EUROPEAN JOURNAL OF CANCER 42 (2006) 1237–1242

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.ejconline.com

Brain cancer stem-like cells

*Toru Kondo**

Centre for Brain Repair, University of Cambridge, Cambridge CB2 2PY, UK
Laboratory for Cell Lineage Modulation, Center for Developmental Biology, RIKEN, 2-2-3 Minatojima, Minamimachi, Chuo-ku, Kobe 650-0047, Japan

ARTICLE INFO

Article history:
Received 23 January 2006
Accepted 23 January 2006
Available online 2 May 2006

Keywords:
Cancer stem-like cells
CD133
Side population
Notch
Sonic hedgehog
Wnt

ABSTRACT

Both stem cells and cancer cells are thought to be capable of unlimited proliferation. Moreover, many tumours and cancer cell lines express stem cell markers, including adenosine triphosphate (ATP)-binding cassette transporters, by which the cells pump out specific fluorescent dyes as well as anti-cancer drugs, suggesting either that cancer cells resemble stem cells or that cancers contain stem-like cells. Using the common characteristics of brain tumour cells and neural stem cells, several research groups have succeeded in identifying stem-like cells (cancer stem-like cells) in brain tumours and brain cancer cell lines. The purified cancer stem-like cells, but not the other cancer cells, self-renew and form tumours when transplanted in vivo. Thus, cancer stem-like cells in brain tumours might be a crucial target for anti-brain tumour therapy.

1. Introduction

Recent progress in stem cell research might bring us closer to being able to regenerate cells or tissues lost by injury or illness. Stem cells are defined as cells that self-renew indefinitely and also give rise to differentiated cells.[1] During the last decade, it has been revealed that almost all tissues contain tissue-specific stem cells, which continuously generate the residential differentiated cells responsible for tissue functions and homeostasis.[2] Neural stem cells (NSCs) in the central nervous system (CNS), for example, self-renew and give rise to neurones, astrocytes and oligodendrocytes throughout life.[3,4] If our own NSCs can repair the damaged brain, they would be the best cells for therapy.

There is increasing evidence that malignant tumours, such as leukaemias, breast cancers and brain cancers, contain the cells that maintain the characteristics of tissue-specific stem cells and are malignant.[5–20] Malignant gliomas, for example, contain both proliferating cells expressing stem cell markers and differentiating cells expressing either neuronal markers or glial markers, raising the possibility that the tumours may contain NSC-like cells.[21–23] This idea is supported by recent findings that malignant gliomas can be generated from both NSCs and glial lineage cells,[24–26] such as oligodendrocyte precursor cells (OPCs) or astrocytes, which can behave as NSCs in appropriate conditions.[27–33]

There is other evidence that malignant tumours might contain cancer stem-like cells (CSCs). Although many anti-cancer drugs have been used to eliminate cancers, some cancer cells usually survive and the cancer recurs, indicating that the surviving cells are not only resistant to such anti-cancer drugs but are also malignant. It was shown that various ATP binding cassette (ABC) transporters, such as the protein encoded by the multi-drug resistant gene (MDR), the multi-drug resistant protein (MRP), and the breast cancer resistant protein (BCRP1), contribute to drug resistance in cancers.[34,35] Interestingly, some of these transporters are also expressed in many kinds of normal stem cells. BCRP1, for example, excludes the fluorescent dye Hoechst 33342, identifying a side population (SP), which is enriched for stem cells.[36–38] Together, these findings

* Tel./fax: +81 78 306 3172.
E-mail address: tkondo@cdb.riken.jp.
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2006.01.038

suggest that cancers might contain a SP that is enriched for cells with the characteristics of CSCs.

Using these common characteristics of NSCs and brain cancer cells, several groups have demonstrated that such stem-like cells, but not the other cells in brain tumours or brain cancer cell lines, can self-renew, express well-known NSC markers, such as Nestin, and form tumours when transplanted in vivo,[11,13–19] suggesting that malignant brain cancers contain CSCs and that stem cells might be the primary target of tumourigenesis. For effective therapy against brain tumours, it is crucial to characterise brain CSCs and find ways to kill them.

2. The origin of brain cancers

It has traditionally been thought that brain cancers arise either from differentiated neural cells or from their precursor cells that acquire oncogenic mutations. However, since NSCs have been discovered in the adult CNS,[39,40] it has been speculated that NSCs might be a principal target of such mutations. This speculation is supported by many findings. Firstly, most malignant brain tumours, including glioblastoma multiforme (GBM) and medulloblastoma, are immunolabelled for both NSC markers, such as Nestin, Bmi1, and Sox2, and differentiation markers, including the neuronal marker microtubule associated protein (MAP) 2, the astrocyte marker glial fibrillary acidic protein (GFAP), and the oligodendrocyte marker galactocerebroside.[21–24] Secondly, whereas NSCs survive and proliferate throughout life, differentiated neural cells do not, suggesting that NSCs have more chance to accumulate oncogenic mutations.[5–9] Finally, using a combination of transgenic mice and a retrovirus system, two groups have elegantly demonstrated that Nestin-positive NSCs and GFAP-positive astrocytes formed malignant gliomas in vivo: Holland and colleagues infected transgenic mice that express the receptor of the avian leukosis virus (ALV) from either a nestin or a gfap promoter, with recombinant ALVs encoding oncogenic genes, such as platelet-derived growth factor (PDGF) receptor beta, or activated Akt, or activated Ras, and found glioblastomas in the brain.[24,26] De Pinho and colleagues overexpressed a constitutively active form of epidermal growth factor (EGF) receptor in either NSCs or astrocytes from Ink4a/Arf−/− mice, transplanted them into the brain, and found that the cells formed high-grade gliomas.[25] Taken together, these findings suggest that these oncogenic mutations in either NSCs or astrocytes are sufficient to cause malignant gliomas. It is of interest that such transformed astrocytes acquire the expression of both Nestin and A2B5 (a well-known marker of rat OPCs) and lost GFAP expression because it has been shown that both OPCs and astrocytes can behave as multipotent NSCs[27–33] and that transformed OPCs can form malignant gliomas in vivo.[41] Therefore OPCs might be the source of at least some malignant gliomas (Fig. 1).

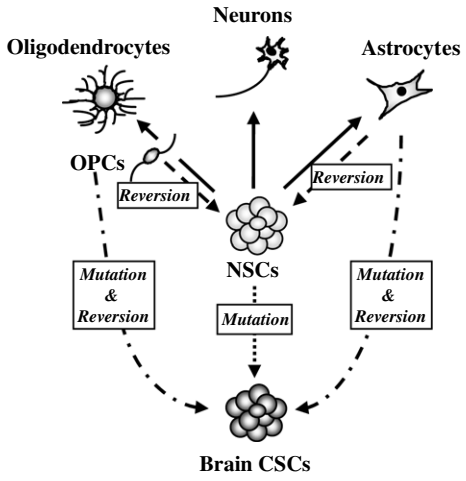

Fig. 1 – Possible origins of brain cancer stem-like cells (CSCs). If neural stem cells (NSCs) and glial lineage cells, such as oligodendrocyte precursor cells (OPCs) or astrocytes, which can behave as NSCs in appropriate conditions, acquired oncogenic mutations, they could become brain CSCs and form malignant brain tumours.

3. Preparation of brain CSCs

3.1. *Purification of CSCs from brain tumours*

Recently, several groups succeeded in isolating CSCs from both medulloblastomas and glioblastomas.[11,13–19] They cultured dissociated tumour samples and expanded the cells in serum-free medium containing basic fibroblast growth factor (bFGF) and EGF. The cells formed floating aggregates (neurospheres) just as NSCs do in the same conditions. These aggregates self-renewed in culture and expressed NSC markers, such as Nestin, CD133, and Notch, as well as differentiation markers, such as MAP2, GFAP and myelin proteins. Moreover, such aggregates formed malignant tumours when transplanted in vivo. Together, these findings indicated that both medulloblastomas and glioblastomas contain cancer-initiating NSC-like cells. Using an immunopurification with anti-CD133 antibody that recognises many kinds of stem cells,[42] Derks and colleagues purified brain CSCs from human medulloblastomas and GBMs.[14,18] They demonstrated that as few as 100 CD133-positive GBM cells can form tumours in NOD-SCID brain, suggesting that CD133 is an excellent marker of brain CSCs, as well as of normal stem cells (Fig. 2). Therefore, it is of great interest to identify any specific markers expressed in CD133-positive CSCs.

3.2. *Purification of CSCs from brain tumour cell lines*

Cancer cell lines might be alternative sources of CSCs. Many cancer cell lines can be maintained indefinitely in culture and form tumours like the original one when transplanted in vivo. Because many such cell lines were derived from single cancer cells, it seems likely that they do not contain any contaminating normal stem cells, such as haematopoietic stem cells, bone marrow (BM)-derived mesenchymal stem cells, or NSCs, all of which are recruited to tumours in vivo.[43–47] BM-derived mesenchymal stem cells, for example, promote angiogenesis and support tumourigenesis; when they are eliminated in vivo, the growth of a transplanted tumour is significantly inhibited,[42] suggesting that both endogenous (CSCs) and exogenous stem cells (normal stem cells) contribute to tumourigenesis in vivo. Moreover, brain CSCs from human GBM can form neurospheres in the presence of bFGF and

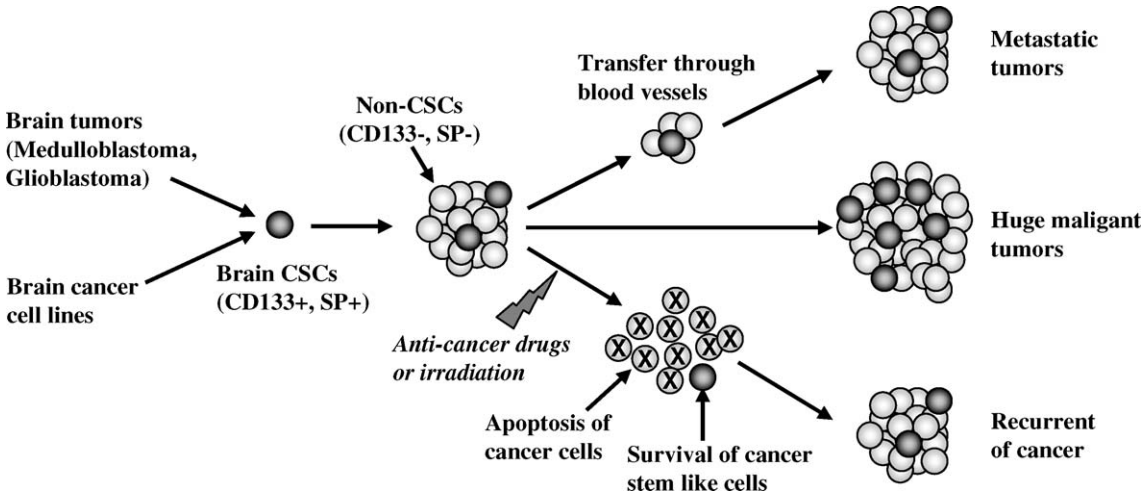

Fig. 2 – Brain cancer stem-like cells (CSCs) and tumorigenesis. Brain CSCs self-renew, produce non-CSCs (cancer cells) and form malignant tumours. Cell aggregates containing CSCs form metastatic tumours. Anti-cancer drugs and irradiation cause cancer cells to die by apoptosis, however CSCs survive and regenerate cancer.

EGF, even after they have been cultured in the medium with 10% serum or in the serum-free medium without both cytokines for 14 d, suggesting that CSCs in GBM can be maintained in normal culture conditions, in which all normal NSCs quickly lose their multipotentiality and differentiate into neurones and glia.[19] Thus these findings make cell lines attractive models to investigate the characteristics of CSCs.

In fact two groups have shown that many cancer cell lines contain CSCs.[16,17] Using Hoechst 33342 staining and flow cytometry, they found that a number of established cancer cell lines, including rat and human glioma cell lines and human neuroblastoma cell lines, all of which have been maintained in culture for decades, contain a small SP. They demonstrated that the SP cells, but not the non-SP cells, self-renewed in culture, were resistant to the anti-cancer drug mitoxantrone, and formed tumours when transplanted in vivo (Fig. 2). Thus, the SP in cancer cell lines contains cells with characteristics of both stem cells and cancer cells.

4. Signalling pathways involved in the maintenance of brain CSCs

Because both cancer cells and normal stem cells can proliferate indefinitely, both types of cells might share the mechanism for self-renewal.[8,48] For example, signalling pathways, activated by PDGF, EGF, bFGF, insulin-like growth factor, Notch, Hedgehog (Hh) and Wnt are important for the proliferation of NSCs and many cancer cells. I focus on three of these pathways – those activated by Notch, Wnt/Frizzled (Frz) and Hh, all of which are involved in brain tumourigenesis.

4.1. *Notch signalling*

Notch receptors are involved in a number of biological functions, including cell proliferation, differentiation, survival and tumourigenesis.[49] There are four known mammalian Notch receptors, Notch 1–4, and five ligands, Delta-like-ligand (Dll) 1, 3 and 4, and Jagged1 and 2. Following the activation, Notch is cleaved in its extracellular region by metalloproteases and in its intracellular region by presenilins (PS), releasing the Notch intracellular domain (NICD) from the plasma membrane. The NICD then translocates into the nucleus, associates with the CSL transcription factor CBF1/RBP-Jk, and activates a number of target genes, including the hairy and enhancer-of-split (Hes) genes (Fig. 3(a)). It has been shown that the inactivation of Notch signalling leads to serious developmental defects: Jagged1, Notch1, Notch2, and PS1and 2 knockout mice are all embryonically or perinatally lethal.[50–52]

There is accumulating evidence that Notch activation not only maintains the multipotentiality of NSCs but also promotes their differentiation into astrocytes.[53,54] Inactivation of Hes1 or Hes5 causes both precocious neuronal differentiation and the reduction of Muller glial cell production in retina.[55,56] Moreover, it was shown that Notch signalling is strongly activated in both primary human gliomas and a number of glioma cell lines.[57] Depletion of Notch1, Dll1, or Jagged1 by RNAi blocks glioma proliferation in vivo and in vitro.[58] Together, these findings suggest that Notch signalling is involved in gliomagenesis, as well as in normal brain development.

4.2. *Wnt signalling*

The Wnt family of secreted proteins co-ordinates diverse developmental processes, including cell proliferation and fate decisions.[59–61] In mammals, there are 20 Wnt members, 10 Wnt receptors (called Frz) and 5 soluble forms of Frz, which are natural inhibitors of Wnt signalling. Once Frz is activated, β-catenin, which is a central player in canonical Wnt signalling, accumulates in the nucleus and induces the expression of Wnt target genes, including *c-myc* and *cyclin D1*, by associating with LEF/TCF transcription factors (Fig. 3(b)). The non-canonical Wnt signalling pathway activates calcium/calmodulin dependent protein kinase and protein kinase C, although the molecular details are still uncertain.[59–61]

Wnt signalling is also crucial for CNS development. Wnt1 and 3a, Frz5 and 8, and β-catenin, for example, are expressed

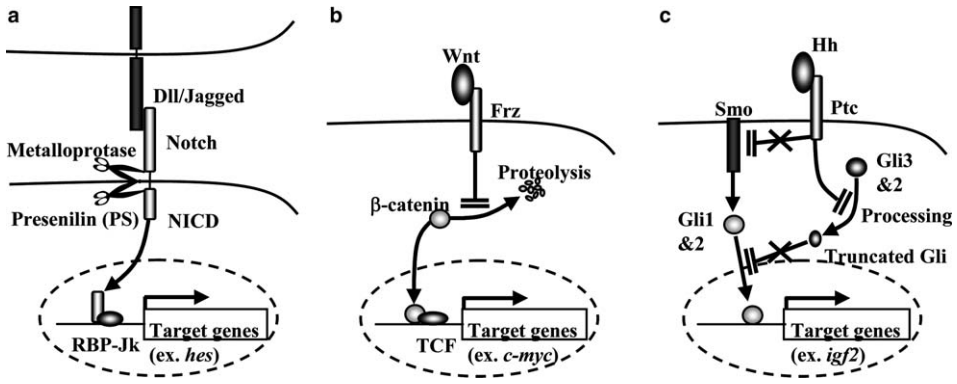

Fig. 3 – Notch, Wnt, Hh signalling pathways. (a) Notch, (b) Wnt/Frz, or (c) Hh/Ptc/Smo signalling pathway activates a number of genes, which regulate cell proliferation and cell fates. The constitutive activation of the pathways leads to abnormal central nervous system (CNS) development and brain tumourigenesis.

in the ventricular and subventricular zones (VZ/SVZ) in the developing brain.[62–65] Inactivation of Wnt1, Wnt3a, or β-catenin causes developmental brain defects.[61,66,67] Moreover, overexpression of a stabilised form of β-catenin in neural precursor cells caused a hyperplasia of lateral ventricles.[65] Some factors in the Wnt signalling pathway, including β-catenin and axin1 (an inhibitor in the pathway), are mutated in medulloblastomas.[68,69] Thus these findings suggest that hyper-activation of Wnt signalling may promote brain tumourigenesis.

4.3. *Hh signalling*

Hh signalling is also involved in proliferation, development and tumourigenesis.[70,71] In mammals, there are three Hh members, Sonic, Desert and Indian, all of which are secreted proteins. When Sonic Hh (Shh), for example, binds to the Patched1 (Ptc1) transmembrane receptor, another transmembrane protein, Smoothened (Smo), which is normally restrained by Ptc, is relieved and activates the zinc-finger transcription factor Gli. Activated Gli accumulates in the nucleus and induces the expression of target genes, including *wnt*, *insulin-growth factor 2 (igf2)*, and *pdgf receptor* α (Fig. 3(c)). There are three Gli transcription factors in mammals. Gli1 and 2 function as activators of Shh signalling, whereas the cleaved form of either Gli2 or Gli3 antagonises the Shh–Gli1/2 signalling pathway. The Shh signalling pathway is essential for CNS development: Shh, Ptc, Gli2 or Gli3 knockout mice die before birth with severe defects in the brain, although Gli1 knockout mice develop normally.[72–75] Conditional inactivation of Smo blocks NSC proliferation in vivo and in vitro.[76] Together with the finding that Glis, Ptc1 and Smo are all expressed in the VZ/SVZ, these observations suggest that Shh signalling may be essential for the maintenance of NSCs.

Ectopic activation of Hh signalling in CNS is likely to lead to brain tumour formation.[77,78] For example, Gli1 is highly activated in many brain cancers,[77] including medulloblastoma, glioblastoma and primitive neuro-ectodermal tumours, some of which also have mutations in Ptc1.[79] It was shown that overexpression of Gli1 in the developing tadpole CNS gives rise to brain tumours.[80] Moreover, cyclopamine, which is a specific inhibitor of Smo, blocks the growth of several primary gliomas, medulloblastomas and glioma cell lines.[80,81] Taken together, these findings suggest that Hh signalling plays an important role in brain tumourigenesis.

5. Conclusion

Although the expression of stem cell markers and the SP phenotype can be used to separate brain CSCs, even the smallest populations of CSCs seem to be contaminated with non-stem cells. Nonetheless, a combination of these methods might greatly enrich CSCs, which are crucial targets for therapy. Once we are able to isolate CSCs, we can study their properties and analyse their gene expression profile using DNA/oligonucleotide microarrays, RT-PCR and cDNA subtraction methods. We could identify the signalling pathways required to maintain CSCs. We could also use the antibody array to identify the cell-surface molecules specific for CSCs[82] and use the cells for drug screening, which may be the most effective way to discover drugs for therapy.

Conflict of interest statement

None declared.

Acknowledgements

I apologise to authors whose works were not referenced because of space limitations. I thank Martin Raff for a critical reading of the manuscript and for helpful comments. T.K. is supported by Merck Sharp and Dohme and the Biotechnology and Biological Science Research Council (BBSRC).

REFERENCES

1. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. *Science* 2000;**287**:1427–30.
2. Weissman IL, Anderson DJ, Gage F. Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. *Annu Rev Cell Dev Biol* 2001;**17**:387–403.

3. Gage F. Mammalian neural stem cells. *Science* 2000;**287**:1433–8.
4. Temple S. The development of neural stem cells. *Nature* 2001;**414**:112–7.
5. Poste G, Greig R. On the genesis and regulation of cellular heterogeneity in malignant tumors. *Invas Metast* 1982;**2**:137–76.
6. Woodruff MF. Cellular heterogeneity in tumors. *Br J Cancer* 1983;**47**:589–94.
7. Sell S, Pierce GB. Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. *Lab Invest* 1994;**70**:6–22.
8. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;**414**:105–11.
9. Tu SM, Lin SH, Logothetis CJ. Stem-cell origin of metastasis and heterogeneity in solid tumours. *Lancet Oncol* 2002;**3**:508–13.
10. Wulf GG, Wang R-Y, Kuehnle I, et al. A leukemic stem cell with intrinsic drug efflux capacity in acute myeloid leukemia. *Blood* 2001;**98**:1166–73.
11. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. *Glia* 2002;**39**:193–206.
12. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;**100**:983–8.
13. Hemmati HD, Nakano I, Lazareff JA, et al. Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci USA* 2003;**100**:15178–83.
14. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003;**63**:5821–8.
15. Galli R, Binda E, Orfanelli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* 2004;**64**:7011–21.
16. Hirschmann-Jax C, Foster AE, Wulf GG, et al. A distinct 'side population' of cells with high drug efflux capacity in human tumor cells. *Proc Natl Acad Sci USA* 2004;**101**:14228–33.
17. Kondo T, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. *Proc Natl Acad Sci USA* 2004;**101**:781–6.
18. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature* 2004;**432**:396–401.
19. Yuan X, Curtin J, Xiong Y, et al. Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* 2004;**23**:9392–400.
20. Trosko JE, Chang CC, Upham BL, Tai MH. Ignored hallmarks of carcinogenesis: stem cells and cell–cell communication. *Ann NY Acad Sci* 2004;**1028**:192–201.
21. Wolf HK, Buslei R, Blumcke I, Wiestler OD, Pietsch T. Neural antigens in oligodendrogliomas and dysembryoplastic neuroepithelial tumors. *Acta Neurophathol* 1997;**94**:436–43.
22. Wharton SB, Chan KK, Hamilton FA, Anderson JR. Expression of neuronal markers in oligodendrogliomas: an immunohistochemical study. *Neuropath Appl Neurobiol* 1998;**24**:302–8.
23. Katsetos CD, Del VL, Geddes JF, et al. Localization of the neuronal class III beta-tubulin in oligodendrogliomas: comparison with Ki-67 proliferative index and 1p/19q status. *J Neuropathol Exp Neurol* 2002;**61**:307–20.
24. Dai C, Celestino JC, Okada Y, Louis DN, Fuller GN, Holland EC. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. *Genes Dev* 2001;**15**:1913–25.
25. Bachoo RM, Maher EA, Ligon KL, et al. Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. *Cancer Cell* 2002;**1**:267–77.
26. Uhrbom L, Dai C, Celestino JC, Rosenblum MK, Fuller GN, Holland EC. Ink4a-Arf loss cooperates with KRas activation in astrocytes and neural progenitors to generate glioblastomas of various morphologies depending on activated Akt. *Cancer Res* 2002;**62**:5551–8.
27. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 1999;**97**:703–16.
28. Kondo T, Raff M. Oligodendrocyte precursor cells reprogrammed to become multipoteintial CNS stem cells. *Science* 2000;**289**:1754–7.
29. Laywell ED, Rakic P, Kukekov VG, Holland EC, Steindler DA. Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc Natl Acad Sci USA* 2000;**97**:13889–94.
30. Gotz M, Malatesta P. Radial glial cells as neuronal precursors: a new perspective on the correlation of morphology and lineage restriction in the developing cerebral cortex of mice. *Brain Res Bull* 2002;**57**:777–88.
31. Belachew S, Chittajallu R, Aguirre AA, et al. Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. *J Cell Biol* 2003;**161**:169–86.
32. Nunes MC, Roy NS, Keyoung HM, et al. Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. *Nat Med* 2003;**9**:439–47.
33. Kondo T, Raff M. Chromatin remodeling and histone modification in the conversion of oligodendrocyte precursors to neural stem cells. *Genes Dev* 2004;**18**:2963–72.
34. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer* 2002;**2**:48–58.
35. Doyle LA, Yang W, Bruzzo LV, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA* 1998;**95**:15665–70.
36. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med* 1996;**183**:1797–806.
37. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* 2001;**7**:1028–34.
38. Zhou S, Morris JJ, Barnes Y, Lan L, Schuetz JD, Sorrentino BP. Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells in vivo. *Proc Natl Acad Sci USA* 2002;**17**:12339–44.
39. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 1992;**255**:1707–10.
40. Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci USA* 1993;**90**:2074–7.
41. Barnett SC, Robertson L, Graham D, Allan D, Rampling R. Oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells transformed with c-myc and H-ras form high-grade glioma after stereotactic injection into the rat brain. *Carcinogenesis* 1998;**19**:1529–37.
42. Uchida N, Buck DW, He D, et al. Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci USA* 2000;**97**:14720–5.
43. Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nat Rev Cancer* 2002;**2**:826–35.

44. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002;**109**:337–46.
45. De Palma M, Venneri MA, Roca C, Naldini L. Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. *Nat Med* 2003;**9**:789–95.
46. Takakura N, Watanabe T, Suenobu S, et al. A role for hematopoietic stem cells in promoting angiogenesis. *Cell* 2000;**102**:199–209.
47. Aboody KS, Brown A, Rainov NG, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc Natl Acad Sci USA* 2000;**97**:12846–51.
48. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer* 2003;**3**:895–902.
49. Radtke F, Raj K. The role of Notch in tumorigenesis: oncogene or tumour suppressor? *Nat Rev Cancer* 2003;**3**:756–67.
50. Swiatek PJ, Lindsell CE, del Amo FF, Weinmaster G, Gridley T. Notch1 is essential for postimplantation development in mice. *Genes Dev* 1994;**8**:707–19.
51. Xue Y, Gao X, Lindsell CE, et al. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum Mol Genet* 1999;**8**:723–30.
52. Krebs LT, Xue Y, Norton CR, et al. Notch signaling is essential for vascular morphogenesis in mice. *Genes Dev* 2000;**14**:1343–52.
53. Tanigaki K, Nogaki F, Takahashi J, Tashiro K, Kurooka H, Honjo T. Notch1 and Notch3 instructively restrict bFGF-responsive multipotent neural progenitor cells to an astroglial fate. *Neuron* 2001;**29**:45–55.
54. Hitoshi S, Alexson T, Tropepe V, et al. Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. *Genes Dev* 2002;**16**:846–58.
55. Ishibashi M, Ang SL, Shiota K, Nakanishi S, Kageyama R, Guillemot F. Targeted disruption of mammalian hairy and Enhancer of split homolog-1 (HES-1) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis, and severe neural tube defects. *Genes Dev* 1995;**9**:3136–48.
56. Hojo M, Ohtsuka T, Hashimoto N, Gradwohl G, Guillemot F, Kageyama R. Glial cell fate specification modulated by the bHLH gene Hes5 in mouse retina. *Development* 2000;**127**:2515–22.
57. Pahlman S, Stockhausen MT, Fredlund E, Axelson H. Notch signaling in neuroblastoma. *Semin Cancer Biol* 2004;**14**:365–73.
58. Purow BW, Haque RM, Noel MW, et al. Expression of Notch-1 and its ligands, Delta-like-1 and Jagged-1, is critical for glioma cell survival and proliferation. *Cancer Res* 2005;**65**:2353–63.
59. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 2004;**20**:781–810.
60. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. *Nat Rev Genet* 2004;**5**:691–701.
61. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005;**434**:843–50.
62. Ikeya M, Lee SM, Johnson JE, McMahon AP, Takada S. Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* 1999;**389**:966–70.
63. Lee SMK, Tole S, Grove E, McMahon AP. A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* 2000;**197**:457–67.
64. Kim AS, Lowenstein DH, Pleasure SJ. Wnt receptors and Wnt inhibitors are expressed in gradients in the developing telencephalon. *Mech Dev* 2001;**103**:167–72.
65. Chenn A, Walsh CA. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* 2002;**297**:365–9.
66. McMahon AP, Bradley A. The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. *Cell* 1990;**62**:1073–85.
67. Brault V, Moore R, Kutsch S, et al. Inactivation of the beta-catenin gene by Wnt1-Cre-mediated deletion results in dramatic brain malformation and failure of craniofacial development. *Development* 2001;**128**:1253–64.
68. Zurawel RH, Chiappa SA, Allen C, Raffel C. Sporadic medulloblastomas contain oncogenic beta-catenin mutations. *Cancer Res* 1998;**58**:896–9.
69. Dahmen RP, Koch A, Denkhaus D, et al. Deletions of AXIN1, a component of the WNT/wingless pathway, in sporadic medulloblastomas. *Cancer Res* 2001;**61**:7039–43.
70. Ruiz i Altaba A, Palma V, Dahmane N. Hedgehog-Gli signalling and the growth of the brain. *Nat Rev Neurosci* 2002;**3**:24–33.
71. Pasca di Magliano M, Hebrok M. Hedgehog signalling in cancer formation and maintenance. *Nat Rev Cancer* 2003;**3**:903–11.
72. Ding Q, Motoyama J, Gasca S, et al. Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. *Development* 1998;**125**:2533–43.
73. Matise MP, Epstein DJ, Park HL, Platt KA, Joyner AL. Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. *Development* 1998;**125**:2759–70.
74. Park HL, Bai C, Platt KA, et al. Mouse Gli1 mutants are viable but have defects in SHH signaling in combination with a Gli2 mutation. *Development* 2000;**127**:1593–605.
75. Palma V, Ruiz i Altaba A. Hedgehog-GLI signaling regulates the behavior of cells with stem cell properties in the developing neocortex. *Development* 2004;**131**:337–45.
76. Machold R, Hayashi S, Rutlin M, et al. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. *Neuron* 2003;**39**:937–50.
77. Ruiz i Altaba A, Sanchez P, Dahmane N. Gli and hedgehog in cancer: tumours, embryos and stem cells. *Nat Rev Cancer* 2002;**2**:361–72.
78. Pasca di Magliano M, Hebrok M. Hedgehog signalling in cancer formation and maintenance. *Nat Rev Cancer* 2003;**3**:903–11.
79. Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* 1997;**277**:1109–13.
80. Dahmane N, Sanchez P, Gitton Y, et al. The sonic hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis. *Development* 2001;**128**:5201–12.
81. Berman DM, Karhadkar SS, Hallahan AR, et al. Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science* 2002;**297**:1559–61.
82. Pavlickova P, Schneider EM, Hug H. Advances in recombinant antibody microarrays. *Clin Chim Acta* 2004;**343**:17–35.