Studies on the Roles of PDGFRA and EGFR in the Classification and Identification of Therapeutic Targets for Human Gliomas

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Abstract

Glioma is the most common type of primary tumor in the adult CNS. In this thesis, we have established a glial genesis-guided molecular classification scheme for gliomas based on the gene modules co-expressed with EGFR (EM) or PDGFRA (PM). This scheme is applicable to gliomas of all major morphological subtypes, and can predict the prognosis of glioma patients. EM/PM glioma subtypes are specifically associated with glial genesis activities, signatures of glial cell types during development or maturation, and known glioma genomic abnormalities. These findings constitute a framework for improving molecular diagnosis and identifying therapeutic targets to combat gliomas.

In addition, we also found that gliomas with high level PDGFRA expression contained nearly all morphological subtypes, which was associated with frequent IDH1 mutation, 1p LOH, 19q LOH, less EGFR amplification, younger age at disease onset and better survival compared to gliomas with low level PDGFRA expression. PDGFRA expression can be induced and maintained by FGF2 in primary glioma cells. Our findings suggest a role of FGF2 in regulating PDGFRA expression in the subset of gliomas with younger age at disease onset and longer patient survival regardless of their morphological diagnosis.

Moreover, we demonstrated that glioma cell proliferation correlated with the extent of surface expression of PDGFRA in both glioma cell lines and their corresponding tumor samples. And the surface PDGFRA expression can be decreased by MEK inhibitor U0126, which results in deviation of PDGFRA from endosomal trafficking and recycling compartment to the Golgi network in a reversible, dose- and time-dependent manner without affecting total PDGFRA expression. U0126 mediated down-regulation of PDGFRA surface expression correlated with diminished cell proliferation. Our findings suggested that the trafficking of PDGFRA in glioma cells is regulated by MEK and ERK activity and can potentially be manipulated to combat glioma growth.

Key words: Gliomas, classification, EGFR, PDGFRA, FGF2, surface expression, cell proliferation

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To my family

Contents

List of original articles in this thesis

This thesis is based on the studies reported in the following papers, referred to in the text by Roman numerals (I – III):

Paper I Yingyu Sun*, Wei zhang*, **Dongfeng Chen***, Junxiong zheng, Henrik lilljebjörn, Yuhong Lv, Liang Ran, Zhaoshi Bo, Charlotte Soneson, Hans Olov Sjögern, Leif G. Salford, Pim J. French, Thoas Fioretos, Tao Jiang, Xiaolong Fan. Glioma molecular classification defined by co-expression modules of EGFR and PDGFRA. *Submitted.* (* Equal contribution)

Paper II **Dongfeng Chen**, Annette Persson, Yingyu Sun, Leif G. Salford, David Gisselsson Nord, Elisabet Englund, Tao Jiang, Xiaolong Fan. Better prognosis of patients with glioma expressing FGF2-dependent PDGFRA expression irrespective of morphological diagnosis. *PLOS ONE, 2013, 8(4): e61556.*

Paper III **Dongfeng Chen**, Duo Zuo, Cheng Luan, Manli Na, Liang Ran, Yingyu Sun, Annette Persson, Elisabet Englund, Leif G. Salford, Erik Renström, Xiaolong Fan*, Enming Zhang*. Glioma cell proliferation controlled by ERK activity-dependent surface expression of PDGFRA. *Submitted.* (* Equal contribution)

Abstract

Glioma is the most common type of primary tumor in the adult central nervous system (CNS). However, the current classification of gliomas is highly subjective and even inaccurate in some cases, which leads to clinical confusion and hinders the development of targeted therapies. EGFR and PDGFRA play crucial roles in glia development and glioma pathogenesis. In this thesis we aim to establish a glial genesis-guided molecular classification scheme for gliomas based on the genes co-expressed with EGFR or PDGFRA and to clarify the clinical relevance and the mechanism of PDGFRA expression in different glioma subtypes. We also aim to investigate the role of cell surface PDGFRA expression in regulating glioma cell proliferation.

In order to establish a glial genesis-guided classification scheme, we identified 69 genes co-expressed with EGFR (EM) or PDGFRA (PM) as classifiers. Using these 69 classifiers, gliomas are clarified into EM (highly expressing EM genes), PM (highly expressing PM genes), and $EM^{low}PM^{low}$ (lowly expressing both EM and PM genes) subtypes in a morphology-independent manner. Our results showed that besides their distinct patterns of genomic alterations, EM gliomas were associated with higher age at diagnosis, poorer prognosis, stronger expression of neural stem cell genes and astrogenesis genes, while PM and $EM^{low}PM^{low}$ gliomas were associated with younger age at diagnosis and better prognosis. PM gliomas were enriched in the expression of oligodendrogenesis genes, whereas $EM^{low}PM^{low}$ gliomas were enriched in the signatures of mature neurons and oligodendrocytes. These findings constitute a framework for improving molecular diagnosis and identifying therapeutic targets to combat gliomas.

To investigate the clinical relevance of PDGFRA in gliomas, the clinical outcomes of gliomas with the top 25% of PDGFRA expression levels (PDGFRA-high) were compared with the gliomas with lowest 25% of PDGFRA expression levels

(PDGFRA-low). We found that PDGFRA-high gliomas contained nearly all morphological subtypes, which was associated with frequent IDH1 mutation, 1p LOH, 19q LOH, less EGFR amplification, younger age at disease onset and better survival compared to PDGFRA-low gliomas. We also found that the PDGFRA expression can be induced and maintained by fibroblast growth factor 2 (FGF2) in primary glioma cells. FGF2-dependent maintenance of PDGFRA expression was concordant with the maintenance of a subset of gliogenic genes and higher rates of cell proliferation. Our findings suggest a role of FGF2 in regulating PDGFRA expression in the subset of gliomas.

To investigate the role of cell surface expression of PDGFRA in regulating cell proliferation, we compared the growth rate of primary glioma cells having high cell surface PDGFRA expression level with the glioma cells having low cell surface PDGFRA expression level. We demonstrated that glioma cell proliferation correlated with the extent of surface expression of PDGFRA in both glioma cell lines and their corresponding tumor samples. We also found that MEK inhibitor U0126 treatment can decrease the surface PDGFRA expression and result in deviation of PDGFRA from endosomal trafficking and recycling compartment to the Golgi network in a reversible, dose- and time-dependent manner without affecting total PDGFRA expression. U0126 mediated down-regulation of PDGFRA surface expression correlated with diminished cell proliferation. Our findings suggested that the trafficking of PDGFRA in glioma cells is regulated by ERK activity and can potentially be manipulated to combat glioma growth.

Populärvetenskaplig sammanfattning på svenska

Gliom är den vanligaste typen av hjärntumörer hos vuxna. Den mikroskopiska klassificeringen av de olika formerna av gliom är subjektiv och i vissa fall t.o.m. felaktig, vilket leder till klinisk oklarhet som kan hindra utvecklingen av terapier som riktas mot speciella egenskaper som kännetecknar speciella typer av gliom. EGFR och PDGFRA är en cellreceptor som har en central roll i utvecklingen av hjärnans normala gliavävnad (vävnad som omger nervcellerna), och har en analog huvudroll i gliavävnadens motsvarande tumör–omvandling till gliom. Denna avhandling syftar till att dels etablera en molekylär klassifikation av gliomen på basen av den stegvisa utmognaden av de olika typerna av normala gliaceller och dess association med gener som uttrycks tillsammans med generna för cellreceptorerna EGFR eller PDGFRA, dels klargöra mekanismen och den kliniska relevansen för uttrycket av PDGFRA i olika typer av gliom, samt studera den roll PDGFRA lokaliserat till cellytan har för reglering av gliomcellers delningshastighet.

För att etablera en molekylär gliomklassificering baserad på den normala gliavävnadens utmognad identifierades 69 gener som var aktiva tillsammans med generna EGFR (EM) respektive PDGFRA (PM). Utan hänsyn till mikroskopiskt utseende klassificeras gliom från olika patienter i 3 grupper: EM (EM-gener uttrycks starkt), PM (PM-gener uttrycks starkt) och $EM^{low}PM^{low}$ (lågt uttryck av både PM- och EM-gener). Resultatet visar att EM-gliom har starkare uttryck av omogna nervcellsgener och gener associerade med en typ av gliaceller (astroglia), sämre prognos för patienten som insjuknar vid högre ålder. PM och $EM^{low}PM^{low}$ är associerade med bättre prognos och lägre ålder vid insjuknandet. PM-gliom har starkare uttryck av gener associerade med en annan typ av gliaceller

(oligodendrocyter) liksom $EM^{low}PM^{low}$. De senare har starkare uttryck av gener associerade med mogna nervceller. Dessa resultat utgör en bas för att förbättra den molkylära diagnosticeringen och för att identifiera molkylära målstrukturer/mekanismer för riktad terapi.

Den kliniska relevansen av PDGFRA-uttryck i gliom har också analyserats genom att jämföra det kliniska utfallet för gliompatienter som har gliom med de 25% högsta uttrycken med patienterna med de 25% lägsta uttrycken. De höguttryckande gliomen innefattar nästan alla mikroskopiskt skilda typer av gliom, har lågt uttryck av EGFR och har ofta vissa specifika genmutationer, och patienterna insjuknade vid lägre ålder och hade bättre prognos än patienterna med lågt PDGFRA-uttryck. En tillväxtfaktor för bindvävsceller (FGF2) visas kunna inducera uttrycket av PDGFRA hos gliomceller i vävnadskultur. Graden av PDGFRA-uttryck på gliomcellers yta visas korrelera med cellernas delningshastighet.

Aktiveringen av ett enzym (MEK) utgör ett viktigt steg i den kaskad av molekylära interaktioner som leder från att en receptor internaliseras till att responderande gener aktiveras i cellens kärna. En hämmare av MEK visas minska PDGFRA-uttrycket på cellytan men däremot inte cellens totala PDGFRA. Det minskade receptoruttrycket på cellytan uppfattas vara beroende på en hämning av signalering av återtransport av internaliserade receptorer till cellytan. Att cellens totala PDGFRA inte minskar kan hänga samman med att en annan väsentlig molekyl (ERK) i de aktuella signalvägarna har visats få kompensatoriskt ökat uttryck som en långsam effekt av MEK-hämmaren. Hämmaren framkallar en minskad delningshastighet hos gliomceller i vävnadsodling, vilket går parallellt med det minskade PDDGFRA-uttrycket på cellytan. Den ökade insikten i den molekylära styrningen av PDGFRA på cellytan och därigenom delningshastigheten kan tänkas öppna för ny terapiutveckling.

Abbreviations

CNS	Central nervous system
GBM	Glioblastoma multiforme
Ig	Immunoglobulin
WHO	World Health Organization
SNP	Single nucleotide polymorphism
CGH	Comparative genomic-hybridization
GEP	Gene expression profiling
TCGA	The Cancer Genome Atlas
YKL40 (CHI3L1)	Chitinase-3-like protein 1
VEGF	Vascular endothelial growth factor
VEGFR2	VEGF receptor 2
PDGF	Platelet-derived growth factor
PDGFRA	PDGF receptor alpha
PDGFRB	PDGF receptor beta
CDK	Cyclin-dependent kinase
MET (HGFR)	Met proto-oncogene (hepatocyte growth factor receptor)
IDH	Isocitrate dehydrogenase
PIK3CA	Phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit 1
TP53 (P53)	Tumor protein p53
EGF	Epidermal growth factor
EGFR	EGF receptor

PTEN	Phosphatase and tensin homolog
CDKN2A	Cyclin-dependent kinase inhibitor 2A
NF1	Neurofibromin 1
DLL3	Delta-like 3 (Drosophila)
OLIG2	Oligodendrocyte lineage transcription factor 2
CD40 (TNFRSF5)	TNF receptor superfamily member 5
LGALS3	Lectin, galactoside-binding, soluble, 3
FDA	Food and Drug Administration
MGMT	O-6-methylguanine-DNA methyltransferase
wt	Wild-type
1p	Short arm of chromosome 1
19q	Long arm of chromosome 19
LOH	Loss of heterozygosity
LGGs	Low-grade giomas
α-KG	α-ketoglutarate
HIF-1α	Hypoxia inducible factor-1 α
NADPH	Nicotinamide adenine dinucleotide phosphate
2-HG	(R)-2-hydroxyglutarate
AML	Acute myeloid leukemia
TET2	Ten-eleven translocation 2
MRS	Magnetic resonance spectroscopy
10q	Long arm of chromosome 10
CCNU	Lomustine
PVC	Procarbazine, vincristine and CCNU
TMZ	Temozolomide

EORTC	European Organisation for Research and Treatment of Cancer
RTOG	Radiation Therapy Oncology Group
NCIC	National Cancer Institute of Canada
NPC	Neural progenitor cell
NSC	Neural stem cell
SVZ	subventricular zone
TK	Tyrosine kinase
TGFα	Transforming growth factor α
PI3K	Phosphatidylinositide 3-kinase
AKT (PKB)	Protein Kinase B
MAPK	Mitogen-activated protein kinase
NSCLC	Non–small cell lung cancer
EGFRvI	Type I EGFR variant deletion mutant
EGFRvI	Type II EGFR variant deletion mutant
EGFRvIII	Type III EGFR variant deletion mutant
EGFRvIV	Type IV EGFR variant deletion mutant
EGFRvV	Type V EGFR variant deletion mutant
mTOR	Mammalian target of rapamycin
CRTC2	CREB-regulated transcription co-activator 2
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B
SFK	Src family kinase
DOCK1 (DOCK180)	Dedicator of cytokinesis 1
IL	Interleukin
LIF	Leukemia inhibitory factor

SH2	Src Homology 2
SHP2 (PTPN11)	Protein tyrosine phosphatase, non-receptor type 11
OPC	Oligodendrocyte precursor cells
bp	Base pair
APC	Astrocytes precursor cells
MADM	Mosaic analysis with double markers
NG2	Neuron-glial antigen 2
RTK	Receptor tyrosine kinase
EM	EGFR module
PM	PDGFRA module
PDGFRA-high	High levels of PDGFRA expression
PDGFRA-low	Lower levels of PDGFRA expression
FGF2	Fibroblast growth factor 2
MEK	Mitogen-activated protein kinase kinase
ERK	Mitogen-activated protein kinase

Introduction

Gliomas

Glioma is referred to tumor that starts in the brain or the spine. The most common site of gliomas is the brain[1]. Symptoms of gliomas depend on which part of the central nervous system (CNS) is affected. Brain gliomas can cause headaches, nausea, volmiting, seizures, and/or visual loss, and cranial nerve disorders as a result of increased intracranial pressure, while spinal cord gliomas can lead to pain, weakness, or numbness in the extremities. Gliomas do not metastasize through the bloodstream, but they can spread via the cerebrospinal fluid and cause "drop metastases" to the spinal cord.

Epidemiology

Gliomas, the most frequent primary brain tumours in adults, make up approximately 30% of all brain and central nervous system tumors and 80% of all malignant brain tumors[2]. The annual incidence is 6 cases per 100 000 [3]. Each year, more than 14 000 new cases are diagnosed in the United States [3].

Glioblastoma multiformes (GBMs) account for approximately 60%-70% of malignant gliomas, anaplastic astrocytomas for 10%-15%, and anaplastic oligodendrogliomas and anaplastic oligoastrocytomas for 10%; less common tumors such as anaplastic ependymomas and anaplastic gangliogliomas account for the rest [3, 4]. The incidence of these tumors has increased slightly over the past two decades, especially in the elderly as a result of improved diagnostic imaging.

Malignant gliomas are 40% more common in men than in women and twice as common in whites as in blacks [3]. The median age of patients at the time of diagnosis is 64 years in the case of GBMs and 45 years in the case of anaplastic

gliomas [3, 5]. Currently, there is no cure for gliomas and most available treatments provide only minor symptomatic relief.

The cause of glioma is unknown, although previous exposure to ionizing radiation is a known risk factor [6]. Radio frequency electromagnetic fields emitted by mobile phones have been suspected to induce gliomas in excessive users of cellular phones. However, the association between radio frequency waves and brain tumours remains unclear [6]. Evidence for an association with head injury, foods containing N-nitroso compounds, occupational risk factors, and exposure to electromagnetic fields is inconclusive [6]. There is suggestive evidence of an association between immunologic factors and gliomas. Patients with atopy have a reduced risk of gliomas [7], and patients with glioblastoma who have elevated immunoglobulin E (IgE levels) appear to live longer than those with normal levels [8]. The importance of these associations is unclear. Gene polymorphisms that affect detoxification, DNA repair, and cell-cycle regulation have also been implicated in the development of gliomas [6].

Approximately 5% of patients with malignant gliomas have a family history of gliomas. Some of these familial cases are associated with rare genetic syndromes, such as neurofibromatosis types 1 and 2, the Li−Fraumeni syndrome (germ-line p53 mutations associated with an increased risk of several cancers), and Turcot's syndrome (intestinal polyposis and brain tumors) [9].

Histological classification of gliomas

Gliomas are classified by subtype and further by grade on the basis of histopathological features. According to the similarity to normal glia cells, gliomas are first classified into astrocytomas (including GBMs), oligodendrogliomas, and ependymomas [4], whose tumor cells show morphological similarity to astrocytes, oligodendrocytes and ependycytes, respectively. Tumors that display similarity with a mixture of these different cells are called mixed gliomas [4].

Gliomas are further separated into grades I through to IV according to the 2007 World Health Organization (WHO) system which is based on the presence or absence of nuclear atypia, mitosis, microvascular proliferation, and necrosis [4].

	Astrocytic tumors	Oligodendroglial tumors	Mixed tumors	Median of Survival (years)
Grade I	Pilocytic astrocytoma	NA	NA	>10
Grade II	Diffuse astrocytoma	Diffuse oligodendroglioma	Diffuse oligoastrocytoma	5-8
Grade III	Anaplastic astrocytoma	Anaplastic oligodendroglioma	Anaplastic oligoastrocytoma	3
Grade IV	GBM	GBM	GBM	1-1.5

Table 1.

Current diagnostic scheme of gliomas according to the 2007 WHO classification of tumors of CNS. NA: Not applicable

The WHO classification of gliomas is prognostic between the grades and subtypes. Grade I gliomas (pilocytic astrocytomas) typically have a good prognosis and more frequently occur in children [10], and grade II gliomas (diffuse astrocytomas) are characterized on histologic examination by hypercellularity. Patients with grade II gliomas have a 5–8-year median survival [10]. Grade III gliomas (anaplastic astrocytoma tumors) are characterized according to hypercellularity, as well as nuclear atypia and mitotic figures. Patients with anaplastic astrocytoma has a 3-year median survival [10]. Grade IV gliomas, also known as GBMs, are characterized according to hypercellularity, nuclear atypia, mitotic figures, and evidence of angiogenesis and/or necrosis [3]. The median

survival for patients with GBM is 12–18 months, and older patients (>60 years of age) typically have a somewhat shorter survival than younger patients [10]. While 90%–95% of GBMs are considered ‘‘primary,’’ 5%–10% of GBMs develop from lower grade gliomas in younger patients and are termed ‘‘secondary’’ [11, 12]. Together with grade III anaplastic astrocytoma, these gliomas consist of the clinical entity termed ‘‘malignant glioma’’.

Oligodendrogliomas are divided into two grades: well-differentiated oligodendrogliomas and oligoastrocytomas (WHO grade II), and anaplastic oligodendrogliomas (WHO grade III). All of these tumors may contain perinuclear halos and a delicate network of branching blood vessels (chicken-wire pattern) [4]. Patients with oligodendrogliomas have a 5-year survival rate of approximately 80% [3].

The problems of histological classification of gliomas

Accurate pathological classification of gliomas is essential because it guides treatment and prognosis. The 2007 WHO classification system is a practical and effective approach of glioma diagnosis for most cases in which it provides a means for placing tumors into specific, relevant prognostic categories. These classification schemes are mainly based on the morphological observations of glioma cells. However, the morphology of cells is dynamic regulated by the actin cytoskeleton that is sensitive to the signals from microenvironments [13]. Same types of glioma in different patients may show distinct cell morphology, which may cause the wrong diagnosis by observers. So this classification schemes are subjective, lack reproducibility, and remain imperfect in their ability to predict individual outcomes [14]. In addition, traditional groupings are only satisfactory for series of cases, and not necessarily adequate predictors of behavior, response to therapy or survival for individual tumors and patients [14].

Some diffuse gliomas are also difficult to place into one of the WHO categories, which can result in diagnostic dilemma, or nebulous diagnoses such as "malignant glioma, not otherwise specified." Many studies have indicated that approximately 20-30% of all gliomas are incorrectly classified [15-18]. For a large proportion of these patients, discrepancies in classification result in different treatment strategies [18] whereas these have been optimized for the different glioma subtypes. Moreover, no specific treatment has been found for any type of gliomas.

These problems clearly indicate the importance of improving the current approach to glioma classification. In this regard, classification for gliomas based on molecular signatures has a greater likelihood of achieving broadly clinical relevance.

Molecular classification of gliomas

The ongoing characterization of the genetic alterations in glioma tumor cells is revealing considerable variability among tumors of the same morphological type and grade. This heterogeneity may contribute to the current limitations in predicting patient survival on the basis of histologic analysis of glioma type and grade alone [10, 15-18] and suggests that classification of certain types and grades of gliomas according to their genetic phenotype will lead to a more accurate prediction of survival and response to therapy [15-18].

High-throughput genomic techniques will accelerate this process. Screening for gene polymorphisms and loss of heterozygosity (LOH) by single nucleotide polymorphism (SNP) microarrays; analyzing chromosomal gains and losses by comparative genomic-hybridization (CGH) arrays; determining global patterns of methylation, acetylation and alternative splicing on microarrays; gene expression profiling (GEP) on microarray; and identifying characteristic proteomic profiles will probably all play a part in the new molecular diagnosis [19-27].

GEP studies have been used to identify subclasses of gliomas based on transcriptomic signatures. It is increasingly clear that these signatures can convey information about prognosis and can, in part, reflect underlying patterns of mutation and signaling alterations. Most recently, transcriptomic signatures have been associated with alterations in DNA sequence and copy number [28] as well as with proteomic markers of key signaling pathways [29] and with patterns of DNA methylation [30]. These studies aimed either to identify differentially expressed genes among morphologically subtypes or to define prognostically distinct molecular subtypes of gliomas.

Identification of differentially expressed genes among morphologically defined gliomas

The earliest GEP studies were performed mainly to identify differentially expressed genes among morphologically defined gliomas. Such genes were found in low-grade versus high-grade astrocytomas [31], high-grade oligodendrogliomas versus GBM [32, 33], primary versus secondary GBM [34-36], adult versus pediatric GBM [37], or a variety of morphologically defined glioma subtypes [34, 35, 38]. Using primarily hierarchical clustering on differentially expressed genes, transcriptomal profiles of individual tumors were shown to be most similar to those from the same diagnostic subtype. These studies demonstrated that morphological differences among gliomas are reflected at the mRNA transcript level and that differentially expressed genes could be utilized to distinguish among morphologically defined subtypes.

However, discordance between morphological diagnosis and GEP-defined molecular subtype was frequent, likely due in part to inclusion of morphologically non-typical gliomas. Moreover, the relatively small sample sizes and lack of data on known prognostic covariates prohibited comprehensive multivariable analyses. Particularly for the earlier studies, the prognostic impact of GEP signatures could not be validated in large, external data sets [39].

GEP-guided prognostically distinct molecular classification

The primary clinical interest of a novel molecular classification of gliomas is whether it can improve the current clinical practice. To achieve this goal, The Cancer Genome Atlas (TCGA) piolt project, established by the US National Cancer Institute and National Human Genome Research Institute in December 2005, with the mission of understanding 'the molecular basis of cancer through the application of genome analysis technologies,' selected GBM as its first cancer type for study, based on its uniformly poor prognosis and limited treatment options. Two studies have currently provided the foundation for classification of GBM subtypes [28, 40].

The study by Phillips *et al*. identified three distinct subtypes of high-grade gliomas based on prognosis-related gene expression signatures [40]. This approach clustered high-grade gliomas into three distinct subtypes termed as proneural, proliferative, and mesenchymal gliomas. The proneural subtype was defined by genes implicated in neurogenesis, composed predominantly of non-GBM, and associated with significantly better survival for the patients than either of the other two tumor subtypes. In contrast, the proliferative and mesenchymal gene signatures contained markers of proliferation and extracellular matrix/invasion-related genes, respectively, and were both associated with poor outcome. Prognostic significance of molecular subtype was validated in an independent cohort of 184 gliomas of various histological subtypes. Of note, although this investigation was the first to use the terms proneural, proliferative, and mesenchymal, the categories delineated by Phillips e*t al.* are similar to an earlier scheme of prognostically relevant high-grade glioma subclassification [41], and the mesenchymal signature described contains markers, such as YKL40 and VEGF, previously reported to distinguish GBMs from lower-grade tumors [34, 42, 43].

In the second study, based on stable unbiased gene expression clusters among glioma samples, Verhaak *et al.* classified 176 classifiable GBMs out of 202 TCGA GBM samples into proneural, neural, classic and mesenchymal subtypes, which were subsequently validated using previously published data from 260 independent samples [28]. Each of the four subtypes was ultimately defined by the expression signature of a 210-gene list. By incorporating the available copy number and sequence data, three of the four subtypes were found to harbor distinct molecular alterations. Specifically, the proneural subtype was enriched for amplifications of PDGFRA, CDK6, CDK4, and MET; IDH1 and PIK3CA/ PIK3R1 mutations; and loss of heterozygosity (LOH) or mutation of TP53. Of note, this subtype contained the highest percentage of young patients, likely due in part to the high frequency of IDH1 mutation in this subtype. However, the proneural subtype was not associated with improved prognosis. The classical subtype was enriched for amplification of EGFR and loss of PTEN and CDKN2A, whereas the mesenchymal subtype harbored mutations and/or loss of NF1, TP53, and CDKN2A.

Although the number of subtypes identified by the Verhaak *et al.* and Phillips *et al.* studies is different, the proneural and mesenchymal classifications identified using distinct methodologies and sample sets are the most stable and concordant [44]. For instance, both groups identified proneural class expression of DLL3 and OLIG2 and mesenchymal class expression of CD40 and YKL40, the latter of which appears to be a potential serum protein marker of prognosis in GBM patients [45]. Moreover, a subset of the genes represented in these subtypes is represented in a nine-gene panel shown to predict outcome in glioblastoma, as increased expression of mesenchymal genes such as YKL40 and LGALS3 combined with decreased expression of a proneural gene, OLIG2, were associated with typical short-term survival compared with longer-term survivors [46].

Other studies have also identified characteristic gene expression signatures among high-grade gliomas [32, 47-49] or all major glioma subtypes [50, 51], and found correlations between expression signatures and the prognoses of patients and the cellular and genomic abnormalities of gliomas.

These studies have made important steps forward in establishing an objective molecular classification for gliomas. However, further investigation is needed to optimize the selection of classifiers so as to reflect the full range of tumor pathogenesis from low- to high-grade gliomas.

Barriers for clinical application

Up to now, only a few molecular classifiers based on GEP have been established as diagnostic tests, and the FDA has only cleared two assays for diagnostic application: Agendia's MammaPrint [52] and Pathwork Diagnostics' Tissue of Origin [53]. Several factors prohibit the molecular classifiers from practical application. First, the method is technically demanding. Although DNA microarrays are now well established in laboratory, the quality control issues for using DNA microarrays, as diagnostic tests can be quite difficult to achieve. Second, defining a molecular classifier needs mathematics methods, which are often a "black box" for clinicians. The difficulty of understanding the concept of the mathematical method constitutes a psychological hurdle for clinicians to accept as part of the diagnostic routine. Third, a new diagnostic test must have apparent advantages over existing methods. For example, in gastrointestinal cancer, the stage classification is dominant, and most prognostic factors, including the gene expression profile, are too weak for practical use. The first two barriers against using DNA microarrays for diagnostic purposes are more easily to overcome, but the third point is critical and is the main reason for why there are not more established diagnostic tests using these new technologies.

Current known genetic hallmarks in gliomas

Recent developments in anti-cancer drug research have resulted in a new type of diagnostic approach that is often called "personalized medicine." The goal of personalized medicine is the selection of patients with particular molecular diagnoses for treatment with a specific anti-cancer drug. Some molecular targeted drugs, such as trastuzumab or gefitinib, are already used for patients who undergo routine diagnostic tests that involve selection based on aberrations of target genes [54-56]; the selection process allows for the treatment of patients who will successfully respond to the therapy. Because most anti-cancer drugs in the pharmaceutical pipeline are molecularly targeted, personalized medicine will definitely be important for the treatment of gliomas as well [54-56].

Many molecular changes in gliomas have been extensively studied for possible clinical applications. Several studies have indicated a strong correlation between isocitrate dehydrogenase (IDH) mutations, 1p/19q co-deletion, methylation of the promoter region of the methylguanine methyltransferase (MGMT) gene, EGFR amplification and the malignant potential of gliomas [57-61].

IDH mutations

The recent identification of glioma-associated mutations in the IDH1 and IDH2 has added new insight to evolving notions of molecular stratification in malignant gliomas [62]. Point mutations in either IDH1 or IDH2 have been demonstrated in most WHO grade II and III diffuse gliomas and secondary GBMs but are rare in primary GBMs [63, 64].

GBMs with IDH1 mutations are phenotypically and genotypically distinct from IDH1 wild-type (wt) GBMs [65]. Over 60% of IDH1 mutant GBMs are localized in the frontal lobe and the peak incidence occurs in the third decade of life. IDH1 mutant GBMs share radiographic features with grade II and III gliomas and often have cystic or diffuse components more often than IDH1 wt GBMs.

IDH1 mutant GBMs also have a higher frequency of MGMT promoter methylation and TP53 mutation [65].

Several studies suggest that IDH1/2 mutation may be an early event in IDH1/2 mutant neoplasms. IDH1 or IDH2 mutations occur in 70% or more of low-grade gliomas (LGGs) [64]. The majority of LGGs with TP53 mutations or 1p/19q co-deletion have IDH mutations [66]. This possibility is also supported by recent sequencing analysis of *IDH1* and *TP53* genes in a separate study [65]. IDH2 mutations are rare and occur mainly in oligodendrogliomas [64]. IDH mutations are strongly associated with 1p/19q co-deletion and MGMT promoter methylation in anaplastic oligodendrogliomas [67]. A total of 90% to 100% of 1p/19q co-deleted gliomas also harbor IDH1 or 2 mutations [66, 68].

Whether mutant IDH results in a loss of tumor suppressor function or acts as an oncogene is an area of intense research. The exact mechanisms associated with tumorigenesis and improved prognoses are yet to be defined. Mutant IDH enzyme produces decreased cytoplasmic levels of α-ketoglutarate (α-KG) and NADPH [69, 70]. Resulting decreased cytosolic α-KG may stabilize hypoxia inducible factor-1 α (HIF-1α) facilitating cellular proliferation [69, 70]. It has been demonstrated that mutant cells contained extremely high levels of (R)-2-hydroxyglutarate (2-HG) [71], which was also found in primary IDH1 mutant gliomas and in the serum of IDH mutant acute myeloid leukemia (AML) patients [72, 73]. This indicated an oncogenic role of IDH mutant in cancer development.

Work on the downstream biological effects of IDH1/2 mutation expression has focused largely on the inhibition of α-KG-dependent dioxygenases by 2-HG. This diverse group of enzymes regulates a broad range of physiological processes, including hypoxic sensing, histone demethylation, demethylation of hypermethylated DNA, fatty acid metabolism, and collagen modification, among others [74]. Several studies have provided evidence to demonstrate that several of these functions are influenced by the expression of mutated IDH1/2 [63, 64, 69-

72]. IDH1 mutant gliomas exhibit a global DNA hypermethylation state, termed the glioma CpG island methylator phenotype (G-CIMP) [30]. Furthermore, this methylation phenotype correlated with a gene expression signature comprised of a limited set of down-regulated genes that discriminated between IDH1 mutant and wt proneural tumors. This hypermethylation state may be caused in part by the 2-HG-mediated inhibition of the α-KG-dependent ten-eleven translocation 2 (TET2) [75, 76]; the resultant decrease in 5-hydroxymethylcytosine was also observed in GBM specimens [75].

Despite our incomplete understanding of mutant IDH biology, the mutant status of the IDH1/2 genes may serve as an important prognostic indicator. Specifically, patients with anaplastic astrocytoma [62-64, 77] and GBM [64] harboring mutant IDH1 show a significantly longer overall survival compared with wt IDH1 counterparts and are younger at disease presentation, and this survival benefit has also been observed in grade II gliomas [77]. Patients with G-CIMP^{+} GBM also experience a similar survival benefit [30]. In addition, a comprehensive genomic and clinical analysis of GBMs harboring mutant and wt IDH1 suggests that, while histopathologically similar, these tumors may represent disease processes far more disparate than has been appreciated. Specifically, IDH1 mutant tumors display less contrast enhancement, less peritumoral edema, larger initial size, greater cystic components, and a greater likelihood of frontal lobe involvement compared with IDH1 wt tumors [65].

Several methods have been developed to assess IDH1 mutant protein status [78] or its 2-HG by-product [79] in clinical settings. While 2-HG is easily detected in the serum of AML patients, the correlation between serum 2-HG and tumor mutation status may be weaker in glioma patients [78]. It may also be possible to monitor the presence of 2-HG non-invasively using magnetic resonance spectroscopy (MRS) imaging of the brain [80].

Together, these studies provide overwhelming evidence for the clinical relevance of IDH1 status in gliomas from grades II to IV and support the view that its status should be incorporated into the current WHO histopathological scheme for every glioma analyzed [63]. Despite their histological similarities, IDH1mutant and wt GBMs are clearly distinct diseases, and understanding the biological basis behind the differences in their natural histories will surely be a major area of focus in the field.

1p/19q co-deletion

Co-deletion of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) has been frequently reported in gliomas during the last decade. The frequency of 1p/19q co-deletion is approximately 80%-90% in grade II oligodendrogliomas and 50%–70% in grade III oligodendrogliomas [81], which indicates the strong association between 1p/19q co-deletion and oligodendroglial lineage gliomas [82, 83]. The 1p/19q co-deletion has also been identified in mixed glial tumors (oligoastrocytomas), albeit in a lower proportion than in pure oligodendrogliomas [84]. Moreover, these oligoastrocytomas with 1p/19q co-deletion seem to behave more like oligodendrogliomas than comparable tumors lacking the co-deletion. But 1p/19q co-deletions are uncommon in GBMs [85, 86] and the clinical association between 1p/19q co-deletion and GBMs is still controversial [86, 87].

Although the 1p and 19q regions have been extensively mapped and many genes have been evaluated as candidate tumor suppressor genes, no tumorigenic genes have currently been definitively identified. In most cases, deletions seem to represent complete chromosomal arm loss, which might be the result of an unbalanced centromeric translocation of 1p and 19q [88, 89]. Even though the genes on 1p and 19q remain unidentified, many correlations have been made regarding 1p/19q co-deletion. For example, gliomas with 1p/19q co-deletion frequently show classic histology [83, 90, 91] and frequently have IDH1 and IDH2

mutations [58, 64, 68]. Anaplastic oligodendrogliomas with 1p/19q co-deletions preferentially have a proneural gene expression profile [92]. This profile, which is partly characterized by expression of neuronal genes, is over-represented among low-grade gliomas and might predict therapeutic response in GBMs [93]. However, 1p/19q co-deletion correlates inversely with TP53 mutations, 10q deletions, and amplification of EGFR [94].

Many studies have demonstrated the potential therapeutic value of 1p/19q co-deletions for glioma treatment. A recent study showed that patients with anaplastic oligodendrogliomas carrying 1p/19q co-deletion treated with PCV (procarbazine, CCNU, vincristine) had better-improved survival outcomes compared to the patients treated with temozolomide (TMZ) [95]. The long-term follow-up data from the European Organization for Research and Treatment of Cancer (EORTC) [96] and Radiation Therapy Oncology Group (RTOG) [97] trials testing radiotherapy vs radiotherapy plus adjuvant or neoadjuvant PCV in anaplastic oligodendrogliomas indicated that 1p/19q co-deletion is both prognostic and predictive of improved outcomes with PCV chemotherapy [84, 98]. Such data suggest that this marker is more useful as an indicator of tumor vulnerability to a broad range of therapeutic options than as a specific predictor of chemosensitivity. As a result, assessment of 1p and 19q status has been widely implemented in the neuro-oncological management of patients with anaplastic oligodendroglioma [99].

Thus, the 1p/19q status in oligodendroglial tumours has been frequently examined over the past 10 years. This molecular signature denotes a clinically distinct tumor type with progression, prognosis, and treatment responses that are different from those for other gliomas [81, 100]. Although the mechanisms by which 1p/19q co-deletion generates these clinical differences remain unclear, given the range of survival outcomes and challenge of reproducibly classifying astrocytomas, mixed

oligoastrocytomas, and oligodendrogliomas, 1p/19q co-deletion has become an important biomarker and predictor for glioma diagnosis and prognosis.

MGMT methylation

MGMT is a DNA repair enzyme that repairs O^6 alkyl guanine adducts [101]. Chemotherapy-induced alkylation at O^6 position triggers cytotoxicity and apoptosis. Tumor cells that express high levels of the MGMT repair protein may thereby counteract the therapeutic effect of alkylating agents, including nitrosourea compounds and temozolomide that are most commonly used for the treatment of malignant gliomas [102]. MGMT promoter methylation is frequently associated with other prognostic biomarkers: 1p19q co-deletion and IDH mutations. A strong association of MGMT hypermethylation with 1p/19q co-deletions [102-104] and IDH1 mutations [77] has been observed in oligodendroglial neoplasms. The co-incidence of MGMT promoter hypermethylation and IDH1 mutations has also been frequently found in diffusely infiltrating astrocytic gliomas [77].

MGMT is epigenetically inactivated via hypermethylation of the 5′-CpG island in approximately 40% of primary GBMs and over 70% of secondary GBMs [105]. MGMT promoter methylation is also observed in 50% of the diffuse and anaplastic astrocytomas as well as approximately 70% of the oligodendroglial and mixed tumors [105]. Aberrant methylation of CpG islands in the MGMT promoter region results in epigenetic silencing of gene transcription [106, 107]. Among malignant gliomas, however, the MGMT promoter methylation patterns are highly heterogeneous among tumors and it is unknown which particular CpG sites or combinations thereof need to be methylated for silencing the gene and conveying benefit from alkylating agent therapy. The various assays that are in current use to evaluate MGMT status assess different numbers of CpGs at distinct locations within the MGMT promoter, typically between 3 and 20 of a total of 97 CpGs [108, 109].

The clinical value of MGMT as a molecular marker is for predicting the patient's survival outcome regarding the response of malignant gliomas to alkylating chemotherapy using either nitrosourea compounds [110], temozolomide [111], or a combination of both [112]. In the EORTC/National Cancer Institute of Canada (NCIC) 22981/26981 trial [56, 111], patients with a methylated MGMT promoter survived significantly longer than patients without methylated MGMT promoter after treated with radiotherapy and temozolomide [56, 111]. Even though data from EORTC/NCIC 22981/26981 trial and another clinical study [113] showed that MGMT promoter methylation was predictive for longer survival only in patients who received temozolomide, a recent clinical study reported that MGMT promoter methylation may also be predictive of response to radiotherapy and associated with longer survival in the absence of adjuvant chemotherapy in glioblastoma patients [114].

While the prognostic role of MGMT in GBM patients not treated with chemotherapy is a matter of debate, recent data from the NOA-04 and the EORTC 26951 trials both showed that MGMT promoter methylation predicted prolonged survival irrespective of the initial treatment, i.e., radiotherapy, chemotherapy or a combination of both [67, 115]. However, the prognostic role of MGMT promoter methylation in patients with low-grade gliomas is unclear. One study showed that MGMT promoter methylation was a negative prognostic factor for progression of gliomas whereas no correlation with survival in low-grade gliomas [116]. In contrast, a phase II study of low-grade gliomas reported that patients with MGMT promoter methylated tumor had a better outcome than patients with tumor without MGMT promoter methylation after temozolomide treatment [117]. So the role of MGMT promoter methylation as a predictive biomarker of temozolomide sensitivity is still controversial. These results suggest MGMT promoter methylation may be a predictive biomarker in certain patient subsets.

Therefore, MGMT promoter methylation can be considered as an important clinical molecular marker in neuro-oncology. While treatment decisions in the routine setting are not yet based on this marker, the MGMT promoter methylation status is now used as an important stratification or selection parameter in ongoing clinical trials.

EGFR pathway alterations

EGFR signaling has the fundamental roles in regulating the differentiation of neural stem cells (NSCs) and neural progenitor cells (NPCs) [118, 119] and glia genesis [120, 121]. EGFR signaling through regulating NOTCH pathway maintain the balance between the NSC and NPC numbers in the subventricular zone (SVZ) [118]. Enhanced EGFR signaling *in vivo* results in the expansion of the NPC pool, and reduces NSC number and self-renewal. This occurs through a non-cell-autonomous mechanism involving EGFR-mediated regulation of Notch signalling [118]. Inhibition of EGFR signaling induces the neuronal differentiation of glial progenitors *in vivo* [120]. Furthermore, activation EGFR triggers quiescent astrocytes into reactive astrocytes [121].

In contrast, deregulated EGFR signaling (cell-surface EGFR overexpression, autocrine activation and EGFR gene mutation) contributes to the formation of many epithelial malignancies in humans [122, 123]. The oncogenic role of EGFR has been functionally validated in both cell culture–based systems and animal models with several tumor types including GBM tumorigenesis [122, 124, 125].

Normal EGFR pathway

EGFR belongs to ErbB/EGFR family, which consists of four members in mammals (EGFR, also known as ErbB1 or HER1; ErbB2, also known as HER2/neu; ErbB3, also known as HER3; and ErbB4, also known as HER4) that are thought to diverge from a common ancestral receptor [126, 127]. The structure

of each of the members comprises: a ligand-binding ectodomain with 2 cysteine-rich regions; a single transmembrane region; and, a cytoplasmic tyrosine kinase (TK) domain [128].

These four receptors utilize 13 different ligands to convert extracellular cues into intracellular signals [127]. The most common ligands for HER receptors are members of epidermal growth factors (EGF, such as heparin binding EGF-like growth factor, amphiregulin, epiregulin, betacellulin) and transforming growth factor α (TGFα) [129]. Interestingly, there is no known ligand for ERBB2, which is believed to undergo ligand-independent activation [126].

Binding of a cognate ligand to the ligand-binding site of HER receptors induces receptor homo- or hetero-dimerization, resulting in a conformational change that activates the intracellular TK domain. This results in autophosphorylation of the cytoplasmic tail of the receptor, which activates downstream signalings (such as phosphatidylinositol 3-kinase (PI3K)/AKT and the Ras-Raf-mitogen-activated protein kinase (MAPK) pathways) [126], and induces the transcription of genes controlling multiple cellular responses [126]. ErbB/EGFR family mediate various cellular processes, including cell division, migration, adhesion, differentiation, and apoptosis [130]. Because so many fundamental cellular processes are regulated by the EGFR signaling, deregulation of these components can lead to cancer and other diseases [126].

Deregulation of EGFR signaling in gliomas

EGFR overexpression

Deregulation of EGFR signaling is associated with poor prognosis in various tumor types, including breast cancer, head and neck cancer, prostate cancer, non–small cell lung cancer (NSCLC), and gliomas [131-136]. There are multiple mechanisms that can lead to deregulation of EGFR signaling in gliomas.

Of these mechanisms, increased EGFR abundance is frequently found in primary GBMs and can be caused by gene amplification, increased translation of the EGFR gene, or both. EGFR amplification occurs in 40%-70% of primary GBMs, but is not observed in lower-grade astrocytomas [132, 137], which indicates that EGFR activation may drive tumorigenesis in primary GBMs. Focal EGFR amplification occurs usually at an extremely high level (>20 copies) [124]. All primary GBMs with EGFR gene amplification have concurrent EGFR protein overexpression, but only a subset (70%-90%) of tumors with EGFR protein overexpression also show EGFR gene amplification, indicating that a fraction of GBM tumors show increased receptor abundance in the absence of gene amplification [138]. EGFR overexpression in primary GBMs is occasionally accompanied by increased abundance of its cognate ligands, EGF and TGFα. This suggests the existence of an autocrine loop that results in unregulated chronic EGFR signaling [139].

EGFR mutations

In addition to increases in receptor and ligand abundance, activating mutations of EGFR have also been found in GBMs. TCGA consortium has identified EGFR as the fourth most highly mutated gene in GBM tumors [140]. EGFR mutations can occur in extracellular domain and the cytoplasmic tail of the receptor [141-145].

A number of deletion mutations that occur in the EGFR extracellular domain are exclusively found in GBMs. These include the mutants that encode the EGFR type I and type II variants (EGFRvI and vII) [144, 145], which give rise to truncated proteins that are believed to be oncogenic. Other point mutations that also reside primarily in the extracellular region of EGFR are identified in ~14% of GBMs [142]. These mutations include R84K and A265V/D/T at the domain I/II interface, and P545L and G574V at the domain II/IV interface. Interestingly, these mutants are constitutively active but still capable of binding ligand [142]. Moreover, EGFR kinase domain mutations commonly found in NSCLCs are

rare in GBM, whereas extracellular mutations that are common in GBMs occur rarely in NSCLCs [134, 142]. However, the molecular basis of the organ site specificity of these mutations and their functional consequences remain unknown.

The cytoplasmic tail deletion mutants EGFRvIV and vV are also found exclusively in GBMs [141]. These mutations are thought to occur at a low frequency (~15% of EGFR-overexpressing GBMs) and may exhibit defects in receptor internalization. However, EGFRvIV and vV can still bind ligand and have the potential to modulate oncogenic signaling pathways commonly elicited by wt EGFR [146].

EGFRvIII mutant

The most common and best-studied EGFR mutation found in GBM is the type III EGFR variant deletion mutant (EGFRvIII), which is also found in NSCLCs, breast, and prostate cancers, albeit at much lower frequencies than in GBMs [147, 148]. This mutation has not been observed in normal tissue [148], but has been found in 20%-30% of overall GBM patients and 50%-60% in patients with EGFR amplification GBM [149-151]. However, EGFRvIII is not reported to be as prevalent in the secondary GBMs. Moreover, clinical studies have shown a correlation between the presence of the EGFRvIII receptor and poor prognosis in patients with GBM [152].

EGFRvIII is generated from a deletion of 801 base pairs in exons 2–7 of EGFR gene [153]. This deletion removes 267 amino acid from the extracellular domain, creating a junction site between exons 1 and 8 and a new glycine residue [154, 155]. The molecular mass of EGFRvIII is approximately 145 kDa [156]. This mutant has similarities to the v-ErbB transforming protein of avian erythroblastosis virus, which also is an EGFR-related auto-activating oncogene generated by a large extracellular deletion [157].

The disarrangement in the extracellular domain of EGFRvIII results in crucial changes in the functional characteristics of the receptor. Although the truncated extracelluar domain is unable to bind any known EGFR ligand [158, 159], the receptor shows constitutive tyrosine kinase activity [160]. While the strength of its constitutive kinase activity remains controversial [160-162], it is generally accepted that this constitutive activation is important for its pro-oncogenic effects because a kinase-deficient EGFRvIII is unable to confer a similar oncogenic advantage [132, 162, 163].

EGFRvIII shows defective internalization, resulting in its constitutive localization to the plasma membrane [162]. EGFRvIII is internalized at a much slower rate than unstimulated wt EGFR [164], and the small amount of internalized EGFRvIII receptors is not transported to the lysosome for degradation but rather recycled back to the cell surface [165]. So it is likely that the signaling potency of EGFRvIII is increased by its ability to prolong kinase activity and downstream signaling through its inefficient endocytosis and degradation and rapid recycling [132, 162, 165, 166].

Another mechanism for enhanced EGFRvIII signaling is through forcing EGFRvIII to form homodimers or heterodimers with either the EGFR or ErbB2 [158, 161, 167-170]. Moreover, EGFRvIII positive glioma cells can secrete microvesicles delivering EGFRvIII into surrounding EGFRvIII negative cells, thereby ‘‘passing on” EGFRvIII-mediated signaling and enhanced tumourigenicity [171].

EGFRvIII activates several downstream pathways, but a considerable amount of evidence indicates that it preferentially activates the PI3K/AKT signal transduction pathway [172-176]. EGFRvIII expression is tightly correlated with the activation of downstream targets of PI3K/AKT, including the mammalian target of rapamycin (mTOR), the forkhead box (FOX) transcription factor family and S6 [177]. EGFRvIII could activate CRTC2 via the PI3K/AKT pathway,

which in turn leads to stimulation of the NF-κB pathway and resistance to chemotherapy [178]. Selective activation of the PI3K/AKT pathway by EGFRvIII is also thought to mediate the resistance to radiation in EGFRvIII-positive GBM [177, 179-183]. Moreover, EGFRvIII signaling via the PI3K/AKT pathway may be facilitated by associated loss or mutation of the PTEN gene, which occurs in approximately 40% of patients with EGFRvIII mutant GBM [184-187].

EGFRvIII is also reported to co-operatively work with Src family kinases (SFKs) to enhance GBM tumorigenicity [188, 189]. Genetic or pharmacological inhibition of SFKs inhibited cell motility *in vitro* and growth of EGFRvIII-expressing GBM xenografts *in vivo* [188, 190]. EGFRvIII signaling leads to the phosphorylation of Tyr772 on SFKs and thereby activates dedicator of cytokinesis 1 (DOCK1; also known as DOCK180), a guanine nucleotide exchange factor with roles in cell motility, survival and proliferation [190]. Genetic ablation of DOCK1 blocked the EGFRvIII-mediated tumorigenicity of GBM cells [190].

The EGFRvIII signaling is also thought to be associated with the enhanced signaling of angiogenesis in GBM cells. The tumorigenicity of GBM cell lines can be increased by EGFRvIII transfection [191, 192]. Genetically inhibiting the NF-κB pathway could reverse this increased tumorigenicity and concurrently lead to reducation in the VEGF and interleukin-8 (IL-8) expression and with decrease in tumor angiogenesis [191, 192]. In contrast, EGFRvIII transfection could significantly increase IL-8 expression through the NF-κB pathway in GBM cells. RNA-interference-mediated knockdown of the IL-8 pathway or the NF-κB pathway inhibited GBM xenograft growth and attenuated angiogenesis [191],

Furthermore, EGFRvIII could activate multiple receptors in glioma cells such as MET, platelet-derived growth factor receptor beta (PDGFRB) and VEGF receptor 2 (VEGFR2) [193-195], all of which regulate the cell proliferation. EGFRvIII has also been showed to stimulate the production of cytokines, including IL-6 and

leukemia inhibitory factor (LIF) [196]. Importantly, these cytokines can activate overexpressed wt EGFR in neighboring GBM cell, which led to enhanced GBM cell proliferation [196]. Thus, EGFRvIII contributes to the growth of surrounding GBM cells through bystander effect. This indicates that EGFRvIII actively contributes to the heterogeneity of GBM by acting indirectly on EGFRvIII negative neighboring cells. This hypothesis is consistent with the observation that wt EGFR amplification and EGFRvIII expression are usually co-exist in GBM samples.

Finally, both EGFRvIII and wt EGFR/ErbB family proteins have been identified in the nucleus and are thought to drive proliferation and DNA damage repair through both transcriptional and signaling functions [197]. Moreover, EGFR is also observed to translocate to the mitochondria [198]. All these provide evidence that the contributions of EGFR malignancy may not be limited to its conventional cell membrane location.

PDGFRA pathway

PDGF ligands

Platelet-derived growth factor (PDGF) was discovered in the mid-1970s and is an approximately 30 kDa dimeric glycoprotein composed of two chains [199, 200]. Each chain is encoded by an individual gene located on chromosomes 7, 22, 4, and 11, respectively [201]. There are four identified genes encoding the PDGF monomer chains: *PDGF-A, PDGF-B*, *PDGF-C* and *PDGF-D* [200, 202-204]. The products of these genes dimerize and form five PDGF proteins: PDGF-AA, -AB, -BB, -CC, and –DD [205].

PDGF target a broad spectrum of mesoderm-derived cells, such as fibroblasts, pericytes, smooth muscle cells, or mesangial cells and glial cells [206]. PDGFs are major mitogens for connective tissue cells and glia cells, play a number of critical roles in normal embryonic development, cellular differentiation, and

response to tissue damage, as well as in pathologic processes, such as wound healing, inflammation, and neoplasms [207-210].

The receptor for PDGF (PDGFR) belongs to class III family of RTK. Two genes, *PDGFRA* and *PDGFRB*, encoding the alpha-type and beta-type PDGFRs, are highly homologous and share a common architecture [211]. They consist of five N-terminal Ig-like domains, which bind to various isoforms of PDGF, a single transmembrane domain and a split protein tyrosine kinase domain. PDGFRA binds to PDGF-AA, PDGF-BB and PDGF-AB, whereas PDGFRB binds with high affinity to PDGF-BB and PDGF-AB [212]. Upon activation by PDGF, these receptors dimerise, and are "switched on" by auto-phosphorylation of several sites on their cytosolic domains, which serve to mediate binding of cofactors and subsequently activate signal transduction [213].

PDGFRA

The gene encoding PDGFRA is located at chromosome 4q11-12, which spans 23 exons and encodes a transmembrane protein composed of five IgG-like domains in the extracellular region, a transmembrane domain, an ATP binding site and a hydrophilic kinase insert domain in the intracellular portion [214].

Ligand-activated PDGFRA promotes its interaction with and activation of SH2 domain–containing proteins, including SFKs, phosphotyrosine phosphatase SHP2, PI3K, and PLCγ [215, 216]. In particular, PI3K has been identified as the major effector of PDGFRA signaling *in vitro* and *in vivo* [216-218]. SFKs and PLCγ contribute to some but not all PDGFRA functions [217-219], whereas SHP2 is not required for cell survival during Xenopus embryogenesis [217].

Involvement of PDGFRA pathway in glia genesis

Many studies show that PDGFRA is required for the genesis of oligodendrocyte precursor cells (OPCs) [220, 221]. PDGFRA regulates the timing of oligodendrocyte maturation through controlling the cell cycle progression of OPCs

[220]. PDGFRA signaling promotes OPC proliferation and therefore regulates the OPC number combining with PI3K and PLC-γ pathways [220, 222]. Furthermore, PDGFRA-expressing B cells in SVZ have been demonstrated functioning as progenitors of neurons and oligodendrocytes *in vivo* [221]. Increased PDGF signaling in these cells stimulates their proliferation and blocks their ability to give rise to differentiated progeny, causing them to form tumor-like growths resembling astrocytomas. Interestingly, similar PDGFRA-expressing astrocytes are found to exist in the adult human SVZ [221].

Involvement of PDGFRA pathway in glioma pathogenesis

PDGFRA has been reported overexpressed and/or activated in many tumor types, such as gliomas [223, 224], gastrointestinal stromal tumors [225], medulloblastomas [226], sarcomas [227], ovarian tumors [228] and lung cancers [229]. PDGFRA activation in cancer occurs as a consequence of gene amplification [223, 224], chromosomal rearrangements [230], mutations [28, 231], or autocrine/paracrine engagement [232]. The receptors mediate signals critical to cell growth and survival [233], transformation [234], migration [235], and vascular permeability [236]. Inhibition of PDGFRA signaling resulted in a reversion of transformed phenotype in glioma cell lines [237], or a reversion from high-grade to lower grade tumor histology in mouse model [238].

PDGFRA amplification is identified in 15% of all gliomas and is enriched in the proneural subtype of GBMs [28, 40]. A recent study showed that *PDGFRA* amplification was detected in 27 (16.3%) of 166 diffuse astrocytomas, significantly more frequent than in diffuse oligodendrogliomas (3 [2.6%] of 115). The vast majority of diffuse astrocytomas showed IDH1/2 mutations and/or *PDGFRA* amplification (154 [93%] of 166). Mean survival of diffuse astrocytoma patients with *PDGFRA* amplification was similar to that with IDH1/2 mutations[224]. Another study reported that *PDGFRA* amplifications were more frequent in anaplastic astrocytomas than in diffuse astrocytomas,

oligodendrogliomas, and oligoastrocytomas [239]. Approximately 11% of GBMs are reported with *PDGFRA* amplification that is the second most frequent RTK gene amplification in GBMs. However, one study shows that some gliomas with *PDGFRA* gene amplification do not display PDGFRA protein expression [240]. Moreover, the pattern of PDGFR-pathway activation at the protein level even in the absence of *PDGFRA* amplification is also reported in GBM [29]. So the mechanism of regulating *PDGFRA* gene amplification in gliomas should be further investigated.

The *PDGFRA* gene rearrangements and mutations are also found in human gliomas. Deep sequencing analysis of GBM has found several point mutations of the Ig-like domain [28]. Recent work shows that 40% of GBMs with PDGFRA amplification harbor an intragenic deletion, termed PDGFRA$^{\Delta 8,\ 9}$ [241], in which an in-frame deletion of 243 base pairs (bp) of exons 8 and 9 leads to a truncated extracellular domain [230]. In addition, in-frame gene fusion of the extracellular domain of KDR/VEGFR-2 and the kinase and intracellular domains of PDGFRA has also been identified, and both the PDGFRA$^{\Delta 8,\ 9}$ and KDR-PDGFRA mutant proteins were constitutively active and transforming and could be inhibited with inhibitors of PDGFRA [230]. Tumors with these two types of PDGFRA rearrangement displayed histological features of oligodendroglioma [230].

Of the additional ways to activate PDGFR signaling, PDGF ligands (A–D) are up-regulated in 30% of glioma surgical samples and cell lines [237, 242]. In clinical glioma specimens, PDGFRA and PDGF-A are overexpressed in tumor cells, while PDGFRB is only expressed in endothelial and peri-endothelial compartments [243]. Specific activation of PDGFRA signaling by infusion of PDGF-A proteins, which only bind to PDGFRA in PDGFRA-positive type B NSCs in the SVZ, leads to glioma-like growth of these cells in adult brain [221]. PDGF-B, which binds to both PDGFRA and PDGFRB, is an oncoprotein that causes glioma formation in

the brain [244]. In mice, PDGFB overexpression in neonatal CNS or adult CNS, either by the transgenic approach on a p53-/- background [245], or by the retroviral gene transfer approach [244, 246, 247], generated glioma-like tumor growth. Interestingly, tumors generated in these models all expressed PDGFRA [245, 246, 248]. The intratumoral co-expression of PDGF and PDGFR suggests that both autocrine and paracrine loops play roles in gliomas.

However, the occurrence and clinical relevance of PDGFRA expression in different glioma subtypes is debated and controversial as both enhanced as well as negative expression patterns have been reported in GBM [240, 249, 250]. Recently, enriched PDGFRA expression was reported in the proneural GBM subtype which was seen to be associated with a better prognosis compared to the other molecular subtypes of GBM [28, 40], while other studies reported no correlation between PDGFRA expression and clinical-pathological parameters of glioma patients [240].

Cellular origins of gliomas

It has been accepted that cancer occurs as a consequence of genetic and epigenetic alterations in a differentiated cell. These alterations could provide a proliferative advantage and ultimately lead to uncontrolled growth and spread of the malignant cells. This theory suggests that tumors, such as gliomas, arise from terminally differentiated astrocytes and oligodendrocytes that obtain accumulated mutations and therefore "de-differentiate" into a less differentiated phenotype [251-253]. However, this hypothesis has not been adequately tested. This theory also fails to explain adequately the origin of mixed glioma, the oligoastrocytoma.

As in other cancers, the continued interest in cellular origins of gliomas is stimulated by the possibility that an improved understanding of cellular origin will help identify fundamental pathways and lineage dependencies that could

represent novel diagnostic and therapeutic targets [254]. However, the cellular origins of gliomas remain controversial. Some studies showed that glioma might originate from NSCs. Successful isolation of tumor cells with stem cell features (termed as cancer stem cells) from human gliomas [255] implies NSCs as the cell of origin. However, such NSC-like features of malignant glioma cells could be acquired during transformation rather than reflect the nature of the original cell type [254]. Nevertheless, another study reported that introducing genes such as v-myc and H-Ras into human fetal NSCs led to tumorigenic transformation of NSCs, which resulted in heterogeneous glial tumors with some characteristics of cancer stem cells (small numbers of nestin-positive neural stem-like cells) [256].

Further evidence supporting the NSC origin of glioma was obtained from mouse genetic studies. The inactivation of tumor suppressor genes TP53 and NF1 or the expression of a mutant form of TP53 in NSCs consistently led to glioma formation in mouse models, and the physical locations of tumors appeared to associate with the SVZ where adult NSCs reside [257-259].

Although many studies have demonstrated the role of NSCs in gliomagenesis, glial progenitors may be as more plausible cellular origins of gliomas because they are much more susceptible to neoplastic transformation [260]. Adult glial progenitors have the proliferative and self-renewing capacity that is needed to form malignant tumors [260]. Glial precursors can also be found throughout the brain and can behave in a malignant manner when overstimulated with high levels of growth factors such as PDGF and EGF [246, 261, 262]. Moreover, several studies have showed that the glial progenitors including astrocytes precursor cells (APCs) or OPCs might directly transform into glioma cells [263-265].

Recently, the OPC origin of glioma has been successfully and convincingly demonstrated in mouse models [266, 267]. In this study by using mosaic analysis with double markers (MADM) techniques, a mouse genetic mosaic system was generated to analyze aberrations in individual cell lineages before the final

transformation, which allows for the screening of the cell of origin. After initiating p53/Nf1 mutations sporadically in NSCs, mutant NSCs and all of their progeny at pre-malignant stages were analyzed. Only mutant NSCs generated neoplastic oligodendrocyte precursor cells (OPCs), which were PDGFRA-positive. All other NSCs-derived cell types, including NSCs themselves, remained mostly unaffected by the disruption of the two tumor suppressive pathways. When p53/Nf1 inactivation is targeted specifically to OPCs, tumors form as NSCs-derived gliomas. Interestingly, these tumors acquired the expression of NSCs genes, which could be misleading during analysis in further stages of the tumor development. The findings demonstrate that, in p53/Nf1 mutation-driven gliomas, mutations may initially occur in either NSCs or OPCs, but only OPCs provide the suitable cellular context needed for transformation.

Even though murine studies have been helpful in clarifying glioma origins, it remains to be verified whether the findings can be fully extended to human disease. For example, human and mouse OPCs may be biologically similar, as recent study has demonstrated conserved mechanisms of oligodendroglioma formation through disruption of asymmetric division of $NG2^{+}$ OPCs in mouse verbB/$p53^{+/-}$-induced oligodendrogliomas and in human oligodendrogliomas [268]. However, in adult mice, the SVZ is a prominent regenerative zone including many types of stem cells, but the analogous region in adult human brains may not harbor similar numbers or types of stem cells [269]. However, it is still important to continue to model human disease in mice considering the powerful and mature technologies that make sophisticated genetic targeting possible. These approaches will be invaluable to explore clinical observations such as the distinct differences in location between IDH1 mutant and wt GBMs [65].

Finally, given the diversity of histological subtypes, various subsets of molecular patterns and subclasses, and increasingly number of stem and progenitor

cells in the brain, further studies will be required to clarify the origins of malignant gliomas and the mechanisms that drive their pathogenesis.

This thesis

Background

The current WHO classification scheme for glioma is highly subjective and cannot clearly diagnose a substantial fraction of gliomas due to their atypical histology. Transcriptome-based molecular classification is expected to overcome the limitations of morphological diagnosis and to develop new treatment strategies in an era of personalized medicine. However, challenges in defining molecular classifiers limit the development and clinical application of molecular classification of gliomas.

EGFR and PDGFRA are two RTKs that govern cell fate specification, cell proliferation and migration in NSCs compartment and glial development [270, 271]. EGFR is frequently amplified, mutated and overexpressed in GBMs [28, 141]. Enforced EGFR signaling results in expansion and blocked differentiation of OPCs [272].

Contrary to EGFR, PDGFRA is a characteristic marker for widely distributed OPCs, depletion of PDGFRA signaling resulted in diminished generation of oligidendrocytes [221]. Autocrine stimulation of PDGFRA signaling is suggested to be important for glioma initiation and progression [243, 244]. However, compared to frequent amplification and mutation of EGFR, alterations of PDGFRA are less frequent in glioma genomes [273]. These findings indicated distinct expression pattern of EGFR and PDGFRA in gliomas. Therefore, EGFR and PDGFRA may serve as the candidates for molecular classification of gliomas.

Aims of the present studies

The overall goal of the present study was to investigate the role of EGFR and PDGFRA in the classification and identification of therapeutic targets for human gliomas, in particular, to study the clinical relevance of PDGFRA expression and trafficking.

Specific aims of the studies

Paper I

To establish a glial genesis-guided molecular classification scheme for gliomas based on the genes co-expressed with EGFR or PDGFRA

Paper II

To clarify the mechanism of PDGFRA expression and the clinical relevance of PDGFRA expression in diffierent glioma subtypes

Paper III

To investigate the role of cell surface PDGFRA expression in regulating glioma cell proliferation.

Results and discussion

Glioma molecular classification defined by co-expression modules of EGFR and PDGFRA (Paper I)

Using 69 genes that are co-expressed with EGFR (EGFR module, EM) or PDGFRA (PDGFRA module, PM), we have shown here that adult low- and high-grade gliomas from disparate institutions and ethnic backgrounds can be classified into EM, PM and $EM^{low}PM^{low}$ subtypes. This classification scheme is not guided by the morphological diagnosis of glioma or the survival outcome of the patients. EM gliomas are associated with enriched expression of genes involved in the NSC compartment and astrogenesis, and they occur predominantly in patients older than 50 years and with an overall survival period of less than 2 years. Conversely, PM gliomas are associated with enriched expression of genes regulating oligodendrogenesis and occur predominantly in patients younger than 50 years and with significantly longer survival time. $EM^{low}PM^{low}$ gliomas are enriched in mature neuron and oligodendrocyte signatures with ages at diagnosis and survival outcomes similar to the PM gliomas. The three glioma subtypes show unique patterns of genomic alteration. Our findings suggest that EM, PM and $EM^{low}PM^{low}$ glioma subtypes might represent biologically separate entities with distinct cellular origins, genetic alterations and prognoses.

Previous classifications have identified genes that were expressed highly variably between glioma samples [28, 49-51] or that correlated significantly with the survival outcome of glioma patients [40, 41, 274]. The relevance of those classifiers to glioma pathogenesis and the relationships between the classifiers were largely unknown. Based on the significance of EGFR and PDGFRA signaling in glial genesis and glioma development, we chose these two genes as a basis for our identification of co-expression networks to classify gliomas.

The co-expression signatures of EM and PM were found to be reproducible in three independent glioma data sets from three countries (China, USA and the Netherlands) that contain all major morphological subtypes and grades. These data sets differ in their composition of patients and controls, in the measurement platforms used, and likely also in the details of biopsy processing. Subsets of EM were enriched in murine astrocyte precursor cells and subsets of PM were enriched in oligodendrocyte precursor cells. Furthermore, chromosomal regions encompassing subsets of EM or PM genes were recurrently and concomitantly altered in glioma genomes. Gene products of EM and PM are predicted to form protein-protein interaction networks. Thus, EM and PM networks likely reveal the inherent organization of glioma transcriptomes. Although only a small subset of EM and PM genes are currently known to be involved in glial development and glioma genesis [275, 276], our findings indicate that coherent functions of EM and PM genes are involved in the pathogenesis of EM and PM glioma subtypes respectively, and that hub genes of the EM and PM networks could be candidate therapeutic targets for the respective glioma subtypes.

Between the EM and PM glioma subtypes, we found differential expression of SVZ astrocyte markers, genes regulating astrogenesis or oligodendrogenesis, and the signatures of astrocytes, oligodendrocytes or neurons during development or at maturation. EM gliomas show gene expression patterns related to NSCs and their progenitors along the astrogenesis pathway, PM along the OPC differentiation pathway, and $EM^{low}PM^{low}$ along the oligodendrocyte and neuron maturation pathway.

In summary, the EM/PM classification scheme described here is applicable to gliomas of all major morphological subtypes, and can predict the prognosis of glioma patients. EM/PM glioma subtypes are specifically associated with glial genesis activities, signatures of glial cell types during development or maturation, and known glioma genomic abnormalities. This suggests that signaling pathways

specific for CNS cell lineages and differentiation stages are differentially involved in, and may account for, the etiology of EM/PM glioma subtypes. Our findings create a new framework towards establishing molecular diagnostic tools and identifying new therapeutic targets to combat gliomas.

Better prognosis of patients with glioma expressing FGF2-dependent PDGFRA irrespective of morphological diagnosis (Paper II)

Using two large data sets consisting of 648 glioma samples of all major morphological subtypes and grades, we found that under the supervision of morphological diagnosis, PDGFRA expression was enriched in low-grade gliomas compared to high-grade gliomas. However, all morphological subtypes were represented among gliomas with enriched PDGFRA expression. The top 25% of gliomas with high levels of PDGFRA expression (PDGFRA-high) were significantly associated with concomitant *IDH1* mutation, higher frequency of deletions at 1p and 19q, lower frequency of EGFR amplification, younger age at diagnosis and better patient survival, compared to the below 25% of gliomas with lower levels of PDGFRA expression (PDGFRA-low).

However, different mechanisms may account for high-level PDGFRA expression in gliomas. *PDGFRA* gene amplification and mutation in gliomas with high expression levels was reported previously [230, 241]. Our findings showed that in adulthood gliomas as analyzed in this report, amplification of *PDGFRA* gene was unlikely the main cause of PDGFRA overexpression in gliomas. Our findings in cell culture studies and expression analysis in glioma samples supported the hypothesis that PDGFRA expression was dependent on the niche factors in gliomas. However, correlated expression between FGF2 and PDGFRA at the mRNA level was not observed in either the Rembrandt or GSE16011 data sets. FGF2-dependent PDGFRA expression as observed in our study is likely an indirect effect; FGF2-dependent PDGFRA expression is probably applicable only to a specific subset of gliomas.

FGF2 is widely expressed in normal brain astrocytes and also in gliomas [249, 277-280]. In large numbers of glioma samples, our results showed that the pattern of FGF2 expression was similar to that of PDGFRA expression. In the low-passage cell lines tested in our study, FGF2 was able to induce and maintain PDGFRA expression *in vitro*. Moreover, Maintenance of PDGFRA expression was concordant with the expression of a subset of gliogenic genes. We speculate that FGF2 mediated signaling can potentially be manipulated to suppress PDGFRA expression and thereby inhibit niche factor-dependent glioma growth.

FGF2-dependent PDGFRA expression appears to be a converged mechanism in normal glial development and glioma genesis. Enhanced signaling of FGF2 or PDGFRA results in proliferation but blocked differentiation of OPCs towards oligodendrocytes [248, 281, 282]. Although it is beyond the scope of this report, we speculate that additional features governing differentiation and proliferation of oligodendrocyte lineage can be detected in gliomas with enriched PDGFRA expression.

Glioma cell proliferation controlled by ERK activity-dependent surface expression of PDGFRA

In this study we demonstrate that glioma cell proliferation correlated with the extent of surface expression of PDGFRA in both glioma cell lines and their corresponding tumor samples. We also find that MEK inhibitor U0126 treatment can decrease the surface PDGFRA expression and result in deviation of PDGFRA from endosomal trafficking and recycling compartment to the Golgi network in a reversible, dose- and time-dependent manner without affecting total PDGFRA expression. U0126 mediated down-regulation of PDGFRA surface expression correlates with diminished cell proliferation.

PDGFRA signaling is likely dependent on PDGFRA endocytosis because endocytosis of receptor into endosomes (where the MEK signal molecules reside

and ERK molecules are activated) is required for the activation of signaling [283]. However, our findings suggest a causal relationship in the reverse direction, with surface PDGFRA expression being regulated by the activity of MEK-ERK. Our results show that the number of PDGFRA molecules in plasma membrane decreases following enhanced ERK activity. Consequently, the ability of glioma cells to respond to their microenvironment may be blunted. Moreover, MEK and ERK are aberrantly active in gliomas and in many other cancers. Our findings indicate a new possibility that the trafficking of the receptors can be targeted by the signaling activities of the receptors.

MEK might regulate PDGFRA surface expression via the activation of ERK molecules, which in turn translocate to the nucleus to activate RTK related transcriptional programs; surface expression of PDGFRA could be a consequence of the activated transcriptional programs [284]. In this scenario, PDGFRA surface expression increases in proportion to total PDGFRA expression, which is both inefficient and slow. Our findings suggest the existence of more efficient mechanism to regulate PDGFRA surface expression via controlling the intracellular trafficking system. Our data clearly show that the MEK inhibitor U0126 down-regulated surface PDGFRA expression within 6 hours without noticeably changing total PDGFRA expression. Surprisingly, after an initial drop in ERK phosphorylation in response to U0126 treatment, a strong enhancement was seen after 18 hrs. This U0126 induced positive feedback of ERK activity has also been observed in hepatocellular carcinoma cells [285]. Moreover, our findings suggest that inhibition of MEK coupled with a strong positive feedback of ERK activity may in turn regulate steady-state RTK trafficking, resulting in a re-localization of PDGFRA from internalizing and recycling endosomes to the Golgi apparatus.

Previous reports demonstrate that Ras-PI3K, but not MEK, signaling regulates the trafficking of signal molecules between the cytoplasm and nucleus [286]. In

contrast, MEK but not PI3K signals regulate cellular global trafficking events, i.e. tubulin nucleating [287, 288] or actin remodeling [289, 290]. Both these processes are of fundamental importance for trafficking of signal proteins between the plasma membrane and cytosolic compartments. Furthermore, our findings demonstrate that the MEK signaling could directly regulate the trafficking-related endocytic and recycling processes. U0126 treatment significantly decreased co-localization of PDGFRA molecules to clathrin and caveolin, but increased PDGFRA localization to the Golgi apparatus, as assessed by co-localization with giantin. This re-localization of PDGFRA coincided with diminished localization of PDGFRA to EEA1-positive early endosomes and RAB11-positive recycling endosomes. These results clearly show that positive feedback of ERK activity deviates PDGFRA from the intracellular recycling trafficking network to the Golgi apparatus. These findings are compatible with the localization and generation of Ras-Raf-MEK-ERK complexes in the early endosome compartment [291]. These results indicate an interesting scenario in that in addition to PDGFRA trafficking in gliomas, this mechanism might be applicable to the trafficking of other RTKs in other cancers, thereby providing a new approach to design cancer therapy strategy.

Conclusions and future perspectives

The current thesis has firstly focused on establishing a glial genesis-guided molecular classification scheme for gliomas based on the gene modules co-expressed with EGFR (EM) or PDGFRA (PM). From our results we can conclude that 1) EM/PM classification scheme can be successfully established using 69 genes that are co-expressed with EGFR or PDGFRA; 2) the EM/PM classification scheme is applicable to gliomas of all major morphological subtypes, and can predict the prognosis of glioma patients; 2) EM/PM glioma subtypes are specifically associated with glial genesis activities, signatures of glial cell types during development or maturation, and known glioma genomic abnormalities.

As discussed above, EGRFR and PDGFRA are both key regulators in normal and malignant glia genesis. That is the critical reason why we choose them to identify classifiers for glioma classification. Not only RTKs but also other key regulators in glia genesis may also be good candidates for using molecular classification for gliomas. Furthermore, identifying the crucial therapeutic targets for each subtype of glioma under EM/PM classification should be the missions in future.

Secondly, we also have made effort to clarify the clinical relevance of PDGFRA in gliomas and the mechanism of regulating PDGFRA expression in primary glioma cells. We found that 1) PDGFRA expression was enriched in low-grade gliomas compared to high-grade gliomas; 2) gliomas with high level PDGFRA expression are associated with concomitant *IDH1* mutation, higher frequency of deletions at 1p and 19q, lower frequency of EGFR amplification, younger age at diagnosis and better patient´s survival; 3) amplification of *PDGFRA* gene was unlikely the main cause of PDGFRA overexpression in gliomas; 4) FGF2 can induce and maintain PDGFRA expression in primary glioma cells.

Even though our data showed that gliomas with high level PDGFRA expression have better patient's survival, the abnormal expression of PDGFRA was only

found in gliomas but not in non-tumor samples. So the reason why abnormal PDGFRA expression can lead to good clinical outcome needs to be found out in further studies. In addition, how FGF2 maintain and induce PDFDRA should also be further studied. Moreover, FGF2-dependent PDGFRA expression should also be verified in vivo.

Thirdly, we also investigated the role of cell surface expression of PDGFRA in regulating glioma cell proliferation. Our results showed that 1) glioma cell proliferation correlated with the extent of surface expression of PDGFRA in both glioma cell lines and their corresponding tumor samples; 2) MEK inhibitor U0126 treatment can decrease the surface PDGFRA expression and result in deviation of PDGFRA from endosomal trafficking and recycling compartment to the Golgi network in a reversible, dose- and time-dependent manner without affecting total PDGFRA expression; 3) U0126 mediated down-regulation of PDGFRA surface expression correlated with diminished cell proliferation.

In our study we demonstrated that gliomas with high level PDGFRA expression have better patient's survival. We also demonstrated that glioma cells with high cell surface expression level of PDGFRA have stronger capacity of cell proliferation. It seems these two findings are conflictive. There are many possible explanations for this confliction. However, the best way to clear the conflict is to clarify the potential mechanisms of regulating these two findings. Moreover, the mechanism of U0126 decreasing PDGFRA cell surface expression in glioma cells should be further studied.

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References

1. Mamelak AN, Jacoby DB: Targeted delivery of antitumoral therapy to glioma and other malignancies with synthetic chlorotoxin (TM-601). *Expert opinion on drug delivery* 2007, 4(2):175-186.

2. Goodenberger ML, Jenkins RB: Genetics of adult glioma. *Cancer genetics* 2012, 205(12):613-621.

3. Dolecek TA, Propp JM, Stroup NE, Kruchko C: CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. *Neuro-oncology* 2012, 14 Suppl 5:v1-49.

4. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P: The 2007 WHO classification of tumours of the central nervous system. *Acta neuropathologica* 2007, 114(2):97-109.

5. Fisher JL, Schwartzbaum JA, Wrensch M, Wiemels JL: Epidemiology of brain tumors. *Neurologic clinics* 2007, 25(4):867-890, vii.

6. Group IS: Brain tumour risk in relation to mobile telephone use: results of the INTERPHONE international case-control study. *International journal of epidemiology* 2010, 39(3):675-694.

7. Linos E, Raine T, Alonso A, Michaud D: Atopy and risk of brain tumors: a meta-analysis. *Journal of the National Cancer Institute* 2007, 99(20):1544-1550.

8. Wrensch M, Wiencke JK, Wiemels J, Miike R, Patoka J, Moghadassi M, McMillan A, Kelsey KT, Aldape K, Lamborn KR *et al*: Serum IgE, tumor epidermal growth factor receptor expression, and inherited polymorphisms associated with glioma survival. *Cancer research* 2006, 66(8):4531-4541.

9. Farrell CJ, Plotkin SR: Genetic causes of brain tumors: neurofibromatosis, tuberous sclerosis, von Hippel-Lindau, and other syndromes. *Neurologic clinics* 2007, 25(4):925-946, viii.

10. Wen PY, Kesari S: Malignant gliomas in adults. *The New England journal of medicine* 2008, 359(5):492-507.

11. Biernat W, Huang H, Yokoo H, Kleihues P, Ohgaki H: Predominant expression of mutant EGFR (EGFRvIII) is rare in primary glioblastomas. *Brain pathology* 2004, 14(2):131-136.

12. Ohgaki H, Kleihues P: Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. *Journal of neuropathology and experimental neurology* 2005, 64(6):479-489.

13. Hall A: Rho GTPases and the actin cytoskeleton. *Science* 1998, 279(5350):509-514.

14. van den Bent MJ: Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. *Acta neuropathologica* 2010, 120(3):297-304.

15. Louis DN: Molecular pathology of malignant gliomas. *Annual review of pathology* 2006, 1:97-117.

16. Mason WP, Cairncross JG: Invited article: the expanding impact of molecular biology on the diagnosis and treatment of gliomas. *Neurology* 2008, 71(5):365-373.

17. Rao RD, Uhm JH, Krishnan S, James CD: Genetic and signaling pathway alterations in glioblastoma: relevance to novel targeted therapies. *Frontiers in bioscience : a journal and virtual library* 2003, 8:e270-280.

18. Sathornsumetee S, Rich JN: Designer therapies for glioblastoma multiforme. *Annals of the New York Academy of Sciences* 2008, 1142:108-132.

19. Lindblad-Toh K, Tanenbaum DM, Daly MJ, Winchester E, Lui WO, Villapakkam A, Stanton SE, Larsson C, Hudson TJ, Johnson BE *et al*: Loss-of-heterozygosity analysis of small-cell lung carcinomas using single-nucleotide polymorphism arrays. *Nature biotechnology* 2000, 18(9):1001-1005.

20. Yeakley JM, Fan JB, Doucet D, Luo L, Wickham E, Ye Z, Chee MS, Fu XD: Profiling alternative splicing on fiber-optic arrays. *Nature biotechnology* 2002, 20(4):353-358.

21. Albertson DG, Pinkel D: Genomic microarrays in human genetic disease and cancer. *Human molecular genetics* 2003, 12 Spec No 2:R145-152.

22. Hoque MO, Lee CC, Cairns P, Schoenberg M, Sidransky D: Genome-wide genetic characterization of bladder cancer: a comparison of high-density single-nucleotide polymorphism arrays and PCR-based microsatellite analysis. *Cancer research* 2003, 63(9):2216-2222.

23. Liotta LA, Espina V, Mehta AI, Calvert V, Rosenblatt K, Geho D, Munson PJ, Young L, Wulfkuhle J, Petricoin EF, 3rd: Protein microarrays: meeting analytical challenges for clinical applications. *Cancer cell* 2003, 3(4):317-325.

24. Shi H, Wei SH, Leu YW, Rahmatpanah F, Liu JC, Yan PS, Nephew KP, Huang TH: Triple analysis of the cancer epigenome: an integrated microarray system for assessing gene expression, DNA methylation, and histone acetylation. *Cancer research* 2003, 63(9):2164-2171.

25. Shendure J, Ji H: Next-generation DNA sequencing. *Nature biotechnology* 2008, 26(10):1135-1145.

26. Meyerson M, Gabriel S, Getz G: Advances in understanding cancer genomes through second-generation sequencing. *Nature reviews Genetics* 2010, 11(10):685-696.

27. Chin L, Hahn WC, Getz G, Meyerson M: Making sense of cancer genomic data. *Genes & development* 2011, 25(6):534-555.

28. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP *et al*: Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer cell* 2010, 17(1):98-110.

29. Brennan C, Momota H, Hambardzumyan D, Ozawa T, Tandon A, Pedraza A, Holland E: Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. *PloS one* 2009, 4(11):e7752.

30. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP *et al*: Identification of a CpG island methylator

phenotype that defines a distinct subgroup of glioma. *Cancer cell* 2010, 17(5):510-522.

31. Rickman DS, Bobek MP, Misek DE, Kuick R, Blaivas M, Kurnit DM, Taylor J, Hanash SM: Distinctive molecular profiles of high-grade and low-grade gliomas based on oligonucleotide microarray analysis. *Cancer research* 2001, 61(18):6885-6891.

32. Nutt CL, Mani DR, Betensky RA, Tamayo P, Cairncross JG, Ladd C, Pohl U, Hartmann C, McLaughlin ME, Batchelor TT *et al*: Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer research* 2003, 63(7):1602-1607.

33. Shirahata M, Iwao-Koizumi K, Saito S, Ueno N, Oda M, Hashimoto N, Takahashi JA, Kato K: Gene expression-based molecular diagnostic system for malignant gliomas is superior to histological diagnosis. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2007, 13(24):7341-7356.

34. Godard S, Getz G, Delorenzi M, Farmer P, Kobayashi H, Desbaillets I, Nozaki M, Diserens AC, Hamou MF, Dietrich PY *et al*: Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. *Cancer research* 2003, 63(20):6613-6625.

35. Shai R, Shi T, Kremen TJ, Horvath S, Liau LM, Cloughesy TF, Mischel PS, Nelson SF: Gene expression profiling identifies molecular subtypes of gliomas. *Oncogene* 2003, 22(31):4918-4923.

36. Tso CL, Freije WA, Day A, Chen Z, Merriman B, Perlina A, Lee Y, Dia EQ, Yoshimoto K, Mischel PS *et al*: Distinct transcription profiles of primary and secondary glioblastoma subgroups. *Cancer research* 2006, 66(1):159-167.

37. Faury D, Nantel A, Dunn SE, Guiot MC, Haque T, Hauser P, Garami M, Bognar L, Hanzely Z, Liberski PP *et al*: Molecular profiling identifies prognostic subgroups of pediatric glioblastoma and shows increased YB-1 expression in tumors. *Journal of*

clinical oncology : official journal of the American Society of Clinical Oncology 2007, 25(10):1196-1208.

38. van den Boom J, Wolter M, Kuick R, Misek DE, Youkilis AS, Wechsler DS, Sommer C, Reifenberger G, Hanash SM: Characterization of gene expression profiles associated with glioma progression using oligonucleotide-based microarray analysis and real-time reverse transcription-polymerase chain reaction. *The American journal of pathology* 2003, 163(3):1033-1043.

39. Subramanian J, Simon R: What should physicians look for in evaluating prognostic gene-expression signatures? *Nature reviews Clinical oncology* 2010, 7(6):327-334.

40. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L *et al*: Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer cell* 2006, 9(3):157-173.

41. Freije WA, Castro-Vargas FE, Fang Z, Horvath S, Cloughesy T, Liau LM, Mischel PS, Nelson SF: Gene expression profiling of gliomas strongly predicts survival. *Cancer research* 2004, 64(18):6503-6510.

42. Fuller GN, Hess KR, Rhee CH, Yung WK, Sawaya RA, Bruner JM, Zhang W: Molecular classification of human diffuse gliomas by multidimensional scaling analysis of gene expression profiles parallels morphology-based classification, correlates with survival, and reveals clinically-relevant novel glioma subsets. *Brain pathology* 2002, 12(1):108-116.

43. Sallinen SL, Sallinen PK, Haapasalo HK, Helin HJ, Helen PT, Schraml P, Kallioniemi OP, Kononen J: Identification of differentially expressed genes in human gliomas by DNA microarray and tissue chip techniques. *Cancer research* 2000, 60(23):6617-6622.

44. Huse JT, Phillips HS, Brennan CW: Molecular subclassification of diffuse gliomas: seeing order in the chaos. *Glia* 2011, 59(8):1190-1199.

45. Iwamoto FM, Hottinger AF, Karimi S, Riedel E, Dantis J, Jahdi M, Panageas KS, Lassman AB, Abrey LE, Fleisher M *et al*: Serum YKL-40 is a marker of prognosis and disease status in high-grade gliomas. *Neuro-oncology* 2011, 13(11):1244-1251.

46. Colman H, Zhang L, Sulman EP, McDonald JM, Shooshtari NL, Rivera A, Popoff S, Nutt CL, Louis DN, Cairncross JG *et al*: A multigene predictor of outcome in glioblastoma. *Neuro-oncology* 2010, 12(1):49-57.

47. Horvath S, Zhang B, Carlson M, Lu KV, Zhu S, Felciano RM, Laurance MF, Zhao W, Qi S, Chen Z *et al*: Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. *Proceedings of the National Academy of Sciences of the United States of America* 2006, 103(46):17402-17407.

48. Liang Y, Diehn M, Watson N, Bollen AW, Aldape KD, Nicholas MK, Lamborn KR, Berger MS, Botstein D, Brown PO *et al*: Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proceedings of the National Academy of Sciences of the United States of America* 2005, 102(16):5814-5819.

49. Murat A, Migliavacca E, Gorlia T, Lambiv WL, Shay T, Hamou MF, de Tribolet N, Regli L, Wick W, Kouwenhoven MC *et al*: Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. *J Clin Oncol* 2008, 26(18):3015-3024.

50. Gravendeel LA, Kouwenhoven MC, Gevaert O, de Rooi JJ, Stubbs AP, Duijm JE, Daemen A, Bleeker FE, Bralten LB, Kloosterhof NK *et al*: Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. *Cancer research* 2009, 69(23):9065-9072.

51. Li A, Walling J, Ahn S, Kotliarov Y, Su Q, Quezado M, Oberholtzer JC, Park J, Zenklusen JC, Fine HA: Unsupervised analysis of transcriptomic profiles reveals six glioma subtypes. *Cancer research* 2009, 69(5):2091-2099.

52. Cardoso F, Van't Veer L, Rutgers E, Loi S, Mook S, Piccart-Gebhart MJ: Clinical application of the 70-gene profile: the MINDACT trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2008, 26(5):729-735.

53. Mentrikoski MJ: The who and when of molecular testing for tumors of unknown primaries: one resident's perspective. *American journal of clinical pathology* 2012, 138(1):162-164.

54. Franceschi E, Cavallo G, Lonardi S, Magrini E, Tosoni A, Grosso D, Scopece L, Blatt V, Urbini B, Pession A *et al*: Gefitinib in patients with progressive high-grade gliomas: a multicentre phase II study by Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO). *British journal of cancer* 2007, 96(7):1047-1051.

55. Carcaboso AM, Elmeliegy MA, Shen J, Juel SJ, Zhang ZM, Calabrese C, Tracey L, Waters CM, Stewart CF: Tyrosine kinase inhibitor gefitinib enhances topotecan penetration of gliomas. *Cancer research* 2010, 70(11):4499-4508.

56. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U *et al*: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *The New England journal of medicine* 2005, 352(10):987-996.

57. Mizoguchi M, Yoshimoto K, Ma X, Guan Y, Hata N, Amano T, Nakamizo A, Suzuki SO, Iwaki T, Sasaki T: Molecular characteristics of glioblastoma with 1p/19q co-deletion. *Brain tumor pathology* 2012, 29(3):148-153.

58. Jiang H, Ren X, Cui X, Wang J, Jia W, Zhou Z, Lin S: 1p/19q codeletion and IDH1/2 mutation identified a subtype of anaplastic oligoastrocytomas with prognosis as favorable as anaplastic oligodendrogliomas. *Neuro-oncology* 2013, 15(6):775-782.

59. Ichimura K: Molecular pathogenesis of IDH mutations in gliomas. *Brain tumor pathology* 2012, 29(3):131-139.

60. Lopez-Gines C, Gil-Benso R, Ferrer-Luna R, Benito R, Serna E, Gonzalez-Darder J, Quilis V, Monleon D, Celda B, Cerda-Nicolas M: New pattern of EGFR amplification in glioblastoma and the relationship of gene copy number with gene

expression profile. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2010, 23(6):856-865.

61. Juratli TA, Kirsch M, Geiger K, Klink B, Leipnitz E, Pinzer T, Soucek S, Schrock E, Schackert G, Krex D: The prognostic value of IDH mutations and MGMT promoter status in secondary high-grade gliomas. *Journal of neuro-oncology* 2012, 110(3):325-333.

62. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL *et al*: An integrated genomic analysis of human glioblastoma multiforme. *Science* 2008, 321(5897):1807-1812.

63. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W *et al*: Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta neuropathologica* 2009, 118(4):469-474.

64. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ *et al*: IDH1 and IDH2 mutations in gliomas. *The New England journal of medicine* 2009, 360(8):765-773.

65. Lai A, Kharbanda S, Pope WB, Tran A, Solis OE, Peale F, Forrest WF, Pujara K, Carrillo JA, Pandita A *et al*: Evidence for sequenced molecular evolution of IDH1 mutant glioblastoma from a distinct cell of origin. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011, 29(34):4482-4490.

66. Kim YH, Nobusawa S, Mittelbronn M, Paulus W, Brokinkel B, Keyvani K, Sure U, Wrede K, Nakazato Y, Tanaka Y *et al*: Molecular classification of low-grade diffuse gliomas. *The American journal of pathology* 2010, 177(6):2708-2714.

67. Wick W, Hartmann C, Engel C, Stoffels M, Felsberg J, Stockhammer F, Sabel MC, Koeppen S, Ketter R, Meyermann R *et al*: NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009, 27(35):5874-5880.

68. Labussiere M, Idbaih A, Wang XW, Marie Y, Boisselier B, Falet C, Paris S, Laffaire J, Carpentier C, Criniere E *et al*: All the 1p19q codeleted gliomas are mutated on IDH1 or IDH2. *Neurology* 2010, 74(23):1886-1890.

69. Thompson CB: Metabolic enzymes as oncogenes or tumor suppressors. *The New England journal of medicine* 2009, 360(8):813-815.

70. Kloosterhof NK, Bralten LB, Dubbink HJ, French PJ, van den Bent MJ: Isocitrate dehydrogenase-1 mutations: a fundamentally new understanding of diffuse glioma? *The lancet oncology* 2011, 12(1):83-91.

71. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC *et al*: Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 2009, 462(7274):739-744.

72. Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, Sasaki M, Jin S, Schenkein DP, Su SM *et al*: Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *The Journal of experimental medicine* 2010, 207(2):339-344.

73. Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA, Cross JR, Fantin VR, Hedvat CV, Perl AE *et al*: The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer cell* 2010, 17(3):225-234.

74. Loenarz C, Schofield CJ: Expanding chemical biology of 2-oxoglutarate oxygenases. *Nature chemical biology* 2008, 4(3):152-156.

75. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT *et al*: Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer cell* 2011, 19(1):17-30.

76. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS *et al*: IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 2012, 483(7390):479-483.

77. Sanson M, Marie Y, Paris S, Idbaih A, Laffaire J, Ducray F, El Hallani S, Boisselier B, Mokhtari K, Hoang-Xuan K *et al*: Isocitrate dehydrogenase 1 codon 132 mutation

is an important prognostic biomarker in gliomas. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009, 27(25):4150-4154.

78. Capper D, Zentgraf H, Balss J, Hartmann C, von Deimling A: Monoclonal antibody specific for IDH1 R132H mutation. *Acta neuropathologica* 2009, 118(5):599-601.

79. Sahm F, Capper D, Pusch S, Balss J, Koch A, Langhans CD, Okun JG, von Deimling A: Detection of 2-hydroxyglutarate in formalin-fixed paraffin-embedded glioma specimens by gas chromatography/mass spectrometry. *Brain pathology* 2012, 22(1):26-31.

80. Pope WB, Prins RM, Albert Thomas M, Nagarajan R, Yen KE, Bittinger MA, Salamon N, Chou AP, Yong WH, Soto H *et al*: Non-invasive detection of 2-hydroxyglutarate and other metabolites in IDH1 mutant glioma patients using magnetic resonance spectroscopy. *Journal of neuro-oncology* 2012, 107(1):197-205.

81. Cairncross G, Jenkins R: Gliomas with 1p/19q codeletion: a.k.a. oligodendroglioma. *Cancer journal* 2008, 14(6):352-357.

82. Jeuken JW, von Deimling A, Wesseling P: Molecular pathogenesis of oligodendroglial tumors. *Journal of neuro-oncology* 2004, 70(2):161-181.

83. McDonald JM, See SJ, Tremont IW, Colman H, Gilbert MR, Groves M, Burger PC, Louis DN, Giannini C, Fuller G *et al*: The prognostic impact of histology and 1p/19q status in anaplastic oligodendroglial tumors. *Cancer* 2005, 104(7):1468-1477.

84. Aldape K, Burger PC, Perry A: Clinicopathologic aspects of 1p/19q loss and the diagnosis of oligodendroglioma. *Archives of pathology & laboratory medicine* 2007, 131(2):242-251.

85. Jansen M, Yip S, Louis DN: Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers. *Lancet neurology* 2010, 9(7):717-726.

86. Clark KH, Villano JL, Nikiforova MN, Hamilton RL, Horbinski C: 1p/19q testing has no significance in the workup of glioblastomas. *Neuropathology and applied neurobiology* 2013.

87. Boots-Sprenger SH, Sijben A, Rijntjes J, Tops BB, Idema AJ, Rivera AL, Bleeker FE, Gijtenbeek AM, Diefes K, Heathcock L *et al*: Significance of complete 1p/19q co-deletion, IDH1 mutation and MGMT promoter methylation in gliomas: use with caution. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2013, 26(7):922-929.

88. Griffin CA, Burger P, Morsberger L, Yonescu R, Swierczynski S, Weingart JD, Murphy KM: Identification of der(1;19)(q10;p10) in five oligodendrogliomas suggests mechanism of concurrent 1p and 19q loss. *Journal of neuropathology and experimental neurology* 2006, 65(10):988-994.

89. Jenkins RB, Blair H, Ballman KV, Giannini C, Arusell RM, Law M, Flynn H, Passe S, Felten S, Brown PD *et al*: A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. *Cancer research* 2006, 66(20):9852-9861.

90. Smith JS, Alderete B, Minn Y, Borell TJ, Perry A, Mohapatra G, Hosek SM, Kimmel D, O'Fallon J, Yates A *et al*: Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. *Oncogene* 1999, 18(28):4144-4152.

91. Smith JS, Perry A, Borell TJ, Lee HK, O'Fallon J, Hosek SM, Kimmel D, Yates A, Burger PC, Scheithauer BW *et al*: Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2000, 18(3):636-645.

92. Ducray F, Idbaih A, de Reynies A, Bieche I, Thillet J, Mokhtari K, Lair S, Marie Y, Paris S, Vidaud M *et al*: Anaplastic oligodendrogliomas with 1p19q codeletion have a proneural gene expression profile. *Molecular cancer* 2008, 7:41.

93. Sulman EP, Guerrero M, Aldape K: Beyond grade: molecular pathology of malignant gliomas. *Seminars in radiation oncology* 2009, 19(3):142-149.

94. Nutt CL: Molecular genetics of oligodendrogliomas: a model for improved clinical management in the field of neurooncology. *Neurosurgical focus* 2005, 19(5):E2.

95. Lassman AB, Iwamoto FM, Cloughesy TF, Aldape KD, Rivera AL, Eichler AF, Louis DN, Paleologos NA, Fisher BJ, Ashby LS *et al*: International retrospective study of over 1000 adults with anaplastic oligodendroglial tumors. *Neuro-oncology* 2011, 13(6):649-659.

96. van den Bent MJ, Carpentier AF, Brandes AA, Sanson M, Taphoorn MJ, Bernsen HJ, Frenay M, Tijssen CC, Grisold W, Sipos L *et al*: Adjuvant procarbazine, lomustine, and vincristine improves progression-free survival but not overall survival in newly diagnosed anaplastic oligodendrogliomas and oligoastrocytomas: a randomized European Organisation for Research and Treatment of Cancer phase III trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2006, 24(18):2715-2722.

97. Intergroup Radiation Therapy Oncology Group T, Cairncross G, Berkey B, Shaw E, Jenkins R, Scheithauer B, Brachman D, Buckner J, Fink K, Souhami L *et al*: Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy alone for pure and mixed anaplastic oligodendroglioma: Intergroup Radiation Therapy Oncology Group Trial 9402. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2006, 24(18):2707-2714.

98. Kouwenhoven MC, Gorlia T, Kros JM, Ibdaih A, Brandes AA, Bromberg JE, Mokhtari K, van Duinen SG, Teepen JL, Wesseling P *et al*: Molecular analysis of anaplastic oligodendroglial tumors in a prospective randomized study: A report from EORTC study 26951. *Neuro-oncology* 2009, 11(6):737-746.

99. Abrey LE, Louis DN, Paleologos N, Lassman AB, Raizer JJ, Mason W, Finlay J, MacDonald DR, DeAngelis LM, Cairncross JG *et al*: Survey of treatment recommendations for anaplastic oligodendroglioma. *Neuro-oncology* 2007, 9(3):314-318.

100. Reifenberger G, Louis DN: Oligodendroglioma: toward molecular definitions in diagnostic neuro-oncology. *Journal of neuropathology and experimental neurology* 2003, 62(2):111-126.

101. Gerson SL: MGMT: its role in cancer aetiology and cancer therapeutics. *Nature reviews Cancer* 2004, 4(4):296-307.

102. van den Bent MJ, Dubbink HJ, Sanson M, van der Lee-Haarloo CR, Hegi M, Jeuken JW, Ibdaih A, Brandes AA, Taphoorn MJ, Frenay M *et al*: MGMT promoter methylation is prognostic but not predictive for outcome to adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors: a report from EORTC Brain Tumor Group Study 26951. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009, 27(35):5881-5886.

103. Mollemann M, Wolter M, Felsberg J, Collins VP, Reifenberger G: Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. *International journal of cancer Journal international du cancer* 2005, 113(3):379-385.

104. Brandes AA, Nicolardi L, Tosoni A, Gardiman M, Iuzzolino P, Ghimenton C, Reni M, Rotilio A, Sotti G, Ermani M: Survival following adjuvant PCV or temozolomide for anaplastic astrocytoma. *Neuro-oncology* 2006, 8(3):253-260.

105. Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W, Hegi ME: MGMT promoter methylation in malignant gliomas: ready for personalized medicine? *Nature reviews Neurology* 2010, 6(1):39-51.

106. Fouse SD, Costello JF: Epigenetics of neurological cancers. *Future oncology* 2009, 5(10):1615-1629.

107. Nagarajan RP, Costello JF: Epigenetic mechanisms in glioblastoma multiforme. *Seminars in cancer biology* 2009, 19(3):188-197.

108. Everhard S, Tost J, El Abdalaoui H, Criniere E, Busato F, Marie Y, Gut IG, Sanson M, Mokhtari K, Laigle-Donadey F *et al*: Identification of regions correlating MGMT promoter methylation and gene expression in glioblastomas. *Neuro-oncology* 2009, 11(4):348-356.

109. Nakagawachi T, Soejima H, Urano T, Zhao W, Higashimoto K, Satoh Y, Matsukura S, Kudo S, Kitajima Y, Harada H *et al*: Silencing effect of CpG island hypermethylation and histone modifications on O6-methylguanine-DNA methyltransferase (MGMT) gene expression in human cancer. *Oncogene* 2003, 22(55):8835-8844.

110. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG: Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *The New England journal of medicine* 2000, 343(19):1350-1354.

111. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L *et al*: MGMT gene silencing and benefit from temozolomide in glioblastoma. *The New England journal of medicine* 2005, 352(10):997-1003.

112. Herrlinger U, Rieger J, Koch D, Loeser S, Blaschke B, Kortmann RD, Steinbach JP, Hundsberger T, Wick W, Meyermann R *et al*: Phase II trial of lomustine plus temozolomide chemotherapy in addition to radiotherapy in newly diagnosed glioblastoma: UKT-03. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2006, 24(27):4412-4417.

113. Weller M, Felsberg J, Hartmann C, Berger H, Steinbach JP, Schramm J, Westphal M, Schackert G, Simon M, Tonn JC *et al*: Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009, 27(34):5743-5750.

114. Rivera AL, Pelloski CE, Gilbert MR, Colman H, De La Cruz C, Sulman EP, Bekele BN, Aldape KD: MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. *Neuro-oncology* 2010, 12(2):116-121.

115. van den Bent MJ, Dubbink HJ, Marie Y, Brandes AA, Taphoorn MJ, Wesseling P, Frenay M, Tijssen CC, Lacombe D, Idbaih A *et al*: IDH1 and IDH2 mutations are prognostic but not predictive for outcome in anaplastic oligodendroglial tumors: a report of the European Organization for Research and Treatment of Cancer Brain Tumor Group. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2010, 16(5):1597-1604.

116. Komine C, Watanabe T, Katayama Y, Yoshino A, Yokoyama T, Fukushima T: Promoter hypermethylation of the DNA repair gene O6-methylguanine-DNA methyltransferase is an independent predictor of shortened progression free survival in patients with low-grade diffuse astrocytomas. *Brain pathology* 2003, 13(2):176-184.

117. Kesari S, Schiff D, Drappatz J, LaFrankie D, Doherty L, Macklin EA, Muzikansky A, Santagata S, Ligon KL, Norden AD *et al*: Phase II study of protracted daily temozolomide for low-grade gliomas in adults. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2009, 15(1):330-337.

118. Aguirre A, Rubio ME, Gallo V: Notch and EGFR pathway interaction regulates neural stem cell number and self-renewal. *Nature* 2010, 467(7313):323-327.

119. Williams JP, Wu J, Johansson G, Rizvi TA, Miller SC, Geiger H, Malik P, Li W, Mukouyama YS, Cancelas JA *et al*: Nf1 mutation expands an EGFR-dependent peripheral nerve progenitor that confers neurofibroma tumorigenic potential. *Cell stem cell* 2008, 3(6):658-669.

120. Ju P, Zhang S, Yeap Y, Feng Z: Induction of neuronal phenotypes from NG2+ glial progenitors by inhibiting epidermal growth factor receptor in mouse spinal cord injury. *Glia* 2012, 60(11):1801-1814.

121. Liu B, Chen H, Johns TG, Neufeld AH: Epidermal growth factor receptor activation: an upstream signal for transition of quiescent astrocytes into reactive astrocytes after neural injury. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2006, 26(28):7532-7540.

122. Arteaga CL: Epidermal growth factor receptor dependence in human tumors: more than just expression? *The oncologist* 2002, 7 Suppl 4:31-39.

123. Salomon DS, Brandt R, Ciardiello F, Normanno N: Epidermal growth factor-related peptides and their receptors in human malignancies. *Critical reviews in oncology/hematology* 1995, 19(3):183-232.

124. Stockhausen MT, Broholm H, Villingshoj M, Kirchhoff M, Gerdes T, Kristoffersen K, Kosteljanetz M, Spang-Thomsen M, Poulsen HS: Maintenance of EGFR and

EGFRvIII expressions in an in vivo and in vitro model of human glioblastoma multiforme. *Experimental cell research* 2011, 317(11):1513-1526.

125. Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK, Huang HJ: A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. *Proceedings of the National Academy of Sciences of the United States of America* 1994, 91(16):7727-7731.

126. Hynes NE, Lane HA: ERBB receptors and cancer: the complexity of targeted inhibitors. *Nature reviews Cancer* 2005, 5(5):341-354.

127. Citri A, Yarden Y: EGF-ERBB signalling: towards the systems level. *Nature reviews Molecular cell biology* 2006, 7(7):505-516.

128. Karpel-Massler G, Schmidt U, Unterberg A, Halatsch ME: Therapeutic inhibition of the epidermal growth factor receptor in high-grade gliomas: where do we stand? *Molecular cancer research : MCR* 2009, 7(7):1000-1012.

129. Hasselbalch B, Lassen U, Poulsen HS, Stockhausen MT: Cetuximab insufficiently inhibits glioma cell growth due to persistent EGFR downstream signaling. *Cancer investigation* 2010, 28(8):775-787.

130. Yarden Y, Sliwkowski MX: Untangling the ErbB signalling network. *Nature reviews Molecular cell biology* 2001, 2(2):127-137.

131. Chaffanet M, Chauvin C, Laine M, Berger F, Chedin M, Rost N, Nissou MF, Benabid AL: EGF receptor amplification and expression in human brain tumours. *European journal of cancer* 1992, 28(1):11-17.

132. Ekstrand AJ, Sugawa N, James CD, Collins VP: Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. *Proceedings of the National Academy of Sciences of the United States of America* 1992, 89(10):4309-4313.

133. Turner T, Chen P, Goodly LJ, Wells A: EGF receptor signaling enhances in vivo invasiveness of DU-145 human prostate carcinoma cells. *Clinical & experimental metastasis* 1996, 14(4):409-418.

134. Sharma SV, Bell DW, Settleman J, Haber DA: Epidermal growth factor receptor mutations in lung cancer. *Nature reviews Cancer* 2007, 7(3):169-181.

135. Todd R, Wong DT: Epidermal growth factor receptor (EGFR) biology and human oral cancer. *Histology and histopathology* 1999, 14(2):491-500.

136. Verbeek BS, Adriaansen-Slot SS, Vroom TM, Beckers T, Rijksen G: Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts. *FEBS letters* 1998, 425(1):145-150.

137. Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, Burkhard C, Schuler D, Probst-Hensch NM, Maiorka PC *et al*: Genetic pathways to glioblastoma: a population-based study. *Cancer research* 2004, 64(19):6892-6899.

138. Ohgaki H, Kleihues P: Genetic pathways to primary and secondary glioblastoma. *The American journal of pathology* 2007, 170(5):1445-1453.

139. Singh AB, Harris RC: Autocrine, paracrine and juxtacrine signaling by EGFR ligands. *Cellular signalling* 2005, 17(10):1183-1193.

140. Cancer Genome Atlas Research N: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008, 455(7216):1061-1068.

141. Frederick L, Wang XY, Eley G, James CD: Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. *Cancer research* 2000, 60(5):1383-1387.

142. Lee JC, Vivanco I, Beroukhim R, Huang JH, Feng WL, DeBiasi RM, Yoshimoto K, King JC, Nghiemphu P, Yuza Y *et al*: Epidermal growth factor receptor activation in glioblastoma through novel missense mutations in the extracellular domain. *PLoS medicine* 2006, 3(12):e485.

143. Zandi R, Larsen AB, Andersen P, Stockhausen MT, Poulsen HS: Mechanisms for oncogenic activation of the epidermal growth factor receptor. *Cellular signalling* 2007, 19(10):2013-2023.

144. Humphrey PA, Gangarosa LM, Wong AJ, Archer GE, Lund-Johansen M, Bjerkvig R, Laerum OD, Friedman HS, Bigner DD: Deletion-mutant epidermal growth factor

receptor in human gliomas: effects of type II mutation on receptor function. *Biochemical and biophysical research communications* 1991, 178(3):1413-1420.

145. Wong AJ, Ruppert JM, Bigner SH, Grzeschik CH, Humphrey PA, Bigner DS, Vogelstein B: Structural alterations of the epidermal growth factor receptor gene in human gliomas. *Proceedings of the National Academy of Sciences of the United States of America* 1992, 89(7):2965-2969.

146. Peschard P, Park M: From Tpr-Met to Met, tumorigenesis and tubes. *Oncogene* 2007, 26(9):1276-1285.

147. Garcia de Palazzo IE, Adams GP, Sundareshan P, Wong AJ, Testa JR, Bigner DD, Weiner LM: Expression of mutated epidermal growth factor receptor by non-small cell lung carcinomas. *Cancer research* 1993, 53(14):3217-3220.

148. Moscatello DK, Holgado-Madruga M, Godwin AK, Ramirez G, Gunn G, Zoltick PW, Biegel JA, Hayes RL, Wong AJ: Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. *Cancer research* 1995, 55(23):5536-5539.

149. Sugawa N, Ekstrand AJ, James CD, Collins VP: Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas. *Proceedings of the National Academy of Sciences of the United States of America* 1990, 87(21):8602-8606.

150. Frederick L, Eley G, Wang XY, James CD: Analysis of genomic rearrangements associated with EGRFvIII expression suggests involvement of Alu repeat elements. *Neuro-oncology* 2000, 2(3):159-163.

151. Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C *et al*: Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes & development* 2007, 21(21):2683-2710.

152. Feldkamp MM, Lala P, Lau N, Roncari L, Guha A: Expression of activated epidermal growth factor receptors, Ras-guanosine triphosphate, and mitogen-activated protein kinase in human glioblastoma multiforme specimens. *Neurosurgery* 1999, 45(6):1442-1453.

153. Jungbluth AA, Stockert E, Huang HJ, Collins VP, Coplan K, Iversen K, Kolb D, Johns TJ, Scott AM, Gullick WJ *et al*: A monoclonal antibody recognizing human cancers with amplification/overexpression of the human epidermal growth factor receptor. *Proceedings of the National Academy of Sciences of the United States of America* 2003, 100(2):639-644.

154. Yamazaki H, Ohba Y, Tamaoki N, Shibuya M: A deletion mutation within the ligand binding domain is responsible for activation of epidermal growth factor receptor gene in human brain tumors. *Japanese journal of cancer research : Gann* 1990, 81(8):773-779.

155. Humphrey PA, Wong AJ, Vogelstein B, Friedman HS, Werner MH, Bigner DD, Bigner SH: Amplification and expression of the epidermal growth factor receptor gene in human glioma xenografts. *Cancer research* 1988, 48(8):2231-2238.

156. Batra SK, Castelino-Prabhu S, Wikstrand CJ, Zhu X, Humphrey PA, Friedman HS, Bigner DD: Epidermal growth factor ligand-independent, unregulated, cell-transforming potential of a naturally occurring human mutant EGFRvIII gene. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 1995, 6(10):1251-1259.

157. Downward J, Parker P, Waterfield MD: Autophosphorylation sites on the epidermal growth factor receptor. *Nature* 1984, 311(5985):483-485.

158. O'Rourke DM, Nute EJ, Davis JG, Wu C, Lee A, Murali R, Zhang HT, Qian X, Kao CC, Greene MI: Inhibition of a naturally occurring EGFR oncoprotein by the p185neu ectodomain: implications for subdomain contributions to receptor assembly. *Oncogene* 1998, 16(9):1197-1207.

159. Voldborg BR, Damstrup L, Spang-Thomsen M, Poulsen HS: Epidermal growth factor receptor (EGFR) and EGFR mutations, function and possible role in clinical trials. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 1997, 8(12):1197-1206.

160. Prigent SA, Nagane M, Lin H, Huvar I, Boss GR, Feramisco JR, Cavenee WK, Huang HS: Enhanced tumorigenic behavior of glioblastoma cells expressing a

truncated epidermal growth factor receptor is mediated through the Ras-Shc-Grb2 pathway. *The Journal of biological chemistry* 1996, 271(41):25639-25645.

161. Fernandes H, Cohen S, Bishayee S: Glycosylation-induced conformational modification positively regulates receptor-receptor association: a study with an aberrant epidermal growth factor receptor (EGFRvIII/DeltaEGFR) expressed in cancer cells. *The Journal of biological chemistry* 2001, 276(7):5375-5383.

162. Huang HS, Nagane M, Klingbeil CK, Lin H, Nishikawa R, Ji XD, Huang CM, Gill GN, Wiley HS, Cavenee WK: The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling. *The Journal of biological chemistry* 1997, 272(5):2927-2935.

163. Nagane M, Coufal F, Lin H, Bogler O, Cavenee WK, Huang HJ: A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis. *Cancer research* 1996, 56(21):5079-5086.

164. Bublil EM, Yarden Y: The EGF receptor family: spearheading a merger of signaling and therapeutics. *Current opinion in cell biology* 2007, 19(2):124-134.

165. Grandal MV, Zandi R, Pedersen MW, Willumsen BM, van Deurs B, Poulsen HS: EGFRvIII escapes down-regulation due to impaired internalization and sorting to lysosomes. *Carcinogenesis* 2007, 28(7):1408-1417.

166. Han W, Zhang T, Yu H, Foulke JG, Tang CK: Hypophosphorylation of residue Y1045 leads to defective downregulation of EGFRvIII. *Cancer biology & therapy* 2006, 5(10):1361-1368.

167. Hwang Y, Chumbalkar V, Latha K, Bogler O: Forced dimerization increases the activity of DeltaEGFR/EGFRvIII and enhances its oncogenicity. *Molecular cancer research : MCR* 2011, 9(9):1199-1208.

168. Luwor RB, Zhu HJ, Walker F, Vitali AA, Perera RM, Burgess AW, Scott AM, Johns TG: The tumor-specific de2-7 epidermal growth factor receptor (EGFR) promotes

cells survival and heterodimerizes with the wild-type EGFR. *Oncogene* 2004, 23(36):6095-6104.

169. Ge H, Gong X, Tang CK: Evidence of high incidence of EGFRvIII expression and coexpression with EGFR in human invasive breast cancer by laser capture microdissection and immunohistochemical analysis. *International journal of cancer Journal international du cancer* 2002, 98(3):357-361.

170. Montgomery RB, Guzman J, O'Rourke DM, Stahl WL: Expression of oncogenic epidermal growth factor receptor family kinases induces paclitaxel resistance and alters beta-tubulin isotype expression. *The Journal of biological chemistry* 2000, 275(23):17358-17363.

171. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J: Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nature cell biology* 2008, 10(5):619-624.

172. Mizoguchi M, Betensky RA, Batchelor TT, Bernay DC, Louis DN, Nutt CL: Activation of STAT3, MAPK, and AKT in malignant astrocytic gliomas: correlation with EGFR status, tumor grade, and survival. *Journal of neuropathology and experimental neurology* 2006, 65(12):1181-1188.

173. Li B, Yuan M, Kim IA, Chang CM, Bernhard EJ, Shu HK: Mutant epidermal growth factor receptor displays increased signaling through the phosphatidylinositol-3 kinase/AKT pathway and promotes radioresistance in cells of astrocytic origin. *Oncogene* 2004, 23(26):4594-4602.

174. Mendelsohn J, Baselga J: The EGF receptor family as targets for cancer therapy. *Oncogene* 2000, 19(56):6550-6565.

175. Wikstrand CJ, McLendon RE, Friedman AH, Bigner DD: Cell surface localization and density of the tumor-associated variant of the epidermal growth factor receptor, EGFRvIII. *Cancer research* 1997, 57(18):4130-4140.

176. Wikstrand CJ, Hale LP, Batra SK, Hill ML, Humphrey PA, Kurpad SN, McLendon RE, Moscatello D, Pegram CN, Reist CJ *et al*: Monoclonal antibodies against

EGFRvIII are tumor specific and react with breast and lung carcinomas and malignant gliomas. *Cancer research* 1995, 55(14):3140-3148.

177. Choe G, Horvath S, Cloughesy TF, Crosby K, Seligson D, Palotie A, Inge L, Smith BL, Sawyers CL, Mischel PS: Analysis of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma patients in vivo. *Cancer research* 2003, 63(11):2742-2746.

178. Tanaka K, Babic I, Nathanson D, Akhavan D, Guo D, Gini B, Dang J, Zhu S, Yang H, De Jesus J *et al*: Oncogenic EGFR signaling activates an mTORC2-NF-kappaB pathway that promotes chemotherapy resistance. *Cancer discovery* 2011, 1(6):524-538.

179. Johns TG, McKay MJ, Cvrljevic AN, Gan HK, Taylor C, Xu H, Smyth FE, Scott AM: MAb 806 enhances the efficacy of ionizing radiation in glioma xenografts expressing the de2-7 epidermal growth factor receptor. *International journal of radiation oncology, biology, physics* 2010, 78(2):572-578.

180. Learn CA, Hartzell TL, Wikstrand CJ, Archer GE, Rich JN, Friedman AH, Friedman HS, Bigner DD, Sampson JH: Resistance to tyrosine kinase inhibition by mutant epidermal growth factor receptor variant III contributes to the neoplastic phenotype of glioblastoma multiforme. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2004, 10(9):3216-3224.

181. Bianco R, Shin I, Ritter CA, Yakes FM, Basso A, Rosen N, Tsurutani J, Dennis PA, Mills GB, Arteaga CL: Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. *Oncogene* 2003, 22(18):2812-2822.

182. Chakravarti A, Chakladar A, Delaney MA, Latham DE, Loeffler JS: The epidermal growth factor receptor pathway mediates resistance to sequential administration of radiation and chemotherapy in primary human glioblastoma cells in a RAS-dependent manner. *Cancer research* 2002, 62(15):4307-4315.

183. Moscatello DK, Holgado-Madruga M, Emlet DR, Montgomery RB, Wong AJ: Constitutive activation of phosphatidylinositol 3-kinase by a naturally occurring

mutant epidermal growth factor receptor. *The Journal of biological chemistry* 1998, 273(1):200-206.

184. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ *et al*: Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *The New England journal of medicine* 2005, 353(19):2012-2024.

185. Gottschalk AR, Basila D, Wong M, Dean NM, Brandts CH, Stokoe D, Haas-Kogan DA: p27Kip1 is required for PTEN-induced G1 growth arrest. *Cancer research* 2001, 61(5):2105-2111.

186. Li DM, Sun H: PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells. *Proceedings of the National Academy of Sciences of the United States of America* 1998, 95(26):15406-15411.

187. Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, Parsons R: Somatic mutations of PTEN in glioblastoma multiforme. *Cancer research* 1997, 57(19):4183-4186.

188. Lu KV, Zhu S, Cvrljevic A, Huang TT, Sarkaria S, Ahkavan D, Dang J, Dinca EB, Plaisier SB, Oderberg I *et al*: Fyn and SRC are effectors of oncogenic epidermal growth factor receptor signaling in glioblastoma patients. *Cancer research* 2009, 69(17):6889-6898.

189. Cvrljevic AN, Akhavan D, Wu M, Martinello P, Furnari FB, Johnston AJ, Guo D, Pike L, Cavenee WK, Scott AM *et al*: Activation of Src induces mitochondrial localisation of de2-7EGFR (EGFRvIII) in glioma cells: implications for glucose metabolism. *Journal of cell science* 2011, 124(Pt 17):2938-2950.

190. Feng H, Hu B, Jarzynka MJ, Li Y, Keezer S, Johns TG, Tang CK, Hamilton RL, Vuori K, Nishikawa R *et al*: Phosphorylation of dedicator of cytokinesis 1 (Dock180) at tyrosine residue Y722 by Src family kinases mediates EGFRvIII-driven glioblastoma tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America* 2012, 109(8):3018-3023.

191. Bonavia R, Inda MM, Vandenberg S, Cheng SY, Nagane M, Hadwiger P, Tan P, Sah DW, Cavenee WK, Furnari FB: EGFRvIII promotes glioma angiogenesis and growth through the NF-kappaB, interleukin-8 pathway. *Oncogene* 2012, 31(36):4054-4066.

192. Wu JL, Abe T, Inoue R, Fujiki M, Kobayashi H: IkappaBalphaM suppresses angiogenesis and tumorigenesis promoted by a constitutively active mutant EGFR in human glioma cells. *Neurological research* 2004, 26(7):785-791.

193. Huang PH, Mukasa A, Bonavia R, Flynn RA, Brewer ZE, Cavenee WK, Furnari FB, White FM: Quantitative analysis of EGFRvIII cellular signaling networks reveals a combinatorial therapeutic strategy for glioblastoma. *Proceedings of the National Academy of Sciences of the United States of America* 2007, 104(31):12867-12872.

194. Garnett J, Chumbalkar V, Vaillant B, Gururaj AE, Hill KS, Latha K, Yao J, Priebe W, Colman H, Elferink LA *et al*: Regulation of HGF expression by DeltaEGFR-mediated c-Met activation in glioblastoma cells. *Neoplasia* 2013, 15(1):73-84.

195. Pillay V, Allaf L, Wilding AL, Donoghue JF, Court NW, Greenall SA, Scott AM, Johns TG: The plasticity of oncogene addiction: implications for targeted therapies directed to receptor tyrosine kinases. *Neoplasia* 2009, 11(5):448-458, 442 p following 458.

196. Inda MM, Bonavia R, Mukasa A, Narita Y, Sah DW, Vandenberg S, Brennan C, Johns TG, Bachoo R, Hadwiger P *et al*: Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. *Genes & development* 2010, 24(16):1731-1745.

197. Wang SC, Hung MC: Nuclear translocation of the epidermal growth factor receptor family membrane tyrosine kinase receptors. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2009, 15(21):6484-6489.

198. Boerner JL, Demory ML, Silva C, Parsons SJ: Phosphorylation of Y845 on the epidermal growth factor receptor mediates binding to the mitochondrial protein cytochrome c oxidase subunit II. *Molecular and cellular biology* 2004, 24(16):7059-7071.

199. Westermark B, Wasteson A: A platelet factor stimulating human normal glial cells. *Experimental cell research* 1976, 98(1):170-174.

200. Heldin CH, Westermark B, Wasteson A: Platelet-derived growth factor: purification and partial characterization. *Proceedings of the National Academy of Sciences of the United States of America* 1979, 76(8):3722-3726.

201. Fredriksson L, Li H, Eriksson U: The PDGF family: four gene products form five dimeric isoforms. *Cytokine & growth factor reviews* 2004, 15(4):197-204.

202. Ek B, Heldin CH: Characterization of a tyrosine-specific kinase activity in human fibroblast membranes stimulated by platelet-derived growth factor. *The Journal of biological chemistry* 1982, 257(17):10486-10492.

203. Li X, Ponten A, Aase K, Karlsson L, Abramsson A, Uutela M, Backstrom G, Hellstrom M, Bostrom H, Li H *et al*: PDGF-C is a new protease-activated ligand for the PDGF alpha-receptor. *Nature cell biology* 2000, 2(5):302-309.

204. Bergsten E, Uutela M, Li X, Pietras K, Ostman A, Heldin CH, Alitalo K, Eriksson U: PDGF-D is a specific, protease-activated ligand for the PDGF beta-receptor. *Nature cell biology* 2001, 3(5):512-516.

205. Heldin CH, Eriksson U, Ostman A: New members of the platelet-derived growth factor family of mitogens. *Archives of biochemistry and biophysics* 2002, 398(2):284-290.

206. Heldin CH, Westermark B: Mechanism of action and in vivo role of platelet-derived growth factor. *Physiological reviews* 1999, 79(4):1283-1316.

207. Heldin CH, Westermark B: Platelet-derived growth factor: mechanism of action and possible in vivo function. *Cell regulation* 1990, 1(8):555-566.

208. Smith TC: Toleration and bioavailability of gemfibrozil in healthy men. *Proceedings of the Royal Society of Medicine* 1976, 69 Suppl 2:24-27.

209. Reigstad LJ, Varhaug JE, Lillehaug JR: Structural and functional specificities of PDGF-C and PDGF-D, the novel members of the platelet-derived growth factors family. *The FEBS journal* 2005, 272(22):5723-5741.

210. Goritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisen J: A pericyte origin of spinal cord scar tissue. *Science* 2011, 333(6039):238-242.

211. Cao Y, Cao R, Hedlund EM: R Regulation of tumor angiogenesis and metastasis by FGF and PDGF signaling pathways. *Journal of molecular medicine* 2008, 86(7):785-789.

212. Kazlauskas A, Cooper JA: Autophosphorylation of the PDGF receptor in the kinase insert region regulates interactions with cell proteins. *Cell* 1989, 58(6):1121-1133.

213. Valius M, Kazlauskas A: Phospholipase C-gamma 1 and phosphatidylinositol 3 kinase are the downstream mediators of the PDGF receptor's mitogenic signal. *Cell* 1993, 73(2):321-334.

214. Kawagishi J, Kumabe T, Yoshimoto T, Yamamoto T: Structure, organization, and transcription units of the human alpha-platelet-derived growth factor receptor gene, PDGFRA. *Genomics* 1995, 30(2):224-232.

215. Heldin CH, Ostman A, Ronnstrand L: Signal transduction via platelet-derived growth factor receptors. *Biochimica et biophysica acta* 1998, 1378(1):F79-113.

216. Rosenkranz S, DeMali KA, Gelderloos JA, Bazenet C, Kazlauskas A: Identification of the receptor-associated signaling enzymes that are required for platelet-derived growth factor-AA-dependent chemotaxis and DNA synthesis. *The Journal of biological chemistry* 1999, 274(40):28335-28343.

217. Van Stry M, Kazlauskas A, Schreiber SL, Symes K: Distinct effectors of platelet-derived growth factor receptor-alpha signaling are required for cell survival during embryogenesis. *Proceedings of the National Academy of Sciences of the United States of America* 2005, 102(23):8233-8238.

218. Klinghoffer RA, Hamilton TG, Hoch R, Soriano P: An allelic series at the PDGFalphaR locus indicates unequal contributions of distinct signaling pathways during development. *Developmental cell* 2002, 2(1):103-113.

219. Tallquist M, Kazlauskas A: PDGF signaling in cells and mice. *Cytokine & growth factor reviews* 2004, 15(4):205-213.

220. McKinnon RD, Waldron S, Kiel ME: PDGF alpha-receptor signal strength controls an RTK rheostat that integrates phosphoinositol 3'-kinase and phospholipase Cgamma pathways during oligodendrocyte maturation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2005, 25(14):3499-3508.

221. Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, VandenBerg S, Alvarez-Buylla A: PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron* 2006, 51(2):187-199.

222. Fruttiger M, Karlsson L, Hall AC, Abramsson A, Calver AR, Bostrom H, Willetts K, Bertold CH, Heath JK, Betsholtz C *et al*: Defective oligodendrocyte development and severe hypomyelination in PDGF-A knockout mice. *Development* 1999, 126(3):457-467.

223. Mao X, Hamoudi RA: Molecular and cytogenetic analysis of glioblastoma multiforme. *Cancer genetics and cytogenetics* 2000, 122(2):87-92.

224. Motomura K, Mittelbronn M, Paulus W, Brokinkel B, Keyvani K, Sure U, Wrede K, Nakazato Y, Tanaka Y, Nonoguchi N *et al*: PDGFRA gain in low-grade diffuse gliomas. *Journal of neuropathology and experimental neurology* 2013, 72(1):61-66.

225. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A *et al*: PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003, 299(5607):708-710.

226. MacDonald TJ, Brown KM, LaFleur B, Peterson K, Lawlor C, Chen Y, Packer RJ, Cogen P, Stephan DA: Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease. *Nature genetics* 2001, 29(2):143-152.

227. Oda Y, Wehrmann B, Radig K, Walter H, Rose I, Neumann W, Roessner A: Expression of growth factors and their receptors in human osteosarcomas. Immunohistochemical detection of epidermal growth factor, platelet-derived growth factor and their receptors: its correlation with proliferating activities and p53 expression. *General & diagnostic pathology* 1995, 141(2):97-103.

228. Matei D, Chang DD, Jeng MH: Imatinib mesylate (Gleevec) inhibits ovarian cancer cell growth through a mechanism dependent on platelet-derived growth factor receptor alpha and Akt inactivation. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2004, 10(2):681-690.

229. Reinmuth N, Liersch R, Raedel M, Fehrmann F, Fehrmann N, Bayer M, Schwoeppe C, Kessler T, Berdel W, Thomas M *et al*: Combined anti-PDGFRalpha and PDGFRbeta targeting in non-small cell lung cancer. *International journal of cancer Journal international du cancer* 2009, 124(7):1535-1544.

230. Ozawa T, Brennan CW, Wang L, Squatrito M, Sasayama T, Nakada M, Huse JT, Pedraza A, Utsuki S, Yasui Y *et al*: PDGFRA gene rearrangements are frequent genetic events in PDGFRA-amplified glioblastomas. *Genes & development* 2010, 24(19):2205-2218.

231. Velghe AI, Van Cauwenberghe S, Polyansky AA, Chand D, Montano-Almendras CP, Charni S, Hallberg B, Essaghir A, Demoulin JB: PDGFRA alterations in cancer: characterization of a gain-of-function V536E transmembrane mutant as well as loss-of-function and passenger mutations. *Oncogene* 2013.

232. Matei D, Emerson RE, Lai YC, Baldridge LA, Rao J, Yiannoutsos C, Donner DD: Autocrine activation of PDGFRalpha promotes the progression of ovarian cancer. *Oncogene* 2006, 25(14):2060-2069.

233. Yao R, Cooper GM: Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science* 1995, 267(5206):2003-2006.

234. Huang JS, Huang SS, Deuel TF: Transforming protein of simian sarcoma virus stimulates autocrine growth of SSV-transformed cells through PDGF cell-surface receptors. *Cell* 1984, 39(1):79-87.

235. Yu J, Moon A, Kim HR: Both platelet-derived growth factor receptor (PDGFR)-alpha and PDGFR-beta promote murine fibroblast cell migration. *Biochemical and biophysical research communications* 2001, 282(3):697-700.

236. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R: PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *The American journal of pathology* 1990, 136(6):1235-1246.

237. Lokker NA, Sullivan CM, Hollenbach SJ, Israel MA, Giese NA: 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 tumors. *Cancer research* 2002, 62(13):3729-3735.

238. Shih AH, Dai C, Hu X, Rosenblum MK, Koutcher JA, Holland EC: Dose-dependent effects of platelet-derived growth factor-B on glial tumorigenesis. *Cancer Res* 2004, 64(14):4783-4789.

239. Puputti M, Tynninen O, Sihto H, Blom T, Maenpaa H, Isola J, Paetau A, Joensuu H, Nupponen NN: Amplification of KIT, PDGFRA, VEGFR2, and EGFR in gliomas. *Molecular cancer research : MCR* 2006, 4(12):927-934.

240. Martinho O, Longatto-Filho A, Lambros MB, Martins A, Pinheiro C, Silva A, Pardal F, Amorim J, Mackay A, Milanezi F *et al*: Expression, mutation and copy number analysis of platelet-derived growth factor receptor A (PDGFRA) and its ligand PDGFA in gliomas. *British journal of cancer* 2009, 101(6):973-982.

241. Clarke ID, Dirks PB: A human brain tumor-derived PDGFR-alpha deletion mutant is transforming. *Oncogene* 2003, 22(5):722-733.

242. Smith JS, Wang XY, Qian J, Hosek SM, Scheithauer BW, Jenkins RB, James CD: Amplification of the platelet-derived growth factor receptor-A (PDGFRA) gene occurs in oligodendrogliomas with grade IV anaplastic features. *Journal of neuropathology and experimental neurology* 2000, 59(6):495-503.

243. Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin CH, Westermark B, Nister M: 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 research* 1992, 52(11):3213-3219.

244. Dai C, Celestino JC, Okada Y, Louis DN, Fuller GN, Holland EC: PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. *Genes & development* 2001, 15(15):1913-1925.

245. Hede SM, Hansson I, Afink GB, Eriksson A, Nazarenko I, Andrae J, Genove G, Westermark B, Nister M: GFAP promoter driven transgenic expression of PDGFB in the mouse brain leads to glioblastoma in a Trp53 null background. *Glia* 2009, 57(11):1143-1153.

246. Assanah M, Lochhead R, Ogden A, Bruce J, Goldman J, Canoll P: Glial progenitors in adult white matter are driven to form malignant gliomas by platelet-derived growth factor-expressing retroviruses. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2006, 26(25):6781-6790.

247. Uhrbom L, Hesselager G, Nister M, Westermark B: Induction of brain tumors in mice using a recombinant platelet-derived growth factor B-chain retrovirus. *Cancer Res* 1998, 58(23):5275-5279.

248. Assanah MC, Bruce JN, Suzuki SO, Chen A, Goldman JE, Canoll P: PDGF stimulates the massive expansion of glial progenitors in the neonatal forebrain. *Glia* 2009, 57(16):1835-1847.

249. van der Valk P, Lindeman J, Kamphorst W: Growth factor profiles of human gliomas. Do non-tumour cells contribute to tumour growth in glioma? *Ann Oncol* 1997, 8(10):1023-1029.

250. Hermanson M, Funa K, Koopmann J, Maintz D, Waha A, Westermark B, Heldin CH, Wiestler OD, Louis DN, von Deimling A *et al*: Association of loss of heterozygosity on chromosome 17p with high platelet-derived growth factor alpha receptor expression in human malignant gliomas. *Cancer Res* 1996, 56(1):164-171.

251. Mabon RF, Svien HJ, Adson AW, Kernohan JW: Astrocytomas of the cerebellum. *Archives of neurology and psychiatry* 1950, 64(1):74-88.

252. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A: Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 1999, 97(6):703-716.

253. Sakariassen PO, Immervoll H, Chekenya M: Cancer stem cells as mediators of treatment resistance in brain tumors: status and controversies. *Neoplasia* 2007, 9(11):882-892.

254. Visvader JE: Cells of origin in cancer. *Nature* 2011, 469(7330):314-322.

255. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB: Identification of human brain tumour initiating cells. *Nature* 2004, 432(7015):396-401.

256. Lee JS, Lee HJ, Moon BH, Song SH, Lee MO, Shim SH, Kim HS, Lee MC, Kwon JT, Fornace AJ, Jr. *et al*: Generation of cancerous neural stem cells forming glial tumor by oncogenic stimulation. *Stem cell reviews* 2012, 8(2):532-545.

257. Alcantara Llaguno S, Chen J, Kwon CH, Jackson EL, Li Y, Burns DK, Alvarez-Buylla A, Parada LF: Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer cell* 2009, 15(1):45-56.

258. Wang Y, Yang J, Zheng H, Tomasek GJ, Zhang P, McKeever PE, Lee EY, Zhu Y: Expression of mutant p53 proteins implicates a lineage relationship between neural stem cells and malignant astrocytic glioma in a murine model. *Cancer cell* 2009, 15(6):514-526.

259. Zhu Y, Guignard F, Zhao D, Liu L, Burns DK, Mason RP, Messing A, Parada LF: Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. *Cancer cell* 2005, 8(2):119-130.

260. Canoll P, Goldman JE: The interface between glial progenitors and gliomas. *Acta neuropathologica* 2008, 116(5):465-477.

261. Ellis JA, Castelli M, Bruce JN, Canoll P, Ogden AT: Retroviral delivery of platelet-derived growth factor to spinal cord progenitor cells drives the formation of intramedullary gliomas. *Neurosurgery* 2012, 70(1):198-204; discussion 204.

262. Kim HD, Guo TW, Wu AP, Wells A, Gertler FB, Lauffenburger DA: Epidermal growth factor-induced enhancement of glioblastoma cell migration in 3D arises from an intrinsic increase in speed but an extrinsic matrix- and proteolysis-dependent increase in persistence. *Molecular biology of the cell* 2008, 19(10):4249-4259.

263. Bachoo RM, Maher EA, Ligon KL, Sharpless NE, Chan SS, You MJ, Tang Y, DeFrances J, Stover E, Weissleder R *et al*: Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. *Cancer cell* 2002, 1(3):269-277.

264. Lindberg N, Kastemar M, Olofsson T, Smits A, Uhrbom L: Oligodendrocyte progenitor cells can act as cell of origin for experimental glioma. *Oncogene* 2009, 28(23):2266-2275.

265. Persson AI, Petritsch C, Swartling FJ, Itsara M, Sim FJ, Auvergne R, Goldenberg DD, Vandenberg SR, Nguyen KN, Yakovenko S *et al*: Non-stem cell origin for oligodendroglioma. *Cancer cell* 2010, 18(6):669-682.

266. Liu C, Sage JC, Miller MR, Verhaak RG, Hippenmeyer S, Vogel H, Foreman O, Bronson RT, Nishiyama A, Luo L *et al*: Mosaic analysis with double markers reveals tumor cell of origin in glioma. *Cell* 2011, 146(2):209-221.

267. Sukhdeo K, Hambardzumyan D, Rich JN: Glioma development: where did it all go wrong? *Cell* 2011, 146(2):187-188.

268. Sugiarto S, Persson AI, Munoz EG, Waldhuber M, Lamagna C, Andor N, Hanecker P, Ayers-Ringler J, Phillips J, Siu J *et al*: Asymmetry-defective oligodendrocyte progenitors are glioma precursors. *Cancer cell* 2011, 20(3):328-340.

269. Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai HH, Wong M, Gupta N, Berger MS, Huang E, Garcia-Verdugo JM *et al*: Corridors of migrating neurons in the human brain and their decline during infancy. *Nature* 2011, 478(7369):382-386.

270. Chojnacki A, Mak G, Weiss S: PDGFRalpha expression distinguishes GFAP-expressing neural stem cells from PDGF-responsive neural precursors in the adult

periventricular area. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2011, 31(26):9503-9512.

271. Pastrana E, Cheng LC, Doetsch F: Simultaneous prospective purification of adult subventricular zone neural stem cells and their progeny. *Proceedings of the National Academy of Sciences of the United States of America* 2009, 106(15):6387-6392.

272. Ivkovic S, Canoll P, Goldman JE: Constitutive EGFR signaling in oligodendrocyte progenitors leads to diffuse hyperplasia in postnatal white matter. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2008, 28(4):914-922.

273. Dunn GP, Rinne ML, Wykosky J, Genovese G, Quayle SN, Dunn IF, Agarwalla PK, Chheda MG, Campos B, Wang A *et al*: Emerging insights into the molecular and cellular basis of glioblastoma. *Genes & development* 2012, 26(8):756-784.

274. Lee Y, Scheck AC, Cloughesy TF, Lai A, Dong J, Farooqi HK, Liau LM, Horvath S, Mischel PS, Nelson SF: Gene expression analysis of glioblastomas identifies the major molecular basis for the prognostic benefit of younger age. *BMC Med Genomics* 2008, 1:52.

275. Freeman MR: Specification and morphogenesis of astrocytes. *Science (New York, NY* 2010, 330(6005):774-778.

276. Nicolay DJ, Doucette JR, Nazarali AJ: Transcriptional control of oligodendrogenesis. *Glia* 2007, 55(13):1287-1299.

277. Dono R: Fibroblast growth factors as regulators of central nervous system development and function. *Am J Physiol Regul Integr Comp Physiol* 2003, 284(4):R867-881.

278. Fukui S, Nawashiro H, Otani N, Ooigawa H, Nomura N, Yano A, Miyazawa T, Ohnuki A, Tsuzuki N, Katoh H *et al*: Nuclear accumulation of basic fibroblast growth factor in human astrocytic tumors. *Cancer* 2003, 97(12):3061-3067.

279. Takahashi JA, Mori H, Fukumoto M, Igarashi K, Jaye M, Oda Y, Kikuchi H, Hatanaka M: Gene expression of fibroblast growth factors in human gliomas and meningiomas: demonstration of cellular source of basic fibroblast growth factor

mRNA and peptide in tumor tissues. *Proc Natl Acad Sci U S A* 1990, 87(15):5710-5714.

280. Zagzag D, Miller DC, Sato Y, Rifkin DB, Burstein DE: Immunohistochemical localization of basic fibroblast growth factor in astrocytomas. *Cancer Res* 1990, 50(22):7393-7398.

281. Fortin D, Rom E, Sun H, Yayon A, Bansal R: Distinct fibroblast growth factor (FGF)/FGF receptor signaling pairs initiate diverse cellular responses in the oligodendrocyte lineage. *J Neurosci* 2005, 25(32):7470-7479.

282. Mayer M, Bogler O, Noble M: The inhibition of oligodendrocytic differentiation of O-2A progenitors caused by basic fibroblast growth factor is overridden by astrocytes. *Glia* 1993, 8(1):12-19.

283. Dobrowolski R, De Robertis EM: Endocytic control of growth factor signalling: multivesicular bodies as signalling organelles. *Nature reviews Molecular cell biology* 2012, 13(1):53-60.

284. Lidke DS, Huang F, Post JN, Rieger B, Wilsbacher J, Thomas JL, Pouyssegur J, Jovin TM, Lenormand P: ERK nuclear translocation is dimerization-independent but controlled by the rate of phosphorylation. *The Journal of biological chemistry* 2010, 285(5):3092-3102.

285. Yip-Schneider MT, Klein PJ, Wentz SC, Zeni A, Menze A, Schmidt CM: Resistance to mitogen-activated protein kinase kinase (MEK) inhibitors correlates with up-regulation of the MEK/extracellular signal-regulated kinase pathway in hepatocellular carcinoma cells. *The Journal of pharmacology and experimental therapeutics* 2009, 329(3):1063-1070.

286. Liu JL, Mao Z, LaFortune TA, Alonso MM, Gallick GE, Fueyo J, Yung WK: Cell cycle-dependent nuclear export of phosphatase and tensin homologue tumor suppressor is regulated by the phosphoinositide-3-kinase signaling cascade. *Cancer Res* 2007, 67(22):11054-11063.

287. Colello D, Mathew S, Ward R, Pumiglia K, LaFlamme SE: Integrins regulate microtubule nucleating activity of centrosome through mitogen-activated protein

kinase/extracellular signal-regulated kinase kinase/extracellular signal-regulated kinase (MEK/ERK) signaling. *The Journal of biological chemistry* 2012, 287(4):2520-2530.

288. Watanabe K, Tanimura S, Uchiyama A, Sakamoto T, Kawabata T, Ozaki K, Kohno M: Blockade of the extracellular signal-regulated kinase pathway enhances the therapeutic efficacy of microtubule-destabilizing agents in human tumor xenograft models. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2010, 16(4):1170-1178.

289. Neel NF, Rossman KL, Martin TD, Hayes TK, Yeh JJ, Der CJ: The RalB small GTPase mediates formation of invadopodia through a GTPase-activating protein-independent function of the RalBP1/RLIP76 effector. *Molecular and cellular biology* 2012, 32(8):1374-1386.

290. Houle F, Rousseau S, Morrice N, Luc M, Mongrain S, Turner CE, Tanaka S, Moreau P, Huot J: Extracellular signal-regulated kinase mediates phosphorylation of tropomyosin-1 to promote cytoskeleton remodeling in response to oxidative stress: impact on membrane blebbing. *Molecular biology of the cell* 2003, 14(4):1418-1432.

291. Galperin E, Sorkin A: Endosomal targeting of MEK2 requires RAF, MEK kinase activity and clathrin-dependent endocytosis. *Traffic* 2008, 9(10):1776-1790.

Appendix