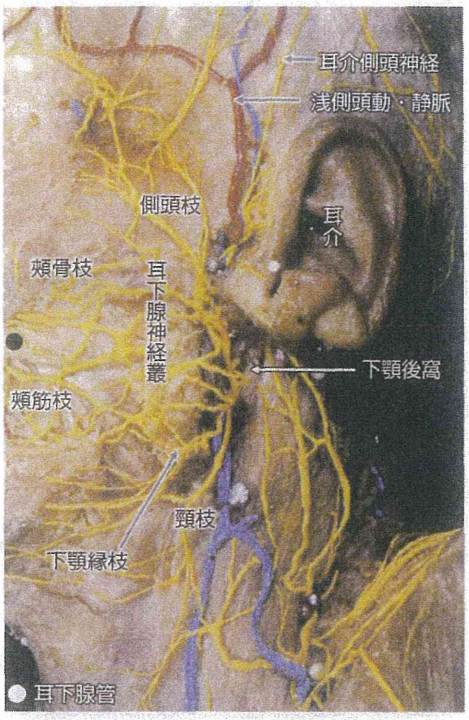
大唾液腺以外の小唾液腺として，上唇，下唇に分布する口唇腺，舌尖部に分布する前舌腺(別名ブランディン・ヌーン腺)(図1)，有郭乳頭および葉状乳頭付近の粘膜下にあるエブネル腺，分界溝より後部の後舌腺，頬粘膜の粘膜下に存在する頬腺，口蓋腺，臼歯腺がある。

図4 顔面神経の分布



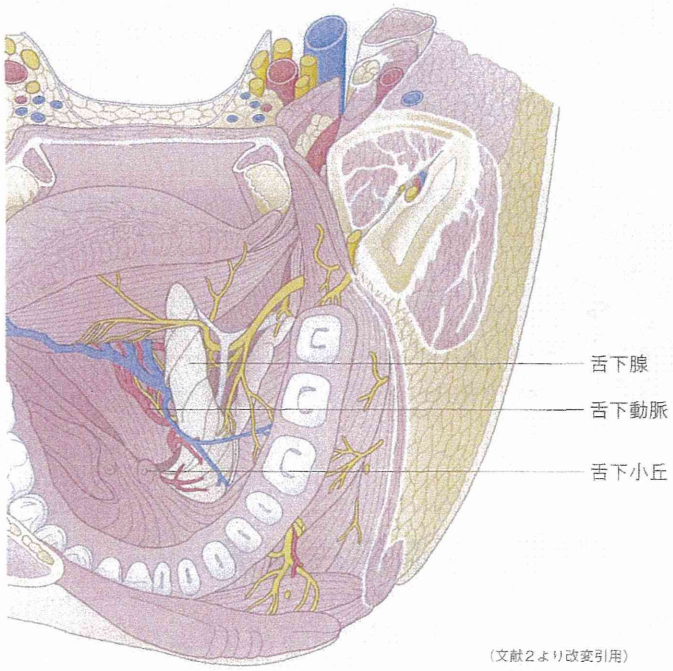
(文献3より改変引用)

図5



[上島幸枝, 北村清一郎：顔面. 鍼灸師・柔道整復師のための局所解剖カラーアトラス(北村清一郎, 熊本賢三編)改訂第2版, p34, 2012, 南江堂より許可を得て改変し転載)]

図6



(文献2より改変引用)

文献

1) Barnes L, et al: WHO Classification Head and Neck Tumours. Oxford Univ. Press UK 2005; 212-4.
2) Ferner H: 臨床応用局所解剖図譜Pernkopf Atlas der topographischen und angewandten Anatomie des Menschen. 第1巻頭部・頸部第2版, 医学書院, 東京, 1980.
3) 清木勘治：小解剖学書改訂第3版. 金芳堂, 東京, 1983.
4) 北村清一郎編著：臨床家のための口腔顎顔面解剖アトラス. 医歯薬出版株式会社, 東京, 2009.

唾液腺疾患（腫瘍）の疫学

中原寛和

唾液腺腫瘍の頻度

全世界における年間の唾液腺腫瘍の頻度は人口10万人あたり0.4～13.4人で，唾液腺悪性腫瘍の頻度は人口10万人あたり0.4～2.6人とされている[1]。米国では，頭頸部癌の6%が唾液腺悪性腫瘍であり，全悪性腫瘍中の0.3%を占めている[1]。

大唾液腺，小唾液腺別頻度および悪性腫瘍の比率

唾液腺腫瘍の大唾液腺，小唾液腺別の頻度については64～80%が耳下腺に発生し，続いて顎下線，小唾液腺に多く，舌下腺の発生頻度は1%以下となっている（表1）。唾液腺腫瘍は良性腫瘍の比率が高いが，全唾液腺腫瘍のなかで悪性腫瘍の比率は21～46%となっている。各々の腺においては，舌下腺では70～90%と悪性腫瘍の比率が高く，顎下線，小唾液腺と比率は低下し，耳下腺では11～32%と最も低いと報告されている[1]。わが国では全唾液腺腫瘍の悪性腫瘍の比率は15.8～43.7%と施設によって異なっている[2-5]。わが国においても耳下腺より顎下線，舌下腺のほうが悪性腫瘍の頻度が高いとの報告が多い[2-5]。

表1 唾液腺腫瘍の頻度と悪性腫瘍の比率

	腫瘍の腺別頻度	悪性腫瘍の比率
耳下腺	64～80%	11～32%
顎下腺	7～11%	41～45%
舌下腺	1%以下	70～90%
小唾液腺	9～23%	50%
全体		21～46%

（文献1より引用）

唾液腺腫瘍の性差，患者平均年齢

唾液腺腫瘍患者の平均年齢は良性では46歳，悪性では47歳となっているが，60～70歳台に分布のピークがある[1]。頻度の高い多形腺腫，粘表皮癌，腺房細胞癌は30～40歳台に多い。組織型により若干の性差があるとされている[1]。わが国においても年齢分布は中年層に多くみられるとの報告が多い[2-5]。全唾液腺腫瘍として性差はないとされているが，良性腫瘍は女性に，悪性腫瘍は男性に多いとする報告も認められる[2-4]。

組織型別頻度

最も発生頻度の高い腫瘍は多形腺腫で全唾液腺腫瘍の50%を占める。続いてワルチン腫瘍が多い。悪性腫瘍では粘表皮癌の発生頻度が最も高いという報告が多い[1]。わが国でも組織別の発生頻度ではほとんどの報告で多形腺腫が最も発生頻度が高いとしている[2-5]。良性腫瘍の組織型は多形腺腫とワルチン腫瘍がほとんどであり，その他の組織型はまれとされている[3]。悪性では粘表皮癌，腺様嚢胞癌，多形腺内癌，腺癌と報告されているものの施設によってばらつきがある[2-5]。悪性腫瘍のなかでは腺様嚢胞癌が最も多いとする報告もある[4]。

文献

1) Barnes L, et al: WHO Classification Head and Neck Tumours. Oxford Univ. Press UK, 2005; 212-4.
2) 安田大栄ほか：唾液腺腫瘍の臨床統計．耳鼻臨床 2004; 97: 611-4.
3) 川野真太郎ほか：唾液腺腫瘍151例の臨床統計的検討．日口外誌 2005; 52: 393-400.
4) 堀之内康文ほか：唾液腺腫瘍160例の臨床統計的検索．日口外誌 1990; 36: 1730-7.
5) 山中康嗣ほか：唾液腺腫瘍90例の臨床統計的検討．口腔腫瘍 1999; 11: 1-10.

唾液腺疾患のMRI

2014年10月1日　第1版第1刷発行

■編　集　柏木伸夫　かしわぎ のぶお

■発行者　鳥羽清治

■発行所　株式会社メジカルビュー社
〒162－0845　東京都新宿区市谷本村町2－30
電話　03（5228）2050（代表）
ホームページ　http://www.medicalview.co.jp/

営業部　FAX 03（5228）2059
E－mail　eigyo@medicalview.co.jp

編集部　FAX 03（5228）2062
E－mail　ed@medicalview.co.jp

■印刷所　図書印刷株式会社

ISBN978－4－7583－1581－4　C3047

Oncogene (2014) 33, 2236–2244

www.nature.com/onc

ORIGINAL ARTICLE

The pleiotrophin-ALK axis is required for tumorigenicity of glioblastoma stem cells

R Koyama-Nasu[1], R Haruta[1], Y Nasu-Nishimura[1], K Taniue[1], Y Katou[2], K Shirahige[2], T Todo[3], Y Ino[3], A Mukasa[3], N Saito[3], M Matsui[1], R Takahashi[1], A Hoshino-Okubo[1], H Sugano[1], E Manabe[1], K Funato[1] and T Akiyama[1]

Increasing evidence suggests that brain tumors arise from the transformation of neural stem/precursor/progenitor cells. Much current research on human brain tumors is focused on the stem-like properties of glioblastoma. Here we show that anaplastic lymphoma kinase (ALK) and its ligand pleiotrophin are required for the self-renewal and tumorigenicity of glioblastoma stem cells (GSCs). Furthermore, we demonstrate that pleiotrophin is transactivated directly by SOX2, a transcription factor essential for the maintenance of both neural stem cells and GSCs. We speculate that the pleiotrophin-ALK axis may be a promising target for the therapy of glioblastoma.

Oncogene (2014) **33,** 2236–2244; doi:10.1038/onc.2013.168; published online 20 May 2013

Keywords: ALK; cancer stem cell; glioblastoma; kinase; pleiotrophin; SOX2

INTRODUCTION

Glioblastoma is one of the most aggressive human cancers with a median survival of around 1 year.[1] Increasing evidence suggests that glioblastoma may arise from the transformation of neural stem/precursor/progenitor cells.[2] Consistent with this idea, glioblastoma cells cultured in serum-free media, which favor the growth of neural stem cells (NSCs), maintain stem-like properties and tumorigenicity.[3] However, when grown in the presence of serum, they undergo irreversible differentiation and lose their tumorigenicity.[3] This finding raises the possibility that differentiation therapy might be effective for glioblastoma.[4]

Almost all cell proliferative signaling involves phosphotransfer cascades, and accordingly protein kinases have been intensely pursued as drug targets. Indeed, a number of small-molecule inhibitors and antibodies targeting kinases are currently being used for cancer treatment.[5] Therefore, one approach to developing differentiation-inducing therapies for glioblastoma would be to identify kinases that regulate their stem-like properties.

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that is bound by the growth factor pleiotrophin or the closely related midkine.[6,7] ALK was initially discovered as a protein fused to nucleophosmin (NPM) in an anaplastic large cell lymphoma (ALCL). This fusion was shown to cause the ligand-independent autophosphorylation and activation of ALK. Studies using mouse models further showed that this NPM-ALK fusion is a primary driver of oncogenesis in ALCL.[8] Moreover, oncogenic fusions or mutations of ALK have also been described in various other cancers, including inflammatory myofibroblastic tumors, non-small cell lung cancer (NSCLC), diffuse large B-cell lymphoma, squamous cell carcinoma of the esophagus and neuroblastoma.[7] Importantly, the ALK inhibitor crizotinib has recently been approved for the treatment of ALK-positive NSCLC.[9,10] In addition, an anti-ALK antibody has been shown to repress the invasive capacity of the glioblastoma cell line U87 (Stylianou *et al.*[11]).

The Sry-related transcription factor SOX2 was identified as a partner of Oct3/4 in embryonic stem cells (ESCs) and is known to be essential for pluripotent cell development.[12–14] SOX2 is also expressed in NSCs and has an important role in neural development and homeostasis of the adult central nervous system.[14,15] On the other hand, it has also been shown that SOX2 is overexpressed in glioblastoma.[16] Furthermore, it has been reported that knockdown of SOX2 by RNA interference (RNAi) suppresses the tumorigenicity of glioblastoma stem cells (GSCs) xenografted into immunodeficient mice.[17]

In the present study, we show that the pleiotrophin-ALK axis is activated by SOX2 and is required for the self-renewal and tumorigenicity of GSCs.

RESULTS

ALK and its ligand pleiotrophin are required for the self-renewal and stem-like properties of GSCs

We established four GSC lines under serum-free conditions, GB2–5, and found that they are of the proneural type.[18,19] These cell lines exhibited enrichment for GSCs as they maintained sphere-forming ability and expressed high levels of the NSC markers, SOX2 and Nestin, as reported previously[3] (Figure 1). The GB2 cell line possesses the highest tumorigenic activity among our GSC lines, and we used these cells to perform an unbiased kinome-wide RNAi screen. We transfected GB2 cells that had been maintained in serum-free medium with a library of small interference RNAs (siRNAs) that target each of 704 kinases and kinase-related genes and then measured the expression levels of the stem cell markers *CD133*[20] and *Lgr5*.[21] We found 15 kinase genes whose suppression affected *CD133* or *Lgr5* expression, including 4 kinases known to be involved in the proliferation of GSCs[22] (Table 1 and Supplementary Table S1). One of these top 15 genes, ALK was also previously reported to be involved in

[1]Laboratory of Molecular and Genetic Information, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan; [2]Laboratory of Genome Structure and Function, Center for Epigenetic Disease, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan and [3]Department of Neurosurgery, The University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan. Correspondence: Professor T Akiyama, Laboratory of Molecular and Genetic Information, Institute for Molecular and Cellular Bioscience, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-0032, Japan.
E-mail: akiyama@iam.u-tokyo.ac.jp
Received 27 June 2012; revised 23 March 2013; accepted 25 March 2013; published online 20 May 2013

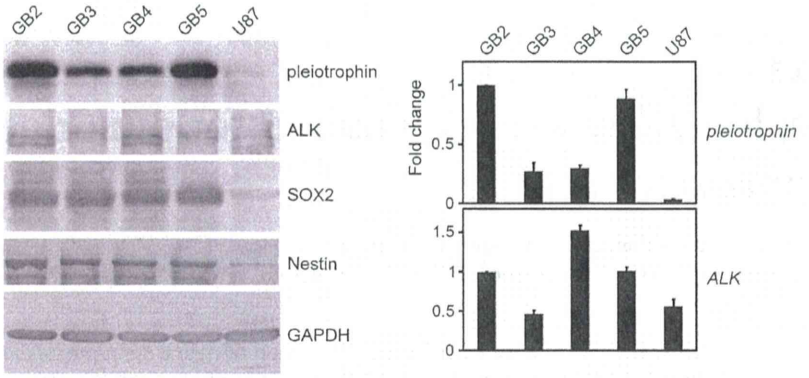


Figure 1. Expression levels of ALK and pleiotrophin in GSCs. GB2–5 cells were maintained in serum-free medium. U87 cells were maintained in serum-containing medium. Cell lysates were subjected to immunoblotting with antibodies to the indicated proteins (left). The mRNA levels of *ALK* or *pleiotrophin* were evaluated by quantitative RT–PCR and shown as the fold change over mRNA levels in GB2 cells (right). Error bars represent the s.d. ($n = 3$).

Table 1. Kinome-wide RNAi screen in GSCs

	Gene symbol	*CD133*	*Lgr5*	*Average*
1	TSSK6	0.55	0.50	0.52
2	GRK4	0.49	0.81	0.65
3	FRK	0.58	0.76	0.67
4	CDC42BPA*	0.45	0.89	0.67
5	PICK1	0.71	0.70	0.71
6	TAL1	0.78	0.64	0.71
7	DVL3	0.80	0.62	0.71
8	PCTK2	0.78	0.65	0.71
9	MARK1	0.70	0.73	0.72
10	OXSR1*	0.75	0.70	0.72
11	FYN*	0.53	0.91	0.72
12	DYRK2	0.76	0.70	0.73
13	MAPKAPK3*	0.68	0.80	0.74
14	**ALK**	**0.69**	**0.80**	**0.75**
15	ETNK2	0.89	0.60	0.75

Abbreviations: GSC, glioblastoma stem cell; RNAi, RNA interference. Note: Average fold changes in the top 15 genes whose knockdown resulted in the greatest change in CD133 and/or Lgr5 expression. ALK data are indicated in bold and the asterisks indicate genes identified in a similar RNAi screen.[22] The entire results are shown in Supplementary Table S1.

glioblastomagenesis.[11,23] However, these studies mainly analyzed the commonly used glioblastoma cell line U87, which does not exhibit any stem-like properties.[3,24] Furthermore, although U87 cells are highly tumorigenic, U87-derived tumors do not show any glioblastoma-specific features.[3,24] We therefore set out to study the role of ALK in the tumorigenicity of GSCs. Lentiviral introduction of a short hairpin RNA (shRNA) targeting ALK resulted in a decrease in both sphere formation and stem cell marker expression (Figures 2a and b). Although the levels of *nestin* mRNA were downregulated, the levels of Nestin protein did not change drastically, probably due to the stability of the Nestin protein in GB2 cells.

We next examined whether the ALK ligand pleiotrophin is required for the stem-like properties of GSCs. We found that pleiotrophin was expressed at high levels in GB2–5 cells compared with U87 cells and mainly as an 18-kDa protein (Figure 1). When pleiotrophin expression was knocked down by shRNA, both sphere formation and expression levels of the stem cell markers examined were suppressed (Figures 2a and b). We found that GSCs infected with a lentivirus expressing an shRNA targeting ALK or pleiotrophin had increased levels of the neural marker mitogen-activated protein kinase 2 (*MAP2*; Supplementary Figure S1A). By contrast, knockdown of either ALK or pleiotrophin resulted in decreased expression of the astrocyte marker glial fibrillary acidic protein (*GFAP*) and the oligodendrocyte marker *Olig2* (Supplementary Figure S1A). We observed that knockdown of either ALK or pleiotrophin did not cause apoptosis (Supplementary Figure S1B) or any drastic morphological change (Supplementary Figure S1C). In addition, knockdown of pleiotrophin resulted in a decrease in ALK protein and mRNA levels, suggesting that pleiotrophin stimulates not only ALK kinase activity but also *ALK* gene expression. These results suggest that ALK and pleiotrophin are important for the self-renewal and stem-like properties of GSCs.

ALK and pleiotrophin activate the Myc and ESC-like transcriptional programs in GSCs

To study the role of the pleiotrophin-ALK axis in GSCs, we investigated the gene expression profiles of GB2 cells in which either ALK or pleiotrophin expression had been suppressed by siRNA. DNA microarray analyses revealed that the MAP kinase, phosphoinositide 3-kinase (PI3-kinase) and Janus kinase/signal transducer and activator of transcription factor (JAK/STAT) pathways are activated in GSCs (Figure 2c and Supplementary Tables S2–S4). Pleiotrophin, but not ALK, was also found to activate the Wnt signaling pathway. We found about a 30% overlap in the genes whose expression was reduced by suppression of ALK and pleiotrophin (Figure 2d and Supplementary Tables S2–S4). Furthermore, we found that downstream target genes of ALK and pleiotrophin overlap with those enriched in ESCs, which are known to be overexpressed in poorly differentiated tumors, including glioblastoma[25] (Figure 2d, upper panel and Supplementary Tables S2–S4). It had been previously reported that the NPM-ALK fusion protein induces Myc expression.[26] We also found that ALK and pleiotrophin target genes overlap those targeted by Myc and its related proteins, the Myc module, which has been reported to account for most of the similarity between ESCs and cancer cells[27] (Figure 2d, lower panel and Supplementary Tables S2–S4). These results suggest that ALK and pleiotrophin may confer a more aggressive oncogenic phenotype to glioblastomas by activating the Myc and ESC-like transcriptional programs.

ALK and pleiotrophin are critical for the tumorigenicity of GSCs

It has been suggested that the stem-like properties of GSCs are indispensable for their tumorigenicity.[3,28] We therefore attempted to clarify the involvement of the pleiotrophin-ALK axis in the tumorigenicity of GSCs. We took GB2 cells containing a

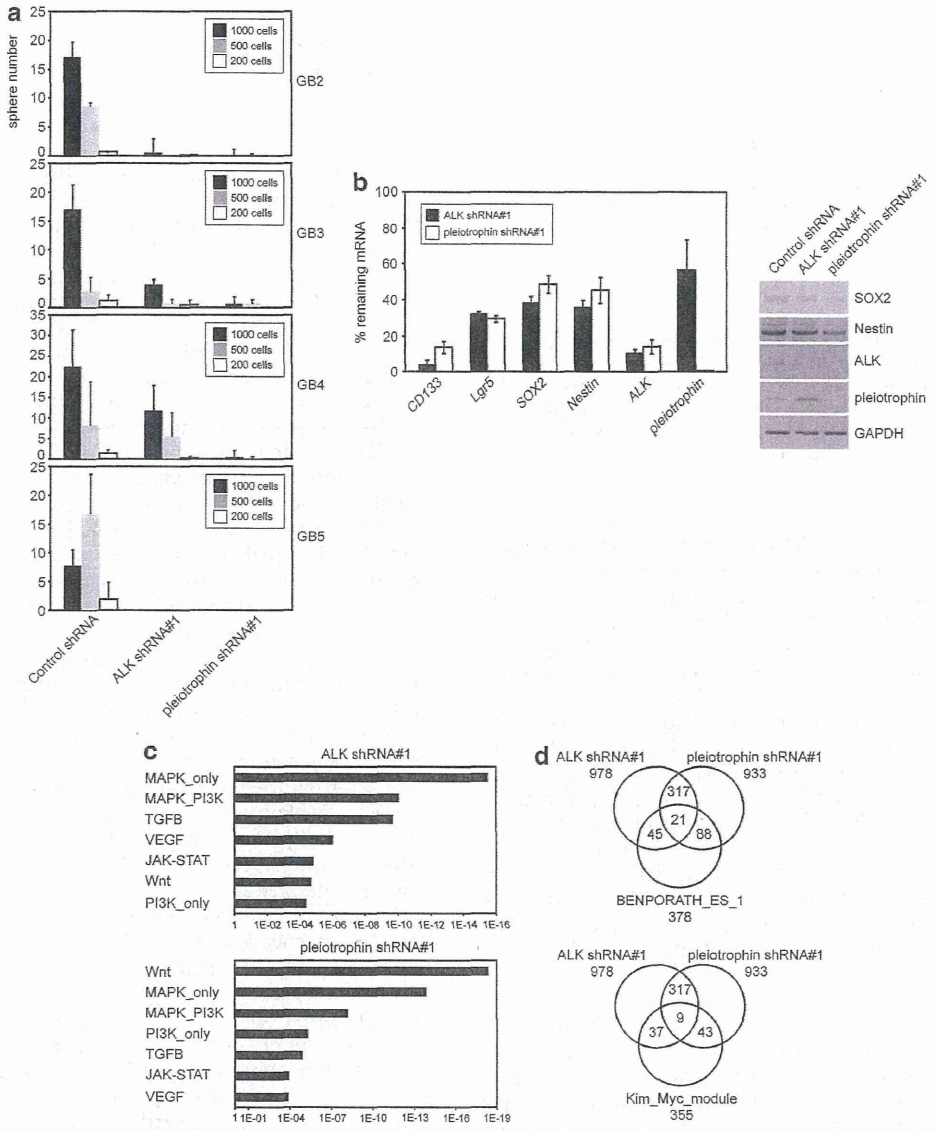


Figure 2. ALK and pleiotrophin are required for the self-renewal and stem-like properties of GSCs. (**a**) GB2–5 cells maintained in serum-free medium were infected with a lentivirus expressing an shRNA-targeting ALK or pleiotrophin. Three days after lentivirus infection, cells were trypsinized and plated at the indicated cell numbers into 96-well tissue culture plates. Two weeks after plating, the number of spheres was counted. Error bars represent the s.d. ($n = 7–8$). (**b**) GB2 cells maintained in serum-free medium were infected with a lentivirus expressing an shRNA-targeting ALK or pleiotrophin. One week after infection, the mRNA levels of the indicated genes were evaluated by quantitative RT–PCR and shown as the percentage of the remaining mRNA compared with cells expressing control shRNA (left). Error bars represent the s.d. ($n = 3$). Cell lysates were subjected to immunoblotting with antibodies to the indicated proteins (right). CD133 and Lgr5 could not be detected by immunoblotting because of their low expression levels. (**c**) GB2 cells maintained in serum-free medium were treated as described in panel (**b**). One week after infection, expression data were generated using HG-U133 plus 2.0 GeneChips. Bar graph represents signaling pathways downregulated by knockdown of ALK (upper) and pleiotrophin (lower). (**d**) Venn diagram showing the overlap among genes downregulated by knockdown of ALK (ALK_signature: ALK shRNA#1), those downregulated by knockdown of pleiotrophin (PTN_signature: pleiotrophin shRNA#1) and those enriched in ESCs (ES signature: BENPORATH_ES_1) (upper) or those targeted by Myc and its related proteins (Myc human: Kim_Myc_module) (lower). The significance of the overlap between each pair of signature is shown in Supplementary Table S4 (hypergeometric *P*-value).

lentivirus-delivered shRNA that stably suppresses ALK or pleiotrophin expression and intracranially transplanted these into immunodeficient mice. Mice receiving the ALK- or pleiotrophin-suppressed cells were found to survive longer than those transplanted with GB2 cells infected with a control lentivirus (Figure 3). Histopathological analysis of the tumor xenografts demonstrated that silencing of either ALK or pleiotrophin inhibited glioblastoma progression, whereas control GB2 cells formed invasive glioblastoma (Supplementary Figure S2). Taken together, these results suggest that the pleiotrophin-ALK axis maintains the tumorigenicity of GSCs.

High expression of pleiotrophin in GSCs

Aberrant activation of ALK by oncogenic fusion or mutation can drive tumorigenesis.[7] However, we could not identify any

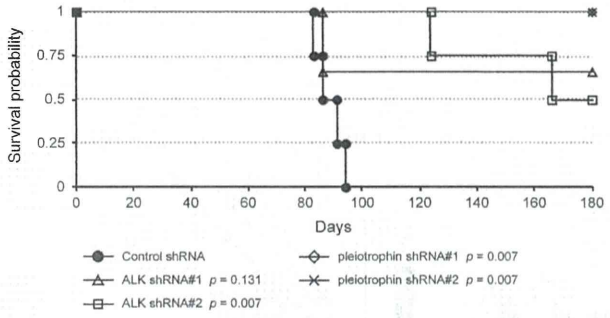


Figure 3. ALK and pleiotrophin are critical for the tumorigenicity of GSCs. GB2 cells maintained in serum-free medium were infected with a lentivirus expressing an shRNA-targeting ALK or pleiotrophin. One week after infection, cells were transplanted into the frontal lobe of immunodeficient mice. *P*-values with comparison to control shRNA by log rank test are shown.

mutation in the *ALK* gene in GB2 cells, consistent with the results of a comprehensive genome analysis reported previously.[29,30] It is known that pleiotrophin is highly expressed in GSCs (Figure 1). We therefore examined the transcriptional control of *pleiotrophin* in GSCs. We found that pleiotrophin protein and mRNA expression levels decreased during serum-induced differentiation, similar to what was observed for the stem cell markers (Figure 4a). Moreover, retinoic acid-induced differentiation also resulted in a decrease in the levels of pleiotrophin[31] (Figure 4b). We also investigated the expression profiles of *pleiotrophin* obtained from a public microarray database.[3] Almost all patient glioblastomas and two GSC lines as well as NSCs expressed substantial levels of *pleiotrophin* (Figure 4c). By contrast, glioma cell lines and two GSC lines cultured in serum-containing medium expressed relatively low levels of *pleiotrophin*, presumably because they had undergone 'differentiation'.[3] These results suggest that high expression of pleiotrophin is a common feature of GSCs.

SOX2 directly transactivates the expression of pleiotrophin in GSCs

To further investigate the mechanisms underlying the expression of *pleiotrophin*, we performed reporter assays using a *luciferase* reporter under the control of the full-length *pleiotrophin* 5′ region (−1401 to +309) and several deletion variants. When transfected into GB2 cells, a reporter containing the region between −251 and +309 showed a substantial level of activity (Figure 5a, left panel). Furthermore, this activity was repressed upon serum-induced differentiation. We therefore attempted to identify the transcription factor(s) involved in modulating this promoter activity. We first searched the region −251 and +309 for transcription factor consensus binding sites and then compared the expression patterns of the selected transcription factors and pleiotrophin using a microarray database.[3] We found that the expression pattern of *pleiotrophin* is similar to that of *SOX2* (Figure 4c). Furthermore, a mutant reporter lacking the SOX2-binding site (−40 to −21) showed reduced promoter activity (Figure 5a, right panel). Chromatin immunoprecipitation analysis demonstrated that endogenous SOX2 was present at the *pleiotrophin* promoter in GSCs (Figure 5b and Supplementary Figure S3A). Consistent with these results, silencing of SOX2 by shRNA resulted in a decrease in the levels of pleiotrophin protein and mRNA (Figure 5c and Supplementary Figure S3B). Similar results were obtained by transfecting two different siRNAs targeting SOX2 (Supplementary Figure S3C). These results suggest that SOX2 stimulates transcription of the *pleiotrophin* gene, thereby maintaining high expression levels of pleiotrophin in GSCs.

DISCUSSION

It has been reported that ALK acquires oncogenic potential when truncated and fused to a partner protein, such as NPM, as can occur via chromosomal rearrangement.[7,8] It has also been reported that ALK is activated by point mutations in its kinase domain in some neuroblastoma.[7,32] Although we could not identify any chromosomal rearrangement or point mutation in ALK in GB2 cells, we found that ALK is highly expressed in GSC lines cultured in serum-free medium, consistent with previous reports.[33] Furthermore, we showed that knockdown of ALK results in a decrease in the self-renewing capacity and tumorigenicity of GB2 cells. We also found that pleiotrophin is overexpressed both in patient glioblastomas and in GSC lines cultured in serum-free medium and that knockdown of pleiotrophin leads to a reduction in the self-renewing capacity and tumorigenicity of GB2 cells. Thus, our results suggest that the pleiotrophin-ALK axis is required for the self-renewal and tumorigenicity of GSCs.

NPM-ALK, the most thoroughly studied ALK-fusion protein, has been reported to signal via the MAP kinase, phospholipase Cγ, PI3-kinase and JAK/STAT pathways.[7] Consistent with these reports, our DNA array analysis revealed that the MAP kinase, PI3-kinase and JAK/STAT pathways are activated in GSCs. Furthermore, we found that ALK and pleiotrophin activate the Myc and ESC-like transcriptional programs, which are known to be associated with more aggressive phenotypes in human cancers.[25,27] Activation of these pathways by ALK has not been previously reported, presumably because earlier studies utilized cultured cell lines grown in serum-containing media that do not exhibit any stem cell-like properties. We found that there was only about a 30% overlap between the genes dependent on ALK and pleiotrophin expression. Furthermore, only pleiotrophin, but not ALK, activated the Wnt signaling pathway. These results appear to be in line with the fact that pleiotrophin signals not only via ALK but also via other receptors, such as RPTPβ/ζ (receptor protein tyrosine phosphatase β/ζ) and N-syndecan.[34,35] Interestingly, pleiotrophin is known to stimulate tyrosine phosphorylation of β-catenin through inactivation of RPTPβ/ζ.[34] Furthermore, pleiotrophin stimulates tumor angiogenesis and remodeling of the microenvironment.[36] Consistent with these findings, we found that repression of pleiotrophin has a more dramatic effect on the tumorigenicity of GSCs than repression of ALK.

It has been reported that SOX2 is involved in the tumorigenesis of several cancers, including lung cancer and breast cancer.[37–39] We found that SOX2 directly transactivates the expression of pleiotrophin in GSCs. Our results appear to be consistent with a previous report showing that knockdown of SOX2 suppresses the tumorigenicity of GSCs in immunodeficient mice.[17] Thus, SOX2-mediated transactivation of pleiotrophin may be important for the tumorigenicity of GSCs. However, knockdown of SOX2 only partially reduced the expression levels of pleiotrophin (Figure 5c and Supplementary Figures 3B and C), and mutation of the SOX2-binding site in the pleiotrophin promoter only partially reduced activity of a luciferase reporter (Figure 5a, right panel). In addition, we found that the levels of pleiotrophin decreased faster than those of SOX2 during serum-induced differentiation (Figure 4a) and that retinoic acid-induced differentiation resulted in a drastic decrease in the expression levels of pleiotrophin but not of SOX2 (Figure 4b). Thus, the pleiotrophin-ALK axis may be regulated by other transcription factors in addition to SOX2.

SOX2 is well known to have critical roles in the maintenance of neural stem and progenitor cells.[14,15] For example, it has been reported that multipotent neural stem-like cells transfected with an siRNA targeting SOX2 express increased neurofilaments but decreased GFAP and Nestin levels.[40] These results suggest that SOX2 inhibits the differentiation of neural stem-like cells into neurons and maintains their stem-like properties. Intriguingly, our results appear to be consistent with these results. We found that

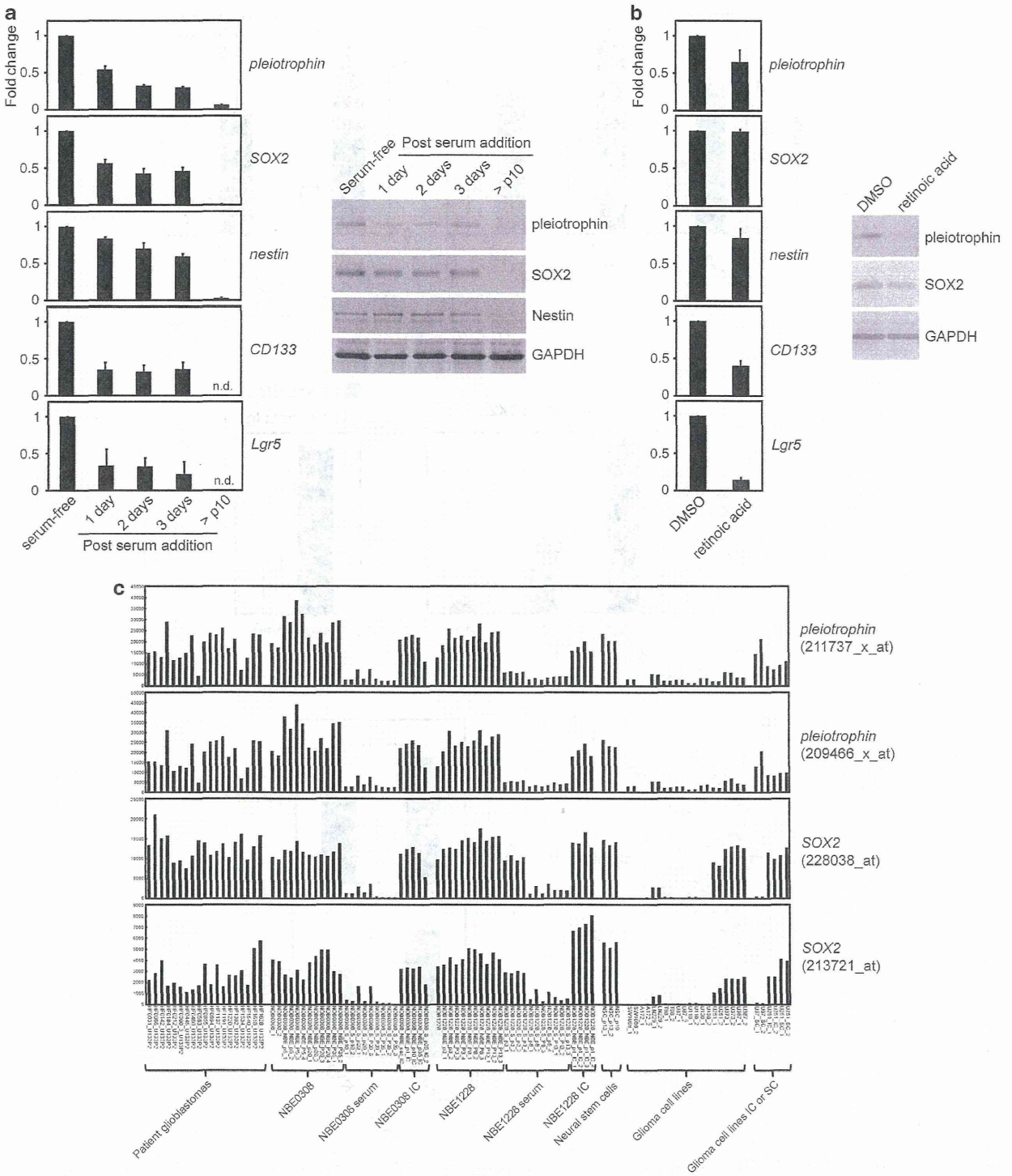


Figure 4. High expression of pleiotrophin in GSCs. (**a**) GB2 cells maintained in serum-free medium were cultured in serum-containing medium for the indicated times. > p10, GB2 cells cultured in serum-containing medium for > 10 passages. The mRNA levels of the indicated genes were evaluated by quantitative RT–PCR and shown as the fold change over mRNA levels in GB2 cells maintained in serum-free medium (left). Error bars represent the s.d. ($n = 3$). ND, not detected. Cell lysates were subjected to immunoblotting with antibodies to the indicated proteins (right). CD133 and Lgr5 could not be detected by immunoblotting because of their low expression levels. (**b**) GB2 cells maintained in serum-free medium were cultured in medium containing retinoic acid for 4 days. The mRNA levels of the indicated genes were evaluated by quantitative RT–PCR and shown as the fold change over the vehicle-treated cells (left). Error bars represent the s.d. ($n = 3$). Cell lysates were subjected to immunoblotting with antibodies to the indicated proteins (right). (**c**) Gene expression profiles of *pleiotrophin* and *SOX2* taken from the public microarray database GSE4536 (Lee *et al.*[3]). Data obtained with two independent probes for each gene are shown. NBE0308 and NBE1228 are GSC lines. Serum, GSCs cultured in serum-containing medium; IC, intracranial injection; SC, subcutaneous injection.

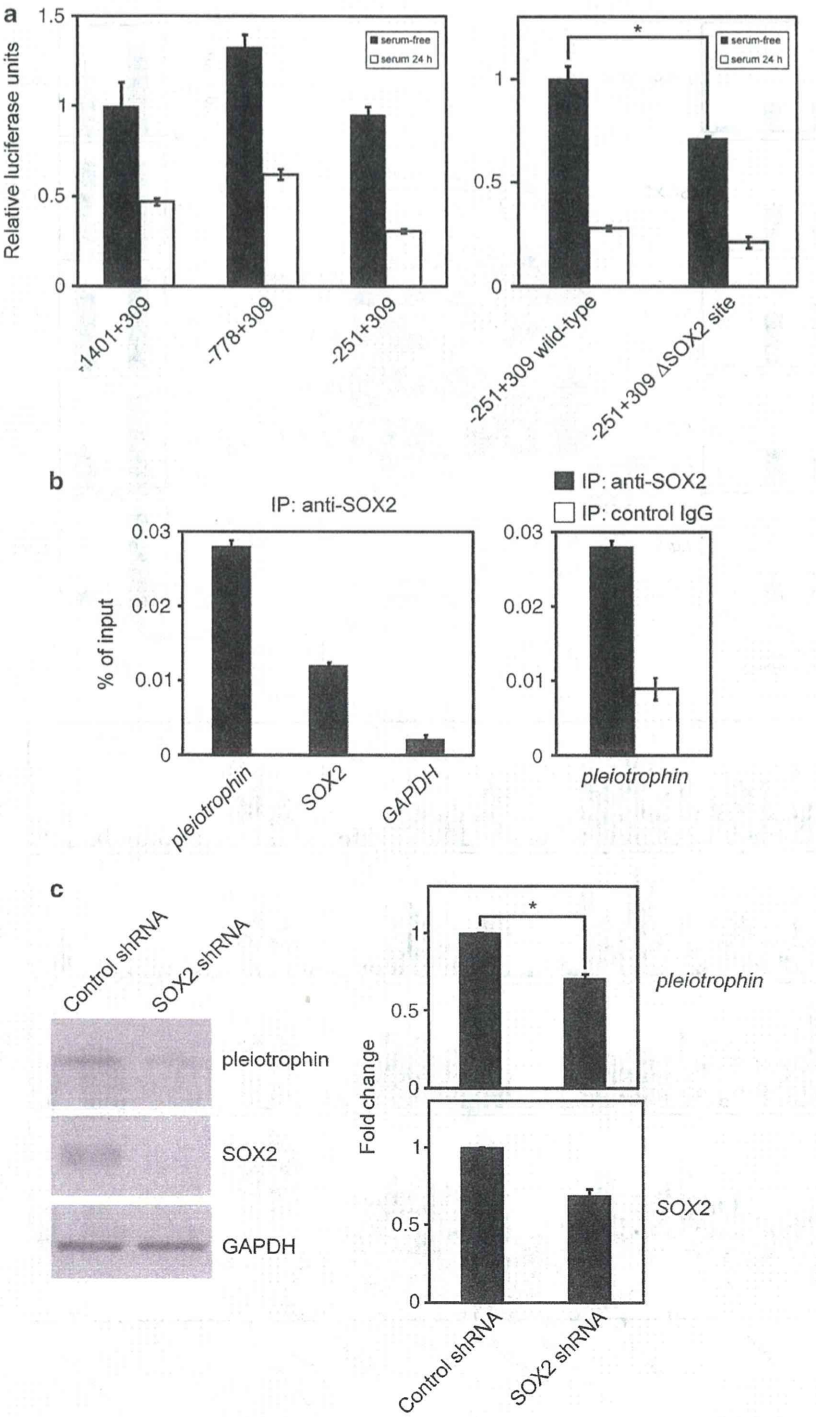


Figure 5. SOX2 directly transactivates the expression of pleiotrophin in GSCs. (**a**) GB2 cells maintained in serum-free medium were transfected with a luciferase reporter driven by the *pleiotrophin* promoter. Luciferase reporter activity is shown. Error bars represent the s.d. ($n = 3$). *$P = 0.0058$ by *t*-test. (**b**) Chromatin immunoprecipitation experiments with anti-SOX2 antibody were performed with GB2 cells maintained in serum-free medium. The precipitated chromatin was amplified by quantitative PCR using primers flanking the indicated gene promoters (*pleiotrophin* and *GAPDH*) or enhancer (*SOX2*) (left). The *SOX2* enhancer was used as a positive control, as SOX2 has been reported to be autoregulated.[50] The *GAPDH* promoter was used as a negative control. Control IgG was used as a negative control (right). Error bars represent the s.d. ($n = 3$). (**c**) GB2 cells maintained in serum-free medium were infected with a lentivirus expressing an shRNA-targeting SOX2. Cell lysates were subjected to immunoblotting with antibodies to the indicated proteins (left). The mRNA levels of the indicated genes were evaluated by quantitative RT–PCR and shown as the fold change over mRNA levels in cells expressing control shRNA (right). Error bars represent the s.d. ($n = 3$). *$P = 0.001$ by *t*-test.

GSCs infected with a lentivirus expressing an shRNA targeting ALK or pleiotrophin have increased MAP2 but decreased GFAP, Olig2 and Nestin levels. These results suggest that the pleiotrophin-ALK axis inhibits neural differentiation of GSCs but maintains their stem-like characteristics. It would be interesting to examine whether the pleiotrophin-ALK axis is also involved in the maintenance of normal neural stem and progenitor cells. SOX2 has also been reported to have essential roles in the neuronal differentiation of subsets of neural stem and progenitor cells.[14,15] For example, it has been reported that SOX2 deficiency causes impaired neurogenesis and neurodegeneration in the adult mouse brain.[41,42] Therefore, it would be interesting to investigate whether the pleiotrophin-ALK axis is also involved in the differentiation of neural stem and progenitor cells.

The ALK inhibitor crizotinib has recently been approved for the treatment of metastatic and late stage ALK-positive NSCLC having translocations of the ALK gene.[9,10] In addition, the ALK inhibitor TAE684 has been reported to inhibit the growth of ALK-positive ALCL, neuroblastoma and NSCLC cell lines.[43] Thus, we speculate that ALK inhibitors may be effective for the treatment of glioblastoma. ALK has been assumed to have a role in the development and function of the central and peripheral nervous system, as ALK is abundantly expressed in the nervous system during mouse embryogenesis,[7] which could raise concerns about the safety of such therapy. However, both ALK- and pleiotrophin-mutant mice survive as long as wild-type mice.[7,44] In addition, although pleiotrophin is highly expressed in NSCs, it seems dispensable for their proliferation *in vivo*.[45] Furthermore, it has been reported that the most common adverse reactions of crizotinib are relatively minor, consisting of vision disorder, nausea, diarrhea, vomiting, edema and constipation.[46] Thus, compounds targeting ALK or pleiotrophin would be expected to have relatively few serious side effects due to their effects on NSCs. In addition, we imagine that antibodies or compounds that specifically target the extracellular domain of ALK or pleiotrophin could also hold promise as novel anti-tumor reagents.

MATERIALS AND METHODS

Tumor specimens and primary tumor cultures

Tumor samples classified as primary glioblastoma were obtained from patients undergoing surgical treatment at the University of Tokyo Hospital with informed consent and as approved by the Institutional Review Board. Tumors were washed and mechanically and enzymatically dissociated into single cells. Tumor cells were cultured in Neurobasal medium (Life Technologies, Carlsbad, CA, USA) containing B27 supplement minus vitamin A (Life Technologies), EGF and FGF2 (20 ng/ml each; Wako Pure Chemical Industries, Osaka, Japan). For *in vitro* differentiation, tumor cells were cultured in DMEM (Dulbecco's modified Eagle's medium)/F-12 medium (Life Technologies) containing 10% fetal bovine serum or 10 μM all-trans retinoic acid (Sigma, St. Louis, MO, USA). U87 and 293FT cells were cultured in DMEM (Nissui, Tokyo, Japan) containing 10% fetal bovine serum. Transfections were performed using Lipofectamine 2000 Reagent, Lipofectamine LTX Reagent or Lipofectamine RNAiMAX Reagent (Life Technologies).

RNAi

Silencer Select Human Extended Druggable Genome siRNA Library was purchased from Life Technologies (the list is given in Supplementary Table S1). The siRNA oligonucleotide sequences were as follows: SOX2#1 (5′-CAGUAUUUAUCGAGAUAAA-3′), and SOX2#2 (5′-AGUG GAAACUUUUGUCGGA-3′).

The shRNA oligonucleotide sequences were as follows: ALK#1 (5′-GGCCU GUAUACCGGAUAAUGA-3′), ALK#2 (5′-GAAUACAGCACCCAAAUCAAG-3′), pleiotrophin#1 (5′-GGAGCUGAGUGCAAGCAAACC-3′), pleiotrophin#2 (5′-GCAACUGGAAGAAGCAAUUUG-3′), and SOX2 (5′-GUAAGAAACAGCAUG GAGAAA-3′).

Quantitative RT–PCR

Total RNA was extracted using the NucleoSpin RNA Clean-up kit (Takara Bio Inc., Shiga, Japan) and reverse-transcribed into cDNA using PrimeScript RT Master Mix (Takara Bio Inc.). Real-time PCR was performed using LightCycler480 SYBR Green I Master and a LightCycler480 Instrument (Roche, Indianapolis, IN, USA). The results were normalized with the detected value for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) or hypoxanthine phosphoribosyltransferase 1 (*HPRT1*). Primers used in real-time PCR were as follows: *GAPDH* forward (5′-GCACCGTCAAGGCTGA GAAC-3′), *GAPDH* reverse (5′-TGGTGAAGACGCCAGTGGA-3′); *HPRT1* forward (5′-GGCAGTATAATCCAAAGATGGTCAA-3′), *HPRT1* reverse (5′-GTCA AGGGCATATCCTACAACAAAC-3′); *CD133* forward (5′-AGTGGCATCGTGCA AACCTG-3′), *CD133* reverse (5′-CTCCGAATCCATTCGACGATAGTA-3′); *nestin* forward (5′-GAGGTGGCCACGTACAGG-3′), *nestin* reverse (5′-AAGCTGAGGG AAGTCTTGGA-3′); *Lgr5* forward (5′-GATTTCCTGCTTGACTTTGAGG-3′), *Lgr5* reverse (5′-GCAGGTGTTCACAGGGTTTG-3′); *SOX2* forward (5′-TTGCTGCCTC TTTAAGACTAGGA-3′), *SOX2* reverse (5′-CTGGGGCTCAAACTTCTCTC-3′); *ALK* forward (5′-CACTCCAGGGAAGCATGG-3′), *ALK* reverse (5′-TCGAAATGGGTT GTCTGGA-3′); *pleiotrophin* forward (5′-AACTGACCAAGCCCAAACCT-3′), *pleiotrophin* reverse (5′-GGTGACATCTTTTAATCCAGCA-3′), *MAP2* forward (5′-TCTCCTGTGTTAAGCGGAAAA-3′), *MAP2* reverse (5′-AATACACTGGGAGC CAGAGC-3′), *GFAP* forward (5′-GACCTGGCCACTGTGAGG-3′), *GFAP* reverse (5′-AGGCAGCCAGGTTGTTCTC-3′), *Olig2* forward (5′-AGCTCCTCAAATCGC ATCC-3′), and *Olig2* reverse (5′-ATAGTCGTCGCAGCTTTCG-3′).

Antibodies

Rabbit polyclonal antibody (pAb) to green fluorescence protein (GFP) and goat pAb to SOX2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit pAbs to ALK were from Cell Signaling Technology (Danvers, MA, USA). Mouse monoclonal antibody (mAb) to pleiotrophin (H-6) was from Santa Cruz Biotechnology. Mouse mAb to Nestin was from R&D systems (Minneapolis, MN, USA). Mouse mAb to GAPDH was from Millipore (Bedford, MA, USA).

Immunoblotting

Cells were lysed in lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 1 mM DTT, 1 mM EDTA, 2 mM Na_3VO_4, 10 mM NaF and protease inhibitors. Lysates were fractionated by SDS–PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and transferred to a PVDF (polyvinylidene difluoride) membrane (Immobilon-P, Millipore). The membrane was subjected to immunoblot analysis using alkaline phosphatase-conjugated anti-mouse immunoglobulin G (IgG) or anti-rabbit IgG (Promega, Madison, WI, USA) as secondary antibodies. Visualization was performed using the NBT/BCIP colorimetric substrate system (Promega).

Lentivirus production

A lentiviral vector (CS-Rfa-CG) expressing an shRNA driven by the H1 promoter was transfected together with the packaging vectors pCAG-HIV-gp and pCMV-VSV-G-RSV-Rev into 293FT cells. All plasmids were kindly provided by H. Miyoshi (RIKEN BioResource Center, Ibaraki, Japan). Virus supernatant was purified by ultracentrifugation at 25 000 r.p.m. for 90 min (SW28 rotor, Beckman Coulter, Brea, CA, USA). Infection efficiency was monitored by GFP expression driven by the CMV promoter.

Intracranial xenograft

One week after lentivirus infection, 1×10^4 cells were injected stereotactically into the right frontal lobe of 5-week-old nude mice (BALB/cAJcl-*nu/nu*, CLEA Japn Inc., Tokyo, Japan), following administration of general anesthesia ($n = 3$ or 4). The injection coordinates were 2 mm to the right of the midline, 1 mm anterior to the coronal suture and 3 mm deep. Mice were monitored for 6 months. Survival of mice was evaluated by Kaplan–Meier analysis. *P*-values were calculated using log rank test. The distribution of tumor cells was analyzed by GFP immunostaining. Tumors were histologically analyzed after hematoxylin and eosin staining. All animal experimental protocols were performed in accordance with the guidelines of the Animal Ethics Committee of the University of Tokyo.

Immunohistochemistry

Three months after injection of cells, brains were fixed in 3.7% buffered formalin, dehydrated and embedded in paraffin. Sections (6 μm) were rehydrated, and endogenous peroxidases were blocked by incubation in