Proc. Indian natn Sci Acad. B69 No.1 pp 49-72 (2003)

Glial Tumorigenesis: Molecular Alterations and Identification of Targets

ANJAN MISRA[1], KUNZANG CHOSDOL[2], TAPASYA SRIVASTAVA[2], PARTHAPRASAD CHATTOPADHYAY[2], A K MAHAPATRA[3], CHITRA SARKAR[4] and SUBRATA SINHA[2*]

[1]*Cancer Center University of California San Francisco, USA, Departments of* [2]*Biochemistry,* [3]*Neurosurgery,* [4]*Pathology, All India Institute of Medical Sciences, New Delhi 110029*

(Received on 16 August 2002; Accepted after revision on 9 December 2002)

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-diamminonedichloroplatinum; *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*, Flourescent 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.

* Corresponding Author: *Email:* sub_sinha@hotmail.com, sub_sinha2000@hotmail.com, *Fax:* 011-6862663, *Tel:* 011-6593314

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

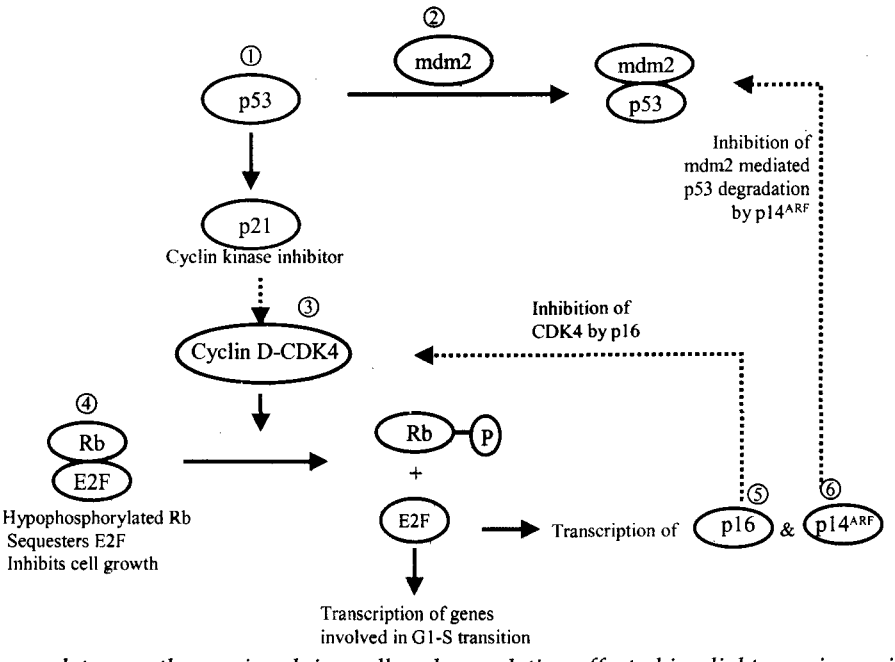

Figure 1 *Some key regulatory pathways involving cell cycle regulation affected in glial tumorigenesis.*
(↑) Indicates amplification/overexpression, (↓) Indicates deletion/mutation.

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 chromosome18q21, 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 immunosupression 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. If 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 200^. 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 (TGFBI)
- 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

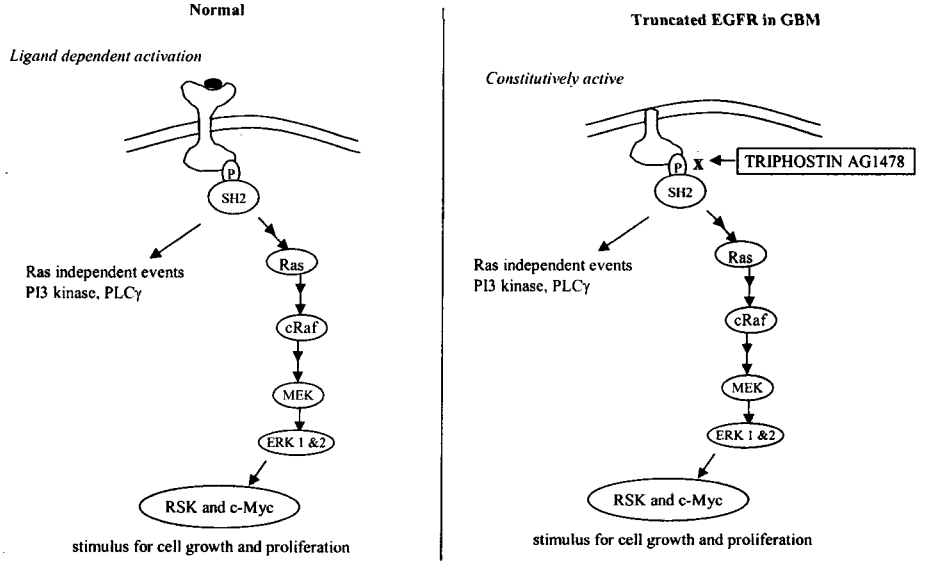

Figure 2 Signal transduction in the normal and mutated EGF receptor.
Normally, intracellular C terminal phosphotyrosine residues of EGFR are phosphorylated only when EGF binds to the extracellular domain. This provides sites for interaction with Src homology 2 (SH2) domain containing adaptor molecules. 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 (Cavanee 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 (Westermark & Heldin 1991, Westermark 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 (Westermark 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-Viciana et al. 1994, Soltoff et al. 1992), Nck (Nishimura et al. 1993), GTPase activating protein of Ras (ras-GAP) (Satoh 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, Westermark 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 to normal brain sections using immunoflourescence (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.

Acknowledgement

The authors wish to acknowledge the DST (Swarnjyanti fellowship to SS), CSIR and ICMR for financial support for their research quoted in this review.

References

Ahr A, Holtrich U, Solbach C, Scharl A, Strebhardt K, Karn T and Kaufmann M 2001 Molecular classification of breast cancer patients by gene expression profiling; *J. Pathol.* 195 312-320

Alizadeh A A, Eisen M B, Davis R E, Ma C, Lossos I S, Rosenwald A, Boldrick J C, Sabet H, Tran T, Yu X, Powell J I, Yang L, Marti G E, Moore T, Hudson J Jr, Lu L, Lewis D B, Tibshirani R, Sherlock G, Chan W C, Greiner T C, Weisenburger D D, Armitage J O, Warnke R and Staudt L M 2000 Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling; *Nature* **403** 503-511

Alonso M, Hamelin R, Kim M, Porwancher K, Sung T, Parhar P, Miller D C, Newcomb E W, 2001 Microsatellite instability occurs in distinct subtypes of pediatric but not adult central nervous system tumours; *Cancer Res.* **61** 2124-2128

Anderson N G, Ahmad T, Chan K, Dobson R, Bundred N J 2001 ZD1839 (Iressa), a novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, potently inhibits the growth of EGFR-positive cancer cell lines with or without erbB2 overexpression; *Int. J. Cancer* **94** 774-782

Armstrong S A, Staunton J E, Silverman L B, Pieters R, den Boer M L, Minden M D, Sallan S E, Lander E S, Golub T R and Korsmeyer S J 2002 MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia; *Nat. Genet.* 30 41-47

Arvidsson A K, Rupp E, Nanberg E, Downward J, Ronnstrand L, Wennstrom S, Schlessinger J, Heldin C H, Claesson-Welsh L 1994. Tyr-716 in the platelet-derived growth factor beta-receptor kinase insert is involved in GRB2 binding and Ras activation; *Mol. Cell Biol.* **14** 6715-6726

Aubry M C, Halling K C, Myers J L, Tazelaar H D, Yang P and Thibodeau S N 2001 DNA mismatch repair genes hMLH1, hMSH2, and hMSH6 are not inactivated in bronchioloalveolar carcinomas of the lung; *Cancer* **92** 2898-2901

Banerjee M, Dinda A K, Sinha S, Sarkar C and Mathur M 1996 c-myc oncogene expression and cell proliferation in mixed oligo-astrocytoma; *Int. J. Cancer* **65** 730-733

Bassi D E, Mahloogi H and Klein-Szanto A J 2000 The proprotein convertases furin and PACE4 play a significant role in tumour progression; *Mol. Carcinog.* **28** 63-69

Battegay E J, Rupp J, Iruela-Arispe L, Sage E H and Pech M 1994 PDGF-BB modulates endothelial proliferation and angiogenesis in vitro via PDGF beta-receptors; *J. Cell Biol.* 125 917-928

Bauman G S, Ino Y, Ueki K, Zlatescu M C, Fisher B J, Macdonald D R, Stitt L, Louis D N, and Cairncross J G 2000 Allelic loss of chromosome 1p and radiotherapy plus chemotherapy in patients with oligodendrogliomas; *Int. J. Radiat. Oncol. Biol. Phys* **48** 825-830

Beer D G, Kardia S L, Huang C C, Giordano T J, Levin A M, Misek D E, Lin L, Chen G, Gharib T G, Thomas D G, Lizyness M L, Kuick R, Hayasaka S, Taylor J M, Iannettoni M D, Orringer M B and Hanash S 2002 Gene-expression profiles predict survival of patients with lung adenocarcinoma; Nat. Med. 8 816-824

Belsches A P, Haskell M D and Parsons S J 1997 Role of c-Src tyrosine kinase in EGF-induced mitogenesis. *Front Biosci.* **2** 501-518

Bennett A M, Tang T L, Sugimoto S, Walsh C T and Neel B G 1994 Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor beta to Ras; *Proc. Natl. Acad. Sci. U S A*, **91** 7335-7339

Bianco C, Tortora G, Bianco R, Caputo R, Veneziani B M, Caputo R, Damiano V, Troiani T, Fontanini G, Raben D, Pepe S, Bianco A R and Ciardiello F 2002 Enhancement of antitumor activity of ionizing radiation by combined treatment with the selective epidermal growth factor receptor-tyrosine kinase inhibitor ZD1839 (Iressa); Clin. Cancer Res. 8 3250-3258

Bongcam-Rudloff E, Nister M, Betsholtz C, Wang J L, Stenman G, Huebner K, Croce C M, and Westermark B 1991 Human glial fibrillary acidic protein: complementary DNA cloning, chromosome localization, and messenger RNA expression in human glioma cell lines of various phenotypes; *Cancer Res.* 51 1553-1560

Buchdunger E, Cioffi C L, Law N, Stover D, Ohno-Jones S, Druker B J and Lydon N B, 2000 Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors; *J Pharmacol. Exp. Ther*, 295 139-145

_____, Zimmermann J, Mett H, Meyer T, Muller M, Druker B J and Lydon N B, 1996 Inhibition of the Abl protein-tyrosine kinase *in vitro* and *in vivo* by a 2-phenylaminopyrimidine derivative; *Cancer Res.* **56** 100-104

Burger P C, Minn A Y, Smith J S, Borell T J, Jedlicka A E, Huntley B K, Goldthwaite P T, Jenkins R B and Feuerstein B G 2001 Losses of chromosomal arms 1p and 19q in the diagnosis of oligodendroglioma. A study of paraffin-embedded sections; *Mod. Pathol*, 14 842-853

Cairncross J G, Ueki K, Zlatescu M C, Lisle D K, Finkelstein D M, Hammond R R, Silver J S, Stark P C, Macdonald D R, Ino Y, Ramsay D A, Louis D N 1998 Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas; *J Natl Cancer Inst.* **90** 1473-1479

Castrilli G, Fabiano A, La Torre G, Marigo L, Piantelli C, Perfetti G, Ranelletti F O and Piantelli M 2002 Expression of hMSH2 and hMLH1 proteins of the human DNA mismatch repair system in salivary gland tumours; *J. Oral. Pathol. Med.* 31 234-238

Cavenee W K 2002 Genetics and new approaches to cancer therapy; *Carcinogenesis* 23 683-686

Chan A S, Leung S Y, Wong M P, Yuen S T, Cheung N, Fan Y W and Chung L P 1998 Expression of vascular endothelial growth factor and its receptors in the anaplastic progression of astrocytoma, oligodendroglioma, and ependymoma; *Am. J. Surg. Pathol.* 22 816-826

Chattopadhyay P, Banerjee M, Sarkar C, Mathur M, Mohapatra A K and Sinha S 1995 Infrequent alteration of the c-myc gene in human glial tumours associated with increased numbers of c-myc positive cells; *Oncogene* 11 2711-2714

_____, Rathore A, Mathur M, Sarkar C, Mahapatra A K and Sinha S 1997 Loss of heterozygosity of a locus on 17p13.3, independent of p53, is associated with higher grades of astrocytic tumours; *Oncogene* 15 871-874

Cheng Y, Pang J C, Ng H K, Ding M, Zhang S F, Zheng J, Liu D G and Poon W S 2000 Pilocytic astrocytomas do not show most of the genetic changes commonly seen in diffuse astrocytomas; *Histopathology* 37 437-444

Chosdol K, Chattopadhyay P and Sinha S, 2002. Molecular pathways of glial tumorigenesis; *Curr. Sci.* 82 648-654

Chu C T, Everiss K D, Wikstrand C J, Batra S K, Kung H J and Bigner D D 1997 Receptor dimerization is not a factor in the signalling activity of a transforming variant epidermal growth factor receptor (EGFRvIII); *Biochem J.* 324 855-861

Claesson-Welsh L, 1994. Platelet-derived growth factor receptor signals. *J Biol Chem.*, **269**, 32023-6.

Cobbs CS, Brenman JE, Aldape K D, Bredt D S and Israel M A 1995 Expression of nitric oxide synthase in human central nervous system tumours; *Cancer Res.* 55 727-730

Collins V P 1995 Gene amplification in human gliomas; *Glia* 15 289-296

Dams E, Van de Klelft E J, Martin J J, Verlooy J and Willems P J 1995 Instability of microsatellites in human gliomas; *Cancer Res.* 55 1547-1549

Debbas M and White E 1993 Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B; *Genes Dev.* 7 546-554

Debinski W, Gibo D M, Slagle B, Powers S K and Gillespie G Y 1999 Receptor for interleukin 13 is abundantly and specifically over-expressed in patients with glioblastoma multiforme; *Int. J. Oncol.* 15 481-486

_____, Slagle-Webb B, Achen M G, Stacker S A, Tulchinsky E, Gillespie G Y and Gibo D M 2001 VEGF-D is an X-linked/AP-1 regulated putative onco-angiogen in human glioblastoma multiforme; *Mol. Med.* 7 598-608

Deininger M H, Meyermann R, Trautmann K, Morgalla M, Duffner F, Grote E H, Wickboldt J, Schluesener H J 2000 Cyclooxygenase (COX)-1 expressing macrophages/microglial cells and COX-2 expressing astrocytes accumulate during oligodendroglioma progression; *Brain Res.* 885 111-116

Dhanasekaran S M, Barrette T R, Ghosh D, Shah R, Varambally S, Kurachi K, Pienta K J, Rubin M A and Chinnaiyan A M 2001 Delineation of prognostic biomarkers in prostate cancer; *Nature* 412 822-826

Dil-Afroze, Misra A, Sulaiman I M, Sinha S, Sarkar C, Mahapatra AK and Hasnain S E 1998 Genetic alterations in brain tumours identified by RAPD analysis; *Gene* 206 45-48

Dong S M, Pang J C, Poon W S, Hu J, To K F, Chang A R, Ng H K 2001 Concurrent hypermethylation of multiple genes is associated with grade of oligodendroglial tumors; *J Neuropathol. Exp. Neurol.* 60 808-816

Doolittle R F, Hunkapiller M W, Hood L E, Devare S G, Robbins K C, Aaronson S A and Antoniades H N 1983 Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor; *Science* 221 275-277

Druker B J, Talpaz M, Resta D J, Peng B, Buchdunger E, Ford J M, Lydon N B, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers C L 2001 Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med.*, 344, 1031-1037

_____, Tamura S, Buchdunger E, Ohno S, Segal G M, Fanning S, Zimmermann J and Lydon N B 1996 Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells; *Nat. Med.* 2 561-566

Egilmez N K and Shmookler-Reis R J 1994 Age-dependent somatic excision of transposable element Tc1 in Caenorhabditis elegans; *Mutat. Res.* 316 17-24

Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin S B and Herman J G 2000 Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents; *N. Engl. J. Med.* 343 1350-1354

Etienne M C, Formento J L, Lebrun-Frenay C, Gioanni J, Chatel M, Paquis P, Bernard C, Courdi A, Bensadoun R J, Pignol J P, Francoual M, Grellier P, Frenay M, Milano G 1998 Epidermal growth factor receptor and labeling index are independent prognostic factors in glial tumour outcome; *Clin. Cancer Res.* 4 2383-2390

Feldkamp M M, Lau N, Rak J, Kerbel R S and Guha A 1999 Normoxic and hypoxic regulation of vascular endothelial growth factor (VEGF) by astrocytoma cells is mediated by Ras; *Int. J. Cancer* **81** 118-124

Fishel R, Lescoe M K, Rao M R S, Copeland N G, Jenkins N A, Garber J, Kane M and Kolodner R 1993 The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer; *Cell* 75 1027-1038

Fleming T P, Saxena A, Clark W C, Robertson J T, Oldfield E H, Aaronson S A, Ali I U, 1992 Amplification and/or overexpression of platelet-derived growth factor receptors and epidermal growth factor receptor in human glial tumours; *Cancer Res.* **52** 4550-4553

Folkman J. 1995. Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenesis; *N. Engl. J. Med.* 333 1757-1763

Forsberg K, Valyi-Nagy I, Heldin CH, Herlyn M and Westermark B 1993 Platelet-derived growth factor (PDGF) in oncogenesis: development of a vascular connective tissue stroma in xenotransplanted human melanoma producing PDGF-BB; *Proc. Natl. Acad. Sci. U S A*, **90** 393-397

Forus A and Myklebost O 1992 A physical map of a 1.3-Mb region on the long arm of chromosome 12, spanning the GLI and LRP loci; *Genomics* **14** 117-120

Fueyo J, Gomez-Manzano C, Bruner J M, Saito Y, Zhang B, Zhang W, Levin V A, Yung W K and Kyritsis A P 1996 Hypermethylation of the CpG island of p16/CDKN2 correlates with gene inactivation in gliomas; *Oncogene* 13 1615-1619

Fuller G N, Rhee C H, Hess K R, Caskey L S, Wang R, Bruner J M, Yung W K, Zhang W, 1999 Reactivation of insulin-like growth factor binding protein 2 expression in glioblastoma multiforme: a revelation by parallel gene expression profiling; *Cancer Res.* **59** 4228-4232

Garber M E, Troyanskaya O G, Schluens K, Petersen S, Thaesler Z, Pacyna-Gengelbach M, van de Rijn M, Rosen G D, Perou C M, Whyte R I, Altman R B, Brown P O, Botstein D, Petersen I 2001 Diversity of gene expression in adenocarcinoma of the lung; *Proc Natl Acad Sci U S A* 98 13784-13789

Ghosh M, Dinda A, Chattopadhyay P, Sarkar C, Bhatia S and Sinha S 1994 Rearranged p53 gene with loss of normal allele in a low-grade nonrecurrent glioma. *Cancer Genetics Cytogenet.* **78** 68-71

Golub T R, Slonim D K, Tamayo P, Huard C, Gaasenbeek M, Mesirov J P, Coller H, Loh M L, Downing J R, Caligiuri M A, Bloomfield C D and Lander E S 1999 Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 286 531-537

Gomori E, Fulop Z, Meszaros I, Doczi T, Matolcsy A, 2002. Microsatellite analysis of primary and recurrent glial tumours suggests different modalities of clonal evolution of tumour cells; *J. Neuropathol. Exp. Neurol.* **61** 396-402

Guha A, Dashner K, Black P M, Wagner J A and Stiles C D 1995 Expression of PDGF and PDGF receptors in human astrocytoma operation specimens supports the existence of an autocrine loop; *Int. J. Cancer* **60** 168-173

Hanash S M, Bobek M P, Rickman D S, Williams T, Rouillard J M, Kuick R, Puravs E, 2002 Integrating cancer genomics and proteomics in the post-genome era; *Proteomics* 2 69-75

_____ 2001 Global profiling of gene expression in cancer using genomics and proteomics; *Curr. Opin. Mol. Ther.* 3 538-545

Hata K and Sakaki Y 1997 Identification of critical CpG sties for repression of L1 transcription by DNA methylation; *Gene* **189** 227-234

Heimberger A B, Learn C A, Archer G E, McLendon R E, Chewning T A, Tuck F L, Pracyk J B, Friedman A H, Friedman H S, Bigner D D and Sampson J H. 2002 Brain Tumors in Mice Are Susceptible to Blockade of Epidermal Growth Factor Receptor (EGFR) with the Oral, Specific, EGFR-Tyrosine Kinase Inhibitor ZD1839 (Iressa); *Clin Cancer Res.* 8 3496-3502

Heinrich M C, Griffith D J, Druker B J, Wait C L, Ott K A and Zigler A J 2000 Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor; *Blood* **96** 925-932

Heldin C H, Ostman A and Ronnstrand L 1998 Signal transduction via platelet-derived growth factor receptors; *Biochim. Biophys. Acta.* **1378** F79-113

_____ 1995 Dimerization of cell surface receptors in signal transduction; *Cell* 80 213-223

Henson J W, Schnitker B L, Correa K M, von Deimling A, Fassbender F, Xu H J, Benedict W F, Yandell D W and Louis D N 1994 The retinoblastoma gene is involved in malignant progression of astrocytomas; *Ann. Neurol.* 36 714-721

Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin C H, Westermark B, Nister M 1992 Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops; *Cancer Res.* 52 3213-3219

Hirano H, Tanioka K, Yokoyama S, Akiyama S and Kuratsu J 2001 Angiogenic effect of thymidine phosphorylase on macrophages in glioblastoma multiforme; *J. Neurosurg.* 95 89-95

Hirose Y, Aldape K, Bollen A, James C D, Brat D, Lamborn K, Berger M, Feuerstein B G, 2001 Chromosomal abnormalities subdivide ependymal tumours into clinically relevant groups; *Am. J Pathol.* 158 1137-1143

Hodgson G, Hager J H, Volik S, Hariono S, Wernick M, Moore D, Nowak N, Albertson D G, Pinkel D, Collins C, Hanahan D and Gray J W 2001 Genome scanning with array CGH delineates regional alterations in mouse islet carcinomas; *Nat. Genet.* **29** 459-464

Holland E C 2001 Gliomagenesis: genetic alterations and mouse models; *Nat. Rev. Genet.* **2** 120-129

Huang H, Colella S, Kurrer M, Yonekawa Y, Kleihues P and Ohgaki H 2000 Gene expression profiling of low-grade diffuse astrocytomas by cDNA arrays; *Cancer Res.* **60** 6868-6874

Huang H S, Nagane M, Klingbeil C K, Lin H, Nishikawa R, Ji X D, Huang C M, Gill G N, Wiley H S, Cavenee W K 1997 The enhanced tumourigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling; *J. Biol. Chem.* **272** 2927-2935

Hughes T R, Roberts C J, Dai H, Jones A R, Meyer M R, Slade D, Burchard J, Dow S, Ward T R, Kidd M J, Friend S H and Marton M J 2000 Widespread aneuploidy revealed by DNA microarray expression profiling; *Nat. Genet.* **25** 333-337

Hui A B, Lo K W, Yin X L, Poon W S and Ng H K 2001 Detection of multiple gene amplifications in glioblastoma multiforme using array-based comparative genomic hybridization; *Lab. Invest.* **81** 717-723

Ichimura K, Bolin M B, Goike H M, Schmidt E E, Moshref A and Collins V P 2000 Deregulation of the p14ARF/MDM2/p53 pathway is a prerequisite for human astrocytic gliomas with G1-S transition control gene abnormalities; *Cancer Res.* **60** 417-424

_____, Schmidt E E, Goike H M and Collins V P 1996 Human glioblastomas with no alterations of the CDKN2A (p16INK4A, MTS1) and CDK4 genes have frequent mutations of the retinoblastoma gene; *Oncogene* **13** 1065-1072

Ino Y, Betensky R A, Zlatescu M C, Sasaki H, Macdonald D R, Stemmer-Rachamimov A O, Ramsay D A, Cairncross J G and Louis D N 2001 Molecular subtypes of anaplastic oligodendroglioma: implications for patient management at diagnosis; *Clin. Cancer Res.* **7** 839-845

_____, Silver J S, Blazejewski L, Nishikawa R, Matsutani M, von Deimling A and Louis D N 1999 Common regions of deletion on chromosome 22q12.3-q13.1 and 22q13.2 in human astrocytomas appear related to malignancy grade; *J. Neuropathol. Exp. Neurol.* **58** 881-885

_____, Zlatescu M C, Sasaki H, Macdonald D R, Stemmer-Rachamimov A O, Jhung S, Ramsay D A, von Deimling A, Louis D N and Cairncross J G 2000 Long survival and therapeutic responses in patients with histologically disparate high-grade gliomas demonstrating chromosome 1p loss; *J. Neurosurg.* **92** 983-990

Jain K C, Chattopadhyay P, Sarkar C, Sinha S and Mahapatra A K 1999 A pilot study of recurrence of human glial tumours in light of p53heterozygosity status; *J. Biosciences* **24** 477-481

Jennings M T, Hart C E, Commers P A, Whitlock J A, Martincic D, Maciunas R J, Moots P L and Shehab T M 1997 Transforming growth factor beta as a potential tumor progression factor among hyperdiploid glioblastoma cultures: evidence for the role of platelet derived growth factor; *J Neuro-Oncol.* **31** 233-254

Joki T, Carroll R S, Dunn I F, Zhang J, Abe T and Black P M 2001 Assessment of alterations in gene expression in recurrent malignant glioma after radiotherapy using complementary deoxyribonucleic acid microarrays; *Neurosurgery* **48** 195-201

Kashishian A, Kazlauskas A and Cooper J A 1992 Phosphorylation sites in the PDGF receptor with different specificities for binding GAP and PI3 kinase *in vivo*; *EMBO J.* **11** 1373-1382

Kavanaugh W M, Turck C W and Williams L T 1995 PTB domain binding to signaling proteins through a sequence motif containing phosphotyrosine; *Science* **268** 1177-1179

Kilic T, Alberta J A, Zdunek P R, Acar M, Iannarelli P, O'Reilly T, Buchdunger E, Black P M, and Stiles C D 2000 Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenylaminopyrimidine class; *Cancer Res.* **60** 5143-5150

Kinzler K W and Vogelstein B 1996 Lessons from hereditary colorectal cancer; *Cell* **87** 159-170

Kleihues P and Cavanee W K, *World Health Organization Classification of Tumours: Pathology and Genetics: Tumours of the Nervous System*, Oxford University Press, 2nd edition, page 9-54

_____ and Ohgaki H 1999 Primary and secondary glioblastomas: from concept to clinical diagnosis; *Neuro-oncol.* **1** 44-51

Kumabe T, Sohma Y, Kayama T, Yoshimoto T and Yamamoto T 1992 Amplification of alpha-platelet-derived growth factor receptor gene lacking an exon coding for a portion of the extracellular region in a primary brain tumour of glial origin; *Oncogene* **7** 627-633

Kumar R, Yoneda J, Bucana C D and Fidler I J 1998 Regulation of distinct steps of angiogenesis by different angiogenic molecules; *Int. J Oncol.* **12** 749-757

Kunwar S, Mohapatra G, Bollen A, Lamborn K R, Prados M and Feuerstein B G 2001 Genetic subgroups of anaplastic astrocytomas correlate with patient age and survival; *Cancer Res.* **61** 7683-7688

Kuruvilla F G, Shamji A F, Sternson S M, Hergenrother P J and Schreiber S L 2002 Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays; *Nature* **416** 653-657

Lakhani S R and Ashworth A 2001 Microarray and histopathological analysis of tumours: the future and the past? *Nat. Rev. Cancer* **1** 151-157

Lakhani S R, O'Hare M J and Ashworth A 2001 Profiling familial breast cancer; *Nat. Med.* **7** 408-410

Leach F S, Nicolades N C, Papadulous N, Liu B, Jen J, Parsons R, Peltomaki P, Sistonen P, Aaltonen L A, Nystrom-Lahti M, Guan X Y, Jhang J, Meltzer P S, Yu J, Kao F, Chen D J, Cerosaletti, Keith Fournier R E, Todd S, Lewis T, Leach R J, Naylor S L, Weissenbach J, Mecklin J P, Jarvinen H, Peterson G M, Hamilton S R, Greene J, Jass J, Watson P, Lynch H T, Trent J N, de la Chapelle A, Kinzler K W and Vogelstein B 1993 Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer; *Cell* **75** 1215-1225

Lechleider R J, Sugimoto S, Bennett A M, Kashishian A S, Cooper J A, Shoelson S E, Walsh C T and Neel B G 1993 Activation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet-derived growth factor receptor; *J. Biol. Chem.* **268** 21478-21481

Li W, Yu J C, Michieli P, Beeler J F, Ellmore N, Heidaran M A and Pierce J H 1994 Stimulation of the platelet-derived growth factor beta receptor signaling pathway activates protein kinase C-delta; *Mol. Cell Biol.* **14** 6727-6735

Li X, Ponten A, Aase K, Karlsson L, Abramsson A, Uutela M, Backstrom G, Hellstrom M, Bostrom H, Li H, Soriano P, Betsholtz C, Heldin CH, Alitalo K, Ostman A and Eriksson U 2000 PDGF-C is a new protease-activated ligand for the PDGF alpha-receptor. *Nat. Cell Biol.* **2** 302-309

Lim P C, Tester D, Cliby W, Ziesmer S C, Roche P C, Hartmann L, Thibodeau S N, Podratz K C and Jenkins R B 1996 Absence of mutations in DNA mismatch repair genes in sporadic endometrial tumours with microsatellite instability; *Clin. Cancer Res.* **2** 1907-1911

Ljubimova J Y, Lakhter A J, Loksh A, Yong W H, Riedinger M S, Miner J H, Sorokin L M, Ljubimov A V and Black K L 2001 Overexpression of alpha4 chain-containing laminins in human glial tumours identified by gene microarray analysis; *Cancer Res.* **61** 5601-5610

Loeb L A and Christians F C 1996 Multiple mutations in human cancers; *Mutat. Res.* **350** 279-286

Lokker N A, Sullivan C M, Hollenbach S J, Israel M A and Giese N A 2002 Platelet-derived Growth Factor (PDGF) Autocrine Signaling Regulates Survival and Mitogenic Pathways in Glioblastoma Cells: Evidence That the Novel PDGF-C and PDGF-D Ligands May Play a Role in the Development of Brain Tumours; *Cancer Res.* **62** 3729-3735

Lopez-Otin C and Overall C M 2002 Protease degradomics: A new challenge for proteomics; *Nat. Rev. Mol. Cell Biol.* **3** 509-519

Lossos I S, Alizadeh A A, Diehn M, Warnke R, Thorstenson Y, Oefner P J, Brown P O, Botstein D and Levy R 2002 Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes; *Proc. Natl. Acad. Sci. USA* **99** 8886-8891

Louis D N and Gusella J F 1995 A tiger behind many doors: multiple genetic pathways to malignant glioma; *Trends Genet.* **11** 412-415

Lowenstein E J, Daly R J, Batzer A G, Li W, Margolis B, Lammers R, Ullrich A, Skolnik EY, Bar-Sagi D and Schlessinger J 1992 The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling; *Cell* **70** 431-442

Lundin D A, Blank A, Berger M S and Silber J R 1998 Microsatellite instability is infrequent in sporadic adult gliomas; *Oncol Res.* **10** 421-428

MacDonald T J, Brown K M, LaFleur B, Peterson K, Lawlor C, Chen Y, Packer R J, Cogen P and Stephan D A 2001 Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease; *Nat. Genet.* **29** 143-152

Maehama T, Taylor G S and Dexon J E 2001 PTEN and myotubularin: novel phosphoinositide phosphatases; *Annu. Rev. Biochem,* **70** 247-279

Marshall C J 1995 Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation; *Cell* **80** 179-185

Mayanil C S, George D, Freilich L, Miljan E J, Mania-Farnell B, McLone D G, Bremer E G, 2001 Microarray analysis detects novel Pax3 downstream target genes. *J. Biol. Chem.,* **276** 49299-49309

Millauer B, Longhi M P, Plate K H, Shawver L K, Risau W, Ullrich A and Strawn L M 1996 Dominant-negative inhibition of Flk-1 suppresses the growth of many tumor types *in vivo*; Cancer Res. **56** 1615-1620

Mills AP Jr. 2002 Gene expression profiling diagnosis through DNA molecular computation; *Trends Biotechnol.* **20** 137-140

Misra A, Chattopadhyay P, Dinda A K, Sarkar C, Mahapatra A K, Hasnain S E, Sinha S, 2000 Extensive intra-tumour heterogeneity in primary human glial tumours as a result of locus non-specific genomic alterations; *J. Neurooncol.* **48** 1-12

_____, Chosdol K, Sarkar C, Mahapatra A K and Sinha S 2001 Alteration of a sequence with homology to human endogenous retrovirus (HERV-K) in primary human glioma: implications for viral repeat mediated rearrangement; *Mutat Res.* **484** 53-59

Miyakawa A, Ichimura K, Schimdt E E and Varmeh-Ziaie S 2000 Multiple deleted regions on the long arm of chromosome 6 in astrocytic tumours; *British J. Cancer* **82** 543-549

Mohapatra G, Bollen A W, Kim D H, Lamborn K, Moore D H, Prados M D, Feuerstein B G, 1998 Genetic analysis of glioblastoma multiforme provides evidence for subgroups within the grade; *Genes Chromosomes Cancer* **21** 195-206

Mori S, Ronnstrand L, Yokote K, Engstrom A, Courtneidge S A, Claesson-Welsh L, Heldin C H 1993 Identification of two juxtamembrane autophosphorylation sites in the PDGF beta-receptor; involvement in the interaction with Src family tyrosine kinases; *EMBO J.* **12** 2257-2264

Moscatello D K, Montgomery R B, Sundareshan P, McDanel H, Wong M Y, Wong A J, 1996 Transformational and altered signal transduction by a naturally occurring mutant EGF receptor; *Oncogene* **13** 85-96

Nagane M, Coufal F, Lin H, Bogler O, Cavenee W K and Huang H J 1996 A common mutant epidermal growth factor receptor confers enhanced tumourigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis; *Cancer Res.* **56** 5079-5086

_____, Levitzki A, Cavenee W K and Huang H J S 2001 Sensitization of human glioblastoma xenografts overexpressing a tumour specific epidermal growth receptor to cisplatin by the A1478 tyrosine kinase inhibitor; *J. Neurosurg.* **95** 472-479

_____, Levitzki A, Gazit A, Cavenee W K and Huang H J 1998 Drug resistance of human glioblastoma cells conferred by a tumour-specific mutant epidermal growth factor receptor through modulation of Bcl-XL and caspase-3-like proteases; *Proc. Natl. Acad. Sci. USA* **95** 5724-5729

Nielsen T O, West R B, Linn S C, Alter O, Knowling M A, O'Connell J X, Zhu S, Fero M, Sherlock G, Pollack J R, Brown P O, Botstein D, van de Rijn M. 2002 Molecular characterisation of soft tissue tumours: a gene expression study; *Lancet* **359** 1301-1307

Nishikawa R, Ji X D, Harmon R C, Lazar C S, Gill G N, Cavenee W K and Huang H J 1994 A mutant epidermal growth factor receptor common in human glioma confers enhanced tumourigenicity; *Proc. Natl. Acad. Sci. U S A* **91** 7727-7731

_____, Li W, Kashishian A, Mondino A, Zhou M, Cooper J and Schlessinger J 1993 Two signaling molecules share a phosphotyrosine- containing binding site in the platelet-derived growth factor receptor; *Mol. Cell Biol.* **13** 6889-6896

Panayotou G, Fry M J and Waterfield M D 1992 Multi-enzyme complexes associated with receptors during signal transduction; *J C R Acad. Sci. III.* **314**(9 Suppl) 11-20

Park J E, Keller G A and Ferrara N. 1993 The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF; *Mol. Biol. Cell* **4** 1317-1326

Park S H, Jung K C, Ro J Y, Kang G H and Khang S K 2000 5' CpG island methylation of p16 is associated with absence of p16 expression in glioblastomas; *J. Korean Med. Sci.*.**15** 555-559

Penn S G, Rank D R, Hanzel D K and Barker D L, 2000 Mining the human genome using microarrays of open reading frames; *Nat. Genet.* **26** 315-318

Perou C M, Sorlie T, Eisen M B, van de Rijn M, Jeffrey S S, Rees C A, Pollack J R, Ross D T, Johnsen H, Akslen L A, Fluge O, Pergamenschikov A, Williams C, Zhu S X, Lonning P E, Borresen-Dale A L, Brown P O and Botstein D 2000 Molecular portraits of human breast tumours; *Nature* **406** 747-752

Perry J R, Louis D N and Cairncross J G 1999. Current treatment of oligodendrogliomas. *Arch. Neurol.* **56** 434-436

Perry J R 2001 Oligodendrogliomas: clinical and genetic correlations; *Curr. Opin. Neurol.* **14** 705-710

Platten M, Wick W, Wild-Bode C, Aulwurm S, Dichgans J, Weller M. 2000 Transforming growth factors beta(1) (TGF-beta(1)) and TGF-beta(2) promote glioma cell migration via Up-regulation of alpha(V)beta(3) integrin expression; *Biochem Biophys Res Commun.* **268** 607-611

Pohl U, Cairncross J G and Louis D N 1999 Homozygous deletions of the CDKN2C/p18INK4C gene on the short arm of chromosome 1 in anaplastic oligodendrogliomas; *Brain Pathol.* **9** 639-643

Pomeroy S L, Tamayo P, Gaasenbeek M, Sturla L M, Angelo M, McLaughlin M E, Kim J Y, Goumnerova L C, Black P M, Lau C, Allen J C, Zagzag D, Olson J M, Curran T, Wetmore C, Biegel J A, Poggio T, Mukherjee S, Rifkin R, Califano A, Stolovitzky G, Louis D N, Mesirov J P, Lander E S and Golub T R 2002 Prediction of central nervous system embryonal tumour outcome based on gene expression; *Nature* **415** 436-442

Prigent S A, Nagane M, Lin H, Huvar I, Boss G R, Feramisco J R, Cavenee W K, Huang H S 1996 Enhanced tumourigenic behavior of glioblastoma cells expressing a truncated epidermal growth factor receptor is mediated through the Ras-Shc-Grb2 pathway; *J. Biol. Chem.* **271** 25639-25645

Rainov N G, Dobberstein K U, Bahn H, Holzhausen H J, Lautenschlager C, Heidecke V, and Burkert W 1997 Prognostic factors in malignant glioma: influence of the overexpression of oncogene and tumour-suppressor gene products on survival; *J Neurooncol* **35** 13-28

Ramaswamy S and Golub T R 2002 DNA microarrays in clinical oncology; *J. Clin. Oncol.* **20** 1932-1941

Ramirez I, Tebar F, Grau M and Soley M 1995 Role of heterotrimeric G-proteins in epidermal growth factor signalling; *Cell Signal* **7** 303-311

Rasheed B K, Wiltshire R N, Bigner S H and Bigner D D 1999 Molecular pathogenesis of malignant gliomas; *Curr. Opin. Oncol.* **11** 162-167

Reifenberger G, Reifenberger J, Ichimura K, Meltzer PS, Collins VP, 1994. Amplification of multiple genes from chromosomal region 12q13-14 in human malignant gliomas: preliminary mapping of the amplicons shows preferential involvement of CDK4, SAS, and MDM2. *Cancer Res.* **54** 4299-4303

Reyes-Mugica M, Rieger-Christ K, Ohgaki H, Ekstrand B C, Helie M, Kleinman G, Yahanda A, Fearon E R, Kleihues P and Reale M A 1997 Loss of DCC expression and glioma progression; *Cancer Res.* 57 382-386

Ribom D, Andrae J, Frielingsdorf M, Hartman M, Nister M and Smits A 2002 Prognostic value of platelet derived growth factor alpha receptor expression in grade 2 astrocytomas and oligoastrocytomas; *J. Neurol. Neurosurg. Psychiatry.* **72** 782-787

Rickman D S, Bobek M P, Misek D E, Kuick R, Blaivas M, Kurnit D M, Taylor J, Hanash S M 2001 Distinctive molecular profiles of high-grade and low-grade gliomas based on oligonucleotide microarray analysis; *Cancer Res.* **61** 6885-6891

Roberts C J, Nelson B, Marton M J, Stoughton R, Meyer M R, Bennett H A, He Y D, Dai H, Walker W L, Hughes T R, Tyers M, Boone C and Friend S H 2000 Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles; *Science* 287 873-880

Rodriguez-Viciana P, Warne P H, Dhand R, Vanhaesebroeck B, Gout I, Fry M J, Waterfield M D and Downward J 1994 Phosphatidylinositol-3-OH kinase as a direct target of Ras; *Nature* 370 527-532

Ronnstrand L, Mori S, Arridsson A K, Eriksson A, Wernstedt C, Hellman U, Claesson-Welsh L and Heldin C H 1992. Identification of two C-terminal autophosphorylation sites in the PDGF beta-receptor: involvement in the interaction with phospholipase C-gamma. *EMBO J.* 11 3911-3919

Rozakis-Adcock M, McGlade J, Mbamalu G, Pelicci G, Daly R, Li W, Batzer A, Thomas S, Brugge J and Pelicci P G 1992. Association of the Shc and Grb2/Sem5 SH2-containing proteins is implicated in activation of the Ras pathway by tyrosine kinases; *Nature* 360 689-692

Rubio M P, Correa K M, Ueki K, Mohrenweiser H W, Gusella J F, von Deimling A, Louis D N 1994 The putative glioma TSG on chromosome 19q maps between APOC2 and HRC; *Cancer Res.* 54 4760-4763

Sallinen S L, Sallinen P K, Haapasalo H K, Helin H J, Helen P T, Schraml P, Kallioniemi O P and Kononen J 2000 Identification of differentially expressed genes in human gliomas by DNA microarray and tissue chip techniques; *Cancer Res.* 60 6617-6622

Sarkar C, Rathore A, Chattopadhyay P, Mahapatra A K and Sinha S 2000 Role of 17p13.3 chromosomal region in determining p53 protein immunopositivity in human astrocytic tumors; *Pathology* 32 84-88

_____, Chattopadhyay P, Ralte AM, Mahapatra AK and Sinha S. Loss of heterozygosity of a locus on the chromosomal region 17p 13.3 is associated with increased cell proliferation in astocytic tumors; *Cancer Genetics and Cytogenetics*, in press

Sasaki H, Zlatescu M C, Betensky R A, Ino Y, Cairncross J G and Louis D N 2001 PTEN is a target of chromosome 10q loss in anaplastic oligodendrogliomas and PTEN alterations are associated with poor prognosis; *Am. J. Pathol.* 159 359-367

Satoh T, Fantl W J, Escobedo J A, Williams L T and Kaziro Y 1993 Platelet-derived growth factor receptor mediates activation of ras through different signaling pathways in different cell types; *Mol. Cell Biol.* 13 3706-3713

Schlegel J, Merdes A, Stumm G, Albert F K, Forsting M, Hynes N, Kiessling M 1994 Amplification of the epidermal-growth-factor-receptor gene correlates with different growth behaviour in human glioblastoma; *Int. J. Cancer* 56 72-77

Schlessinger J 1993 How receptor tyrosine kinases activate Ras; *Trends Biochem. Sci.* 18 273-275

Schmidt E E, Ichimura K, Messerle K R, Goike H M and Collins V P 1997 Infrequent methylation of CDKN2A(MTS1/p16) and rare mutation of both CDKN2A and CDKN2B(MTS2/p15) in primary astrocytic tumours; *Br. J. Cancer* 75 2-8

_____, _____, Reifenberger G, Collins V P 1994 CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas; *Cancer Res.* 54 6321-6324

Schwechheimer K, Huang S and Cavenee W K 1995 EGFR gene amplification—rearrangement in human glioblastomas; *Int. J. Cancer* 62 145-148

Sehgal A 1998 Molecular changes during the genesis of human gliomas; *Semin Surg Oncol.* 14 3-12

Shibuya M, Ito N and Claesson-Welsh L 1999 Structure and function of vascular endothelial growth factor receptor-1 and -2; *Curr. Top Microbiol. Immunol.* 237 59-83

Shin K H and Park J G 2000 Microsatellite instability is associated with genetic alteration but not with low levels of expression of the human mismatch repair proteins hMSH2 and hMLH1; *Eur. J. Cancer* 36 925-931

Shipp M A, Ross K N, Tamayo P, Weng A P, Kutok J L, Aguiar R C, Gaasenbeek M, Angelo M, Reich M, Pinkus G S, Ray T S, Koval M A, Last K W, Norton A, Lister T A, Mesirov J, Neuberg D S, Lander E S, Aster J C, Golub T R 2002 Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning; *Nat. Med.* 8 68-74

Sjoblom T, Shimizu A, O'Brien K P, Pietras K, Dal Cin P, Buchdunger E, Dumanski J P, Ostman A and Heldin C H 2001 Growth inhibition of dermatofibrosarcoma protuberans tumours by the platelet-derived growth factor receptor antagonist STI571 through induction of apoptosis; *Cancer Res.* **61** 5778-5783

Smith J S, Perry A, Borell T J, Lee H K, O'Fallon J, Hosek S M, Kimmel D, Yates A, Burger P C, Scheithauer B W and Jenkins R B 2000 Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas; *J. Clin. Oncol.* **18** 636-645

_____, Wang X Y, Qian J, Hosek S M, Scheithauer B W, Jenkins R B, James C D, 2000 Amplification of the platelet-derived growth factor receptor-A (PDGFRA) gene occurs in oligodendrogliomas with grade IV anaplastic features; *J. Neuropathol. Exp. Neurol.* **59** 495-503

Snijders A M, Nowak N, Segraves R, Blackwood S, Brown N, Conroy J, Hamilton G, Hindle A K, Huey B, Kimura K, Law S, Myambo K, Palmer J, Ylstra B, Yue J P, Gray J W, Jain A N, Pinkel D and Albertson D G 2001 Assembly of microarrays for genome-wide measurement of DNA copy number; *Nat. Genet.* **29** 263-264

Soltoff S P, Rabin S L, Cantley L C and Kaplan D R 1992 Nerve growth factor promotes the activation of phosphatidylinositol 3-kinase and its association with the trk tyrosine kinase; *J. Biol. Chem.* **267** 17472-17477

Sorkin A 2001 Internalization of the epidermal growth factor receptor: role in signalling; *Biochem. Soc. Trans.* **29** 480-484

Sorlie T, Perou C M, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen M B, van de Rijn M, Jeffrey S S, Thorsen T, Quist H, Matese J C, Brown P O, Botstein D, Eystein Lonning P and Borresen-Dale A L 2001 Gene expression patterns of breast carcinomas distinguish tumour subclasses with clinical implications; *Proc. Natl. Acad. Sci. U S A* **98** 10869-10874

Steck P A, Pershouse M A, Jasser S A, Yung W K, Lin H, Ligon A H, Langford L A, Baumgard M L, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng D H, Tavtigian S V, 1997 Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers; *Nat. Genet.* **15** 356-362

Suzuki H, Gabrielson E, Chen W, Anbazhagan R, van Engeland M, Weijenberg M P, Herman J G and Baylin S B 2002 A genomic screen for genes upregulated by demethylation and histone deacetylase inhibition in human colorectal cancer; *Nat. Genet.* **31** 141-149

Takahashi J A, Fukumoto M, Igarashi K, Oda Y, Kikuchi H and Hatanaka M 1992 Correlation of basic fibroblast growth factor expression levels with the degree of malignancy and vascularity in human gliomas; *J. Neurosurg.* **76** 792-798

Tang C K, Gong X Q, Moscatello D K, Wong A J and Lippman M E 2000 Epidermal growth factor receptor vIII enhances tumourigenicity in human breast cancer; *Cancer Res.* **60** 3081-3087

Thommen R, Humar R, Misevic G, Pepper M S, Hahn A W, John M, Battegay E J 1997 PDGF-BB increases endothelial migration on cord movements during angiogenesis *in vitro; J. Cell Biochem.* **64** 403-413

Twamley G M, Kypta R M, Hall B and Courtneidge S A 1993 Src family tyrosine kinases and the response to platelet-derived growth factor; *Adv. Second Messenger Phosphoprotein Res.* **28** 187-194

Twamley-Stein G M, Pepperkok R, Ansorge W and Courtneidge S A 1993 The Src family tyrosine kinases are required for platelet-derived growth factor-mediated signal transduction in NIH 3T3 cells; *Proc. Natl. Acad. Sci. U S A.* **90** 7696-7700

van 't Veer L J, Dai H, van de Vijver M J, He Y D, Hart A A, Mao M, Peterse H L, van der Kooy K, Marton M J, Witteveen A T, Schreiber G J, Kerkhoven R M, Roberts C, Linsley P S, Bernards R and Friend S H 2002 Gene expression profiling predicts clinical outcome of breast cancer; *Nature* **415** 530-536

Varlet P, Guillamo J S, Nataf F, Koziak M, Beuvon F and Daumas-Duport C 2000 Vascular endothelial growth factor expression in oligodendrogliomas: a correlative study with Sainte-Anne malignancy grade, growth fraction and patient survival; *Neuropathol. Appl. Neurobiol.* **26** 379-389

von Deimling A, Louis D N, von Ammon K, Petersen I, Hoell T, Chung R Y, Martuza R L, Schoenfeld D A, Yasargil M G and Wiestler O D 1992 Association of epidermal growth factor receptor gene amplification with loss of chromosome 10 in human glioblastoma multiforme; *J. Neurosurg.* **77** 295-301

_____, _____, _____, _____, Wiestler O D, and Seizinger B R 1992 Evidence for a tumour suppressor gene on chromosome 19q associated with human astrocytomas, oligodendrogliomas, and mixed gliomas; *Cancer Res.* **52** 4277-4279

Wang D, Huang H J, Kazlauskas A and Cavenee W K 1999 Induction of vascular endothelial growth factor expression in endothelial cells by platelet-derived growth factor through the activation of phosphatidylinositol 3-kinase; *Cancer Res.* **59** 1464-1472

Watanabe K, Tachibana O, Sata K, Yonekawa Y, Kleihues P and Ohgaki H 1996 Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas; *Brain Pathol.* **6** 217-223

Waterfield M D, Scrace GT, Whittle N, Stroobant P, Johnsson A, Wasteson A, Westermark B, Heldin C H, Huang J S and Deuel T F 1983 Platelet-derived growth factor is structurally related to the putative transforming protein p28sis of simian sarcoma virus; *Nature* **304** 35-39

Watson M A, Perry A, Budhjara V, Hicks C, Shannon W D and Rich K M 2001 Gene expression profiling with oligonucleotide microarrays distinguishes World Health Organization grade of oligodendrogliomas; *Cancer Res.* 61 1825-1829

Welsh J B, Sapinoso L M, Su A I, Kern S G, Wang-Rodriguez J, Moskaluk C A, Frierson H F Jr, and Hampton G M 2001 Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer; *Cancer Res.* 61 5974-5978

Westermark B 1993 PDGF and its receptors in human tumour cells; in *Biology of Platelet Derived Growth Factor*, 5, B. Westermark and C. Sorg., eds. Krager, Basel; pp. 146-162

_____ and Heldin C H 1991 Platelet-derived growth factor in autocrine transformation; *Cancer Res.* 51 5087-5092

_____ and Nister M 1995 Molecular genetics of human glioma; *Curr. Opin. Oncol.* 7 220-225

Wikstrand C J, Reist C J, Archer G E, Zalutsky M R and Bigner D D 1998 The class III variant of the epidermal growth factor receptor (EGFRvIII): characterization and utilization as an immunotherapeutic target; *J. Neurovirol.* **4** 148-158

Wong A J, Ruppert J M, Bigner S H, Grzeschik C H, Humphrey P A, Bigner D S and Vogelstein B 1992 Structural alterations of the epidermal growth factor receptor gene in human gliomas; *Proc. Natl. Acad. Sci. U S A* 89 2965-2969

Woodruff R C and Nikitin A G 1995 P DNA element movement in somatic cells reduces the life span in Drosophila melanogaster: evidence in support of the somatic muataion theory of aging; *Mutat. Res.* 338 35-42

Yao Y, Kubota T, Sato K, Kitai R, Takeuchi H and Arishima H 2001 Prognostic value of vascular endothelial growth factor and its receptors Flt-1 and Flk-1 in astrocytic tumours; *Acta Neurochir (Wien)* 143 159-166

Yarden Y 2001 The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities; *Eur. J. Cancer* 37 Suppl 4 S3-8

Yokote K, Mori S, Hansen K, McGlade J, Pawson T, Heldin C H and Claesson-Welsh L 1994 Direct interaction between Shc and the platelet-derived growth factor beta-receptor.*J Biol Chem.* 269 15337-15343

Zhu D, Caveney S, Kidder G M and Naus C C 1991 Transfection of C6 glioma cells with connexin 43 cDNA: analysis of expression, intercellular coupling, and cell proliferation; *Proc. Natl. Acad. Sci. U S A,* 88 1883-1887

Zuber J, Tchernitsa O I, Hinzmann B, Schmitz A C, Grips M, Hellriegel M, Sers C, Rosenthal A and Schafer R 2000 A genome-wide survey of RAS transformation targets; *Nat Genet* 24 144-152