

Glial Tumorigenesis: Molecular Alterations and Identification of Targets

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Glia are the supporting cells of the brain and much more numerous than neurons, with which they share a common cellular lineage. Glial tumours are the commonest primary brain tumours and the most frequent cause of solid tumours in young adults. The increasing sophistication of modern therapeutic regimens unfortunately has not been reflected in the outcome of the patients suffering from gliomas. Most gliomas arise from astrocytes and this review will restrict itself to astrocytic tumours, namely low-grade diffuse astrocytoma (DA), anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM), that form part of a lineage of progression, with tumours of a lower grade tending to recur as higher grade. Molecular model building studies have basically looked at alterations including overexpression, mutations, loss and rearrangements of known growth factors, oncogenes and tumour suppressor genes corresponding to a defined tumour grade. Many changes associated with the formation of DA, AA and GBM are fairly well characterized. There is also molecular evidence of two types of GBM – primary (de novo) and secondary (arising from lower grades). Mostly, the utilization of this extensive knowledge of tumour molecular biology for therapeutic purposes is still at the experimental stages, though some drugs are in clinical trials with promising results. This review discusses three targets, the Epidermal Growth Factor Receptor (EGFR), Platelet Derived Growth Factor Receptor (PDGFR) and Vascular Endothelial Growth Factor (VEGF) that show promise for intervention in these tumours. It also discusses the role of array technology in the study of glioma. It is expected that this knowledge-based approach will lead to more effective and less toxic management strategies for Glial tumours.

Key Words: Glioma, Oncogene, Tumour Suppressor gene, EGFR, PDGF, Genetics, Genomic instability, Arrays, Molecular targets

List of abbreviations

AA, Anaplastic astrocytoma; AKT, also known as protein kinase B; CDDP, Cis-diamminedichloroplatinum; CDK, Cyclin Dependent Kinase c-kit alias; SCFR, Stem Cell Factor Receptor; CNTF, Ciliary neurotrophic factor; COX, Cyclooxygenase; DA, low-grade diffuse astrocytoma; DCC, Deleted-in-colon carcinoma; EGF, Epidermal growth factor; EGFR, Epidermal growth factor receptor; Erk, Extracellular signal-regulated kinase; FGF, Fibroblast growth factor; FISH, Fluorescent in-situ hybridization; GAP, GTPase activating protein; GBM, Glioblastoma multiforme; GFAP, Glial fibrillary acidic protein; HERV, Human endogenous retrovirus; hMLH1, human mutL homolog; hMSH2, human mutS homolog; IL, interleukin; ILR, interleukin receptor; LOH, Loss of heterozygosity; MAPK, Mitogen activated protein kinase; MGMT, O6-methylguanine-DNA methyltransferase; MMAC, Mutated in multiple advanced cancers; MMR, mismatch repair; MSI, Microsatellite instability; NO, Nitric oxide; NOS, Nitric oxide synthase; OA, Oligoastrocytoma; OG, Oligodendroglioma; PA, Pilocytic astrocytoma; PDGF, Platelet derived growth factor; PDGFR, Platelet derived growth factor receptor; PI3, Phosphatidylinositol 3-kinase; PLC, gamma- Phospholipase C-gamma; PTEN, Phosphatase and tensin homologue; RAPD, Random amplification of polymorphic DNA; RAS, Rat sarcoma viral oncogene homolog; Rb, Retinoblastoma; SH2, Src homology 2; TSG, Tumour suppressor gene; TGF, beta-Transforming growth factor-beta; VEGF, Vascular endothelial growth factor.

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Introduction

Glial cells are the supportive cells of the central nervous system that are considerably more numerous than neurons. Tumours arising from the glial cells are called gliomas. Glial cells comprise primarily astrocytes, oligodendrocytes and ependymal cells. This review is restricted to glial tumours arising from cells of the astrocytic lineage. These are also called astrocytic tumours. Gliomas are the commonest solid tumour in the young adults. According to WHO classification the astrocytic tumours are graded into 4 grades (Kleihues & Cavanee 2000). *Pilocytic astrocytomas* (PA) (WHO grade I) are a group of astrocytomas that are distinguishable from other astrocytomas by their distinctive pathologic appearance and almost invariably of benign behavior. They typically occur in children and young adults and usually located in the cerebellum. These tumours are usually not a part of the progression scheme of the other tumours of astrocytic lineage discussed in the review. This is substantiated by observations that the genetic alterations in pilocytic astrocytomas differ from those of DA (Cheng et al. 2000).

Low-grade Diffuse Astrocytomas (DA) (WHO grade II), in general are commoner in young adults. The cells are relatively more differentiated and have fewer proliferating cells as compared to the higher grades. These tumours are often manageable by surgery, although recurrence and progression to a higher grade is almost a rule. Recurrent tumours are often found to be less differentiated than the primary tumours. In spite of improved management protocols, the mean survival time of DA is about 6 years and hence the tumour is by no means benign. *Anaplastic astrocytomas* (AA) (WHO grade III) and *Glioblastoma multiforme* (GBM) (WHO grade IV) are more malignant and have poor prognosis. AAs show a marked increase in mitosis and have more pleomorphic cells. GBMs have abnormal cells like giant cells, and show increased vascularity and necrosis. In GBM, survival drops considerably as compared to DA to about one year. The review will mainly discuss these three grades of astrocytic tumours (DA, AA or GBM) and the term glioma will refer to these tumours unless stated otherwise. One other tumour type, *oligodendroglioma*, will also be discussed. These

neoplasms arise from oligodendroglia, and usually have a better outcome than DA, though there are higher grade tumours where the prognosis would be worse. Cells from astrocytomas resemble immature astrocytes while those of oligodendrogliomas resemble immature oligodendrocytes. Two alternative explanations are possible regarding the cell of origin of the tumours. The cell type of origin could be the one closest to the phenotype of the final tumour e.g. astrocytes for astrocytoma. However it is also possible that dedifferentiation of mature cells occurs, and gives rise to neoplastic cells (Holland 2001). Oligodendroglia and astrocytes have a common precursor. A group of tumours that has both the astrocytic and oligodendroglial components is called mixed glioma (*oligoastrocytoma*) and malignant mixed (*malignant oligoastrocytoma*) glioma. As the name specifies, one is more malignant than the other. Such tumours can also progress to GBM.

Glial tumorigenesis involves several genes and studies elucidating the molecular pathways involved 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. Several genes encode key regulatory elements in the cell cycle. As will be evident from this review, there are several oncogenes and TSGs some of which are involved in the formation of low-grade DA, others in the transition from DA to AA, and yet others in the formation of oligodendrogliomas and oligoastrocytomas. Others are involved in the final progression to GBM. Finally the whole process is quite dynamic as is evident from the tendency (fortunately not seen in all the cases) of all the tumour types to progress to GBM. It is also important to keep in mind that multiple molecular aberrations may be involved in producing a common histological end point.

In this review the initial sections will focus on the genetic alterations reported for the formation of WHO Grade II, III and IV astrocytomas (DA, AA and GBM) and the models of tumour formation and progression that can be inferred from the same. Subsequent sections will focus on how this knowledge helps in our understanding of the more

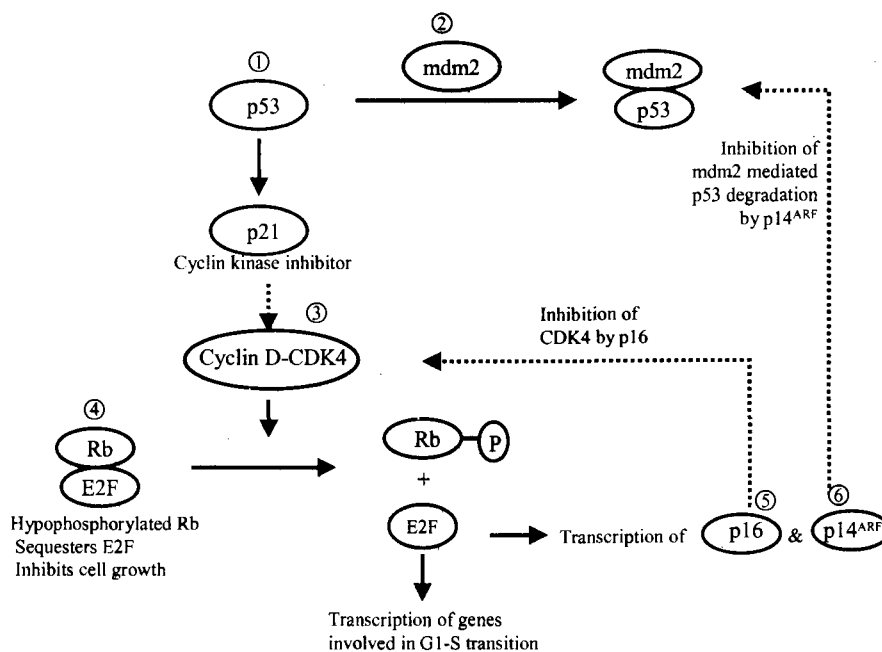


Figure 1 Some key regulatory pathways involving cell cycle regulation affected in glial tumorigenesis.

(↑) Indicates amplification/overexpression, (↓) 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%.
2. mdm2 (↑)- reduces p53 activity in some tumours.
3. p14^{ARF} inhibits mdm2 mediated degradation of p53. When p14^{ARF} is lost (more in AA and GBM), p53 activity is reduced.
4. CyclinD-CDK4 (↑) affected in some GBM. The cyclinD-CDK4 complex phosphorylates Rb leading to E2F release and G1-S transition.
5. Rb mutation/loss or phosphorylation by cyclin D-CDK4 complex decreases the Rb-E2F complex, essential for controlling cell division.
6. 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 cyclinD-CDK4 complex.

— Indicates positive influence.

----- Indicates negative influence.

(Derived from Chosdol K 2002)

complex issues related to tumorigenesis. These include the importance of molecular variation within a histological sub class in determining tumour behaviour, genomic instability in tumours, specific drug design and the role of array technology in elucidating pathways of neoplasia.

Formation of WHO Grade II Diffuse Astrocytomas (DA)

The presence of autocrine and paracrine loops resulting from the overproduction of growth factors and/or their receptors is an important feature of DA. These include PDGF and its receptors (Hermanson et al. 1992), FGF2 and CNTF (Takahashi et al. 1992, Holland 2001). There is an excess of growth factor and its receptor produced in the same cell resulting in autocrine stimulation and increased activity of downstream pathways of these

receptors including those that lead to the RAS and AKT pathways (Holland 2001). PDGF over expression correlates with inactivation of the p53 gene (Debbas & White 1993). It is possible that p53 inactivation prevents the induction of apoptosis resulting from inappropriate and excessive mitogenic stimuli. Activating mutations of oncogenes and amplification of specific regions of DNA carrying oncogenes are not very common in DA. However, we have also demonstrated that 3' rearrangements of the c-myc oncogene in some low-grade gliomas are associated with increased expression of the protein (Chattopadhyay et al. 1995).

The p53 gene on chromosome 17p13.1 encodes tumour suppressor protein p53. Genetic changes leading to the inactivation of p53, usually by the mutation of one allele and the chromosomal loss of the other allele, occurs in approximately one third of

DA, AA and GBMs (Louis & Gusella 1995). p53 can be inactivated by other means as well, The MDM2 gene is a downstream inhibitor of p53 that binds to and degrades the protein. MDM2 amplification sometimes occurs in glioma (Reifenberger et al. 1993, Reifenberger et al. 1994). The p14^{ARF}, a downstream regulator of p53, inhibits the MDM2 mediated degradation of p53 protein (Ichimura et al. 2000). Thus p53 activity may be abrogated by the loss of p14^{ARF} (figure 1). Combining all the known mechanisms for inactivation of p53 activity, Ichimura et al. (2000) conclude that approximately two-thirds of all gliomas have p53 inactivation and the percentage is similar for DA, AA and GBM. These figures are approximately twice of what is observed with p53 mutation and further confirm that while p53 inactivation is very important for tumorigenesis, it is not a major factor in tumour progression. Our laboratory has also established this by molecular typing of primary tumours as well as follow up of the affected patients and the study of recurrent tumours (Ghosh et al. 1994, Jain et al. 1999).

Deletion mapping shows that two regions on chromosome 22q (22q12.3-22q13.1 and 22q13.2) are lost in approximately 20-30% of astrocytomas, suggesting the presence of other astrocytoma related TSGs at this location (Rubio et al. 1994, Ino et al. 1999).

Transition from Diffuse Astrocytomas (DA) to Anaplastic Astrocytomas (AA)

A number of genetic changes have been identified for this transition. These include the inactivation of TSGs on chromosome 13q13, 9p21, 19q and amplification of chromosome 12q, which are rare in low-grade astrocytomas.

The Retinoblastoma gene (Rb) has been well studied as a prototype of its class. The Rb protein in its hypophosphorylated form inhibits cell cycle progression by sequestering E2F and rendering 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. Disruption of Rb function is a feature of higher tumour grades (AA and GBM), where it is significantly more (70%) frequent than in DA (25%). This could occur either by deletion or mutation of the Rb gene or by mutation of its upstream and downstream regulators (Schmidt

et al. 1994, Schmidt et al. 1997). Only part of the inactivation is due to directly demonstrable losses of the chromosomal region 13q13 (observed in approximately one third of the tumours). Such alterations are again more frequent in higher-grade tumours (Henson et al. 1994). The cyclin D2-CDK4 complex phosphorylates most Rb protein during late G1 stage. An upstream Rb regulator protein, p16^{INK4A} (CDKN2A), binds to CDK4 and prevents the formation of active complex with cyclin D protein and prevents Rb phosphorylation (figure 1). Inactivating mutations or deletions of p16^{INK4A} have been observed to be more frequent in AA and GBM (Schmidt et al. 1994, Schmidt et al. 1997). The p15^{INK4B} TSG is also located in the 13q13 region.

Von Deimling et al. (1992) have identified a putative tumour suppressor gene on chromosome 19q that appears to be unique to glial tumours. The region of common deletion has been identified to be as narrow as 150 kb (Smith et al. 2000).

Chromosome 12q has several other genes like MDM2, SAS, GADD, GLI and A2MR, which undergoes amplification during the genesis of late stages of AA and early stages of GBM (Collin 1995, Reifenberger et al. 1994, Forus & Myklebost 1992). Another region in which deletions are seen in AA and GBM is on chromosome 6q (Miyakawa et al. 2000).

There is increasing awareness of the importance of *epigenetic changes* leading to heritable alterations in gene expression during tumorigenesis. Silencing of TSGs in the absence of deletions or mutations can occur due to methylation. It has been shown that hypermethylation of the CpG island of p16/CDKN2 correlates with gene inactivation in gliomas (Fueyo et al. 1996). Cases that are immunonegative for the protein but show no structural changes in the gene are likely to have hypermethylation of the gene or its promoter (Park et al. 2000). Similar mechanisms may be operative in other TSGs as well.

Formation of GBM

Based on molecular changes and histological and clinical observations, two major types of GBM have been postulated (Kleihues & Ohgaki 1999). One is the *de novo* variety, which is characterized by an amplified EGFR gene (Watanabe et al. 1996) (table 1). These are also known as primary gliomas. Such tumours also have a short clinical

Table 1 Genetic alterations in different grades of glioma.

Astrocytoma development and progression with involvement of various tumour suppressor genes and oncogenes. Symbols (-) for loss/mutation of TSG, (+) for amplification/ over-expression of oncogene and (N) for No change. GBM is formed either *de novo* (primary) or progresses from lower grade (secondary) through acquisition of additional mutation. These two pathways have distinct molecular alterations.

I. Deletion/mutation of tumor suppressor gene	Diffuse Astrocytoma	Anaplastic astrocytoma	Glioblastoma multiforme	
			Secondary	Primary
1. P53 gene (17p13)	(-)	(-)	(-)	(N)
2. Rb gene (13q13)	(-)	(-, -)	(-, -)	(N)
3. PTEN gene (10q24)	(N)	(N)	(N)	(-, -)
4. Chromosome 9p21 (CDKN2A/Band p14 gene)	(N)	(-)	(-)	(-)
5. Chromosome 22q	(-)	(-)	(-)	(N)
6. Chromosome 19q	(-)	(-)	(-)	(N)
7. Chromosome 6q	(N)	(-)	(-)	(N)
8. Chromosome 17p13.3	(N)	(-)	(-)	(?)
II. Amplification/overexpression of Oncogene/growth factors				
1. PDGF/PDGFR	(+)	(+)	(+)	(N)
2. FGF2	(+)	(+,+)	(+,+)	(N)
3. CNGF	(+)	(+)	(+)	(N)
4. Chromosome 12q (CDK4, MDM2, GLI)	(N)	(+)	(+,+)	(N)
5. EGFR (7p12)	(N)	(N)	(+)	(+)
6. IL13R	(N)	(N)	(+)	(+)
7. c-myc	(+)	(+)	(+)	(+)

history (less than 3 months). De novo (primary) tumours are supposed to be more malignant (Etienne et al. 1998), though there are some contradictory reports on this score. The other type, which develops due to progression from a lower grade, i.e. from DA, is known as secondary gliomas. These typically have a mutated p53 gene.

von Deimling et al. 1992 first showed that the critical changes unique to the formation of GBM are the inactivation of a TSG on chromosome 10q and amplification of the EGFR gene. The EGFR gene is amplified in approximately 40-70% of all GBMs. Often the EGFR gene is altered giving rise to a truncated product consisting of a constitutively active cytoplasmic domain of the protein. The use of the EGFR gene as a target for therapy has been further described in the section on specific molecular targets.

Sixty to eighty-five percent of GBMs show loss of chromosome 10, with most cases showing allelic loss of the entire chromosome (Louis & Gusella 1995). Loss of chromosome 10 is mostly observed in GBMs

exhibiting EGFR gene amplification (von Deimling et al. 1992). The PTEN TSG has been isolated by the mapping of homozygous deletion on chromosome 10q. The PTEN protein has phosphatase activity (Maehama et al. 2001). It removes one phosphate from PIP3 (phosphatidylinositol triphosphate) and blocks its growth stimulating pathways and allows cell suicide to proceed, keeping cell population in check. Loss of PTEN function 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. PTEN has been shown to be the same as MMAC1, (Steck et al. 1997). Loss of Heterozygosity (LOH) of chromosome 10 was associated with a significantly reduced overall survival in patients with AA and GBM.

Other examples of TSGs in GBM include the Deleted-in-colon carcinoma (DCC) on chromosome 18q21, which is shown to be deleted in 53% of GBM (Reyes-Mugica et al. 1997). Loss of C4-2 expression, identified by Differential Display

PCR, in GBM but not in normal brain tissue studied, indicates that it may function as a potential brain tumour suppressor (Sehgal 1998). Cobbs et al. (1995) detected increased expression of nitric oxide synthase (NOS) in astrocytic tumours with the highest level of expression found in higher grade tumours, and suggested association of nitric oxide (NO) production with the pathophysiological processes important to these tumours. Both endothelial and brain NOS were affected. 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 (Zhu D 1991). Connexin 43 has been shown to be affected in these tumours in these tumours. In addition to EGFR amplification and loss of chromosome 10, two third of GBMs are shown to be associated with Rb related deregulation of cell cycle checkpoint (Ichimura et al. 1996) and many tumours also showed over expression of IL13R (Debinski et al. 1999).

TGF-beta promotes clonal proliferation of hyperploid tumour cells and also causes immunosuppression of the host (Jennings et al. 1997). Platten et al. (2000) have shown the role of TGF-beta in glioma cells migration and abrogation of TGF-beta mediated promotion of tumour cells migration by using echistatin, an anti-alpha(V)beta(3) integrin antibody. Since TGF-beta has dual role as growth regulatory and immunomodulatory properties, treatment strategies may be developed against it.

VEGF expression, leading to angiogenesis is also an important part of gliomagenesis. This has been discussed in some detail in the section on specific molecular targets in glioma.

Molecular Variation within a Histological Sub-class and Relation to Tumour Behaviour

Tumour classification is based on histology and the local grade of a tumour is attributed to cell type, differentiation, nuclear morphology, degree of mitosis, vascularity, presence of necrotic tissue etc. The purpose of tumour classification was to provide a universally acceptable index, which would correlate with tumour behavior, response to therapy, propensity for recurrence and overall prognosis. By and large, histological classification has held its ground very well, and its

indispensability is borne out by its central role in management and choice of therapeutic regimens. However, as studies become more refined there is increasing data on the atypical behaviour of tumours with reference to grade, e.g., in the case astrocytic tumours, DA with a significantly shorter and GBM with significantly longer survival time than average. There are reports that different brain tumours e.g. GBM (Mohapatra et al. 1998), AAs (Kunwar et al. 2001), ependymomas (Hirose et al. 2001) etc could be sub-classified based on genetic alterations. Studies correlating these genetic changes to prognosis and therapeutic response are ongoing, and not yet fully conclusive. However there are some studies linking molecular changes with phenotypic alterations associated with tumour behaviour.

In our laboratory (Chattopadhyay et al. 1997), using the techniques of microsatellite mapping, we identified a tumour suppressor locus, telomeric to p53 (at 17p 13.3), which was altered in about 40% of high-grade astrocytomas, but not in DA. At the genetic level, alterations in this locus were independent of the p53. However these were associated with p53 over expression much more significantly than alteration of the p53 gene itself (Sarkar et al. 2000). The high-grade tumours having 17p13.3 alterations had a significantly higher proliferation index than those without (Sarkar et al. in press). Similarly there are suggestions that de novo GBMs (with amplified EGFR gene, no p53 mutations and short clinical history) have a worse outcome than those GBM progressing from a lower grade (with p53 mutations and no EGFR amplification). However the results are not conclusive. In the past few years an attempt has been made to use micro array technology to further sub-classify tumours to get a clearer picture of the processes involved, as is discussed in a subsequent section.

Oligodendrogliomas

Oligodendrogliomas (OG) are the most chemosensitive of all human tumours of glial origin (Perry et al 1999). Several genes are altered in OGs e.g., ERCC6, PTEN, DMBT1 (Sasaki et al. 2001), COX (Deininger et al. 2000), VEGF (Varlet et al. 2000), are being targeted for treatment. The DNA repair enzyme O6-methylguanine-DNA methyltransferase

(MGMT) inhibits tumour cell killing by alkylating agents. MGMT activity is controlled by promoter methylation which silences the gene and make tumour cells sensitive to alkylating agents. The MGMT promoter was found to be methylated in OGs (Esteller et al. 2000, Dong et al 2001). PCV (Procarbazine, Cyclohexylchloroethylnitrosurea (Lomustine), Vincristine) is the chemotherapy of choice for OGs (Cairncross et al. 1998). The molecular predictor for better response to PCV is loss of 1p and 19q in OG (Cairncross et al 1998, Bauman et al. 2000, Ino et al. 2001, Burger et al. 2001). OG with loss of 1p had a 100% success rate with PCV treatment (Ino et al. 2001) in a larger series of homozygous patients. The use of effective chemotherapy for OGs is a promising advance. However this has not helped beyond prediction of prognosis since OG patients are treated with PCV irrespective of their 1p, 19q status. The gene(s) on 1p and 19q are still elusive (Smith et al. 2000, Pohl et al. 1999) but this has underscored the need of looking for such marker in other sub types of gliomas.

There is also a class of tumours with mixed oligodendroglial and astrocytic components the oligoastrocytomas (OAs). There is a difference between the two components of a mixed glioma regarding the expression of different oncoproteins e.g. the c-myc protein which is a function of their proliferation and differentiation status (Banerjee et al. 1996). Some OGs display necrosis and pseudopalisading, which are also features of GBM (Perry 2001). Should OAs be tested for 1p deletion and if 1p is deleted in an OA, should that patient be treated with chemotherapy? This is done in several centers. Many Astrocytomas also show both 1p and 19q loss or 1p alone. An interesting study on only 7 patients with histologically confirmed AA or mixed anaplastic tumours demonstrated a dramatic response to chemotherapeutic treatment and prolonged survival was reported based on 1p LOH (Ino et al. 2000). However loss of 1p does not distinguish all astrocytomas with potential for better response to therapy and longer survival (Cairncross et al. 1998, Ino et al. 2001).

Genomic Instability in Gliomas

Genomic instability is known to be a feature of malignant change from time Boveri thought that alterations in the genetic material are a hallmark of

cancer. As our techniques for studying genomic instability become more refined, various distinct manifestations of genomic instability have been studied. These include cytogenetically observable entities like aneuploidy, hypoploidy and translocations, more specifically defined aberrations as shown by FISH. At the molecular level, these would include loss of heterozygosity, hemi and nullizygosity, DNA amplifications, deletions and rearrangements, microsatellite instability and an increased frequency of point mutations. Most of the examples of molecular model building as classically exemplified by the model of colon cancer, as proposed by Vogelstein and his co-workers (Kinzler & Vogelstein 1996), show a step by step increase in alterations in genes which significantly affect phenotype (e.g., oncogenes and tumour suppressor genes) with increased grade of tumour. On the other hand the mutator hypothesis (Loeb & Christians 1996) proposes that inability to maintain genetic integrity is one of the first molecular defects in a cell, which subsequently leads to alterations in the oncogenes and tumour suppressor genes. A corollary of this is that low-grade tumours would have a high rate of genomic change and show a high degree of intra-tumour genetic heterogeneity, especially if one assays for these phenomena by methods, which do not select for genetic changes, which influence phenotype. In fact such alterations would be more in low-grade tumours because of the chances that a high-grade tumour would be a product of a single 'well adapted' rapidly growing clone for which hypermutability would in fact be a disadvantage.

The shifting phenotype of the cancer cell which leads to the rapid development of therapeutic resistance is a consequence of this increased mutation rate. It is therefore important to tackle the increased mutability of tumours in addition to designing newer approaches for specific molecular targets. It is still too early to see the beginnings of a rational therapeutic strategy based on the high mutation rate. However if hypermutability is a primary defect of cancer cells, it needs to be addressed if one is to tackle the development of therapeutic resistance that could be a problem even with the newly developed specific molecular therapeutics. It is of importance to see whether the

mutator hypothesis actually manifests during tumorigenesis. In our laboratory we have attempted to determine whether the corollaries of the mutator hypothesis can be demonstrated to be valid in astrocytic tumours. In order to do this we have used DNA fingerprinting techniques to identify genomic changes without being selective for the loci, which have an influence on the tumour phenotype (Chosdol et al. 2002). A comparative RAPD profile of tumour and normal DNA was demonstrated to be very effective in identifying and characterizing changes in tumour DNA (Dil-Afroze et al. 1998). We could also demonstrate increased intra-tumour genetic heterogeneity in Grade II DA as compared to Grade IV GBM (Misra et al. 2000). Some of the altered sequences have been shown to be recombinogenic in nature e.g., sequences derived from Human Endogenous Retrovirus-K (HERV-K), (Misra et al. 2001). More studies are required on this aspect of genomic instability if the root cause of the altered phenotype is to be tackled.

The most studied manifestation of genomic instability in tumours is microsatellite instability (MSI), which manifests as a marked increase of expansion and contraction of microsatellite repeats. This is usually associated with defects in the mismatch repair (MMR) genes, hMSH2 and hMLH1. Germ line defects in these genes are a hallmark of cancers like Hereditary Non Polyposis Colon Cancer (Fishel et al., Leach et al. 1993). However the defect may be acquired during tumorigenesis as several studies have shown the association of MSI with higher grade in several tumour types. However this association has not been well established in gliomas (Lundin et al. 1998, Alonso et al. 2001 and Dams et al. 1995) and no certain correlation has been established with tumour progression or grade in these tumours. Also MMR defects are not found in the majority of the tumours studied (Aubry et al. 2001, Castrilli et al. 2002, Lim et al. 1996 and Shin & Park 2000). A study on gliomas by Gomori et al. (2002), revealed that MSI was associated with primary tumours but not with either tumour progression or recurrence. Unpublished results from our lab suggest that no loss of MMR expression occurs in gliomas and moreover, the hMSH2 levels seem to increase in high-grade, GBM, as compared to low-grade, DA.

Genomic instability has been associated with alterations in methylation. Gradual decline in methylation over a period of time could lead to a state where some full length transposable elements (LINE or SINE repeats) would lose their CpG methylation to a critical level and become active (Hata et al. 1997), This leads to genomic instability due to massive transposition and recombination events, mimicking irreparable but non-lethal DNA damage due to genomic instability in non-essential regions. Such events have been observed in *Drosophila* (Woodruff et al. 1995) and *C. elegans* (Egilmez et al. 1994).

Array Technology in Elucidating Molecular Pathways in Gliomas

Southern blotting and Northern blotting techniques have dominated molecular biology for the past two decades. With time, array technology provided a faster and more sensitive technique for performing the equivalent of hundreds of thousands of Southern/Northern blots at a time from a very small quantity of sample and in a limited time.

Essentially there are 3 major types of arrays, *DNA array* (Snijder et al. 2001, Hodgson et al. 2001), *expression (cDNA or oligo) arrays* (Golub et al. 1999, Perou et al. 2000, Watson et al. 2001) and *tissue array* (Sallinen et al. 2000). There are also other arrays e.g. small molecule array (Kuruvilla et al. 2002), protein array (Hanash et al. 2002) for specialized use of array technology.

The use of a DNA array with 58 known candidate oncogenes to test 7 primary GBMs and 7 GBM cell lines (Hui et al. 2001) showed that apart from detection of frequency of those oncogene amplification in the samples, they could identify a single amplified oncogene in a region where several candidate oncogene reside. 12q13-21 has candidate oncogenes WNT1, GL1, CDK4/SAS, MDM2. Hui et al. (2001) could identify amplification of CDK4/SAS and MDM2 in a sample that has normal WNT1 and GL1. This is not possible by a single hybridization without using arrays.

Huang et al. (2000) identified 7 over-expressed and 11 under-expressed (50% > and < than the controls) genes in low-grade diffuse astrocytoma cases with an 1176 cancer associated gene array (commercially available from Clontech). They

validated their data with semiquantitative RT-PCR and immunostaining.

In a very small study 4 primary GBM and their recurrent tumours after radiotherapy were compared for gene expression profile by Joki et al (2001). They concluded that paracrine growth factors expression is diminished as a result of reduction of vascular density after radiation treatment. In contrast, growth factors that are part of an autocrine loop, showed enhanced expression.

Sallinen et al. (2000) used cDNA array and tissue array to study WHO grade II, III and IV astrocytomas and observed alteration of expression of more number of genes in grade IV than in the lower grade astrocytomas. IGFBP2 was most distinct progression related over expressed gene in their study. They validated their data with immunostaining on a tissue array. Previously IGFBP was reported to be consistently over-expressed in GBM but not in other gliomas (AA, OA & OG). This study identified IGFBP2 overexpression by cDNA array and confirmed the array result by Northern and Western blotting (Fuller et al. 1999).

Ljubimova et al. 2001, used a 11,004 gene array (Incyte Genomics, St. Louis, MO) and identified 2 groups of upregulated genes in GBM (a) growth factor related genes and (b) extracellular matrix related genes. They also identified a host of genes that are down regulated in GBM, up or down regulated in AA only. They also noted that despite

normal histological appearance, gene expression profile of tissue immediately adjacent to a GBM was like that of its respective primary GBM. EGFR and Laminin, blood vessels basement membrane proteins were consistently over-expressed in both high and low-grade gliomas. They confirmed their data by semi-quantitative RT-PCR and/or immunocytochemistry. Two different Laminins, isoforms 8 and 9 had differential spatial expression in glioma. The former was present in blood vessels of GBM and normal tissue adjacent to it while the later was mainly in the blood vessels of low-grade gliomas. The former group had a shorter mean time (4.3 months) of tumour recurrence than the later group.

The power of better classification came from the increased number of genes that could be profiled with later version of arrays. Affymetrix first came out with a ~12K gene chip that has been used in many major studies in the field. Rickman et al. (2001) used an Affymetrix ~12K gene chip to study 19 grade I, 5 grade II and 21 GBM and reported that expression profile of a set of 360 genes could provide a signature profile (by cluster analysis) that can distinguish the low and high-grade tumours. As expected, these genes include previously known as well as unknown genes. Some of the genes showing marked changes (more than 5 fold) in expression are depicted in table 2. The same chip was used to study 7 oligodendrogliomas of 2 different Grades

Table 2 *Microarray analysis of altered gene expression in GBM as compared to normal brain (NB).*

This table has been compiled from data published by Rickman et al 2001 and Sallimen et al 200⁰. The expression of a number of genes has been shown to be altered, however only those showing marked changes (< > 5 times normal) are depicted. (Derived from Chosdol K et al. 2002).

Upregulation (> 5 fold INCREASE) in GBM versus Normal brain:

- Transforming growth factor β induced gene (TGFB1)
- Filamin A, α (actin-binding protein-280) (FLNA)
- Midkine (MDK)
- Thymidylate synthetase (TYMS)
- Plasminogen activator inhibitor-1 (PAI-1)
- Fibronectin
- Leukemia inhibitory factor (LIF)
- Integrin α 3
- Insulin like growth factor binding protein 2 (IGFBP2)
- Hexabrachion (tenascin C, cytotactin) (HXB)
- Cyclin-dependent kinase 4 (CDK4)
- Oncogene Tls/Chop, fusion-activated (TLS/CHOP)
- Vascular endothelial growth factor (VEGF)
- Activator 1 40 kD subunit (RFC40)
- Insulin like growth factor binding protein 3 (IGFBP3)
- Caveolin-1

Downregulation (> 5 fold DECREASE) in GBM versus Normal brain:

- Chondroitin sulfate proteoglycan 2 (versican) (CSPG2)
- Thrombospondin 4 (THBS4)
- P53-induced gene-10 (PIG10)
- T-lymphoma invasion and metastasis inducing (TIAM1)
- Tissue inhibitor of metalloproteinase 4 (TIMP 4)
- Cyclin-dependent kinase 4 inhibitor D (p19INK4d)
- Receptor tyrosine kinase (SKY)
- Neuroendocrine Drosophila discs large (NE-dlg)

(Watson et al. 2001) and expression profile of 196 genes together could identify the WHO oligodendroglioma classification accurately.

There are no major studies in glioma so far to predict a tumour group or subgroup based on its expression profile but several other tumour types have been used for this purpose (Lakhani & Ashworth 2001, Ramaswamy & Golub 2002). The idea is to ask the program to classify them by hierarchical clustering (Perou et al. 2000) or 'train' the analysis program (Mills 2002, Shipp et al. 2002). The analysis can be done by studying the basic pattern of gene expression of grades or subgroups of tumours, based on a large number of samples and then asking the program to predict the group or subgroup of an unknown tumour. Different possible prognostic marker or therapeutic targets have been identified using this approach in a variety of tumours e.g., PDGFRA and RAS proteins in medulloblastoma (MacDonald et al. 2001), paediatric embryonal brain tumours (Pomeroy et al. 2002), leukemias (Golub et al. 1999, Armstrong et al. 2001) lymphoma (Alizadeh et al. 2000, Shipp et al. 2002), prostate cancer (Welsh et al. 2001, Dhanasekharan et al. 2001), breast cancer (Perou et al. 2000, Sorli et al. 2001, Lakhani et al. 2001, Ahr et al. 2001, van't Veer et al. 2002), lung adenocarcinoma (Garber et al. 2001, Beer et al. 2002) and soft tissue tumours (Nielsen et al. 2002).

Microarrays can reveal a downstream target gene in a pathway (Zuber et al. 2000, Roberts et al. 2000, Mayanil et al. 2001, Lossos et al. 2002). It also scans genome wide promoter methylation pattern (Suzuki et al. 2002) and even wide spread aneuploidy (Hughes et al. 2000).

The correlation of the results of DNA and RNA arrays is a major gap. Integration is currently done based on sequence data, which keeps on changing with assembly and reassembly of human genome sequence every once in a while. Microarrays of open reading frames (Penn et al. 2000) can solve the problem. Proteomics (Hanash 2001, Hanash et al. 2002) or protein degradomics (Lopez & Overall 2002) is another recent trend to identify potential targets for therapy in cancer. Involvement of protein convertases e.g., furin and PACE4 in tumour progression (Bassi et al. 2000) came from proteomics. However to the best of our knowledge

there is not much published literature on the proteomics of gliomas. While at this stage it is difficult to make a definitive statement on the contribution of array technology to our understanding of gliomas, it is hoped that in addition to identifying novel pathways and combinations of molecular alterations leading to gliomagenesis, such studies would lead to a comprehensive molecular classification of gliomas.

Specific Molecular Targets in Glioma

Of the interesting leads for pharmacological intervention that have come up from our knowledge of molecular alterations in gliomas, maximum progress has been made for the EGFR and PDGFR mediated pathways. These are discussed in this section.

EGFR

As discussed earlier, the single most observed phenomenon of genetic aberration specific to grade IV astrocytoma (GBM), is amplification of EGFR gene. The EGFR gene is amplified in 40-76% of gliomas, and is indicative of de novo (primary) GBM type (Watanabe et al. 1996, Rainov et al. 1997). Some studies show that it is associated with poor prognosis (Schlegel et al. 1994, Etienne et al. 1998). EGFR amplification at DNA level is always correlated with over expression of EGFR mRNA and EGFR protein (Wikstrand et al. 1998). In most cases the gene gets rearranged during amplification creating variant EGFRs in tumour (Wong et al. 1992, Wikstrand et al. 1998), but not in normal tissues including brain. The commonest of such variant is a 801bp deletion of coding sequence generating a mutant, named as del2-7 or deltaEGFR or viiIEGFR mutant by different groups (Schwechheimer et al. 1995, Wikstrand et al. 1998, Rasheed et al. 1999). This is detected in up to 60% of GBMs and 20% AAs (Wikstrand et al. 1998).

Normally, when EGF binds to EGFR, its intracellular C terminal phosphotyrosine residues are phosphorylated (Ramirez et al. 1995, Yarden, 2001). This provides sites for interaction with Src homology 2 (SH2) domain containing adaptor molecules e.g., Shc and Grb2 (Kavanaugh et al. 1995, Belsches et al. 1997). They recruit and activate Ras. When Ras is activated at an increased level, it initiates a cascade of mitogenic signalling pathways

including mitogen-activated-protein-kinase (MAPK) and thereby helping cell proliferation (Marshall 1995).

Since EGFR overexpression is associated with the tumour cell proliferation, invasion and poor prognosis. Therapies targeted against EGFR have been shown to be promising by impairing tumour cell proliferation and apoptosis. There are two major class of EGFR inhibitors, one is anti-EGFR antibodies like IMC-225 (cetuximab), a chimeric human-mouse monoclonal IgG1 antibody and the other class is EGFR selective tyrosine kinase inhibitors like OSI-774 (tarceva) and ZD1839 (Iressa).

Anderson et al. (2001) have investigated the effect of ZD1839 (Iressa) in human cancer cells like MDA-MB-231 (Breast), A431 (epidermoid) and SKOV3 (ovarian) and as xenograft in athymic mice and found that it inhibited proliferation in an in-vitro study and growth of implant in in-vivo study. Bianco et al. (2002) have tested antiproliferative and proapoptotic activity of ZD1839 in combination with ionizing radiation in human colon (GEO), ovarian (OVCAR-3), non-small cell lung (A549 & Calu-6) and breast (MCF-7ADR) cancer cell lines and obtained cooperative antiproliferative and proapoptotic effect.

Heimberger et al. (2002) in their study showed that ZD1839 is active against the brain tumour model expressing EGFR but not against the tumour with EGFR vIII. So, tumours coexpressing EGFR vIII need special consideration.

Many studies are going on using anti-EGFR molecules on different tumours including gliomas. Currently anti-EGFR antibody IMC-225 is under Phase II and Phase III clinical evaluation, EGFR-selective tyrosine kinase inhibitors OSI-774 under Phase II and ZD1839 under Phase III clinical evaluation in cancer patients alone or in combination with conventional therapies, like radiotherapy and chemotherapy. And are listed at NCI Clinical trial Database http://cancer.gov/search/clinical_trials/.

The vIII mutant of the EGFR has no extracellular domain, so it cannot bind to the natural ligand EGF (figure 2). However it is observed to be constitutively phosphorylated at a C-terminal tyr residue (Nishikawa et al. 1994, Huang et al. 1997). To make thing worse, receptor down regulation through internalization by wild type EGFR is reduced in case of vIII mutants (Huang et al. 1997, Chu et al. 1997, Sorkin 2001). This suggests that vIII mutant keeps on

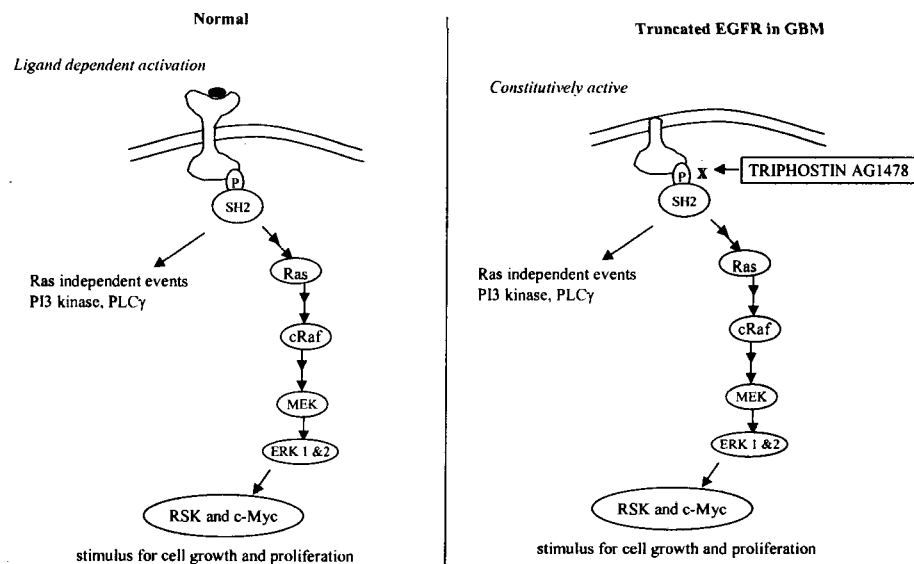


Figure 2 Signal transduction in the normal and mutated EGF receptor.

Normally, intracellular C terminal phosphotyrosine residues of EGFR are phosphorylated only when EGF binds to the extracellular domain. This provides sites for interaction with Src homology 2 (SH2) domain containing adaptor molecules. They recruit and activate Ras that initiates a cascade of mitogenic signalling pathways and thereby helping cell proliferation. The vIII mutant has no extracellular domain and it is observed to be constitutively phosphorylated at a C-terminal tyrosine without extracellular signals leading to uncontrolled cell proliferation.

transducing signal at a lower albeit constitutive level, akin to a dripping faucet. Immuno precipitation studies have co-localized vIII mutants with phosphorylated Src and Grb2 in cell lines (Chu et al. 1997, Prigent et al. 1996, Tang et al. 2000), leading to this notion. What is interesting for vIII mutants is that subsequent activation of Ras and MAPK has been reported to be unique to glioma cells and not in other cells expressing this mutant or wild type EGFR (Chu et al. 1997, Moscatello et al. 1996). This suggested that constitutive phosphorylation mediated signal transduction could be a legitimate and specific target for therapeutic intervention in a substantial number of glioma patients especially in the most aggressive form, the GBM.

Surprisingly, in the first generation experiments, the constitutive expression of vIII mutants in glioma cell line did not make the cells grow faster in culture (Nishikawa et al. 1994). However, these same cells developed into a rapidly growing tumour in immuno-compromised mice, even with a smaller number of cell inoculums as compared to the parental line. When grown intra cerebrally, the mice died earlier due to the rapid growth of the tumour (Nishikawa et al. 1994, Nagane et al. 1996). When the C-terminal tyr residues of vIII mutant were mutated, the increased tumour growth rate was reduced (Huang et al. 1997) indicating that constitutive phosphorylation of the C-terminal tyr residues cause the tumours to grow faster. The proliferation index (Ki67 and BrdU) was higher in these cells but the apoptotic cell death (by TUNEL assay) was significantly reduced. This inhibition was also correlated with higher expression of anti-apoptotic protein Bcl-X_L (Nagane et al. 1996). Many drugs (e.g., cis-diamino-dichloroplatinum (CDDP), taxol, vincristine etc.) exert their anti tumour effect by increasing apoptotic rate by increased activation of caspase-3, an apoptotic mediating protein. It appears that the vIII mutant, by virtue of over expression of the anti apoptotic Bcl-X_L protein, makes these tumours resistant to such drugs, (Nagane et al. 1998). When vIII mutant containing cells were treated with tyrosine kinase inhibitor tryphostin AG1478, that reduced the level

of Bcl-X_L and CDDP that induced caspase 3 activation, they were much more effective together than either of them alone (Nagane et al. 1998, Nagane et al. 2001). This also indicated that disruption of constitutively active tyrosine kinase activity in vIII mutant containing tumours might render them more susceptible to other traditional chemotherapeutic drugs (Cavane 2002).

PDGFR

Over expression of PDGF and/or PDGFR is thought to generate an autocrine loop, thus promoting tumorigenesis (Doolittle et al. 1983, Waterfield et al. 1983). A number of studies have shown that several types of tumours including glioma co-expressed PDGF and PDGF receptors (Westermarck & Heldin 1991, Westermarck 1993). PDGF can be either A, B, C or D isoform (Li et al. 2000, Lokker et al. 2002), where as PDGF receptors form only AA, AB and BB dimers (Lokker et al. 2002). The receptors are activated by ligand induced dimerization that brings the catalytic domains into juxtaposition. Little is known about PDGF-C and PDGF-D so far. While all three combinations of receptors can be induced by PDGF-B, PDGF-A can induce only PDGFR-AA dimer (Westermarck 1995).

The protein-tyr kinase family can be divided into subgroups that have similar structural organization and sequence homology within the kinase domain. The members of type III group of receptor tyr kinase include PDGFR-A & PDGFR-B, CSF-1R, Flt3 and c-kit (Heldin 1995). The receptor dimerization leads to receptor phosphorylation in *trans*. The C terminal tyr molecules of PDGFRs, after autophosphorylation, form attachment sites for the SH2 domain of a number of signal transduction molecules e.g., Src (Mori et al. 1993, Twamley et al. 1993, Twamley-stein et al. 1993), Shb (Claesson-Welsh, 1994), Shc (Yokote et al. 1994, Rozakis-Adcock et al 1992), Grb2 (Arvidsson et al. 1994), PI3 kinase (Panayatou et al. 1992, Rodriguez-Viciano et al. 1994, Soltoff et al. 1992), Nck (Nishimura et al. 1993), GTPase activating protein of Ras (ras-GAP) (Sato et al. 1993, Kashishian et al. 1992), Phosphotyrosine phosphatase-1D (PTP-1D) (Lechleider et al. 1993, Bennett et al. 1994, Li et al. 1994) and PLC-gamma

(Ronnstrand et al. 1992, Westermarck et al. 1995). These proteins trigger several arms of the PDGF signal transduction pathways that are involved in different cellular responses. For examples, Grb2/SOS activates the GTPase Ras, resulting in the activation of a cascade of MAP kinases, such as Erk1 and Erk2, and this contributes to the proliferation arm of the PDGF signalling pathway (Lowenstein et al. 1992, Schlessinger, 1993). PI3 kinase activates yet another kinase, AKT, which is responsible for the survival or the anti-apoptotic arm of PDGF signalling pathways (Heldin et al. 1998). PDGF also stimulates the proliferation and migration of endothelial cells, leading to new blood vessels formation (Forsberg et al. 1993, Battegay et al. 1994, Thommen et al. 1997, Kumar et al. 1998). This action is mediated by stimulating endothelial cells to produce VEGF, a potent angiogenic inducer (Kumar et al. 1998, Wang et al. 1999).

PDGF ligand A and B genes are expressed in almost all glioma cell lines though the most abundant isoform in majority of GBM cell lines is PDGF-A that can induce only PDGFR-AA formation. PDGFR-AA is also over expressed in almost all glioma cell lines (Flemming et al. 1992, Guha et al. 1995, Hermanson et al. 1992). This over expression can be an early event in gliomagenesis since it has been observed in low-grade primary astrocytoma (Guha et al. 1995). Interestingly, a subset of glioma cell lines express PDGF-A receptor (PDGFRA) only and are GFAP (GFAP is an intermediate filament component of the astrocytic cytoskeleton, which is observed in both the perinuclear region and processes of astrocytes, that is to say, GFAP is expressed in cytoplasm.) positive (Bongcam-Rudloff et al. 1991). PDGFR-A is amplified at DNA level in 1-16% of primary GBMs (Fleming et al. 1992, Smith et al. 2000), ~10% of oligodendrogliomas (Smith et al. 2000) and its over expression may (Flemming et al. 1992, Guha et al. 1995) or may not (Hermanson et al. 1992) correlate to the DNA copy number for the gene in GBM. Since it is expressed in a very small number of primary GBMs, somehow it lost its priority to other more frequent amplification event in GBM, namely EGFR. The only report available in literature

(Ribom et al. 2002) suggests that the patients with PDGFR-A amplifications do better than the ones without it. However over-expression even in absence of gene amplification would make it appear to be a promising target.

Two recent findings brought interest in PDGFR-A back into focus: (a) Like vIII mutant of EGFR, PDGFR-A gene amplification causes gene rearrangement leading to formation of a extracellular domain truncated PDGFR-A product which can be constitutively active (Kumabe et al. 1992); and (b) identification of specific PDGFR tyr-kinase inhibitors (Buchdunger et al. 1996, Druker et al. 1996, Bruchdunger et al. 2000), mainly from the CML studies. Gleevec, formerly CGP57148, or imatinib mesylate, or STI-571 specifically inhibits this tyr-kinase, typically within the first three weeks of therapy (Druker et al. 2001). STI-571 also specifically inhibits PDGFR tyr kinase activity both *in vitro* and *in vivo* (Buchdunger et al. 1996, Druker et al. 1996, Bruchdunger et al. 2000) and c-kit activity *in vivo* (Heinrich et al. 2000, Bruchdunger et al. 2000). STI-571 has been shown to inhibit the growth of GBM cells overexpressing PDGFR, injected into the brains of nude mice (Kilic et al. 2000) by induction of apoptosis (Sjoblom et al. 2001). Phase I and phase II clinical trials are on (NCI Clinical trial Database - http://cancer.gov/search/clinical_trials/).

VEGF/VEGF-R

Vascular endothelial growth factor (VEGF) promotes blood vessel formation in early development (vasculogenesis) and has a central role in the growth of new blood vessels (angiogenesis). Blood vessels formation is essential not only for differentiation and development during embryogenesis but also for the wound healing and reproductive function in the adult (Folkman 1995). VEGF family has several members like VEGF-A, VEGF-B, VEGF-C and VEGF-D. VEGF-A is a heparin binding glycoprotein and have four different homodimeric isoform formed by alternative splicing of mRNA. These are VEGF121, VEGF165, VEGF189, VEGF206 (Park et al. 1993).

There are three VEGF receptors like VEGF-R1 (Flt-1), VEGF-R2 (Flk-1) and VEGF-R3 (Flt-4). They have multiple IgG-like extracellular domain and tyrosine kinase activity (Shibuya et al 1999). Millauer et al (1996) have demonstrated the importance of VEGF-R2 (Flk-1) in the tumour angiogenesis and growth in wide range of solid tumours including mammary, ovarian, lung carcinoma as well as GBM by using dominant negative VEGF- receptor. Increased VEGF expression is seen in all GBM (20) and anaplastic oligodendroglioma (3) cases studied (Chan et al 1998). Ubiquitous expression of VEGF-D, a new mammalian member of VEGF family, is found in GBM sections as well as in GBM cell lines as compared to normal brain sections using immunofluorescence (Debinski et al 2001). Likewise, Hirano et al. (2001) in their immunohistochemistry study on GBM sections showed diffuse expression of VEGF. Feldkamp et al (1999) have demonstrated the role of Ras pathway in activation of VEGF secretion in astrocytoma cell lines.

Yao et al. (2001) examined the expression of VEGF and VEGF-receptors Flt-1 immunohistochemically in 50 astrocytic tumours and found that low-grade astrocytoma and glioblastoma multiforme with positive VEGF and Flk-1 expressions had a significantly shorter mean overall survival time than those with negative expressions. Additionally, overexpression of VEGF and Flk-1 were shown to be associated with earlier recurrence in patients with low-grade astrocytomas.

Strategies that interfere with VEGF & VEGF-receptors function have great potential for drug development to block angiogenesis and tumour growth. Anti-VEGF monoclonal antibodies like bevacizumab and VEGF receptor inhibitor like SU5416 are under Phase I and Phase II clinical trial. (NCI Clinical trial Database http://cancer.gov/search/clinical_trials/).

Conclusions

The outlook for glioma patients has not changed substantially despite the modern therapeutic modalities, and the median survival time of GBM is still about a year after diagnosis. However the

new knowledge regarding molecular alterations in gliomas will perhaps change the way we look at these tumours. Knowledge of how genomic instability influences tumours will have important implications for our understanding of drug resistance and metastasis, and perhaps lead to pharmacological measures to deal with the same. Array technology helps in two ways; first by the identification of novel altered targets that could improve diagnosis, prognosis and therapy. However, it is determining the alterations of patterns of genetic changes and gene expression that array technology really scores over 'conventional' molecular biology. The implications of this approach to diagnostics and staging are going to be apparent as the clinical associations of array studies become more and more definitive. The identification of small molecules as well as possibly antibodies to target specific molecular alterations in gliomas like EGFR, PDGFR, VEGF etc. and also the various signalling pathways already show promise and further research will surely lead towards the universal goal of all ligand specific therapies i.e., increased effectiveness and specificity, low side effects and reduced development of drug resistance. Increasing use of combinatorial chemistry approaches should lead to an exponential rise in the number of molecules capable of intervening in the target pathways identified. Advances in structural biology and in our understanding of molecular interactions will further aid in this effort. Genetic markers would be used to identify patients most likely to benefit from therapy. We already have a marker for effective conventional chemotherapy in oligodendrogliomas, though we have yet to understand the basis for the same. Hopefully, the combination of these approaches with surgery and/or radiotherapy would lead to synergistic improvements in the management of gliomas.

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