Title
Brain tumor stem cells as therapeutic targets in models of glioma.

Permalink
https://escholarship.org/uc/item/0nr0q315

Journal
Yonsei medical journal, 51(5)

ISSN
0513-5796

Authors
Laks, Dan Richard
Visnyei, Koppany
Kornblum, Harley Ian

Publication Date
2010-09-01

DOI
10.3349/ymj.2010.51.5.633

Peer reviewed
Brain Tumor Stem Cells as Therapeutic Targets in Models of Glioma

Dan Richard Laks,1 Koppany Visnyei,1 and Harley Ian Kornblum1,2,3,4

1Intellectual and Developmental Disability Research Center, 2Department of Molecular and Medical Pharmacology, 3Department of Pediatrics, 4The Jonsson Comprehensive Cancer Center, UCLA Medical Center, Los Angeles, California, USA.

At this time, brain tumor stem cells remain a controversial hypothesis while malignant brain tumors continue to present a dire prognosis of severe morbidity and mortality. Yet, brain tumor stem cells may represent an essential cellular target for glioma therapy as they are postulated to be the tumorigenic cells responsible for recurrence. Targeting oncogenic pathways that are essential to the survival and growth of brain tumor stem cells represents a promising area for developing therapeutics. However, due to the multiple oncogenic pathways involved in glioma, it is necessary to determine which pathways are the essential targets for therapy. Furthermore, research still needs to comprehend the morphogenic processes of cell populations involved in tumor formation. Here, we review research and discuss perspectives on models of glioma in order to delineate the current issues in defining brain tumor stem cells as therapeutic targets in models of glioma.

Key Words: Brain tumor stem cell, cancer stem cell, glioma, glioblastoma multiforme (GBM), neurosphere, PI3 kinase, Notch, Akt, Rapamycin

INTRODUCTION

The incidence of malignant brain tumors rose steadily over the last quarter of a century in both adults and children.1,6 A proportion of this trend may be due to improved neuro-imaging techniques and access to medical care.7 Familial gene mutations, immune disease, and high dose irradiation are known causes of brain tumors but are likely responsible for a minority of cases. Epidemiological studies and geographic variability in case numbers suggest that the etiology of brain tumors may be associated with environmental factors and exposure to carcinogens.8,9 While brain tumors in the United States constitute a minority of cancer cases, with an incidence of 14.8 brain tumors per 100,000 person years, and roughly half diagnosed as benign, the malignant forms of brain tumor present a devastating prospect of morbidity and mortality.10 The most common malignant brain tumors are gliomas, and within gliomas, glioblastoma multiforme (GBM) are the most common, representing 40% of all primary, malignant central nervous system tumors.11 GBM have a median overall survival of approximately 1 year.12 The term “multiforme” in GBM describes the heterogeneous nature of these neoplasms and their varied histological composition.13 These tumors are characterized by diffuse infiltration into surrounding tissue that prevents complete surgical resection. Currently the standard treatment for GBM is surgical resection...
followed by a combination of radiotherapy and chemotherapy. Due to the poor prognosis of malignant tumors under this regimen of treatment, discovering effective new treatment is a crucial goal of further research. In order to design effective therapy it is imperative that ongoing research aims to understand the molecular pathways essential in tumor proliferation, survival, and invasion. Another emerging field of research is to develop an accurate model of the cell populations and morphogenetic processes that produce the heterogeneous population of cells within a tumor. In both of these fields, brain tumor stem cells represent a central concept that remains to be fully determined and established.

**BRAIN TUMOR STEM CELLS**

Divergent perspectives on the fundamental nature of brain tumor biology fuel a debate that revolves around the theory of brain tumor stem cells (BTSC) as a model of glioma. The cancer stem cell theory posits that only a specific, minority of tumor cells possess the ability to produce a tumor and that these cells may arise from mutations in normal stem or progenitor cells. The brain tumor stem cell theory holds that BTSC produce all the cells of a tumor and therefore represent the essential, specific targets of effective treatment necessary to prevent recurrence. The notion that glioma tumors are caused by transformed neural stem cells was originally fueled by the discovery that brain tumors expressed nestin, an intermediate filament that can be expressed by neural stem cells, although it is also expressed by more limited progenitors as well as by other cells within the body. In this BTSC model, brain tumor stem cells arise from oncogenic mutations in neural stem cells. This hypothesis was supported by several observations: gliomas can arise near the lateral ventricles, a site housing neural stem cells that reside in the subventricular proliferative zone; neural stem cells proliferate enough to make them susceptible to transformation; and neural stem cells and BTSC share essential mechanisms for proliferation and survival.

Evidence for the BTSC model of glioma first came from several laboratories. These studies demonstrated biological similarities between brain tumor initiating cells and neural stem cells through the use of neurosphere cultures. Reynolds and Weiss, et al. originally isolated and enriched neural stem cells from the adult brain through the use of neurosphere cultures. Neural stem cells distinguished themselves from other cells in the brain by their ability to grow as neurospheres (floating spheres of cells) in relatively simple, serum free media with the addition of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), or both. Neurospheres could subsequently differentiate into the multiple lineages of brain cells upon removal of growth factors. In like manner, cells derived from brain tumors form serially passaged clonal neurosphere cultures in serum free media, and, upon removal of growth factors, differentiate into multiple lineages to recapitulate tumor morphologies. In other words, in vitro, BTSC behave in a similar fashion to neural progenitor cells; they respond to the same mitogens, and they express similar markers.

The theory of BTSC was further substantiated when Galli, et al. demonstrated that GBM derived neurosphere cultures were tumorigenic upon xenotransplantation into immunodeficient mice and Singh, et al. demonstrated that tumor cells expressing CD133 (a putative marker of human neural stem cells), when sorted from patient samples, formed tumors in immunodeficient mice while the CD133 negative fraction did not. The theory of BTSC gained acceptance and the model developed that BTSC may originate from transformed neural stem or progenitor cells, and furthermore, are unique amongst other tumor cells in that BTSC possess the capacities to extensively self renew, initiate tumors upon orthotopic transplantation, and give rise to a heterogeneous population of cells such as those found in their parent tumors. More recent studies demonstrated that the ability of glioma tumors to form neurosphere cultures is an independent predictor of clinical outcome. These data provide further evidence that BTSC play a central role in tumor progression and aggressiveness. However, BTSC remains a hypothesis and both the definition and terminology are still debated. Some scientists prefer the less declarative terms “brain tumor initiating cells” or “brain tumor stem like cells”. For the purposes of this paper, we shall use the term brain tumor stem cells.

**MOLECULAR DIAGNOSIS AND THERAPY**

In order to discover effective treatment for malignant glioma, one must seek to characterize and target specific molecular pathways and mechanisms employed by brain tumor stem cells. While gliomas are classified on the basis of histopathological criteria into four grades, in ascending order of malignancy, molecular expression profiling has also been effective at distinguishing subclasses of glioma. Molecular expression profiles provide an advantage by offering valuable insights into the specific oncogenic pathways that drive tumor proliferation and, thereby, produce a more specific characterization of each tumor. Classification of high grade glioma based on molecular expression profiles have classified 3-5 distinct types of malignant tumors that resemble different stages in neurogenesis, predict pa-
tient prognosis, and indicate that activation of the Akt and Notch canonical oncogenic pathways reflect the aggressiveness of these neoplasms.

Many cellular processes involved in regulating neural stem cells are also essential in glioma brain tumor stem cells. For example, certain cell cycle regulators and transcription factors involved in the regulation of neural progenitors, such as c-MYC, OCT-4, BMI-1, Olig-2, and MELK, also regulate brain tumor and putative BTSC proliferation and survival. Similarly, multiple secreted growth factors involved in neural stem cell proliferation, such as EGF, and IGF (insulin like growth factor), bind with receptor tyrosine kinases to activate downstream proliferation and survival pathways in brain tumor initiating cells.

Noteworthy is the PI3 kinase/Akt pathway, a key regulator of signaling via different pathways, including those regulated by EGF and IGF receptors. The PI3 kinase/Akt pathway has received a lot of attention as a target for cancer treatment. Recently, expression of Akt and PI3 kinase activity has been shown to be associated with glioma tumor grade. There are many agents available to researchers that specifically target this pathway, and this area of research promises to change therapeutic strategies utilized in glioma treatment. Rapamycin is a microbial derived therapeutic that acts specifically on mTOR. mTOR is one downstream effector of the Akt pathway which can also act via a feedback loop to influence Akt signaling.

Models of Glioma

Many models exist to explain the etiology and function of the heterogeneous cell populations that form glioma tu-
mors. The hierarchical model of BTSC contrasts with the more established stochastic model of cancer in which variegated cell populations possess equivalent capabilities to form tumors. In the stochastic model of tumors, different populations undergo clonal evolution in competition with each other in a process driven by mutation to form the tumor bulk. It is thought that multiple mutations are required to transform a normal cell into a malignant, cancer cell. Possibly, a mutator phenotype is a requirement to produce malignant cells. In this model, a primary mutation causes genetic instability that drives further mutations; this mutator phenotype eventually produces cancerous cells. In this clonal evolution model of tumors, the diversity of cells within a tumor is not caused by a single BTSC but by a heterogeneous population of genetically distinct cancer cells.

Evidence is accruing that tumors are in a state of genetic flux. Analysis of lymphoblastic leukemia patients revealed that cancer recurrences differed in DNA copy number from their original, primary cancers. Similarly, recurrences of breast cancer tumors were shown to have different mutational profiles than their original, primary tumors. This evidence suggests that tumors possess a heterogeneous population of genetically distinct cells that undergo clonal evolution. The ongoing debate between the cancer stem cell model and the clonal evolution model has been reviewed by Shackleton, et al. Glialoma seem to fit well within the cancer stem cell model because tumorigenic capacity is a relatively rare trait among glioma tumor cells and not a uniform trait as would be predicted by the clonal evolution model. Indeed, glioma BTSC have been shown to demonstrate a hierarchical model capable of generating a diversity of cells. However, genetic diversity has also been discovered within glioma tumors. A study by Shapiro, et al. in 1981 performed karyotypic analysis of different glioma tumors and the cultures derived from them and discovered 3-21 genetically distinct subpopulations within the average glioma tumor with varying chemosensitivities. As this study was done in an age before neurosphere cultures, one cannot determine from this experiment how many genetically distinct, tumorigenic cultures were derived from each tumor. Recently, Piccirillo, et al. isolated two genetically distinct populations of cells from distinct regions of a GBM tumor. However, only one population was tumorigenic, so one cannot assume that multiple populations of cancer stem cells existed in that particular tumor. However, this data does suggest the possibility that genetically distinct BTSC may coexist within the same tumor.

It has been demonstrated that there is considerable genetic variability within populations of neural stem cells in the brain. In fact, it can even be assumed that some genetic variation and instability found in neurosphere cultures represents the genetic variation and instability within the brain. A systems based approach may syncretize the disparate models of glioma in order to address the manifest complexity of these tumors. In contrast to clonal evolution, the complex system model we shall discuss considers the features of adaptive and resistant behavior exhibited by malignant brain tumors to be the emergent properties of a complex adaptive system consisting of multiple brain tumor stem cells. In this model, both genetic and potentially reversible epigenetic changes may explain not only the cellular diversity, but also the increased plasticity these tumors exhibit upon therapeutic intervention.

Cancer has been characterized as a robust, complex system and tumors have been described as a cooperative system of interacting cells. Therefore it is worthwhile to assess cancer as a complex adaptive system. A complex adaptive system is characterized by emergent, global properties that are produced by a requisite diversity of local interactions. These emergent properties are only ascribed to the complex system itself and cannot be reduced to the properties of the individual components of the system. Emergent properties confer the hallmarks of a complex adaptive system: organization, adaptability, and survival. Gliomas fit the essential criteria for a complex adaptive system, they are heterogeneous, self adaptive and self organized. Evidence exists for interactions between BTSC and local environmental cues that play a role in BTSC survival and proliferation. Autocrine and paracrine factors are secreted by brain tumor stem cells to enhance infiltration and migration into surrounding brain tissue. Diffusible factors and adherence cues emitted from surrounding vasculature exert an influence on BTSC proliferation and survival. With all these factors involved in BTSC proliferation, survival, and infiltration, it is conceivable that a diversity of brain tumor stem cells may arise as a complex adaptive system that interacts through diffusible factors and adherence cues. Recently it has been shown in a drosophila model that diverse, adjacent tumor cells can cooperate to produce emergent properties of tumorigenesis and infiltration. To what extent this occurs in human glioma has yet to be determined.

In order to model the tumorigenic process of glioma, it is necessary to ascertain which processes are involved. Besides the brain tumor stem cell model and the clonal evolution model are more complex systems whose roles in glioma are in the realm of possibility (Fig. 1). In order to prioritize therapeutic targets of glioma, it is important to have
Further research is needed to determine whether de-differentiation occurs, whether BTSC can adapt to treatment by switching between different phenotypic states that confer either resistance or growth, whether multiple, genetically distinct brain tumor stem cells exist within each tumor, whether a mixture of the clonal model and the BTSC model co-exist, and to what extent signaling between BTSC, tumor cells, and the niche provides additional therapeutic targets.

To produce a model of glioma with improved diagnostic and therapeutic prediction-value may require a thorough understanding of both the essential molecular and morphogenic processes involved in tumor survival and proliferation. To devise tailored treatment, predictive molecular and genetic diagnostic criteria must be ascertained. Furthermore, it is important to discover whether treatment resistance in glioma is due to intrinsic characteristics of cancer stem cells, mutational evolution, redundant molecular pathways, or to the adaptation of a complex system of multiple brain tumor stem cells. Through the elucidation of glioma tumor biology, research aims to overcome treatment resistance and devise appropriate therapeutic approaches. BTSC may represent an essential target of therapy. Targeting the essential pathways and transcription factors of BTSC may deliver the next generation of improved therapeutic options.

**REFERENCES**

1. Jukich PJ, McCarthy BJ, Surawicz TS, Freels S, Davis FG. Trends in incidence of primary brain tumors in the United States, 1985-1994. Neuro Oncol 2001;3:141-51.
2. Deorah S, Lynch CF, Sibenaller ZA, Ryken TC. Trends in brain cancer incidence and survival in the United States: Surveillance, Epidemiology, and End Results Program, 1973 to 2001. Neurosurg Focus 2006;20:E1.
3. Deltour I, Johansen C, Auvinen A, Feychting M, Klaeboe L, Schüz J. Time trends in brain tumor incidence rates in Denmark, Finland, Norway, and Sweden, 1974-2003. J Natl Cancer Inst 2009;101:1721-4.
4. Pirouzmand F, Sadanand V. The incidence trends of primary...
brain tumors in Saskatchewan from 1970 to 2001. Can J Neurol Sci 2007;34:181-6.
5. Smith MA, Freidlin B, Ries LA, Simon R. Trends in reported incidence of primary malignant brain tumors in children in the United States. J Natl Cancer Inst 1998;90:1269-77.
6. Hess KR, Broglio KR, Bondy ML. Adult glioma incidence trends in the United States, 1977-2000. Cancer 2004;101:2293-9.
7. Schwartzbaum JA, Fisher JL, Alldape KD, Wrensch M. Epidemiology and molecular pathology of glioma. Nat Clin Pract Neurol 2006;2:494-503.
8. Wrensch M, Minn Y, Chew T, Bondy M, Berger MS. Epidemiology of primary brain tumors: current concepts and review of the literature. Neuro Oncol 2002;4:278-99.
9. Fisher JL, Schwartzbaum JA, Wrensch M, Wiemels JL. Epidemiology of brain tumors. Neurol Clin 2007;25:867-90.
10. Buckner JC, Brown PD, O’Neill BP, Meyer FB, Wetmore CJ, Uhlm JH. Central nervous system tumors. Mayo Clin Proc 2007;82:1271-86.
11. Miller CR, Perry A. Glioblastoma. Arch Pathol Lab Med 2007;131:397-406.
12. Daumas-Duport C, Scheithauer B, O’Fallon J, Kelly P. Grading of astrocytomas. A simple and reproducible method. Cancer 1988;62:2152-65.
13. Burger PC, Kleihues P. Cytologic composition of the untreated glioblastoma with implications for evaluation of needle biopsies. Cancer 1989;63:2014-23.
14. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
15. Reynolds BA, Weiss S. A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. J Neurosci 1992;12:4565-74.
16. Nakano I, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 2004;64:7011-21.
17. Nakano I, Clarke ID, Hide T, Dirks PB. Cancer stem cells in nervous system tumors. Oncogene 2004;23:7267-73.
18. Nakano I, Masterman-Smith M, Visneye K, Angenieux B, Ozocho NM, Foran I, et al. Neurosphere formation is an independent predictor of clinical outcome in malignant glioma. Stem Cells 2009;27:980-7.
19. Galli R, Inagaki T, Bignami A, Santamaria A, Vaquero A, Collini S, et al. Distinct transcription profiles of primary and secondary glioblastoma subtypes. J Neurosci Res 2008;86:48-60.
20. Richman DS, Bobek MP, Misiek DE, Kucik R, Blaivas M, Kurnit DM, et al. Distinctive molecular profiles of high-grade and low-grade gliomas based on oligonucleotide microarray analysis. Cancer Res 2001;61:6885-91.
21. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006;9:157-73.
22. Galli R, Inagaki T, Bignami A, Santamaria A, Vaquero A, Collini S, et al. Distinct transcription profiles of primary and secondary glioblastoma subtypes. Cancer Res 2008;66:159-67.
23. Nakano I, Masterman-Smith M, Saigusa K, Paucar AA, Dougherty JD, Rich JN. Turning glioblastoma in the wrong direction. Trends Mol Med 2006;12:456-73.
24. Nakano I, Masterman-Smith M, Saigusa K, Paucar AA, Dougherty JD, Rich JN. Turning glioblastoma in the wrong direction. Trends Mol Med 2006;12:456-73.
Brain Tumor Stem Cells

tie A, et al. Analysis of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma patients in vivo. Cancer Res 2003; 63:2742-6.

45. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2002;2:489-501.

46. Wang G, Kang C, Pu P. Increased expression of Akt2 and activity of PI3K and cell proliferation with the ascending of tumor grade of human gliomas. Clin Neurol Neurosurg 2010;112:324-7.

47. Maira SM, Staufer F, Schnell C, Garcia-Echeverria C. PI3K inhibitors for cancer treatment: where do we stand? Biochem Soc Trans 2009;37:265-72.

48. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, et al. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature 1994;369:756-8.

49. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. Nat Rev Cancer 2004;4:335-48.

50. Chiu MI, Katz H, Berlin V. RAPT1, a mammalian homolog of yeast TOR, interacts with the FKBP12/rapamycin complex. Proc Natl Acad Sci U S A 1994;91:12574-8.

51. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 1994;78:35-43.

52. O’Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 2006;66:1500-8.

53. Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 2006;66:1500-8.

54. Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, et al. Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. PLoS Med 2008;5:e8.

55. Hosoi H, Dilling MB, Liu LN, Dansks MK, Shikata T, Sekulic A, et al. Studies on the mechanism of resistance to rapamycin in human cancer cells. Mol Pharmacol 1998;54:815-24.

56. Efeyan A, Sabatini DM. mTOR and cancer: many loops in one pathway. Curr Opin Cell Biol 2010;22:169-76.

57. Carracedo A, Baselga J, Pandolfi PP. Deconstructing feedback signaling networks to improve anticancer therapy with mTORC1 pathway. Curr Opin Cell Biol 2010;22:169-76.

58. Maira SM, Stauffer F, Schnell C, Garcia-Echeverria C. PI3K signaling and activates Akt. Cancer Res 2006;66:1500-8.

59. Mischel PS, Cloughesy TF. Targeted molecular therapy of GBM. Brain Pathol 2003;13:52-61.

60. Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, et al. Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. PLoS Med 2008;5:e8.

61. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, et al. Down-regulation of Notch-1 and Jagged-1 inhibits prostate cancer cell growth, migration and invasion, and induces apoptosis via inactivation of Akt, mTOR, and NF-kappaB signaling pathways. J Cell Biochem 2010;109:726-36.

62. Kitano H. A robustness-based approach to systems-oriented drug design. Nat Rev Drug Discov 2007;6:202-10.

63. Johannessen TC, Decher OJ, Holland EC. Cancer stem cells and survival pathways. Cell Cycle 2008;7:1371-8.

64. Hambardzumyan D, Decher OJ, Holland EC. Cancer stem cells and survival pathways. Cell Cycle 2008;7:1371-8.

65. Ma J, Meng Y, Kwiatkowski DJ, Chen X, Peng H, Sun Q, et al. Mammalian target of rapamycin regulates murine and human cell differentiation through STAT3/p63/Jagged/Notch cascade. J Clin Invest 2010;120:103-14.

66. Wang Z, Li Y, Baneree S, Kong D, Ahmad A, Nogueira V, et al. Down-regulation of Notch-1 and Jagged-1 inhibits prostate cancer cell growth, migration and invasion, and induces apoptosis via inactivation of Akt, mTOR, and NF-kappaB signaling pathways. J Cell Biochem 2010;109:726-36.

67. Lu C, Shervington A. Chemoresistance in gliomas. Mol Cell Biochem 2008;312:71-80.

68. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005;5:275-84.

69. Hirschmann-Jax C, Bjerkgv R, Tysne D, DNA repair and cancer stem-like cells—potential partners in glioma drug resistance? Cancer Treat Rev 2008;34:558-67.

70. Chung MY, Shervington A. Chemoresistance in gliomas. Mol Cell Biochem 2008;312:71-80.

71. Carson JT, Dinapoli RP, Bronson RT, Miller CB, Shurtleff SA, et al. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science 2008;322:1377-80.

72. Hambardzumyan D, Decher OJ, Holland EC. Cancer stem cells and survival pathways. Cell Cycle 2008;7:1371-8.

73. Hambardzumyan D, Decher OJ, Holland EC. Cancer stem cells and survival pathways. Cell Cycle 2008;7:1371-8.

74. Shackleton M, Quintana E, Fearon ER, Morrison SJ. Heterogeneity of PI3K and cell proliferation with the ascending of tumor grade of human gliomas. Clin Neurol Neurosurg 2010;112:324-7.

75. Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A, et al. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature 2009;461:809-13.

76. Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA, et al. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science 2008;322:1377-80.

77. Shah SP, Khattra J, Prentice L, Pugh T, Burleigh A, et al. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature 2009;461:809-13.

78. Shackleton M, Quintana E, Fearon ER, Morrison SJ. Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell 2009;138:822-9.

79. Shapiro JR, Yung WK, Shapiro WR. Isolation, karyotype, and survival pathways. Cell Cycle 2008;7:1371-8.

80. Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA, et al. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science 2008;322:1377-80.

81. Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Porugot AH, Wiedemeyer R, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science 2007;318:287-90.

82. Shackleton M, Quintana E, Fearon ER, Morrison SJ. Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell 2009;138:822-9.

83. Shapiro JR, Yung WK, Shapiro WR. Isolation, karyotype, and clonal growth of heterogeneous subpopulations of human malignant gliomas. Cancer Res 1980;40:1307-18.

84. Yung WK, Shapiro JR, Shapiro WR. Heterogeneous chemosensitivities of subpopulations of human glioma cells in culture.
Cancer Res 1982;42:992-8.

85. Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 2006;444:761-5.

86. Rehen SK, McConnell MJ, Kaushal D, Kingsbury MA, Yang AH, Chun J. Chromosomal variation in neurons of the developing and adult mammalian nervous system. Proc Natl Acad Sci U S A 2001;98:13361-6.

87. Westra JW, Peterson SE, Yung YC, Mutoh T, Barral S, Chun J. Aneuploid mosaicism in the developing and adult cerebellar cortex. J Comp Neurol 2008;507:1944-51.

88. Sareen D, McMillan E, Ebert AD, Shelley BC, Johnson JA, Meisner LF, et al. Chromosome 7 and 19 trisomy in cultured human neural progenitor cells. PLoS One 2009;4:e7630.

89. Schwab ED, Pienta KJ. Cancer as a complex adaptive system. Med Hypotheses 1996;47:235-41.

90. Kitano H. Cancer as a robust system: implications for anticancer therapy. Nat Rev Cancer 2004;4:227-35.

91. Heppner GH. Tumor heterogeneity. Cancer Res 1984;44:2259-65.

92. Axelrod R, Axelrod DE, Pienta KJ. Evolution of cooperation among tumor cells. Proc Natl Acad Sci U S A 2006;103:13474-9.

93. Grizzi F, Chiriva-Internati M. The complexity of anatomical systems. Theor Biol Med Model 2005;2:26.

94. Grizzi F, Chiriva-Internati M. Cancer: looking for simplicity and finding complexity. Cancer Cell Int 2006;6:4.

95. Ashby W. Requisite variety and its implications for the control of complex systems. Cybernetica 1958;1:83-9.

96. Gilbertson RJ, Rich JN. Making a tumour’s bed: glioblastoma stem cells and the vascular niche. Nat Rev Cancer 2007;7:733-6.

97. Hoelzinger DB, Demuth T, Berens ME. Autocrine factors that sustain glioma invasion and paracrine biology in the brain micro-environment. J Natl Cancer Inst 2007;99:1583-93.

98. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. Cancer Cell 2007;11:69-82.

99. Wu M, Pastor-Pareja JC, Xu T. Interaction between Ras(V12) and scribbled clones induces tumour growth and invasion. Nature 2010;463:545-8.