Emerging concepts in glioma biology: implications for clinical protocols and rational treatment strategies

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Glioblastoma multiforme (GBM), the most common primary central nervous system neoplasm, is a complex, heterogeneous disease. The recent identification of stem cells in murine tumor xenografts that were capable of recapitulating the tumor phenotype adds a new dimension of complexity to the already challenging treatment of patients with GBMs. Although specific cellular and genetic changes are commonly associated with GBM, the mechanism by which those changes occur may have a significant impact on treatment outcome. Of the many bioinformatics techniques developed in recent years, gene expression profiling has become a commonly used research tool for investigating tumor characteristics, and the development of rationally targeted molecular therapies has also accelerated following the initial success of specifically designed inhibitors in the treatment of malignancies. Despite these advances in research techniques and targeted molecular therapies, however, limited clinical impact has been achieved in the treatment of infiltrative malignancies such as GBMs. Thus, further extension in survival of patients with GBMs may require use of multiple analyses of tumors to develop tailored therapies that reflect the inter- and intratumoral heterogeneity of this disease. In this review, the authors briefly consider the potential use of expression profiling combined with mutation analysis in the development of treatment modalities to address the heterogeneity of this complex tumor phenotype.

KEY WORDS • glioblastoma multiforme • microarray • stem cell • targeted therapy

OVERVIEW

Glioblastoma multiforme is the most common primary central nervous system neoplasm, accounting for more than half of all such tumors.^{6,25} Despite advances in survival for many patients with malignancies, the prognosis for those with GBM remains poor, even with modern aggressive intervention, including resection, radiotherapy, and chemotherapy. The overall 2-year survival rate is 25% at best, and 5-year survival rates remain in the low single digits.^{25,42} Thus, it is clear that new treatments are needed to overcome the limitations of conventional therapy. Emerging evidence indicates that patient-specific therapies tailored to the unique biology of an individual's GBM may be required to achieve significant improvements in clinical outcomes. In this article we briefly discuss emerging insights into glioma cell biology and common genetic mutations. In addition, we highlight how understanding the cell biology and gene expression profiles of each patient's GBM could be useful in implementing more effective treatment.

Abbreviations used in this paper: BTSC = brain tumor stem cell; EGFR = epidermal growth factor receptor; GBM = glioblastoma multiforme; JAK/STAT = Janus tyrosine kinase/signal transducer and activator of transcription; MAPK = mitogen-activated protein kinase; NSC = neural stem cell; PDGFR β = platelet-derived growth factor receptor- β ; PI3K = phosphatidylinositol 3-kinase; RB = retinoblastoma; RTK = receptor tyrosine kinase.

ASPECTS OF GBM

Cellular Characteristics

The GBM is a diffuse and infiltrative tumor. Migrating GBM cells can be found several centimeters away from the margins of resection. Accordingly, although tumor recurrence predominantly occurs near the primary resection site, it can arise in distant regions of the brain.^{23,44} The GBM is composed of genetically and morphologically diverse cells, not only among different patients, but also among cells within the same tumor. Furthermore, any therapy that relies on cell division to kill tumor cells is complicated by the fact that only a fraction of glioma cells are actively mitotic during a given treatment window.¹¹

Mounting evidence indicates that, similarly to leukemia and breast cancer, GBM tumors are composed of stem cell-like precursor cells and also more differentiated tumor cells.^{44,45,51} The former, which we will call BTSCs, are endowed with many features of NSCs, including the capacity for self-renewal, the ability to generate daughter cells with different phenotypes from a single mother cell, the capacity to differentiate into a diverse population of cells, and expression of NSC markers.^{45,51} Moreover, BTSCs have been isolated from human GBMs that can form tumors in the brains of mice that bear a morphological resemblance to the

original human tumor.⁴⁵ These BTSCs demonstrate immunoreactivity for CD133, a primitive progenitor cell marker, and appear to be the tumor-initiating cells not only in mouse xenografts but also potentially in spontaneous human GBM. Furthermore, when BTSCs were induced to differentiate in vitro, they retained the ability to form neurospheres when cultured in stem cell-supportive media, an ability that is unique to BTSCs. Thus, BTSCs appear to have the capacity to revert from a terminally differentiated state back to a more primitive state, a characteristic distinct from normal NSCs and consistent with cells found in other malignancies.⁵¹

The ability of cells isolated from human tumors to self-renew and contribute to various cell types in both in vitro and in vivo tumorigenesis models establishes a potential mechanism for the complex heterogeneity characteristic of GBM and highlights the need for therapeutic strategies targeting not only malignant, more differentiated GBM tumor cells, but also GBM stem cells that likely contribute to the inevitable recurrence of this disease. The potential phenotypic differences between GBM stem cells and normal NSCs may be subtle, and may in fact be undetectable by currently used clinical tests. Additionally, the source of BTSCs may not be the tumor itself. In at least one study in which a spontaneous mouse model of GBM was used, it was found that tumor-initiating cells might migrate from a separate location within the brain, only to repopulate an area with a favorable microenvironment for tumor growth.⁵³ Although the combination of invasiveness, the ability to become migratory, and complexity of cellular composition in human GBMs represents a formidable challenge for effective therapy, the identification of BTSCs provides a rational target for new therapies, because these cells appear to be the tumor-initiating component of GBM.

Complex Heterogeneity and Genetic Mutations

Numerous genetic mutations have been identified in GBM that have been postulated to contribute to gliomagenesis. Alterations in tumor suppressors are the genetic lesions most commonly found. Retinoblastoma protein, the *RB-1* gene product, is present ubiquitously and at relatively constant levels throughout the body.¹ In its dephosphorylated state, this protein functions as a tumor suppressor by sequestering molecules that normally promote progression through the cell cycle.^{1,40} Cell proliferative signals, such as RTK activation through ligand binding, in turn promote phosphorylation of RB protein in the nucleus. The progressive state of phosphorylation causes RB to release bound transcription factors such as E2F proteins, allowing them to drive progression of a cell through the mitotic cell cycle. Thus, RB plays a key role in modulating cell division, largely based on its level of phosphorylation. The *RB* gene is commonly mutated or deleted in primary human GBM specimens,¹⁷ thereby implicating RB-mediated cell cycle regulation in the progression of GBM.

Deletion or alteration of the *p53* tumor suppressor is present in 25 to 30% of GBMs^{29,35,48} and has also been extensively studied in these tumors.²² The *p53* gene is not commonly expressed at high levels in healthy tissues; its expression is induced by a variety of mechanisms usually implicated in the induction of DNA damage, like that caused by radiation exposure.^{1,22} When present in the cell, *p53* pro-

motes the transcription and translation of proteins that block progression through the cell cycle, allowing DNA damage to be repaired.²⁷ Loss of *p53* allows cells with DNA damage to progress through the cell cycle unchecked, perpetuating or compounding the DNA damage.^{1,22,46} Direct mutation, deletion, or loss of expression of *p53* is commonly observed in GBMs, as in many other solid tumors.²²

Furthermore, changes in genes upstream of *p53* in this pathway are also commonly found in tumors. Amplification or overexpression of *MDM2* occurs in 10 to 15% of GBMs, resulting in a blunted or absent *p53* response, and has in some studies been associated with poor prognosis in patients with GBM.^{19,38,39,41} The MDM2 binds to and sequesters *p53*, promoting its rapid degradation within the cell, thus preventing transcription of antiproliferative genes.³³ The MDM2 is in turn regulated by another gene product, human p14ARF (called ARF in this paper); ARF antagonizes MDM2, releasing *p53* inhibition to induce cell cycle arrest.³⁷

Although *ARF* is located in the *INK4A* genomic locus and shares exons 2 and 3 with *p16^{INK4A}*, it differs in structure and function due to a frameshift caused by splicing from an alternative exon 1.²⁴ This second gene encoded by the *INK4A* locus, *p16^{INK4A}*, also functions as a tumor suppressor, but with a different mechanism and specificity than ARF. The *p16^{INK4A}* locus indirectly regulates RB by blocking the action of cyclin-dependent kinases, preventing phosphorylation of RB, and maintaining RB-mediated inhibition of cell cycle progression.²² Thus, each of the genes encoded by the *INK4A* locus regulate tumor suppressors, and loss of each has been shown to cause transformation of cells to a malignant phenotype. Therefore, both are considered tumor suppressors and have been implicated in gliomagenesis. Recent studies in mice indicate that ARF may play a more prominent role in gliomagenesis than p16, although there may be differences in the function of these proteins in mice compared with humans.^{22,24}

Separate studies have demonstrated variability of gene expression in different sets of GBMs.^{16,28,32} In particular, tumors expressing the EGFR, which is associated with a particularly poor prognosis, can clearly be identified by microarray expression analysis.³² In other studies, investigators have been able to identify subtypes of GBM based on their molecular signature.^{21,28,32,34} Some of these researchers have further demonstrated that overexpression of specific genes correlates with a more favorable prognosis, although the mechanism by which they were overexpressed was not evaluated. In one study, expression analysis was correlated with copy number changes determined using comparative genomic hybridization, and global gene expression changes were observed in genes not associated with chromosomal regions implicated in gene amplification or deletion.³⁴ Specifically, copy number changes in genes located on chromosome 10 were associated with global gene expression changes. Interestingly, loss of heterozygosity at the chromosomal location 10q23 is a common feature in GBM.

Chromosomal deletions, mutations, and amplifications all play roles in the development of malignancies, including GBMs. Many GBMs may have common features attributable to these genetic changes. Nevertheless, the circuitous path each individual tumor has followed to reach malignant transformation is likely unique to that tumor and patient, and thus may require therapeutic strategies tailored specifically for each patient and GBM.

Aberrant Signal Transduction

In addition to loss of tumor suppressor function, gain of function changes also occur in GBMs and, as in other tumors, often affect signal transduction pathways (Fig. 1). The EGFR is an RTK that is commonly overexpressed in GBMs, and this overexpression typically occurs as a result of gene amplification.^{4,14,52} The EGFR-mediated signal transduction results in activation of a number of downstream pathways including PI3K-AKT and RAS-MAPK, inhibiting apoptosis and driving proliferation.¹⁴ Alternatively, deletion within the *EGFR* coding sequence can result in the expression of a truncated, mutant EGFR protein that signals constitutively.

Other mutations common in GBM affect RTKs other than EGFR. Expression of PDGFR β as well as its ligand is frequently found in GBMs.³¹ The PDGFR β activates RAS-MAPK and PI3K-AKT as well as JAK-STAT signaling, which is involved in cell survival, proliferation, and differentiation.³¹ Specific dysregulation of RAS-MAPK caused by loss of the *NF1* gene in neurofibromatosis Type 1 syndrome has been associated with an increased risk of malignant astrocytomas in affected individuals.⁵³ Therefore, the

RAS-MAPK signaling cascade has been presumed to be important for gliomagenesis. Recent evidence indicates, however, that mutant EGFR and overexpressed wild-type EGFR signal preferentially down divergent pathways in GBM; down RAS-MAPK and PI3K-AKT pathways, respectively.⁵²

Inducible models of tumorigenesis in mice have yielded findings that particular tumors become dependent on specific mutations, and that repression of the initiating oncogenic stimulus can result in widespread apoptosis and tumor regression.^{8,10,18,30} Targeting of specific molecular defects on which tumor cells rely is the goal of many novel therapeutic strategies (Table 1). Many of these strategies, however, depend on comprehensive mutation or expression analysis. Because RTKs and signaling pathways represent attractive targets for molecular therapeutics, distinguishing the nature of pathway activation in individual tumors will be important for the selection of the appropriate therapeutic modality. Again, the pathway to complete malignant transformation is likely unique in every patient and, although similar pathways are implicated in gliomagenesis by changes in various upstream signaling molecules, the overall mechanisms of pathway activation in

Fig. 1. Schematic depicting signaling with the growth factor receptors through the JAK-STAT and RAS pathways. (Signaling occurs through a number of pathways, including JAK-STAT and RAS-MAPK.) Known interactions between specific effectors are indicated by **bold arrows**, and **arrowheads** indicate downstream signaling. Similar schematics could be drawn depicting RTK signaling, but there would be no dependence on JAK activation, because many RTKs can activate STATs directly. Evidence indicates that SHP-2-dependent signaling activates PI3-K or RAS-MAPK differentially from wild-type EGFR or EGFRvIII. GDP = guanosine 5'-diphosphate; GTP = guanosine 5'-triphosphate.

each tumor may play an important role in the ultimate malignant phenotype. Analysis of the pathways involved in individual tumors should therefore be performed to assist in the selection of therapies that address the unique biology of the specific tumor.

For instance, molecular inhibitors of MDM2 have been recently developed for clinical use. These compounds, called “nutlins,” interfere with the interaction between MDM2 and p53, releasing p53 from negative regulation and allowing transcription of cell cycle inhibitory molecules.⁵⁰ Because *MDM2* is the second most commonly amplified gene in GBM, use of nutlins in this disease seems a rational therapeutic strategy. Nevertheless, this approach is likely to be most effective only in patients with *MDM2* overexpression or low *p14^{ARF}* expression. Clinical trials using nutlins as a component of therapy should therefore be designed to include expression and mutation analysis of these two genes as a criterion for patient selection.

Expression Analysis for Rational and Patient-Tailored Therapy

An increasing body of evidence demonstrates the utility of expression profiling in stratification of patients with GBM in terms of tumor classification and survival. Unfortunately, a marker of survival in GBM is a relative term that belies the fact that less than 1% of patients survive more than 5 years.^{25,42} Although identification of markers relevant for patient stratification will undoubtedly contribute to improvements in treatment modality, it is important to expand these studies to include the identification of rational therapeutic targets, which seems a more compelling goal in evaluation of GBM at the transcriptional level.

In addition to intertumoral heterogeneity, studies evaluating differential gene expression within the same tumor have demonstrated intratumoral heterogeneity between core GBM tissue and invasive, proliferating peripheral tumor tissue.¹⁶ Expression differences in one study correlated with differences in the intensity of contrast enhancement on magnetic resonance imaging.¹⁵ This intratumoral hetero-

geneity again highlights the need for multifaceted modalities addressing all aspects of the biology of GBMs. These studies demonstrate the utility of expression profiling in the stratification of patients as well as the selection of targeted molecular and gene therapies used for successful treatment of this disease.

The identification of BTSCs as candidates for the true tumor-initiating cells poses an interesting challenge for conducting routine, high-throughput gene expression analysis of GBMs to tailor therapy to rational targets. It is vital to kill the BTSCs as well as the more differentiated tumor cells to achieve the maximum antitumor effect. Accordingly, it may be critical to purify CD133-positive BTSCs for expression analysis to determine which class of drugs is most likely to kill these cells. In addition, expression analysis could be conducted in the CD133-negative tumor cells to identify a second drug tailored for their elimination.

CONCLUSIONS

In-depth expression analysis with respect to mutation status has not been performed with GBMs to the same extent as with other cancers.^{2,26,49} With the development of molecular inhibitors targeting multiple pathways implicated in gliomagenesis, the combination of expression analysis and mutation status may be the next step in enabling the development of therapeutic modalities better tailored for individual disease characteristics. It is clear that the appropriate drugs for GBM will not be the same for all patients, and that choosing the best drug may require routine expression analysis. Therefore, expression analysis may be most useful as a standard clinical test performed before drug therapy commences to choose rationally patient-tailored drugs that target the appropriate molecular pathways. For instance, expression analysis could be conducted immediately after resection in multiple biopsy samples of core and invasive regions of the tumor, perhaps identified before resection on magnetic resonance imaging.

Implementing expression analysis as a standard clinical test, however, will require significant changes in the way clinicians approach GBM treatment, as well as the development of substantial infrastructure for rapid, high-throughput expression and mutation analysis of tumor tissue. The challenge of interpreting large data sets generated from routine, high-throughput array analysis from individual tumors will require an expansion of expertise from academic, research-oriented studies to wider clinical practice. Despite these obstacles, it is vital to take into account the genetic mutations present in the patient's individual tumor before the use of drugs that target molecular pathways can ever be expected to achieve maximal clinical efficacy. Furthermore, it is important to consider that GBMs are composed of a group of related but genetically distinct tumor cells, containing both BTSCs and differentiated tumor cells, and capitalize on this knowledge by using therapies that target all populations present in the tumor.

References

1. Alberts B, Bray D, Lewis J, et al: **Molecular Biology of the Cell**, ed 3. New York: Garland Publishing, 1994
2. Bullinger L, Dohner K, Bair E, et al: Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. *N Engl J Med* 350:1605–1616, 2004

TABLE 1

*Literature review of currently targeted changes in malignancies**

Authors & Year	Phenotypic or Genetic Change	Targeted Therapy
Carroll, et al., 1997	PDGFR/PDGF overexpression	imatinib mesylate (Gleevec)
Debinski, et al., 1999	IL13R overexpression	immunotoxins
Husain, et al., 2001	IL13R overexpression	immunotoxins
Traxler, et al., 2001	<i>EGFR</i> amplification, <i>EGFRvIII</i>	ZD1839 (IRESSA), OSI-774, PK1166, CI1033, EKB569
Shawver, et al., 2002	<i>EGFR</i> amplification, <i>EGFRvIII</i>	ZD1839 (IRESSA), OSI-774, PK1166, CI1033, EKB569
Ferrara, et al., 2004	VEGF overexpression	antiangiogenesis
Vassilev, et al., 2004	<i>ARF</i> loss	nutlins
	<i>MDM2</i> amplification	nutlins
Chang, et al., 2005	<i>PTEN</i> loss	CCI-779, RAD001
Galanis, et al., 2005	<i>PTEN</i> loss	CCI-779, RAD001
Goudar, et al., 2005	<i>PTEN</i> loss	CCI-779, RAD001
Ohlfest, et al., 2005	VEGF overexpression	antiangiogenesis

* VEGF = vascular endothelial growth factor.

3. Carroll M, Ohno-Jones S, Tamura S, et al: CGP 57148, a tyrosine kinase inhibitor, inhibits the growth of cells expressing BCR-ABL, TEL-ABL, and TEL-PDGFR fusion proteins. **Blood** **90**:4947–4952, 1997
4. Chakravarti A, Seiferheld W, Tu X, et al: Immunohistochemically determined total epidermal growth factor receptor levels not of prognostic value in newly diagnosed glioblastoma multiforme: report from the Radiation Therapy Oncology Group. **Int J Radiat Oncol Biol Phys** **62**:318–327, 2005
5. Chang SM, Wen P, Cloughesy T, et al: Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme. **Invest New Drugs** **23**:357–361, 2005
6. Daumas-Duport C, Scheithauer B, O'Fallon J, et al: Grading of astrocytomas. A simple and reproducible method. **Cancer** **62**: 2152–2165, 1988
7. Debinski W, Gibo DM, Hulet SW, et al: Receptor for interleukin 13 is a marker and therapeutic target for human high-grade gliomas. **Clin Cancer Res** **5**:985–990, 1999
8. Felsher DW, Bishop JM: Reversible tumorigenesis by MYC in hematopoietic lineages. **Mol Cell** **4**:199–207, 1999
9. Ferrara N, Hillan KJ, Gerber HP, et al: Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. **Nat Rev Drug Discov** **3**:391–400, 2004
10. Fisher GH, Wellen SL, Klimstra D, et al: Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. **Genes Dev** **15**:3249–3262, 2001
11. Freese A, O'Rourke D, Judy K, et al: The application of 5-bromodeoxyuridine in the management of CNS tumors. **J Neuro-oncol** **20**:81–95, 1994
12. Galanis E, Buckner JC, Maurer MJ, et al: Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. **J Clin Oncol** **23**:5294–5304, 2005
13. Goudar RK, Shi Q, Hjelmeland MD, et al: Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. **Mol Cancer Ther** **4**:101–112, 2005
14. Grunwald V, Hidalgo M: Developing inhibitors of the epidermal growth factor receptor for cancer treatment. **J Natl Cancer Inst** **95**:851–867, 2003
15. Hobbs SK, Shi G, Homer R, et al: Magnetic resonance image-guided proteomics of human glioblastoma multiforme. **J Magn Reson Imaging** **18**:530–536, 2003
16. Hoelzinger DB, Mariani L, Weis J, et al: Gene expression profile of glioblastoma multiforme invasive phenotype points to new therapeutic targets. **Neoplasia** **7**:7–16, 2005
17. Hu J, Jiang C, Ng HK, et al: Genome-wide allelotyping study of primary glioblastoma multiforme. **Chin Med J (Engl)** **116**: 577–583, 2003
18. Huettner CS, Zhang P, Van Etten RA, et al: Reversibility of acute B-cell leukaemia induced by BCR-ABL1. **Nat Genet** **24**: 57–60, 2000
19. Hunter SB, Abbott K, Varma VA, et al: Reliability of differential PCR for the detection of EGFR and MDM2 gene amplification in DNA extracted from FFPE glioma tissue. **J Neuro-pathol Exp Neurol** **54**:57–64, 1995
20. Husain SR, Joshi BH, Puri RK: Interleukin-13 receptor as a unique target for anti-glioblastoma therapy. **Int J Cancer** **92**: 168–175, 2001
21. Kim S, Dougherty ER, Shmulevich I, et al: Identification of combination gene sets for glioma classification. **Mol Cancer Ther** **1**:1229–1236, 2002
22. Krug U, Ganser A, Koeffler HP: Tumor suppressor genes in normal and malignant hematopoiesis. **Oncogene** **21**:3475–3495, 2002
23. Kuratsu J, Itoyama Y, Uemura S, et al: [Regrowth patterns of glioma—cases of glioma regrew away from the original tumor.] **Gan No Rinsho** **35**:1255–1260, 1989 (Jpn)
24. Kusy S, Larsen CJ and Roche J: p14ARF, p15INK4b and p16INK4a methylation status in chronic myelogenous leukemia. **Leuk Lymphoma** **45**:1989–1994, 2004
25. Lacroix M, Abi-Said D, Fournay DR, et al: A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. **J Neurosurg** **95**:190–198, 2001
26. Lapointe J, Li C, Higgins JP, et al: Gene expression profiling identifies clinically relevant subtypes of prostate cancer. **Proc Natl Acad Sci U S A** **101**:811–816, 2004
27. Lewin B: **Genes VII**. New York: Oxford University Press, 1999
28. Liang Y, Diehn M, Watson N, et al: Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. **Proc Natl Acad Sci U S A** **102**:5814–5819, 2005
29. Louis DN, Holland EC, Cairncross JG: Glioma classification: a molecular reappraisal. **Am J Pathol** **159**:779–786, 2001
30. Marinkovic D, Marinkovic T, Mahr B, et al: Reversible lymphomagenesis in conditionally c-MYC expressing mice. **Int J Cancer** **110**:336–342, 2004
31. Mischel PS, Cloughesy TF: Targeted molecular therapy of GBM. **Brain Pathol** **13**:52–61, 2003
32. Mischel PS, Shai R, Shi T, et al: Identification of molecular subtypes of glioblastoma by gene expression profiling. **Oncogene** **22**:2361–2373, 2003
33. Momand J, Zambetti GP, Olson DC, et al: The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. **Cell** **69**:1237–1245, 1992
34. Nigro JM, Misra A, Zhang L, et al: Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. **Cancer Res** **65**:1678–1686, 2005
35. Ohgaki H, Schauble B, zur Hausen A, et al: Genetic alterations associated with the evolution and progression of astrocytic brain tumors. **Virchows Arch** **427**:113–118, 1995
36. Ohlfest JR, Demorest ZL, Motooka Y, et al: Combinatorial antiangiogenic gene therapy by nonviral gene transfer using the *sleeping beauty* transposon causes tumor regression and improves survival in mice bearing intracranial human glioblastoma. **Mol Ther**: [Epub ahead of print], 2005
37. Pomerantz J, Schreiber-Agus N, Liegeois NJ, et al: The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. **Cell** **92**:713–723, 1998
38. Rainov NG, Dobberstein KU, Bahn H, et al: Prognostic factors in malignant glioma: influence of the overexpression of oncogene and tumor-suppressor gene products on survival. **J Neuro-oncol** **35**:13–28, 1997
39. Reifenberger G, Reifenberger J, Ichimura K, et al: Amplification at 12q13–14 in human malignant gliomas is frequently accompanied by loss of heterozygosity at loci proximal and distal to the amplification site. **Cancer Res** **55**:731–734, 1995
40. Sauerbrey A, Stammler G, Zintl F, et al: Expression of the retinoblastoma tumor suppressor gene (RB-1) in acute leukemia. **Leuk Lymphoma** **28**:275–283, 1998
41. Schiebe M, Ohneseit P, Hoffmann W, et al: Analysis of mdm2 and p53 gene alterations in glioblastomas and its correlation with clinical factors. **J Neuro-oncol** **49**:197–203, 2000
42. Scott JN, Rewcastle NB, Brasher PM, et al: Long-term glioblastoma multiforme survivors: a population-based study. **Can J Neurol Sci** **25**:197–201, 1998
43. Shawver LK, Slamon D, Ullrich A: Smart drugs: tyrosine kinase inhibitors in cancer therapy. **Cancer Cell** **1**:117–123, 2002
44. Silbergeld DL, Chicoine MR: Isolation and characterization of human malignant glioma cells from histologically normal brain. **J Neurosurg** **86**:525–531, 1997

45. Singh SK, Hawkins C, Clarke ID, et al: Identification of human brain tumor initiating cells. **Nature** **432**:396–401, 2004
46. Strachan T, Read AP: **Human Molecular Genetics 1, ed 2**. New York: Wiley, 1999
47. Traxler P, Bold G, Buchdunger E, et al: Tyrosine kinase inhibitors: from rational design to clinical trials. **Med Res Rev** **21**: 499–512, 2001
48. Ushio Y, Tada K, Shiraishi S, et al: Correlation of molecular genetic analysis of p53, MDM2, p16, PTEN, and EGFR and survival of patients with anaplastic astrocytoma and glioblastoma. **Front Biosci** **8**:e281–e288, 2003
49. Valk PJ, Verhaak RG, Beijnen MA, et al: Prognostically useful gene-expression profiles in acute myeloid leukemia. **N Engl J Med** **350**:1617–1628, 2004
50. Vassilev LT, Vu BT, Graves B, et al: In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. **Science** **303**:844–848, 2004
51. Yuan X, Curtin J, Xiong Y, et al: Isolation of cancer stem cells from adult glioblastoma multiforme. **Oncogene** **23**:9392–9400, 2004
52. Zhan Y, O'Rourke DM: SHP-2-dependent mitogen-activated protein kinase activation regulates EGFRvIII but not wild-type epidermal growth factor receptor phosphorylation and glioblastoma cell survival. **Cancer Res** **64**:8292–8298, 2004
53. Zhu Y, Guignard F, Zhao D, et al: Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. **Cancer Cell** **8**:119–130, 2005

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