Molecular pathways of glial tumorigenesis

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Gliomas are the most frequently occurring central nervous system tumours that include astrocytomas, anaplastic astrocytomas and glioblastoma multiforme. In spite of modern therapeutic procedures, the outcome of patients with glial tumours remains unchanged. The development of gliomas, like many other tumours, is a multistep process involving the accumulation of several genetic events which include activation of oncogenes, growth factors and their receptors and the loss of tumour suppressor genes leading to the perturbation of pathways regulating cell growth and differentiation. Genomic instability also develops during the course of glioma formation. There has been an increase in our understanding of the genesis and progression of human astrocytic tumours. This review focuses on various genetic events known to occur in the development and progression of human astrocytic tumours.

THE battle against cancer has been grim and slow. The targets identified in cancer cells have been too few and not different enough from normal cells to be useful in therapeutics so far. Hopefully, with the identification of specific genetic alterations affecting the neoplastic phenotype, this is likely to change. Glial tumours are a class of tumours where the outcome has not improved in spite of modern therapeutic modalities. However our understanding of the molecular changes that occur in glial tumorigenesis has improved considerably in recent years. It is hoped that our knowledge of molecular lesions influencing the tumour phenotype will result in the identification of cellular regulatory pathways that can be specifically targetted in gliomas.

Glial cells

Glial cells are the supportive cells of the central nervous system (CNS) that are 10–50 times more numerous than neurons. These comprise primarily astrocytes, oligodendrocytes and ependymal cells.

Astrocytes

These are stellate cells with numerous, fine branching processes. During development, proliferating neuroblasts migrate along a path indicated by astrocytes. In post-natal life, in addition to mechanical support, astrocytes regulate extracellular fluid and electrolytes balance, trophism and show reactive changes in response to injury and diseases.

Oligodendrocytes

These are small glial cells whose cytoplasmic processes wrap around the axons of neurons to form myelin sheathes that facilitate rapid conduction of neuronal impulse in a manner analogous to the Schwann cells of the peripheral nervous system.

Ependymal cells

These form a single layer of cells lining the ventricular system and the central canal of the spinal cord. They play a role in circulation of cerebrospinal fluid (CSF). Specialized ependymal cells in the choroid plexus regulate CSF production.

Developmentally, both neurons and glial cells originate from a common neuroectodermal precursor cell, which then differentiates into either a neuronal or glial lineage. Differentiation into astrocytes or oligodendroglia occurs further down the glial lineage. Microglia are distinct from the other glial cells, being more akin to macrophages in development and function.

Prevalence of glioma

Neoplasms of the neuroglial cells are called glial tumours/glioma. Glial tumours are one of the commonest primary human brain tumours arising from the astrocytes or oligodendroglial cells or ependymal cells of the brain. In adults, gliomas account for about 2% of the primary malignant tumours[1]. They strike earlier than other tumours, being the commonest solid tumours till the age of 35 years, second only to leukaemias in this age group[2]. Gliomas account for about half of primary intracranial tumours[3] and have morphology and gene expression characteristics similar to astrocytes, oligodendrocytes or a mixture of the two cell types (and their precursors). This review will concentrate on tumours arising from astrocytes.

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Classification and behaviour

The tumours arising from the astrocytes (astrocytic tumours) are histopathologically graded into three types according to their increasing malignancy. These are:

Astrocytomas (WHO grade II)

Astrocytomas (AS) in general are most common in late middle age. They are poorly defined, grey-white tumours, which infiltrate and distort the underlying brain and range from a few centimetres in diameter to enormous lesions that replace substantial parts of the cerebral hemisphere. The cells are relatively more differentiated and have fewer proliferating cells compared to the higher grades. These tumours are often manageable by surgery, although recurrence is frequent. Recurrent tumours are often found to be less differentiated and of higher grade, suggesting their progressive nature[4], and, in spite of improved management protocols, the mean survival time is about six years[5] and hence these tumours are by no means benign.

Anaplastic astrocytomas (WHO grade III)

These are not grossly distinguishable from AS. Microscopically, however, they have anaplastic features such as increased hypercellularity, nuclear and cytoplasmic pleomorphism, and irregularity and nuclear hyperchromatism. Some patients in this group respond well to chemotherapy and/or radiotherapy, while many do not. However, the rate of survival drops considerably compared to AS, the mean survival time being about two years[6].

Glioblastoma multiforme (WHO grade IV)

This is the most malignant type of tumour and has the poorest prognosis. Glioblastoma multiforme (GBM) is distinguished from the other types of AS by its variegated appearance, hence the term multiforme. Foci of necrosis, cysts and haemorrhages are common. The nuclei of the tumour cells are extremely variable in size and shape. The mean survival time is about one year.

Pilocytic astrocytomas (WHO grade I)

These are a group of AS that are distinguishable from other AS by their distinctive pathologic appearance and almost invariably benign behaviour. They typically occur in children and young adults and are usually located in the cerebellum. These tumours are usually not a part of the progression scheme of AS to Anaplastic astrocytomas (AA) to GBM.

Tumours that arise from oligodendroglias are called *oligodendrogliomas* and have an indolent pattern of growth with the median survival rate of five years. Another group of tumours which have both the astrocytic and oligodendroglial components in them are called mixed (*oligoastrocytoma*) and malignant mixed (*malignant oligoastrocytoma*) gliomas, as the names specify, one is more malignant than the other.

An important property of astrocytic tumours is their marked tendency to become more anaplastic with time, so that tumour initially diagnosed as an AS may later on repeat biopsy be graded as a GBM.

Whether malignant or not, brain tumours can cause death by local growth and by affecting the vital surrounding structures. Thus, the distinction between benign and malignant tumours is less important for intracranial tumours than for any other systemic cancer.

Clinical presentation

The location and size of the tumour determines the clinical presentation. Almost all AS in adult occur in the cerebral hemisphere. Patients with gliomas commonly present with headache and/or seizures, or focal neurological alterations that cause weakness of one limb or language disturbances. Most gliomas arise sporadically and are not inherited within families; however patients with gliomas sometimes have a family history of diverse cancer types. Computed tomography (CT) and magnetic resonance imaging (MRI) scans are often used to confirm the diagnosis.

Molecular pathways in glial tumorigenesis

Studies elucidating molecular pathways involved in glial tumorigenesis have basically used the approach of determining alterations in the expression and structure of growth factors, oncogenes or tumour suppressor genes (TSGs) during specific stages of the tumour. Some of these oncogenes and TSGs are involved in the formation of low-grade AS, others in the transition from AS to AA, and yet others in the formation of oligodendrogliomas and oligoastrocytomas, and the remaining in the final progression to GBM.

Formation of WHO grade II AS

AS are associated with several alterations: inactivation of the TP53 TSG, activation of several growth factors such as PDGF (platelet-derived growth factor), FGF2 (fibroblast growth factor) and CNGF (cilliary neurotrophic growth factor) and their receptors and loss of TSG on chromosome 22q.

The *TP53* gene on chromosome 17p13 encodes tumour suppressor protein p53. Inactivation of *TP53*, usually by the mutation of one allele and the chromosomal

loss of the other allele, occurs in approximately one-third of AS, AA and GBM[7]. These are primarily missense mutations and target the evolutionarily conserved domains leading to the loss of *p53*-mediated transcriptional activity[8]. The p53 gene alteration is essential for tumorigenesis, but not a factor in tumour progression, as indicated by a similar frequency of alteration in all grades of tumour. We have also established this by molecular typing of primary tumours and follow up of the affected patients and also by the study of recurrent tumours[9,10]. Studies of *MDM2* oncogene, an upstream inhibitor of p53 function, show that it sometimes undergoes gene amplification[11]. The $p14^{ARF}$, a positive upstream regulator of p53, acts by inhibiting MDM2-mediated degradation of p53 (ref. 12). The loss of $p14^{ARF}$ would lead to decreased availability of wild type p53.

We have also demonstrated 3′ rearrangements of the *c-myc* oncogene in some low-grade gliomas[13], associated with increased expression of the protein. This was a report of 3′ rearrangement of the *c-myc* gene leading to its dysregulation in solid tumours. The expression of *c-myc* was also dependent on cellular differentiation, and more protein was expressed in glial cells which are of astrocytic as compared to oligodendroglial lineage in mixed glioma. Mixed glioma is an interesting tumour, which is a manifestation of the ability of a glial precursor to differentiate into either cell type[14].

Over-production of growth factors such as FGF2, CNTF, PDGF and their receptors, by mechanisms other than genetic mutation is one of the common alterations found in gliomas of all grades[15]. It is frequently seen that there is an excess of growth factors and its receptor produced in the same cell resulting in autocrine stimulation and increased activity of downstream pathways of these receptors. Basic FGF is shown to be detected in most glioma tissues and the expression level increased proportionately with the degree of malignancy, whereas it was undetectable in normal brain tissue[16]. Basic FGF also plays an important role in tumour vascularization as a paracrine angiogenic factor. These growth factor receptors are shown to activate several common signalling pathways, including those that lead to the RAS and AKT pathways in the glioma cell lines and several other tumour cells[15]. PDGF over expression correlates with inactivation of the *TP53* gene[17].

Allelic loss of chromosome 22q is found in approximately 20–30% of AS, suggesting an astrocytoma TSG on this chromosome[18]. Studies using deletion mapping technique on chromosome 22q showed two regions, 22q12.3–22q13.1 and 22q13.2, most likely to be the location of putative TSG[19].

Pilocytic astrocytomas (WHO grade 1), which have been shown to be distinct from the progression pathway of AS to AA to GBM, have also been shown to have distinct genetic lesions, which are not common to AS[20].

Transition from AS to AA

This is associated with inactivation of TSGs on chromosome 13q13, 9p21, 19q and amplification of chromosome 12q, which are rare in low-grade AS.

Loss of chromosome 13q13 occurs in approximately one-third of higher grade tumours and targets the retinoblastoma (Rb) gene[21]. Disruption of Rb function by the mechanisms described below is a feature of higher grade of tumours (AA and GBM), where it is significantly more (70%) frequent than in AS (25%). The Rb protein in its hypophosphorylated form sequesters E2F and renders it unavailable for G1–S transition events. Absence or excessive phosphorylation of the Rb protein releases E2F, which is then available for events leading to cell-cycle progression. The function of the *Rb* gene/product is mainly disrupted in three ways:

1. Deletion and/or mutation of the *Rb* gene, located on chromosome 13q13, occurs approximately in one-third of higher grade tumours.
2. *p16* (CDKN2A)[22], a TSG located on chromosome 9p21 binds to cdk4 protein and prevents the formation of its active complex with cyclin D protein. Cyclin D1-CDK4 has been shown to phosphorylate most Rb sites during late G1. In its hypophosphorylated form, the Rb protein causes cell-cycle arrest at the G1 checkpoint (Figure 1). Loss/mutation of *p16* (CDKN2A) or *p15* (CDKN2B) genes on chromosome 9p21, which occurs more commonly in higher grade tumours[23,24], results in the increased phosphorylation of Rb. This releases the sequestered E2F.
3. Increased expression/gene amplification of cdk4 and cyclinD1 also results in increased phosphorylation of Rb, thus promoting cell-cycle progression. *CDK4* gene (on chromosome 12q) amplification and the associated over expression of cdk4 protein has been shown to occur in gliomas with intact and expressed *p16* gene[25].

Chromosome 12q has several other genes like *MDM2*, *SAS*, *GADD*, *GLI* and *A2MR* which undergo amplification during the genesis of late stages of AA and early stages of GBM[26]. *MDM2* gene located on chromosome 12q13-14 is found to be amplified seventy times higher in GBM compared to normal tissue[27]. *GLI* gene in the same region is also shown to be amplified and over expressed in GBM[28]. Another putative TSG on chromosome 19q13.3 appears to be lost in glial tumours and this loss is seen in all three histological subtypes of malignant gliomas[29]. The region of common deletion has been identified to be as narrow as 150 kb (ref. 30). Another region in which deletions are seen in AA and GBM is on chromosome 6q (ref. 31).

Silencing of TSGs in the absence of deletions or mutations can occur due to methylation. It has been shown

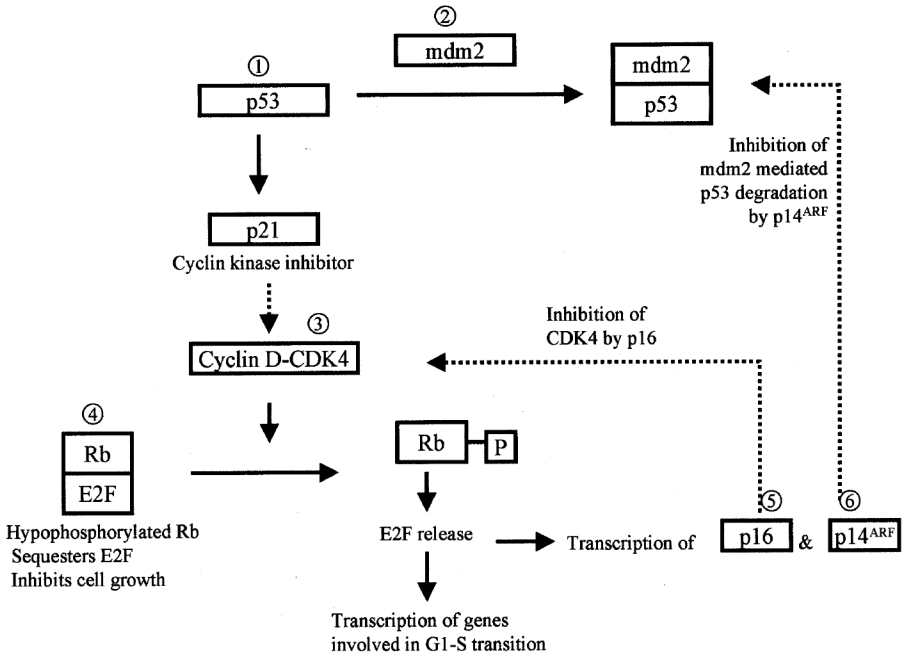

Figure 1. Key regulatory pathways affected in glial tumorigenesis. (↑) Indicates amplification/over expression, (↓) Indicates deletion/mutation. ①, p53-TSG directly affected in 40% of all grades of glioma. However in conjunction with mdm2 (↑) or p14 (↓), p53 activity is affected in 65–75% of all grades of glioma; ②, mdm2 (↑) reduces p53 activity in some tumours; ③, Cyclin D-CDK4 (↑) affected in some GBM. The cyclin D–CDK4 complex phosphorylates Rb leading to E2F release and G1–S transition; ④, The Rb–E2F complex, essential for controlling cell division, is decreased by Rb mutation/loss or phosphorylation by cyclin D–CDK4 complex. Phosphorylation releases E2F and causes G1–S transition; ⑤, p16 is normally induced by E2F as a regulatory mechanism. Loss (seen more in AA and GBM) promotes cell division via increased activity of the cyclin D–CDK4 complex; ⑥, $p14^{ARF}$ inhibits mdm2-mediated degradation of p53. When $p14^{ARF}$ is lost (more in AA and GBM), p53 activity is reduced. — Indicates positive influence; .... Indicates negative influence.

that hypermethylation of the CpG island of p16/CDKN2 correlates with gene inactivation in gliomas[32]. This is true especially in cases that are immunonegative but have no structural changes in the gene[33]. Similar mechanisms may be operative in other TSGs as well.

Malignant progression from AA to GBM

This is associated with inactivation of putative TSG on chromosome 10q and amplification of the *EGFR* gene[34]. The *EGFR* gene is amplified in approximately 40% of all GBM. Often the *EGFR* gene is altered giving rise to a truncated product consisting of a constitutively active cytoplasmic domain of the protein.

Loss of chromosome 10 occurs in 60–85% of GBM, with most cases showing allelic loss of the entire chromosome[7]. GBM exhibiting *EGFR* gene amplification always show loss of chromosome 10 (ref. 34). Mapping of homozygous deletion on chromosome 10q has led to the isolation of a candidate TSG, *PTEN* (contains a phosphatase domain, and its protein product has phosphatase activity), located on 10q23 (ref. 35). The target lipid is PIP3 (phosphatidylinositol triphosphate), one of the cell's major growth-controlled pathways acting both to stimulate cell growth and to block apoptosis. Normally *PTEN* removes one phosphate from PIP3 and blocks its growth-stimulating pathways and allows cell suicide to proceed, keeping cell population in check (Figure 2). Loss of *PTEN* during tumorigenesis presumably keeps PIP3 pathway inappropriately activated allowing the mutated cells to grow unchecked. Loss of *PTEN* function on chromosome 10q might cause genomic instability that enables *EGFR* gene amplification. Recent studies suggest that two other TSGs, *DMBT1* and *LGI1* are also located on chromosome 10. Another candidate TSG, MMAC1, at chromosome 10q23.3 (ref. 36) is found to be mutated in multiple advanced cancers including gliomas. Loss of heterozygosity (LOH) of chromosome 10 was associated with a significantly reduced overall survival in patients with AA and GBM.

A TSG, deleted-in-colon carcinoma (DCC), on chromosome 18q21, is shown to be deleted in 7% of low-grade gliomas and 53% of GBM, suggesting its role in the genesis of GBM[37]. Loss of C4-2 expression, isolated by Differential Display PCR, in GBM but not in normal brain tissue studied, indicates that it may function as a potential brain-tumour suppressor[38]. Cobbs *et al.*[39] detected increased expression of nitric oxide synthase (NOS) in astrocytic tumours with the highest level of

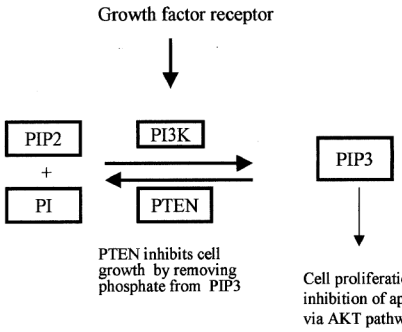

Figure 2. Mechanism of action of *PTEN*. *PTEN*, a tumour suppressor gene on chromosome 10q24, acts as a phosphatase, removes one phosphate from PIP3 and thereby blocks the effect of growth factor receptor signalling via PIP3. Dephosphorylation of PIP3 allows cell suicide to proceed and keeps cell population in check. Loss/mutation of *PTEN* is seen in about 40% GBM, LOH of 10q23 is seen in 70% GBM.

expression found in higher grade tumours, and suggested association of nitric oxide (NO) production with the pathophysiological processes important to these tumours. Connexin, a family of gap junction proteins, plays an important role in cell–cell communication and tissue homeostasis. It is suggested that connexin can act as TSG and loss of its expression may be of importance in tumour progression[40].

Our group has defined a putative TSG locus on chromosome 17p13.3, telomeric to the p53 locus[41]. LOH of this locus is significantly associated with GBM as opposed to AS. While this locus is affected independently of p53 at genetic level, over expression of the p53 protein is more in tumours with LOH of this locus[42]. This p53 protein is unaltered, implying that GBM induced by this pathway have mechanisms to override the apoptotic and cell cycle arrest inducing properties of p53. Such tumours also have markedly higher mitotic indices compared to those without LOH (manuscript under preparation).

In addition to *EGFR* amplification and loss of chromosome 10, two-thirds of GBM are shown to be associated with Rb-related deregulation of cell-cycle checkpoint[25] and many tumours also showed over expression of IL13R (ref. 43). Changes in signal transduction pathways in gliomas are extensively reviewed by Holland[15].

Recently array technology has been used for profiling gene expression in different grades of tumour. cDNA array identified over expression of Insulin-like Growth Factor Binding Protein (IGFBP2)[44]. This gene is normally expressed in foetal cells and turns-off in adult cells indicating aberrant cell differentiation in glioma progression. Rickman *et al.*[45] have compared the transcriptional profile of different tumour stages using oligonucleotide microarray and found evidence of over expression of a number of genes in GBM. Many of these transcripts that were increased in GBM were not previously associated with lower grade gliomas which suggest their involvement in tumour progression. Sallinen *et al.*[46] have used both DNA microarrays and tissue arrays for screening various grades of gliomas for differential expression of genes and observed many gene expression alterations in AA, AS and GBM. Some of the genes in which marked over expression (> 5-fold increase) and reduced expression (< 5-fold decrease) were observed by microarray analysis in GBM compared to normal brain are depicted in Table 1. It is still too early to hypothesize a model on development and progression of glial tumours based on these differential expression results. Some of the genes are obvious candidate genes, like *IGFBP2* and *CDK4* (over expressed) or *p19INK-4d* (down regulated). However a holistic view is still to emerge.

Molecular heterogeneity within a tumour grade – Example of GBM

While molecular heterogeneity is a feature of almost every pathological sub-type of tumours, the situation with GBM is very illustrative. Each individual tumour

Table 1. Microarray analysis of altered gene expression in GBM compared to normal brain (NB). Compiled from data by Rickman *et al.*[45] and Sallinen *et al.*[46]. The expression of a number of genes has been shown to be altered, however only those showing marked changes (⟨and⟩ 5 times normal) are depicted

Upregulation (> 5-fold increase) in GBM versus normal brain
Transforming growth factor β induced gene (TGFBI)
Insulin-like growth factor binding protein 2 (IGFBP2)
Filamin A, α (actin-binding protein-280) (FLNA)
Hexabrachion (tenascin C, cytotactin) (HXB)
Midkine (MDK)
Cyclin-dependent kinase 4 (CDK4)
Thymidylate synthetase (TYMS)
Oncogene Tl1/Chop, fusion-activated (TLS/CHOP)
Plasminogen activator inhibito-1 (PAI-1)
Vascular endothelial growth factor (VEGF)
Fibronectin
Activator 1 40 kD subunit (RFC40)
Leukaemia inhibitory factor (LIF)
Insulin-like growth factor binding protein 3 (IGFBP3)
Integrin α 3
Caveolin-1
Downregulation (> 5-fold decrease) in GBM versus normal brain
Chondroitin sulphate proteoglycan 2 (versican) (CSPG2)
Tissue inhibitor of metalloproteinase 4 (TIMP 4)
Thrombospondin 4 (THBS4)
Cyclin-dependent kinase 4 inhibitor D (p19INK4d)
P53-induced gene-10 (PIG10)
Receptor tyrosine kinase (SKY)
T-lymphoma invasion and metastasis inducing (TIAM1)
Neuroendocrine *Drosophila* discs large (NE-dlg)

Table 2. Genetic alterations in different grades of glioma. Involvement of various tumour suppressor genes and oncogenes in the development and progression of the astrocytic tumour. L, Loss/mutation of TSG; G, Amplification/over expression of oncogene. GBM is formed either *de novo* or progresses from lower grade to higher grade through acquisition of additional mutation. These two pathways have distinct molecular alterations

			Glioblastoma multiforme	
Alteration	Astrocytoma	Anaplastic astrocytoma	Progressive	*De novo*
Deletion/mutation of tumour suppressor gene				
P53 gene (17p13)	L	L	L	–
Rb gene (13q13)	L	LL	LL	–
PTEN gene (10q24)	–	–	–	LL
Chromosome 9p21 (CDKN2A/Band *p14* gene)	–	L	L	L
Chromosome 22q	L	L	L	–
Chromosome 19q	L	L	L	–
Chromosome 6q	–	L	L	–
Chromosome 17p13.3	–	L	L	?
Amplification/over expression of oncogene/growth factors				
PDGF/PDGFR	G	G	G	–
FGF2	G	GG	GG	–
CNGF	G	G	G	–
Chromosome 12q (CDK4, MDM2, GLI)	–	G	GG	–
EGFR (7p12)	–	–	–	G
IL13R	–	–	G	G
c-myc	G	G	G	G

shows only a few of all the described genetic changes. This enables us to define the several distinct molecular pathways resulting in tumour formation and progression (Table 2). One subset of the GBM shows involvement of TP53 inactivation along with activation of the PDGF system. This occurs in significantly younger adults who are often known to have a history of a previous, lower grade AS. Another subset with *EGFR* gene amplification typically occurs in older patients who do not have a history of preceding lower grade AS. *EGFR* gene amplification-associated GBM might arise either *de novo*, or rapidly from a pre-existing tumour[7]. Loss of the *PTEN* gene is often seen in such tumours. Notably, *EGFR* gene amplification almost never occurs in GBM with TP53 mutation or loss of chromosome 17p (refs 47 and 48). A third subset of GBM arises from oligodendrogliomas and oligoastrocytomas (allelic loss of chromosome 1p and 19q, affecting 40–80%)[27,34,49], and has its own unique genetic antecedents. Anaplastic oligodendrogliomas and oligoastrocytomas additionally show allelic losses of chromosomes 9p and 10q loci, which are probably integral to glioma progression[27].

Genomic instability in gliomas

Genomic instability is reported to be determined by assessing variation in microsatellite repeats in randomly selected loci. Using this limited definition of the phenomenon, genomic instability is not related to glioma behaviour[50]. There is no information about the exact stage in glial tumorigenesis where this phenomenon is critical and important in tumour behaviour. However genomic instability may have many aspects, ranging from cytogenetically observable changes to molecular rearrangements (including amplifications and deletions), increased rate of point mutations, etc. We have used DNA fingerprinting techniques including Southern Blotting with multilocus probes[51] and RAPD analysis[52] to demonstrate and characterize multiple alterations in the glial tumour genome. These locus-nonspecific methods provided true estimate of the extent of genomic changes as opposed to determining changes in oncogenes and TSGs, which affect phenotype and are subject to selection during tumour evolution. We have shown extensive intra-tumour genetic heterogeneity in gliomas, which is more in lower grades[53]. An increased level of intra-tumour genetic heterogeneity, which does not influence the tumour, is the evidence in favour of the mutator hypothesis[54]. The genomic instability of a tumour may be seen in focal areas and become another manifestation of intra-tumour heterogeneity. We have demonstrated that instability of 9p, which has loci for p16, p15 and $p14^{ARF}$, can occur in circumscribed region of pituitary adenoma[55]. This is also true for high and low grades of glioma (manuscript communicated).

Genotype-phenotype co-relation

Efforts have been made to chalk out the molecular pathways involved in glial tumorigenesis, and to define the pathways involved in *de novo*, progressive and recurrent tumours of various stages. However, these schemes are simplistic, even though certain associations have been determined. Clinical trials and follow-up studies using patients whose tumours have undergone a molecular staging in addition to conventional work up are required before genetic data can explain tumour behaviour. This will also throw up a number of drug targets specific for tumour sub-types with defined molecular lesions. This will help to achieve the ultimate goal for cancer therapy, which is maximum efficacy with minimum toxicity.

1. Report, American Cancer Society, Atlanta, 1994.
2. Martuza, R. L., Seizinger, B. R., Jacoby, L. B., Ronaku, G. A. and Gusella, R., *Trends Neurosci.*, 1988, **11**, 22–27.
3. Shapiro, W. R., Shapiro, J. R. and Walker, R. W., in *Clinical Oncology* (eds Abeloff, M. D., Armitage, J. O. and Lichter, A. S.), Churchill Livingstone, NY, 1995, pp. 851–912.
4. El-Azouzi, M. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 7186–7190.
5. Shaw, E. G. *et al.*, *Int. J. Radiat. Oncol. Biol. Phys.*, 1989, **16**, 663–668.
6. Shapiro, W. R., *Semin. Oncol.*, 1986, **13**, 38–45.
7. Louis, D. N. and Gusella, J. F., *Trends Genet.*, 1995, **11**, 412–415.
8. Cho, Y., Gorina, S., Jeffrey, P. D. and Pavletich, N. P., *Science*, 1994, **265**, 346–355.
9. Jain, K. C., Chattopadhyay, P., Sarkar, C., Sinha, S., Mahapatra, A. K., *J. Biosci.*, 1999, **24**, 477–481.
10. Ghosh, M., Dinda, A., Chattopadhyay, P., Sarkar, C., Bhatia, S. and Sinha, S., *Cancer Genet. Cytogenet.*, 1994, **78**, 68–71.
11. Reifenberger, G., Liu, L., Ichimura, K., Schmidt, E. E. and Collins, V. P., *Cancer Res.*, 1993, **53**, 2736–2739.
12. Ichimura, K., Bolin, M. B., Goike, H. M., Schmidt, E. E., Moshref, A. and Collins, V. P., *ibid*, 2000, **60**, 417–424.
13. Chattopadhyay, P., Banerjee, M., Sarkar, C., Mathur, M., Mohapatra, A. K. and Sinha, S., *Oncogene*, 1995, **11**, 2711–2714.
14. Banerjee, M., Dinda, A. K., Sinha, S., Sarkar, C. and Mathur, M., *Int. J. Cancer*, 1996, **65**, 730–733.
15. Holland, E. C., *Nat. Rev. Genet.*, 2001, **2**, 120–129.
16. Takahashi, J. A., Fukumoto, M., Igarashi, K., Oda, Y., Kikuchi, H. and Hatanaka, M., *J. Neurosurg.*, 1992, **76**, 792–798.
17. Debbas, M. and White, E., *Genes Dev.*, 1993, **7**, 546–554.
18. Rubio, M. P., Correa, K. M., Ueki, K., Mohrenweiser, H. W., Gusella, J. F., von Deimling, A. and Louis, D. N., *Cancer Res.*, 1994, **54**, 4760–4763.
19. Ino, Y., Silver, J. S., Blazejewski, L., Nishikawa, R., Matsutani, M., von Deimling, A. and Louis, D. N., *J. Neuropathol. Exp. Neurol.*, 1999, **58**, 881–885.
20. Cheng, Y. *et al.*, *Histopathology*, 2000, **37**, 437–444.
21. Henson, J. W. *et al.*, *Ann. Neurol.*, 1994, **36**, 714–721.
22. Kamb, A. *et al.*, *Science*, 1994, **264**, 436–440.
23. Schmidt, E. E., Ichimura, K., Reifenberger, G. and Collins, V. P., *Cancer Res.*, 1994, **54**, 6321–6324.
24. Schmidt, E. E., Ichimura, K., Messerle, K. R., Goike, H. M. and Collins, V. P., *Br. J. Cancer*, 1997, **75**, 2–8.
25. Ichimura, K., Schmidt, E. E., Goike, H. M. and Collins, V. P., *Oncogene*, 1996, **13**, 1065–1072.
26. Collin, V. P., *Glia*, 1995, **15**, 289–296.
27. Reifenberger, G., Reifenberger, J., Ichimura, K., Meltzer, P. S. and Collins, V. P., *Cancer Res.*, 1994, **54**, 4299–4303.
28. Forus, A. and Myklebost, O., *Genomics*, 1992, **14**, 117–120.
29. Louis, D. N. and Gusella, J. F., *Trends Genet.*, 1995, **11**, 412–415.
30. Smith, J. S. *et al.*, *Genes Chromosomes Cancer*, 2000, **29**, 16–25.
31. Miyakawa, A., Ichimura, K., Schimdt, E. E. and Varmeh-Ziaie, S., *Br. J. Cancer*, 2000, **82**, 543–549.
32. Fueyo, J. *et al.*, *Oncogene*, 1996, **13**, 1615–1619.
33. Park, S. H. *et al.*, *J. Korean Med. Sci.*, 2000, **15**, 555–559.
34. von Deimling, A. *et al.*, *J. Neurosurg.*, 1992, **77**, 295–301.
35. Maehama, T., Taylor, G. S. and Dexon, J. E., *Annu. Rev. Biochem.*, 2001, **70**, 247–279.
36. Steck, P. A. *et al.*, *Nature Genet.*, 1997, **15**, 356–362.
37. Reyes-Mugica, M. *et al.*, *Cancer Res.*, 1997, **57**, 382–386.
38. Sehgal, A., *Semin. Surg. Oncol.*, 1998, **14**, 3–12.
39. Cobbs, C. S., Brenman, J. E., Aldape, K. D., Bredt, D. S. and Israel, M. A., *Cancer Res.*, 1995, **55**, 727–730.
40. Zhu, D., Caveney, S., Kidder, G. M. and Naus, C. C., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 1883–1887.
41. Chattopadhyay, P., Rathore, A., Mathur, M., Sarkar, C., Mahapatra, A. K. and Sinha, S., *Oncogene*, 1997, **15**, 871–874.
42. Sarkar, C., Rathore, A., Chattopadhyay, P., Mahapatra, A. K. and Sinha, S., *Pathology*, 2000, **32**, 84–88.
43. Debinski, W., Gibo, D. M., Slagle, B., Powers, S. K. and Gillespie, G. Y., *Int. J. Oncol.*, 1999, **15**, 481–486.
44. Fuller, G. N. *et al.*, *Cancer Res.*, 1999, **59**, 4228–4232.
45. Rickman, D. S. *et al.*, *ibid*, 2001, **61**, 6885–6891.
46. Sallinen, S. L. *et al.*, *ibid*, 2000, **60**, 6617–6622.
47. Rasheed, B. K., McLendon, R. E., Herndon, J. E., Friedman, H. S., Friedman, A. H., Bigner, D. D. and Bigner, S. H., *Cancer Res.*, 1994, **54**, 1324–1330.
48. von Deimling, A., von Ammon, K., Schoenfield, D., Wiestler, O. D., Seizinger, B. R. and Louis, D. N., *Brain Pathol.*, 1993, **3**, 19–26.
49. Kraus, J. A. *et al.*, *J. Neuropathol. Exp. Neurol.*, 1995, **54**, 91–95.
50. Lundin, D. A., Blank, A., Berger, M. S. and Silber, J. R., *Oncol. Res.*, 1998, **10**, 421–428.
51. Joshi, A. R., Sinha, S., Dil-Afroze, Sulaiman, I. M., Banerji, A. K. and Hasnain, S. E., *Indian J. Biochem. Biophys.*, 1996, **33**, 455–457.
52. Dil-Afroze, Misra, A., Sulaiman, I. M., Sinha, S., Sarkar, C., Mahapatra, A. K. and Hasnain, S. E., *Gene*, 1998, **206**, 45–48.
53. Misra, A., Chattopadhyay, P., Dinda, A. K., Sarkar, C., Mahapatra, A. K., Hasnain, S. E. and Sinha, S., *J. Neurooncol.*, 2000, **48**, 1–12.
54. Loeb, L. A., in *Cancer Surveys, Genetic Instability in Cancer* (eds Tooze, J. *et al.*), Cold Spring Harbor Laboratory Press, 1996, vol. 28, pp. 329–342.
55. Jotwani, G., Misra, A., Chattopadhyay, P., Sarkar, C., Mahapatra, A. K. and Sinha, S., *Cancer Genet. Cytogenet.*, 2001, **125**, 41–45.

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