PhD thesis

INVESTIGATION OF PROGNOSTIC MARKERS IN ASTROCYTOMA GRADES II-IV

By

Nato Narsia, MD

Supervisor: Jiri Ehrmann, MD, PhD

Laboratory of Molecular Pathology and Department of Clinical and Molecular Pathology, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University

Field of study: Pathological Anatomy and Forensic Medicine

Olomouc 2017

DECLARATION

I hereby declare that I am the author of this thesis and I have performed all the work myself unless otherwise specified below.

Differential gene expression analysis of astrocytoma subtypes II-IV in my thesis was performed with the guidance from Pradeep Ramagiri, PhD, Institute of Cancer and Genomic Sciences, University of Birmingham, UK. Contributions to the research by others were acknowledged in the thesis.

Olomouc, June, 2017 Nato Narsia, MD

ACKNOWLEDGEMENTS

It is my great pleasure to thank the following persons who provided me support and guidance during this work:

I owe my deepest gratitude to my supervisor Jiri Ehrmann, MD, PhD for providing continuous support and supervision of my PhD study, and without whom this work would not have been possible. My sincere thanks go to Prof. Zdeněk Kolář, MD, PhD, CSc; and Prof. Paul Murray, Institute of Cancer and Genomic Sciences, University of Birmingham, UK; for their invaluable support and providing excellent facilities for the research work.

I thank my colleagues Pradeep Ramagiri, PhD, Institute of Cancer and Genomic Sciences, University of Birmingham, UK., for providing guidance and support in gene expression analysis, and Maha Ibrahim, PhD, Institute of Cancer and Genomic Sciences, University of Birmingham, UK., for technical support for DDR1 IHC study.

I cordially thank the whole staff of the Laboratory of Molecular Pathology and the Department of Clinical and Molecular Pathology who showed generous help and sympathy. I wish to thank the technical staff of the Laboratory of Immunohistochemistry and ISH for their kind and skillful technical assistance.

I also would like to thank Katerina Ciskova, Gvantsa Kharaishvili,, and Dana Simkova for their logistical support and valuable suggestions.

My deepest gratitude go to my teacher, Professor George Burkadze, Department of Pathological Anatomy, Tbilisi State Medical University, Georgia, for providing the opportunity to start a career in molecular pathology.

My most sincere thanks to my family, mother Olga Maglakelidze, sister Elene Narsia, and son Nick, for their patience and never ending support from Georgia. I would like to also thank my friends and relatives, who despite the distance, have provided me constant support and encouragement during my stay in Olomouc and Birminghxam.

This work was supported by grants NPU I LO1304 and RVO: 61989592 from the Czech Ministry of Education and LF_2016_013.

Table of contents

1. INTRODUCTION

This study is focused on investigating the genetic changes in astrocytoma grades II-IV and identifying potential prognostic factors using existing RNA sequencing data. The prognostic relevance of selected genes is further evaluated using an independent cohort of grade II-IV astrocytoma samples.

1.1. Astrocytoma

Astrocytoma is the most prevalent form of brain tumor (Ohgaki and Kleihues, 2005). For the year 2012, crude incidence rate for brain cancer in the world is 3.9 which were lower than in Europe (8.6) (GLOBOCAN, 2012). In Czech Republic crude incidence rate for brain cancer is lower than in Europe (8.1) and similar crude incidence rate for brain cancer was observed in Olomouc region (8.0) for the year 2012 (SVOD, 2016). Crude mortality rate for brain cancer in the world for the year 2012 was 2.7 which was half than what was observed in Europe (6.1), in Czech Republic (6.0), and in Olomouc (6.6) (GLOBOCAN, 2012 and SVOD, 2016).

1.2. Histopathological classification of astrocytoma

Early classifications of brain tumours relied on comparing tumour features with those of normal tissue, tumours with cells resembling astrocytes were termed astrocytomas and those with cells resembling oligodendrocytes were called oligodendrogliomas. A variety of classification schemes have been put forward over the years (Martin-Villalba et al., 2008), but World Health Organization (WHO) classification and grading system is used most widely (Louis et al., 2007). This classification is based on histological features including cellularity, mitotic activity, nuclear atypia, vascularity and necrosis. WHO classification also recognizes four prognostic grades and a variety of histological subtypes of which the most common tumours are astrocytomas (60–70%), oligodendrogliomas (10–30%), ependymomas (<10%), and less common histopathology types, e.g. gangliogliomas and gangliocytomas (Figure 1).

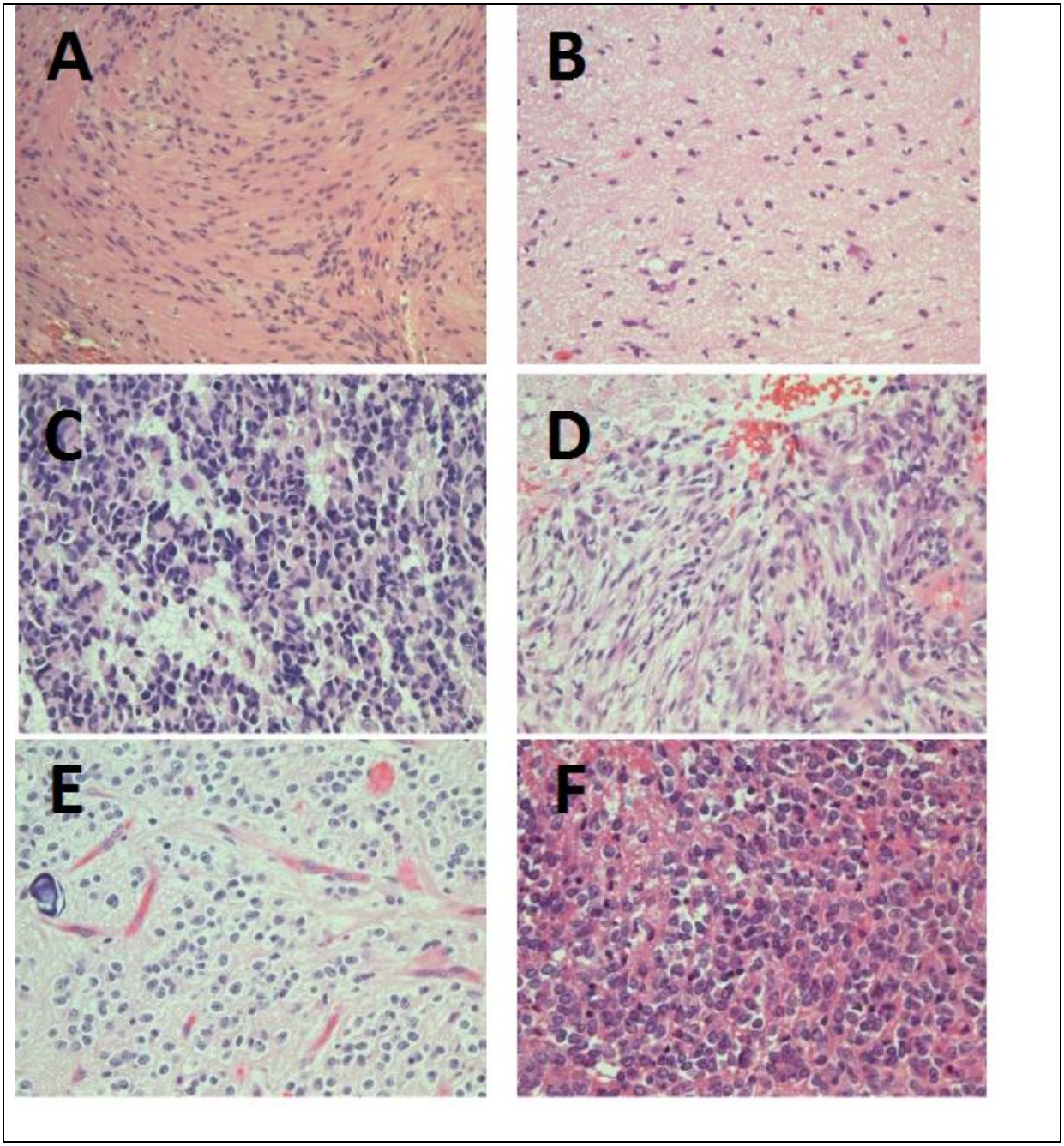

Figure 1. Histopathology classification of gliomas. (A) WHO grade I Pilocytic astrocytoma showing compact bundles of "piloid"/elongated cells containing nuclei with minimal atypia; (B) WHO grade II astrocytoma with increased cellularity and occasional atypical nuclei and some cells with enlarged cytoplasm; (C) WHO grade III astrocytoma showing darkly stained nuclei with increased cytoplasm and occasional mitoses; (D) WHO grade IV glioblastoma showing tumour necrosis without pseudopalisading; (E) WHO grade II oligodenoglioma showing perinuclear haloes, "chickenwire" vasculature and microcalcification; (F) WHO grade III oligodendroglioma showing an increased cellularity retaining roundness of nuclei associated with cell necrosis, note the mild infiltration by neutrophils. (Figure reproduced from Walker et al., 2011).

WHO grade I Pilocytic astrocytomas are slow growing and often cystic astrocytomas comprising 5–6% of all gliomas (Scheithauer et al., 2008). Majority of the Pilocytic astrocytomas arise in the cerebellum, hypothalamus and third ventricular region, most common in children and young adults. They are usually curable by surgical excision.

Diffuse gliomas arise in the cerebral hemispheres and are named due to their marked propensity to infiltrate the surrounding brain parenchyma, irrespective of grade, and include astrocytomas of WHO grades II, III and IV. These infiltrating cells are refractory to conventional therapy and may contribute to treatment failure (Lefranc et al., 2005). Astrocytomas WHO grade II represent 10–15% of all astrocytic tumours. WHO grade II diffuse astrocytoma (AS II) is a slowly growing, invasive, semi-benign astrocytoma that is frequently diagnosed in younger patients aged between 20 and 45 years with an average age of 35 years (Tonn JC, 2006). Histologically diffuse astrocytoma is not clearly distinguishable from surrounding normal tissue making it difficult for surgical resection (Kelly, 2010). Recurrence in most patients is observed after few years, often progressed to more malignant AS III or grade IV Glioblastoma GBM IV (Ohgaki and Kleihues, 2009, Ohgaki and Kleihues, 2013, Johnson et al., 2013). Patients with AS II have a median survival of between five to eight years (Lind-Landstrom et al., 2012).

Anaplastic astrocytoma (AS III) is a fast growing, invasive, malignant astrocytoma. The tumour cells also vary in size and shape in comparison to AS II and previously described to have increased mitotic activity (Louis et al., 2007). Mean age of patients diagnosed with AS III is 45 years. Patients undergo surgical resection, followed by chemotherapy and/or radiotherapy, as preferred means of treatment (Ohgaki and Kleihues, 2013, Johnson et al., 2013). Around 24% of the patients with Anaplastic astrocytoma has overall survival rate of five years (Smoll and Hamilton, 2014) with a median survival between one to four years (Nuño et al., 2013). More often AS III) is progressed into grade IV Glioblastoma after few years (Ohgaki and Kleihues, 2013, Johnson et al., 2013).

WHO grade IV Glioblastoma (GBM IV) is a very fast growing and the most malignant type of astrocytoma. GBM IV can be characterized by the presence of necrosis and/or vascular proliferation. Glioblastoma IV can be genetically distinguishable into two different types: 1. Primary GBM (pGBM IV), that occur rapidly without prior occurrences of lower grade astrocytoma (Ohgaki and Kleihues, 2009, Ohgaki and Kleihues, 2013) and constitute

majority of the GBM IV. Average age of patients with pGBM IV is 62 years. 2. Secondary GBM (sGBM IV), constitute only 5% of the total GBM IV cases and progress gradually from lower grade astrocytoma over several years (Sturm et al., 2014). Patients with sGBM IV are often younger than patients with pGBM IV, with an average age of 42 years (Ohgaki and Kleihues, 2009). Primary GBMs are histologically indistinguishable from secondary GBM. However, they are distinguishable at the molecular level by IDH mutations. Low grade astrocytoma also has mutations in IDH1 or IDH2. Treatment regimen in GBM is usually surgical resection followed by chemotherapy and/or radiotherapy. Poor survival rate is observed in patients with GBM with less than 5% of patients survive longer than five years (Sturm et al., 2014). Intensive combination therapy is observed to increase the average survival from 13 weeks with surgical resection alone to 15 months (Taveras et al., 1962).

1.3. Molecular biology of astrocytoma

Recent advances in technologies in genetics have identified a number of molecular markers that characterize astrocytoma beyond their histologic criteria. Few such markers have been used in the diagnosis, prognosis, and prediction of astrocytoma subtypes some of which have proven difficult to distinguish on morphological criteria alone. Three molecular markers in particular are widely used in the management of high-grade astrocytoma, namely, 1p/19q chromosomal co-deletion, O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, and isocitrate dehydrogenase (IDH) 1 and 2 mutations. Additionally, a specific mutation of the epidermal growth factor receptor (EGFR) variant III (EGFRvIII) is considered as a potential biomarker in these tumors (Table 1).

1p/19q chromosomal co-deletion is characterized by an unbalanced reciprocal translocation of 19q and 1p. Although the biological role of this marker remains unclear, this translocation has been associated with a better prognosis and a better response to postoperative treatment. This translocation is common in oligodendroglial phenotype. In WHO grade III tumors stratified according to 1p/19q status, sequence and the combination of radiotherapy and chemotherapy are currently being investigated by two international randomized trials (Radiation therapy, 2013_1; Radiation therapy, 2013_2).

Table 1. Molecular markers in glioma clinical practice.

Marker	**Diagnostic**	**Prognostic**	**Predictive**
1p/19q	in nearly all oligodendroglial tumors	WHO II-III	Predicitve for the treatment with radiotherapy and/or alkylating agents in WHO III
IDH	WHO > I; 2° GBM; exclusive for some glioma entities	WHO II-IV	No predictive role
MGMT	No diagnostic role	WHO III-IV	WHO IV; alkylating agents, especially in elderly
EGFRvIII	in ~33% of primary GBM	Not clearly defined	WHO IV; vaccine or targeted therapies (experimental)

*Taken from Hofer et al., 2014.

MGMT gene promoter methylation, which results in loss of methyltransferase repair activity, seems to be a prognostic factor prevalent throughout WHO grades II–IV gliomas. However, the frequency of MGMT gene promoter methylation is shown to decrease while the malignant potential rises (Hofer and Lassman, 2010). Over-expression of the MGMT protein in glioma tissue was associated with resistance to alkylating agents. In newly diagnosed glioblastoma multiforme (GBM) patients, addition of temozolomide (TMZ) chemotherapy to radiotherapy showed improved outcomes (Hegi et al., 2005). Recent studies suggest that the patients, especially elderly, with tumors exhibiting methylated MGMT fared better if they were treated with TMZ alone than those treated with radiotherapy alone (Wick et al., 2012, Malmstrom et al., 2012).

IDH1/2 mutations result in the metabolic re-programming in tumor cells, by assisting in the production of 2-hydroxyglutarate instead of α-ketoglutarate. These mutations, which occur early in gliomagenesis, have been shown to be able to drive increased methylation in gliomas. IDH1/2 mutations occur in over 80% of low-grade gliomas and also in secondary high-grade gliomas and the frequency of these mutations shown to remain same during the progression from WHO grade II to WHO grades III or IV. Primary and secondary GBM are histo-morphologically indistinguishable at diagnosis, and the presence of IDH mutations in secondary GBM provides a good diagnostic tool. IDH mutations in gliomas are associated with better prognosis (Zou et al., 2013).

EFGR variant III (EGFRvIII), present exclusively in tumor tissue and frequently expressed in primary GBM, causes constitutive activation of the receptor's tyrosine kinase activity and enhances tumorigenic behavior (Gan et al., 2013). EGFRvIII presents an ideal target for immunotherapy due to its exclusive localization in tumor tissue and reduces the risk of autoimmune toxicity. There have been randomized trials looking to demonstrate activity of EGFRvIII-targeted vaccination (Sampson et al., 2010, Phase III, 2013).

Although some genetic markers, such as EGFR and IDH1, EGFR and TP53, TP53 and 1p/19q, are mutually exclusive, nearly all with 1p/19q codeletion also harbor IDH1/2 mutations in low-grade and anaplastic gliomas. Also, IDH1 mutations are common in tumors with TP53 mutations or 1p/19q co-deletions (Kloosterhof et al., 2011, Malzkorn et al., 2011).

Genetic alterations or gene expression profiles now acts as an addition to histopathological diagnosis and provides, in some cases, a high concordance with prognosis than histopathology assessment (Freije et al., 2004, Nutt et al., 2003, Yamanaka et al., 2006). More than one molecular subtype each with different prognoses may be associated with a single histological subtype. Sequential accumulation of genetic alterations result in the malignant transformation and subsequently development of gliomas (Furnari et al., 2007, Louis et al., 2007, Reifenberger and Collins, 2004) (Figure 2). Several genetic alterations involve in the malignant transformation and subsequently development of gliomas, including amplification and/or overexpression of oncogenes, loss of tumour suppressor genes, mutations in DNA repair genes, loss of heterozygosity (LOH), and epigenetic mechanisms. Due to these genetic changes tumour cells undergo uncontrolled proliferation, lose control mechanisms of normal cell cycle, evasion of apoptosis in response to genotoxic agents, dysfunctional DNA repair mechanisms, genetic instability, and deregulation of growth factor signaling pathways. The molecular pathogenesis differs between various forms of glioma.

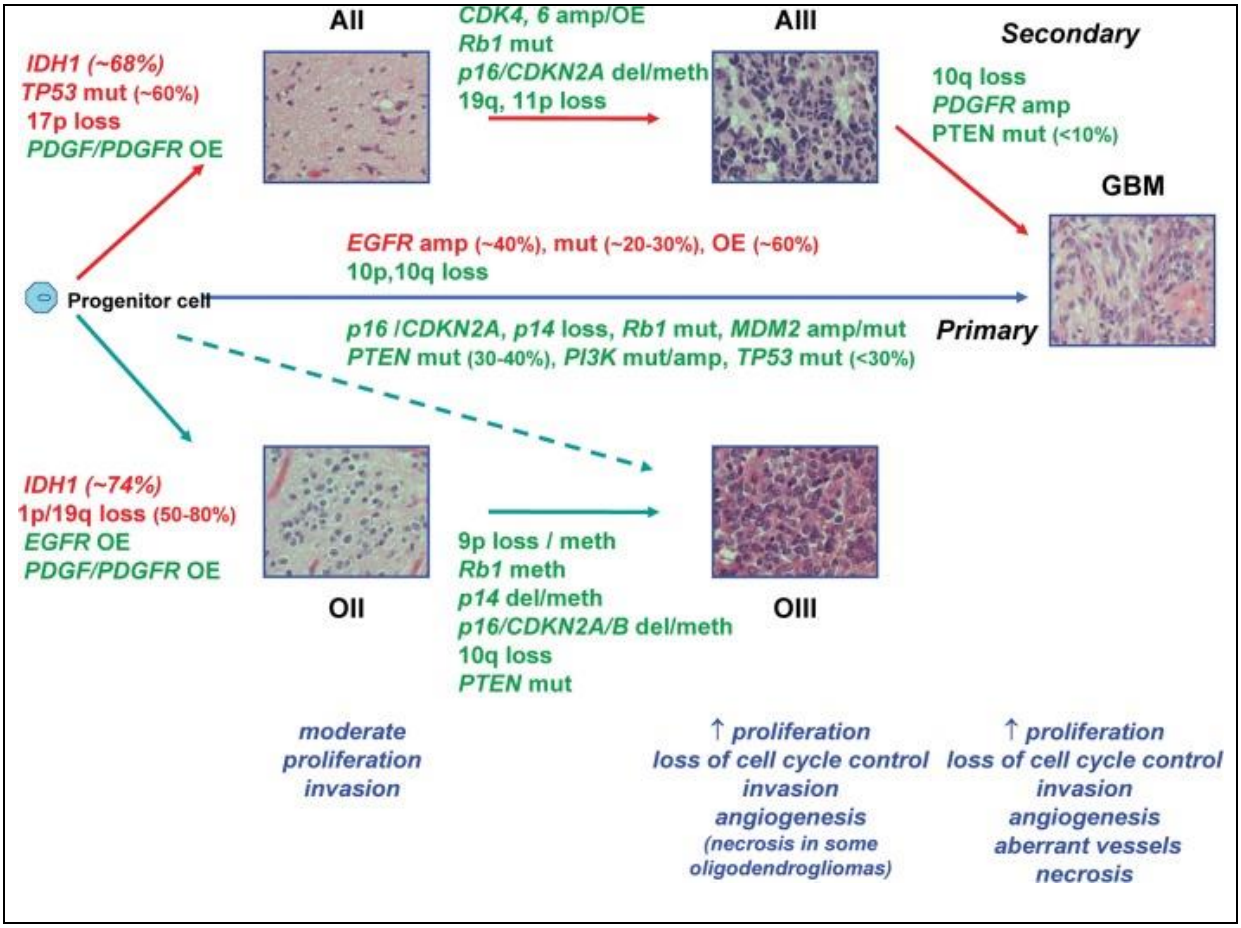

Figure 2. Molecular pathogenesis of adult astrocytic and oligodendroglial neoplasms. The illustration shows the progression of low-grade astrocytomas and oligodendrogliomas to higher grade with sequential accumulation of genetic alterations and impact on the biological properties of these tumours. Genetic alterations seen in lower grade tumours are retained on progression. Anaplastic oligodendrogliomas may arise through progression or de novo, but irrespective of route have similar clinical behaviour and molecular genetic characteristics with 1p/19q loss as their genetic hallmark. Glioblastomas arise *de novo* or progress from lower grade astrocytomas. Although indistinguishable clinically, they may be separated by their spectrum of genetic alterations, but these genetic alterations are not mutually exclusive to either lineage. The most common genetic alterations used to distinguish molecular subtypes of glioma are shown in red. AII, astrocytoma WHO grade II; AIII, astrocytoma WHO grade III; amp, amplification; del, deletion; GBM, glioblastoma WHO grade IV; meth, methylation; mut, mutation; OII, oligodendroglioma WHO grade II; OIII, oligodendroglioma WHO grade III. OE, overexpression (Reproduced from Walker et al., 2011)

1.4. Role of tumor microenvironment in the pathogenesis of glioma

Gliomas are also heterogeneous tumors composed of a complex milieu of tumor cells interacting in close proximity with the components of the tumor microenvironment including stromal cells and extracellular matrix (ECM). It has been shown that the crosstalk between different cell types within the tumor and its surrounding supportive tissue aides in tumor progression (Liotta and Kohn, 2001). Interaction between the invasive tumor cells and the microenvironment results in the remodeling of tumour microenvironment can be visualized in the form of desmoplasia, which will support the tumor growth and tumor progression (Koperek et al., 2011), which are also characteristic hallmarks of GBM (Furnari et al., 2007). Animal xenografts transplanted with purified malignant epithelial cells have shown to form histologically complex tumors, with 80% of the cells being stromal (Dudley et al., 2008). It has been shown that the invasive tumour cells modulate surrounding stroma by releasing several paracrine factors or growth and differentiation factors (GDFs) including fibroblast growth factor, members of the VEGF family, PDGF, EGFR ligands, interleukins, colony-stimulating factors, transforming growth factor β (Mueller and Fusenig, 2004), which can disrupt normal tissue homeostasis and thus result in the inflammatory response and angiogenesis (Baeriswyl and Christofori, 2009).

Although the processes of tumors promoted inflammation and interactions between tumour cells and inflammatory cells are still unclear, deformed immune system has been shown to assist in tumour development and progression (Hu et al., 2005), and chronic inflammation has been shown to be associated with the development of various tumors (Castellsague et al., 2002, Nelson et al., 2002). Infiltrating immune cells, especially macrophages and neutrophils, have shown to provide a regular supply of chemokines, growth factors and proteases, as well as DNA-damaging reactive oxygen and nitrogen species for the tumour development and invasion. These infiltrating macrophages have shown express high levels of proliferation-associated genes, significantly higher than to those from wound or resting peritoneal macrophage profile (De Wever et al., 2004). Activated macrophages produce chemokines such as CXCL12 which attracts immune cells leading to tissue destruction, favoring invasion and metastasis. CXCL12 also promote the growth and survival of cancer cells expressing the CXCR4 receptor and known to activate CXCR4 which may also lead to greatly increased production of tumor necrosis factor-α (TNF-α). CXCL12 has

also been shown to recruit precursor cells for vasculogenesis (Zumsteg and Christofori, 2009).

Macrophage migration inhibitory factor (MIF)

MIF has been shown to inhibit the migration of macrophages and shown to have pro-inflammatory functions (Bloom and Bennett, 1966, Donn and Ray, 2004). Furthermore, in gliomas MIF has been shown to be up-regulated when glioma cells are subject to either hypoxia or hypoglycemia and also involved in several fundamental processes of tumorigenesis, including angiogenesis (Bacher et al., 2003b). A strong correlation between MIF expression and vascular endothelial growth factor (VEGF) expression in human glioblastomas has also been reported (Munaut et al., 2002). Furthermore, overexpression of MIF correlated with higher levels of Interleukin 8 (IL-8) in tumors (Ren et al., 2005, Liao et al., 2010), this is of some interest as IL-8 has shown to play a role in microvessel synthesis in various tumors (Waugh and Wilson, 2008), which has been considered to influence tumor metastasis and prognosis in various human cancers (Thelen et al., 2008). High-grade gliomas such as anaplastic astrocytoma and glioblastoma show a higher degree of microvascular proliferation. *In vitro*, hypoxia shown to induce IL-8 expression by activating two transcription factors, AP-1 and NF-κB in tumor cells (Xu et al., 2004, Maxwell et al., 2007). However, the relationship between MIF and the production of IL-8 by tumor cells in gliomas is largely unknown.

CD74

CD74, a cell membrane receptor for macrophage migration inhibitory factor (MIF), is an MHC class II associated invariant chain molecule expressed on the cell surface in a sub-set of immune cells. CD74 has been shown to be overexpressed in various cancers such as B-cell neoplasms, multiple myeloma, thymic, gastric, renal, non-small cell lung and breast cancers (Datta et al., 2000, Ishigami et al., 2001, Ioachim et al., 1996, Mandal et al., 2007, Burton et al., 2004, Shih et al., 2000, Porter et al., 2003), and has been associated with more aggressive tumor behavior in some of these solid malignancies (Ishigami et al., 2001). In animal models, inactivating CD74 function has resulted in anti-tumor activity (Mark et al.,

2009, Stein et al., 2007, Stein et al., 2004, Chang et al., 2005). Binding of CD74-CD44 complex to MIF ligand results in the recruitment of Src, which then can signal downstream to activate multiple signaling pathways including Ras/MAPK and Akt (Leng et al., 2003, Lue et al., 2006, Lue et al., 2007), which are important modulators of cell proliferation and survival in GBM (Furnari et al., 2007). Recent studies have shown that inactivation of CD74 expression suppresses MIF-induced phosphorylation of MAPK and AKT in U87 cells suggesting the possibility that MIF-CD74 signaling through these pathways may contribute to the TMZ resistance associated with CD74 overexpression (Kitange et al., 2010).

Extracellular matrix (ECM)

ECM, the most abundant component in tumor microenvironment, plays a critical role in modulating tumor cell responses to exogenous influences such as growth factor activation or chemotherapy. Components of the ECM of brain tumors include the basement membrane components, collagen IV, laminin, and fibronectin lining the blood vessels as well as collagen I, tenascin-C, vitronectin, and hyaluronan surrounding the tumor (Bellail et al., 2004, Chintala et al., 1996, Huijbers et al., 2010). Collagen constitutes the scaffold of tumor microenvironment and affects tumor microenvironment such that it regulates ECM remodeling by collagen degradation and re-deposition, and promotes tumor infiltration, angiogenesis, invasion and migration. While collagen was traditionally regarded as a passive barrier to resist tumor cells, it is now evident that collagen is also actively involved in promoting tumor progression. Collagen changes in tumor microenvironment release biomechanical signals, which are sensed by both tumor cells and stromal cells, trigger a cascade of biological events.

Collagen

Collagen, which accounts for one-third of total proteins, consists of a right-handed triple helical structure composed of three polypeptide α-chains with a recurring Gly-X-Y sequence displaying a polyproline-II conformation, where X and Y are usually proline and hydroxyproline respectively (Brodsky and Persikov, 2005, Sweeney et al., 2008). The polypeptide triple helix contains both the collagenous (COL) domain and the non-

collagenous (NC) domains. Collagens can be categorized into classical fibrillar and network-forming collagen, FACITs (fibril-associated collagens with interrupted triple helices), MACITs (membrane-associated collagens with interrupted triple helices), and MULTIPLEXINs (multiple triple-helix domains and interruptions) according to the structure properties of ECM (Shoulders and Raines, 2009). There are 29 different collagen types which are classified into three main classes (Shoulders and Raines, 2009). The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) have identified mutations, at low frequency (<1%) in both GBM tumors and cell lines, in more than 20 different collagen genes using sequencing analysis on glioma samples, however it remains unclear if these mutations are non-functional passenger mutations or drivers that confer a selective survival advantage to tumor cells.

Collagen receptors in gliomas

In brain tumors, collagen has been shown to play three primary functions: 1. Function as scaffolding for adhesion for cells which is critical for cellular processes such as glioma cell migration along collagen tracks and contractility in response to external forces. 2. Function as a reservoir, through a balance of collagen synthesis, posttranslational modifications and degradation, of matricellular proteins, proteoglycans and growth factors (Sweeney et al., 2008), release specific factors in a temporal or dose-dependent manner in response to changing biological context. 3. To act as a ligand for the activation of signal transduction networks required for tumor growth, differentiation and invasion (Leitinger, 2011) through the action of collagen binding receptors.

Discoidin Domain Receptors (DDRs)

Collagens have been shown to activate the DDRs, a class of receptor tyrosine kinases (RTKs), rather than classical growth factor ligands (Fu et al., 2013). Collagen binding to DDRs results in a delayed phosphorylation response that takes several hours to achieve full activation but remains sustained for days without significant downregulation (Fu et al., 2013, Iwai et al., 2013). Two members of DDR family have been identified, namely DDR1 and DDR2. DDR1 is primarily expressed in epithelial cells and is activated by both fibrillar and

basement membrane collagen. DDR2 has been shown to express in mesenchymal cells and is known to bind exclusively to fibrillar collagens and collagen X, and both these receptors have been shown to bind with high affinity to a GVMGFO motif within collagens I, II and III (Leitinger, 2011). The cytoplasmic domains of DDR1 and DDR2 have tyrosine residues that serve as potential phosphorylation sites for receptor activation and these receptors have been shown to be associated with cell proliferation, adhesion, migration and extracellular remodeling (Fu et al., 2013).

Hypoxia and Angiogenesis

The pattern and the extent of vascularization differs between normal tissue and tumor tissue, and several cancers, including astrocytic neoplasms, have been shown to have increased vessel density with an increase in tumour grade (Louis et al., 2007, Wen and Kesari, 2008). Pathogenic vessel formation in tumour tissue, as shown in Figure 3, is a result of cascade of events such as hypoxia, adaptation of tumor cells to rapid growth, metabolic scarcity , alteration of the microvasculature architecture, and synthesis of proangiogenic growth factors and cytokines (Wen and Kesari, 2008, Zadeh and Guha, 2003). Intratumoral differences in the regions of hypoxia, necrosis, and cellular proliferation can critically influence the angiogenic response within the tumor and can also impact distribution of chemotherapy drugs, especially antiangiogenic drugs (Jain, 2003). Characteristic feature of high grade astrocytoma is the presence of anaplastic glial cells, marked mitotic activity, nuclear pleomorphism, dense cellularity, marked increase in microvascular proliferation, and more strikingly the presence of hypoxic necrotic foci often surrounded by pseudopalisading cells (Wen and Kesari, 2008, Bai et al., 2011).

Hypoxic signalling pathways in the dynamic oxygen environment of cancer cells leads to variable expression of molecular markers of hypoxia (e.g. HIF-1α) and its downstream targets (e.g.VEGF), imparting multifaceted angiogenic gene expression in tumours (Bristow and Hill, 2008). Studies based on histological examination and differential expression of angiogenesis-related transcripts revealed a considerable heterogeneity in the microvascular architecture, including the density, shape, magnitude, and pattern of distribution of microvessels, as well as in the molecular signatures among different histological specimens of similar grade and in those from different grades (Godard et al.,

2003, Hendriksen et al., 2009). These results suggest that human brain tumors exhibit variable angiogenic/vasculogenic patterns and that vascular heterogeneity adds another layer of complexity to microenvironmental or metabolic variability in GBM. Understanding the factors involved in metabolic, hypoxic, and angiogenic reprogramming of the cells could prove valuable and provide new insights for diagnosis and therapy.

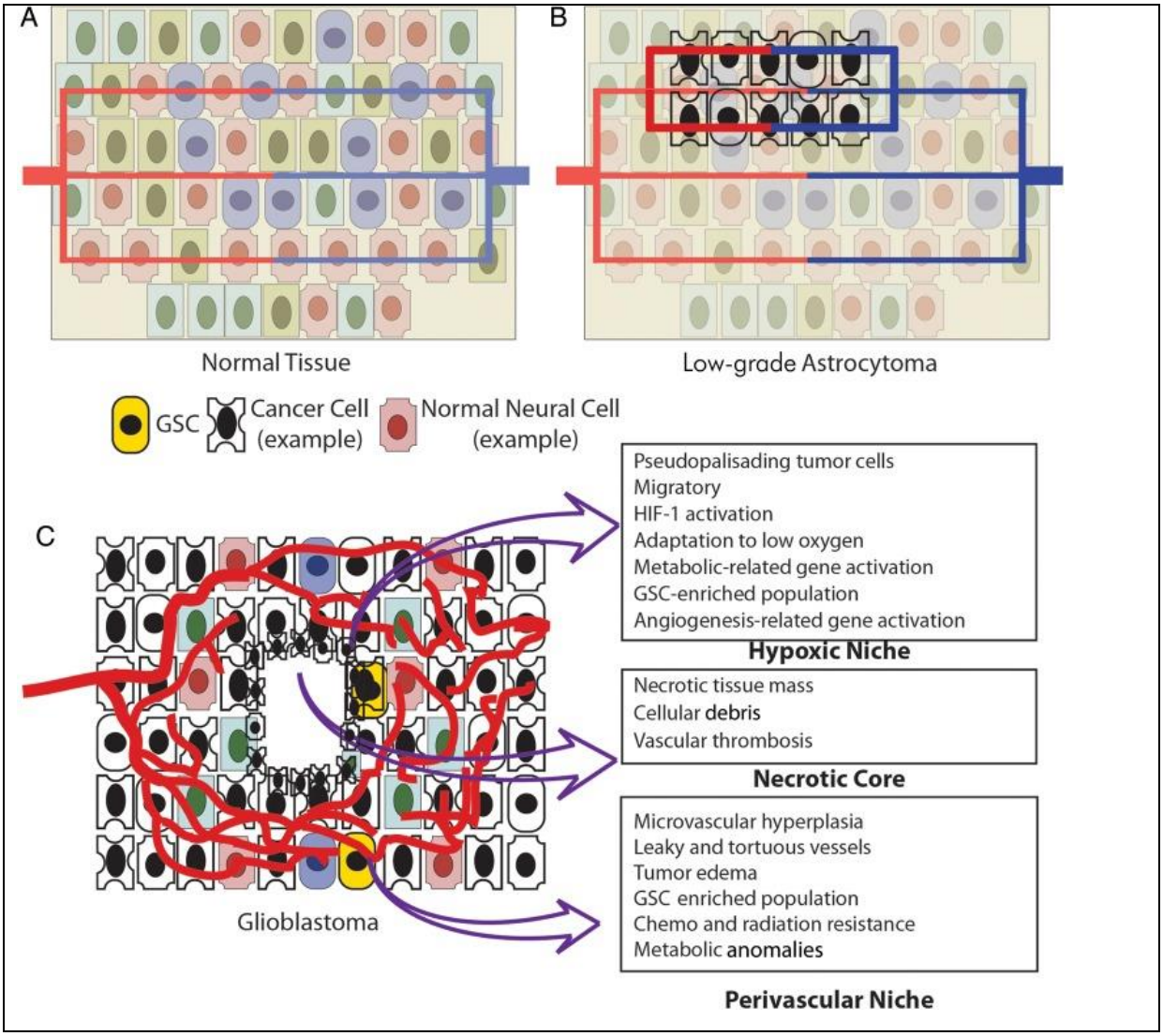

Figure 3. Schematic illustration of the events in glioma development, with respect to vasculature, metabolism, and cellular heterogeneity. (A) Normal tissue displaying regular blood supply. (B) Low grade astrocytoma showing a slight increase in tumour growth due to the increased microvessel proliferation. (C) More heterogeneous microenvironment and abnormal vasculature can be seen in high grade GBM, where each regions have been shown to display different salient features (text in the box) (Reproduced from Vartanian et al., 2014).

1.5. Astrocytoma standard treatment and drug resistance

For patients suffering from glioblastoma, the standard treatment includes maximal safe resection, involved field radiation, and concomitant and adjuvant temozolomide, an alkylating agent that binds to DNA and prevent cell proliferation. Patients who receive a more extensive resection, defined as 78–98 % of contrast enhancing tumor, have shown an improved survival compared to patients who receive a subtotal resection or biopsy (Lacroix et al., 2001, Sanai et al., 2011). Furthermore, combinatorial therapy involving radiation showed a significant improvement in outcomes compared to chemotherapy alone or best conventional care (Walker et al., 1978, Walker et al., 1980), and the standard dosing and fractionation of 60 Gy in 30 daily fractions has been recommended (Chang et al., 1983, Bleehen, 1979, Kristiansen et al., 1981, Shapiro et al., 1989, Coughlin et al., 2000).

For initial management of glioblastoma patients, "Stupp protocol" has become the standard of care as Strupp et al., demonstrated that the concomitant and adjuvant temozolomide had a significant improvement in median survival (14.6 months) compared to patients treated with radiation alone (12.1 months) (Stupp et al., 2005), and also the proportion of patients surviving 5-years after diagnosis was five times higher in the temozolomide group (Stupp et al., 2009). Recent studies have shown that bevacizumab, which targets VEGF and inhibits vascularization in tumour cells, may improve outcomes after disease recurrence, with median progression-free survival of 4–6 months and median overall survival of 8–9 months (Friedman et al., 2009, Kreisl et al., 2009), which compared favorably to historical controls (Wong et al., 1999, Lamborn et al., 2008).

Prospective, randomized trial data defining the utility of these modalities in lower grade astrocytoma such as grade III anaplastic gliomas is lacking as these tumors are more heterogeneous in terms of their behavior, genetics, and response to therapy compared to glioblastomas. Surgical resection has been the part of standard care along with radiotherapy which has been shown to improve the outcome in the lower grade tumours, although a statistically robust subgroup analysis is needed to confirm this observation (Walker et al., 1978, Walker et al., 1980). In anaplastic astrocytoma patients those do not possess a 1p/19q co-deletion, treatment with temozolomide per the Stupp protocol alone has shown better outcomes (Lassman et al., 2011), although in general this treatment is given in combination with radiotherapy (Lamborn et al., 2008).

Although a better understanding of the biology and behavior of these tumors and their response to treatment, a larger case-control and comprehensive study involving the histopathologic, genetic, and epigenetic changes that underlie tumor biology will allow for more tailored treatment of these heterogeneous tumors.

1.6. Gene expression analysis using RNA sequencing

To understand the relationship between genotype and phenotype we need to understand the control of gene expression. Novel technologies such as DNA microarray and RNA sequencing were developed to assess abundance of transcripts in biological samples more reliably. Microarrays use a hybridization method where nucleic acid probes (typically 60-mers) that are covalently bound to glass slides were hybridized to fluorescently labeled target sequences. These hybridized probe-target sequences are scanned and the images are then converted to signal intensities and the data is processed using software specific to the application of the array. However, due to cross-hybridization artefacts and larger sample requirement in the microarray-based methods, RNA-sequencing method has become the favored technique as it provides more quantitatively accurate measurement to obtain absolute transcript abundance, and also detect novel transcripts using relatively low sample input (Wang et al., 2009, Marguerat and Bahler, 2010). Besides gene expression profiling, the utility of RNA-sequencing far exceeds that of a microarray.

RNA-sequencing can characterize exon junctions, and can detect non-coding RNA (Arnvig et al., 2011), single nucleotide polymorphisms, and fusion genes using data from the same experiment (Maher et al., 2009). Furthermore, using updated/refined annotations existing data sets can be re-evaluated as new sequences (Roberts et al., 2013). RNA-sequencing can also detect non-coding RNA (small RNA) with some modifications in sample preparation procedures to exclude larger RNA sequences prior to cDNA generation. Epigenetic processes are now can be examined using RNA-sequencing (Liew et al., 2013). Due to the digital nature of RNA-sequencing, it can achieve higher resolution of differentially expressed genes and provides an unlimited dynamic range of detection. RNA-sequencing costs vary between $400 and up to $1000 per sample.

RNA-sequencing method can be performed on a sample input as little as 10pg of RNA, however a typical gene expression profiling method would require about 200-1000 ng amount of RNA by the current RNA-sequencing platforms. Sample material used in RNA-

sequencing can be fresh, frozen tissue and formalin fixed paraffin embedded material (with some adaptations) (Zhao et al., 2014b). A typical RNA sequencing method involves isolation of total RNA followed by production of cDNA by reverse transcription (Figure 4). This process requires fragmentation and attaching specific sequence linkers (adapters) to the RNA prior to cDNA production. The adapter-ligated sequences are then read on analyzer.

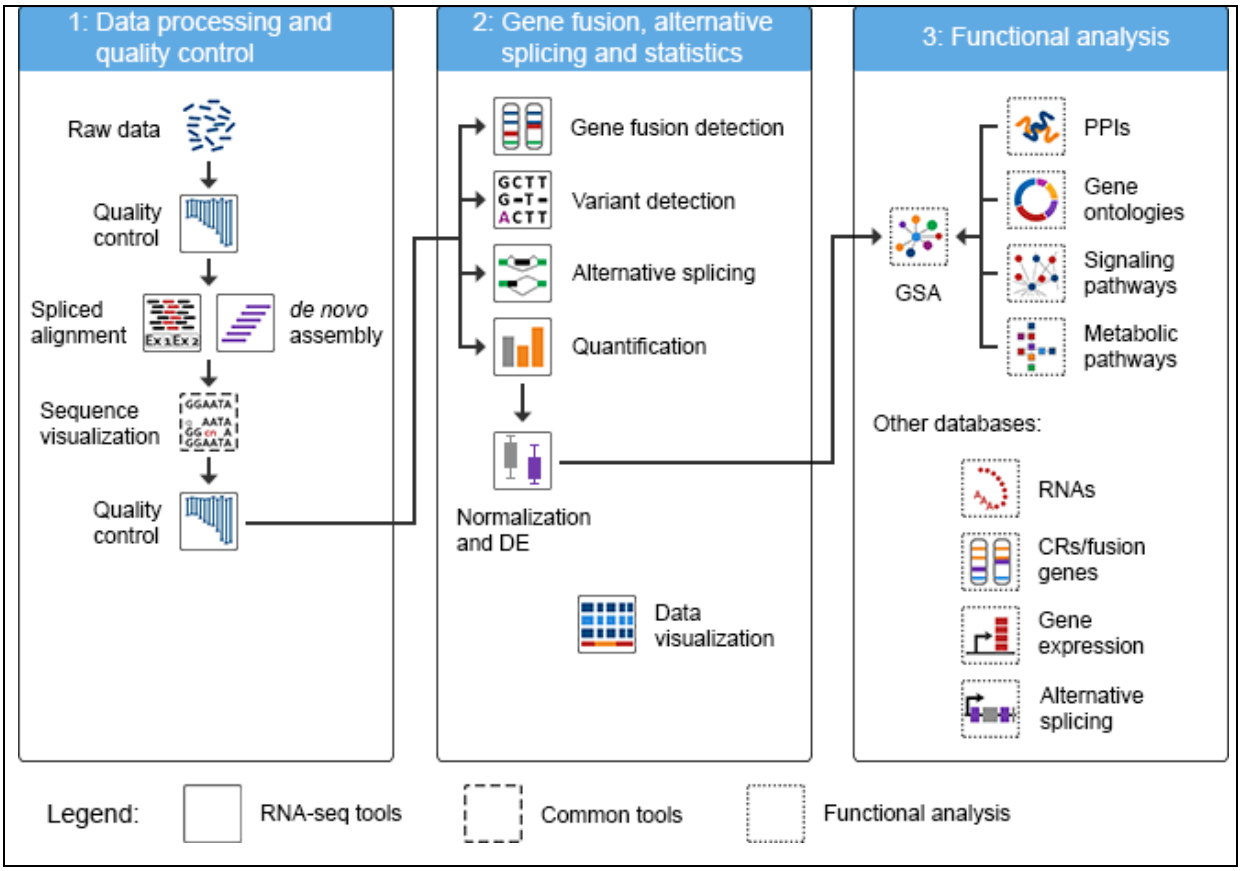

Figure 4. RNA sequencing and data analysis. In recent years, the continuing technical improvements and decreasing cost of next-generation sequencing technology have made RNA sequencing (RNA-sequencing) a popular choice for gene expression studies (Wang et al., 2009, Ozsolak and Milos, 2011). Such sequence-based methods have revolutionized studies of the transcriptome by enabling a wide range of novel applications, including detection of alternative splicing isoforms and transcript fusion detection. In addition, RNA-sequencing has become an attractive alternative to microarrays for the identification of differentially expressed genes between several conditions or tissues (Reproduced from OMICTools; https://omictools.com/RNA-sequencing -category).

Various bioinformatics tools, data resources, and statistical and mathematical models are developed to analyze the data obtained from RNA sequencing method. Some of the data

analysis tools include sequence aligner such as BWA or Rsubread, and Normalization, Quantitative analysis and Differential Expression tools such as Limma (Ritchie et al., 2015), DESeq (Anders and Huber, 2010), Cufflinks (Trapnell et al., 2010), and EdgeR (McCarthy et al., 2012).

Extensive experience and the bioinformatics skills are necessary to analyze RNA-sequencing data. The data analysis techniques vary considerably depending on the type of software used (Trapnell et al., 2012) and purpose of study (Drewe et al., 2013, Zhang et al., 2014).

2. THE AIMS OF THE STUDY

Despite advances in current treatment regimen for patients with diffuse high grade astrocytoma, which includes surgical resection followed by radio- and chemotherapy, a poor overall survival rate is observed in these cases. This poor prognosis is attributed to uncontrolled tumor proliferation and invasion of surrounding brain parenchyma4, and the ability to induce angiogenesis5. A number of ongoing studies are focused on finding potential biomarkers and therapeutic targets to improve survival and prognosis in diffuse high grade astrocytoma. Several clinical factors, including age at diagnosis, surgical resection, and clinical status as assessed by the Karnofsky performance score (KPS), have been established as independent prognostic parameters in glioblastoma patients. The study includes six main parts, in which I aimed to:

1. Investigate similarities and differences in differential gene expression patterns in grade II-IV astrocytoma.
2. Identify potential biomarkers in astrocytoma using gene expression analysis
3. Investigate expression of collagen and DDR1 proteins and their association with the clinicopathological factors in GBM
4. Investigate CD74 protein expression and its association with clinicopathological factors in GBM

3. MATERIALS AND METHODS

3.1. Patients and samples

3.1.1 Samples used in gene expression analysis

RNA sequencing data using in this study was obtained from open resources as detailed in Table 2. No ethical approval was sought for this study as all the data used in this study obtained from published studies which have obtained ethical approval. Detailed information on samples used in RNA sequencing data analysis was shown in Appendix 1.

Table 2. List of samples used in this study.

Sample (WHO grade)	Codes (this study)	Number of samples	GEO Accession
Diffuse Astrocytoma (II)	A1-A34	34	GSE48865
Anaplastic astrocytoma (III)	AA1-AA13	13	
Primary glioblastoma (IV)	pGBM1-pGBM58	58	
Secondary glioblastoma (IV)	sGBM1-sGBM19	19	
Recurrent glioblastoma (IV)	rGBM1-rGBM19	19	
Single cell astrocytes	SC1-SC61	61	GSE6783
Normal brain samples_1	N1-N2	2	GSE62731
Normal brain samples_2	N3-N7	5	GSE49712

3.1.2 Patients and samples used in protein expression analysis and their clinicopathological characteristics

72 untreated patients (15 cases of grade 2 diffuse astrocytoma, 9 grade 3 anaplastic astrocytoma and 48 grade 4 glioblastoma) with clinical data were included in immunohistochemical study. FFPE (Formalin-fixed paraffin embedded) blocks from these astrocytoma patients and 3 normal brain samples were obtained from the archives of Department of Pathology, Faculty of Medicine and Dentistry, Palacky University in Olomouc. Patients were diagnosed between 2007 and 2010 with a maximum follow-up time of 81 months.

Clinical data was extracted from clinical records, including age, gender, KPS and histopathological classification and WHO grade. Clinical data regarding recurrence was not included in the study as this information was missing for the majority of cases. Patient samples were re-evaluated by two independent pathologists (NN & JE) according to the 2007 WHO classification of the central nervous system tumours (Louis et al., 2007). The use of all samples was approved by the Ethics Committee of the University Hospital and the Faculty of Medicine and Dentistry, Palacky University in Olomouc.

3.2. Gene expression analysis

3.2.1 RNA sequencing data analysis

RNA sequencing data analysis was performed with the help from Pradeep Ramagiri, PhD from the Institute of Cancer and Genomic Sciences, the University of Birmingham, UK.

Raw sequence reads were obtained from GEO (Gene Expression Omnibus; RRID: SCR_005012) in the form of FastQ files. Reads from FastQ files were aligned to human reference sequence hg19 (RRID: SCR_006553) using Rsubread aligner (Liao et al., 2013). Mapped sequencing reads were assigned to hg19 refGene using featureCounts (RRID: SCR_012919). RefGene exon coordinates were obtained from UCSC table browser (RRID: SCR_013787). Differentially expressed genes were identified using edgeR (RRID:

SCR_012802)(McCarthy et al., 2012). Genes with read count per million > 1 in at least half of the samples were considered for further analysis. The data was normalized using TMM (trimmed mean of M values) method.

3.2.2 Gene set enrichment analysis

Gene set enrichment analysis was done using Molecular Signatures Database (MSigDB; RRID: SCR_003199) to identify pathways and underlying biological themes. GSEA tool (RRID: SCR_003199) was used to investigate enrichment of 8 gene sets (tumor suppressor genes, oncogenes, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors/co-factors, and cytokine and growth factors) and the cancer census gene list (Futreal et al., 2004) in differentially expressed genes in this study.

3.2.3 Pathway analysis

Pathways enriched in differentially expressed genes were investigated using Molecular Signatures Database (MSigDB; RRID: SCR_003199). MSigDB evaluates the overlap of our genes sets with its collections and provides an estimate of the statistical significance (p and FDR q-value). I have also used cytokine-cytokine interaction pathway (hsa: 04060) and glioma pathway (hsa: 05214) maps from Kyoto Encyclopaedia of genes and genomes (KEGG; RRID: SCR_012773) to highlight key genes from this study.

3.3. Protein expression analysis

3.3.1 Van Geison's method for collagen

For Van Gieson's stain, tissue sections (4μm) were first dewaxed and rehydrated. Slides were then placed on a slide tray and tissue sections encircled using a PAP-PEN. 100 μl of 4% iron alum was pipetted onto the slides ensuring the section was completely covered. After 5 minutes slides were rinsed in tap water and the nuclei counter-stained with Mayer's haematoxylin for 5 minutes. The sections were again washed in warm tap water and placed on a slide tray and 100 μl of Van Gieson's solution applied for 10 minutes. The slides were blot dried with filter paper ensuring no contact with water after this point. Following the Van

Gieson method, stained sections were dehydration by rinsing in 100% ethanol for 30 seconds followed by Histoclear for 5 minutes.

Staining was evaluated by two independent pathologists qualitatively. Areas with malignant glial cells (GFAP positive) distant from necrosis were considered.

3.3.2 Immunohistochemical study

Immunohistochemical staining, evaluation and scoring

Formalin-fixed and paraffin-embedded tissue sections (4μ) were deparaffinized in xylene and rehydrated by washing in serial dilutions of ethanol (96%, 80% and 70%) and rinsed in deionized water. Detailed information on the antigen retrieval procedures, antibodies and their dilutions is provided in Table 3. After antigen retrieval, slides were rinsed in tap and deionized water. For blocking endogenous peroxidase activity, slides were treated with 0.3% hydrogen peroxide solution for 15 min. Sections were washed in deionized water for 5 min, then twice in 0.05M Tris buffer (pH 7.4-7.6), once in Tris buffer with 0.5% Tween solution and incubated with primary antibody for 1 hour at room temperature in a humid chamber. Slides were washed twice in Tris buffer, once in Tris buffer with 0.5% Tween solution, and incubated with secondary antibody (Dual Link, Dako) for 1 hour. After the last washing step in Tris buffer, slides were incubated in substrate solution (DAB), counterstained in hematoxylin, dehydrated through alcohols and xylene and mounted. All antibodies were commercially available and validated by manufacturer.

Table 3. Antibody characteristics and dilutions

Antibody	Clone	Species/ clonallity	Antigen retrieval	Dilution	Positive Control	Manufacturer
CD74	c-2955, LN-2	Mouse monoclonal	Citrate, Buffer pH6,	1:600	Hodgkin lymphoma	Sigma Aldrich
MIF	MAB289, 12302	Mouse monoclonal	Citrate, Buffer pH6, MW	1:600	Hodgkin lymphoma	R&D system
DDR1	D1G6, 5586	Rabbit monoclonal	Citrate, Buffer pH6,	1:250	Normal brain	Cell Signalling

DDR1

DDR1 protein expression was evaluated semi-quantitatively by two independent pathologist (NN and JE) blinded to clinical data. Membranous and cytoplasmic staining was assessed by histoscore method (percentage of positive astrocytoma cells multiplied by intensity of staining (categorized as: 0, absent; 1, weak; 2, moderate; and 3, strong), resulting in histoscore ranged from 0 (minimum) to 300 (maximum)). Score 0 was regarded as negative, cases <185 (median expression score) were considered as low expression, cases with ≥185 as high expression. For each experiment, an additional negative control was included in which primary antibody was replaced by non-immune serum. As positive control for DDR1 antibody staining was used normal brain tissue (Figure 5).

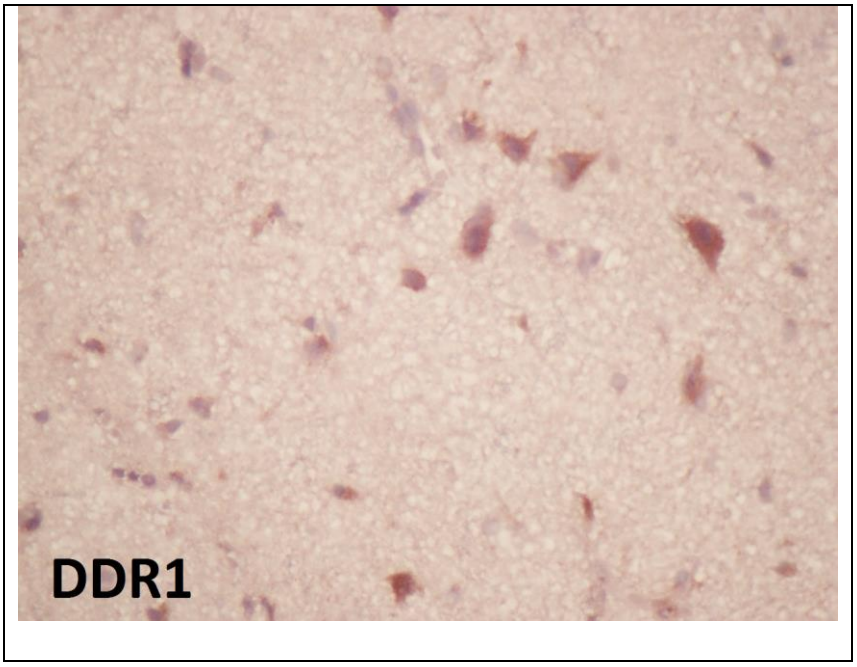

Figure 5. DDR1 staining in normal brain tissue (20X).

MIF and CD74

MIF and CD74 protein expression was evaluated semi-quantitatively by two independent pathologists (NN and JE) blinded to the clinical and histological diagnosis. Immunoreactivity score (IRS) was measured according to the methods described by Hara

et.al.,(Hara and Okayasu, 2004). Percentage of positive cells was estimated and scored into 5 categories defined as follows: 0, all negative; 1+, <25% positive cells; 2+, 25–49%; 3+, 50–74%; and 4+, >75%. The intensity of staining was (scored on a scale 0 to 3) also classified into four groups as follows: 0, negative; 1+, weak; 2+, moderate; and 3+, strong. The intensity level observed in the largest number of positive cells was used for scoring. Immunoreactivity score (ranging between 0 and 12) for each case was obtained by multiplying scores from percentage and intensity. Cases were categorized as low expression group (Hara score ≤ 6 IRS) and high expression group (Hara score > 6 IRS) for statistical analysis. As positive control for both antibody staining was Hodgkin lymphoma (Figure 6).

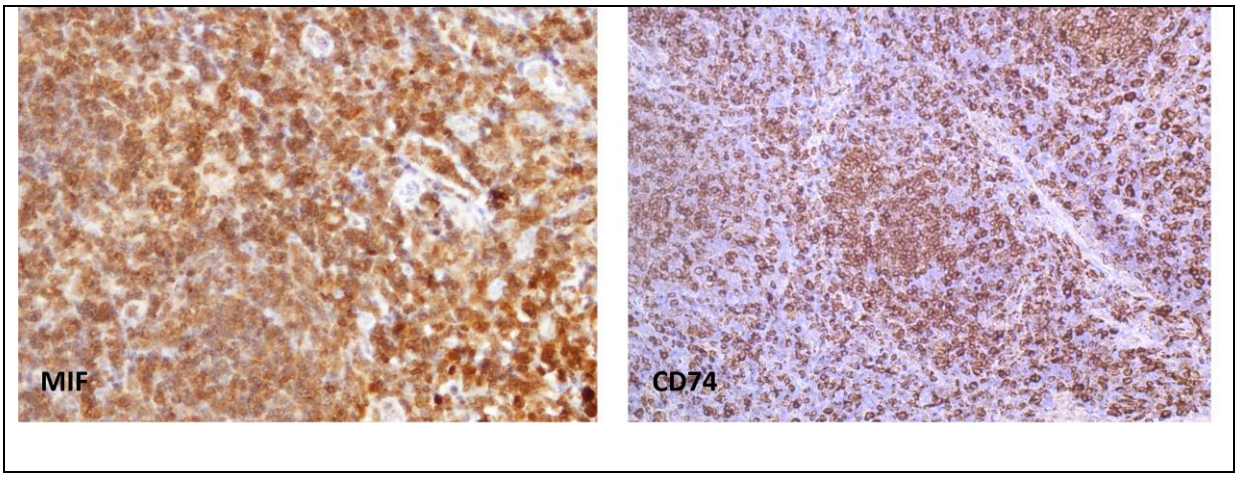

Figure 6. MIF and CD74 staining in Hodgkin lymphoma (20X).

3.4. Statistical analysis

Statistical analysis for gene expression analysis was done using the R language and environment (RRID: SCR_003005). The Heatmap3 program was used to generate heat maps for differentially expressed genes in R (Zhao et al., 2014a).

Immunoreactive scores for CD74 and DDR1 were compared according to each of clinicopathological factors (age, gender, grade, and KPS). Nonparametric comparison between independent groups was carried out using the Mann-Whitney and Kruskal–Wallis test. Overall survival rates were examined by using Kaplan-Meier method (p-values with log-rank test). Uni- and multivariate analysis were performed using Cox regression analysis to examine whether the CD74 expression was a prognostic factor independent from other

clinicopathological factors. Above analyses were performed using the SPSS software (version 20). *P*-values <0.05 were considered statistically significant.

4. RESULTS

4.1. Gene expression profiling of astrocytoma grades II-IV

There is an increased understanding of the pathobiology (Tonn JC, 2006, Parsons et al., 2008, Brennan et al., 2013, Jones et al., 2011, Cancer Genome Atlas Research, 2008), molecular subtypes (Cooper et al., 2010, Noushmehr et al., 2010, Verhaak et al., 2010), and transcriptional regulatory networks (Carro et al., 2009, Deshmukh et al., 2011, Jornsten et al., 2011, Setty et al., 2012, Wang et al., 2013) of astrocytoma over the last two decades. Biomarkers that distinguish between astrocytoma grades have also been reported in previous studies (Hunter et al., 2002, Rickman et al., 2001). Studies involving a comparative analysis of microarray data from AS II, AS III, and GBM IV provided insight into dysregulation of regulatory networks associated with increasing astrocytoma grades (Seifert et al., 2015). However, the majority of these studies have either used no or a low number of normal brain samples in describing gene expression differences between astrocytoma grades (Verhaak et al., 2010, Hunter et al., 2002, Rickman et al., 2001, Seifert et al., 2015).

In this chapter, I described the global gene expression pattern for astrocytoma using publicly available RNA sequencing data from patients with AS II, AS III, and GBM IV, and normal brain samples. I also investigated the genetic similarities and differences between these subtypes and also identified key regulatory networks and signaling pathways that they share and also those that set them apart. Furthermore, I identified key genes that potentially implicate in the pathogenesis of astrocytoma.

I performed RNA sequencing data analysis using reads from FastQ files of the above mentioned data sets. I aligned these reads to human reference sequence using Rsubread aligner as described in Materials and Methods section 2.2.1. Summary of number of RNA sequencing reads (from all the subtypes of Astrocytoma and normal samples) mapped to human reference sequence hg19 was shown in Table 4. Number of mapped reads in single cell RNA sequencing data (Normal SC1- SC61) was ten times lower than that of the other normal and tumour RNA sequencing data. This was expected as the single cell data was obtained from fewer numbers of cells.

Table 4. Number of mapped reads in astrocytoma subtypes and in normal samples (range).

Astrocytoma subtype	**Number of mapped reads (range)**
Astrocytoma (Grade II)	8793303 - 27109750
Anaplastic Astrocytoma (Grade II)	12848932 - 29867560
Primary Glioblastoma (Grade IV)	11156795 - 32043355
Secondary Glioblastoma (Grade IV)	12704542 - 32708433
Recurrent Glioblastoma (Grade IV)	12361499 - 35248857
Normal samples (N1-N7)	25284841 - 71235040
Normal (Single cell)samples (SC1-SC61)	173891 - 2677473

4.1.1 Differential gene expression in astrocytoma

A total of 8936 over-expressed genes and 4699 under-expressed genes were observed in all AS II-IV subtypes when compared to all normal brain samples (log fold change of 2; ($p \leq 0.001$; and FDR ≤ 0.002). Top 100 genes that were differentially expressed in astrocytoma can be seen in Table 5 (for upregulated) and Table 6 (for downregulated).

MKI67, EGFR, Receptor tyrosine kinases (RTK), collagens, G-coupled receptor proteins genes and ligands, home box genes, chemokine receptors and ligands, and PDGF/VEGF growth factor family genes were among the most noticeable groups of genes that are significantly over expressed in astrocytoma. In under expressed genes, Receptor tyrosine kinases (RTK), miRNAs were the largest group genes that are most frequently present in the top 100 genes (17/100, 9/50, and 2/20 top under expressed genes).

Table 5. List of 100 most upregulated genes in astrocytoma grades II-IV

Gene	Log FC*	Gene	Log FC*	Gene	Log FC*
LTF	10.2	C6orf15	7.7	HIST1H1B	7.0
COL3A1	9.9	MMP7	7.6	SIGLEC12	7.0
POSTN	9.5	ABCC3	7.6	PLAU	7.0
PI3	9.4	IGFBP2	7.6	C1QL1	7.0
SAA1	9.4	ALPI	7.6	OSR2	7.0
PLA2G2A	9.3	UBE2C	7.6	BIRC5	7.0
MMP9	8.9	TGFBI	7.6	IBSP	6.9
TOP2A	8.8	FCGBP	7.5	LIF	6.9
H19	8.8	HOXD8	7.5	HJURP	6.9
HOXD13	8.7	SIX6	7.5	MMP14	6.9
MYBL2	8.7	TLX1	7.5	HOXC9	6.9
OR6C2	8.6	LINC00689	7.4	GSC	6.9
HOXD9	8.5	CXCL6	7.4	NDC80	6.9
HOXA10	8.4	MUC2	7.4	NLRP5	6.9
MKI67	8.4	NKX2-5	7.4	TCHHL1	6.9
COL1A2	8.3	COL1A1	7.3	HOXA3	6.9
HOXD11	8.2	GSX1	7.3	CDKN2A	6.9
HOXC10	8.1	TREM1	7.3	DLK1	6.8
CPXM1	8.1	GOLGA6L2	7.3	OR10C1	6.8
MMP1	8.1	NNMT	7.3	ALPK2	6.8
CCL18	8.0	HOXC11	7.3	MXRA5	6.8
RRM2	8.0	MRC2	7.3	COL4A1	6.8
LINC01602	7.9	DLGAP5	7.2	MAGEC2	6.8
MMP13	7.9	MUC17	7.2	BPIFB6	6.8
IGLL5	7.9	IL2RA	7.2	COL5A1	6.8
MMP12	7.8	TROAP	7.2	MDFI	6.7
HOXD10	7.8	F2R	7.2	HOXA7	6.7
PTX3	7.8	PITX1	7.2	CCL20	6.7
COL6A2	7.8	CHI3L2	7.1	GPR50	6.7
FAM111B	7.7	MELK	7.1	MMP2	6.6
CCL7	7.7	ESM1	7.1	SAA2	6.6
AURKB	7.7	NMB	7.1	KIF18B	6.6
DLL3	7.7	DPEP1	7.1		
HOTAIR	7.7	KRT75	7.1		

*FC = Fold change

Table 6. List of 100 most downregulated genes in astrocytoma grades II-IV

Gene	Log FC*	Gene	Log FC*	Gene	Log FC*
GPC5-AS1	-12.1	LOC101928191	-7.7	TMEM212-AS1	-7.0
ARHGAP26-IT1	-10.7	LOC145474	-7.6	SNORD12C	-7.0
NRG3-AS1	-9.7	LOC101929653	-7.6	SNORD63	-7.0
LINC00499	-9.5	MIR5009	-7.6	LOC101927539	-7.0
LINC00710	-9.5	LOC285740	-7.6	LINC01182	-7.0
GPC5-AS2	-9.4	PPP2R2B-IT1	-7.6	MIR4794	-7.0
GNA14-AS1	-9.3	MIR5688	-7.6	SNORA80B	-7.0
LOC102724784	-9.2	FAM155A-IT1	-7.5	MIR3117	-7.0
SNAR-F	-9.1	RRS1-AS1	-7.5	CYCSP52	-7.0
LINC01241	-9.0	CACNA1C-AS4	-7.5	LINC01586	-6.9
MAGI2-AS2	-9.0	MIR3611	-7.4	ZNF630-AS1	-6.9
LOC646029	-8.9	TUSC7	-7.4	SNORA28	-6.9
SNORD128	-8.8	TMEM72	-7.4	AIRN	-6.9
LINC00635	-8.8	MIR4796	-7.4	LOC101927237	-6.9
LOC101927310	-8.8	LOC100303749	-7.4	ZBTB20-AS3	-6.9
MIR8485	-8.7	MIR3128	-7.3	MIR101-1	-6.9
LOC101928786	-8.6	HIST1H4C	-7.3	LOC284344	-6.9
MIR4473	-8.5	MIR4447	-7.3	C6orf7	-6.9
RPS16P5	-8.5	RBMS3-AS1	-7.3	LOC101926933	-6.8
LOC101929124	-8.4	C10orf113	-7.2	LARGE-AS1	-6.8
PWRN3	-8.4	F11-AS1	-7.2	FLJ34503	-6.8
ARHGAP26-AS1	-8.4	MIR548BB	-7.2	LOC100289473	-6.8
LOC101927379	-8.3	STX18-IT1	-7.2	LOC101927437	-6.8
BRD7P3	-8.2	IDI2-AS1	-7.2	LOC101928590	-6.8
LOC729609	-8.2	SNORA70E	-7.2	HIST1H2BB	-6.8
FLJ30679	-8.1	PKIA-AS1	-7.2	MIR215	-6.7
GRID1-AS1	-8.1	CELF2-AS2	-7.2	LOC105376331	-6.7
MIR920	-8.0	GUSBP3	-7.1	TESC-AS1	-6.7
MIR616	-7.9	MIR5096	-7.1	DANT2	-6.7
LOC101929259	-7.9	VAV3-AS1	-7.1	LINC01108	-6.7
HLTF-AS1	-7.9	MIR7157	-7.1	ITPK1-AS1	-6.7
LSAMP-AS1	-7.8	SNORA27	-7.1	CLDN10	-6.7
TMEM108-AS1	-7.8	MIR4529	-7.1		
CTD-2350J17.1	-7.8	GPR171	-7.1		

* FC = Fold change

Molecular functions enriched in differentially expressed genes in astrocytoma grades II-IV

To understand the biological relevance of differentially expressed genes in astrocytoma, I performed gene set enrichment analysis of differentially expressed genes in astrocytoma using gene sets of 8 functional categories as described in section 2.2.2 in Materials and Methods.

This analysis revealed a significant enrichment of tumor suppressor genes, oncogenes, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors/co-factors and cytokine and growth factors (Figure 7) in the over-expressed genes in astrocytoma when compared to normal. Transcriptional factors/co-factors were most significantly enriched in these genes ($p \leq 3.686E^{-170}$).

In case of under-expressed genes, cell differentiation markers, homeodomain proteins, and cytokine and growth factors gene sets were not enriched in the under expressed genes (Figure 7). Tumour suppressor genes were the most significantly enriched genes ($p \leq 8.02E^{-17}$) in under-expressed genes as well.

4.1.2 Similarities and differences in genes expression in astrocytoma grades II-IV

I next performed differential gene expression analysis of astrocytoma grades AS II, AS III, pGBM, sGBM and rGBM separately, which revealed 773, 149, 3963, 280, and 304 over-expressed and 889, 200, 1859, 377, and 390 under-expressed genes, respectively (log fold change of 2, ($p \leq 0.001$, and FDR $\leq$ 0.002). List of 100 most significantly upregulated or down regulated genes in each astrocytoma subtype (II-IV) with fold change is provided in Appendix 3-11.

The number of under-expressed genes was higher that of over-expressed genes in AS II, III, sGBM and rGBM, whereas in pGBM the number of over-expressed genes was more than that of under-expressed genes (Figure 8). The number of differentially expressed genes per sample in primary GBM was higher (3 fold for over-expressed genes) than the rest of the astrocytoma grades.

137 over-expressed genes and 191 under-expressed genes were common to all astrocytoma grades. 50 over-expressed genes and 42 under-expressed genes were found to be unique to AS II (Figures 9-10).

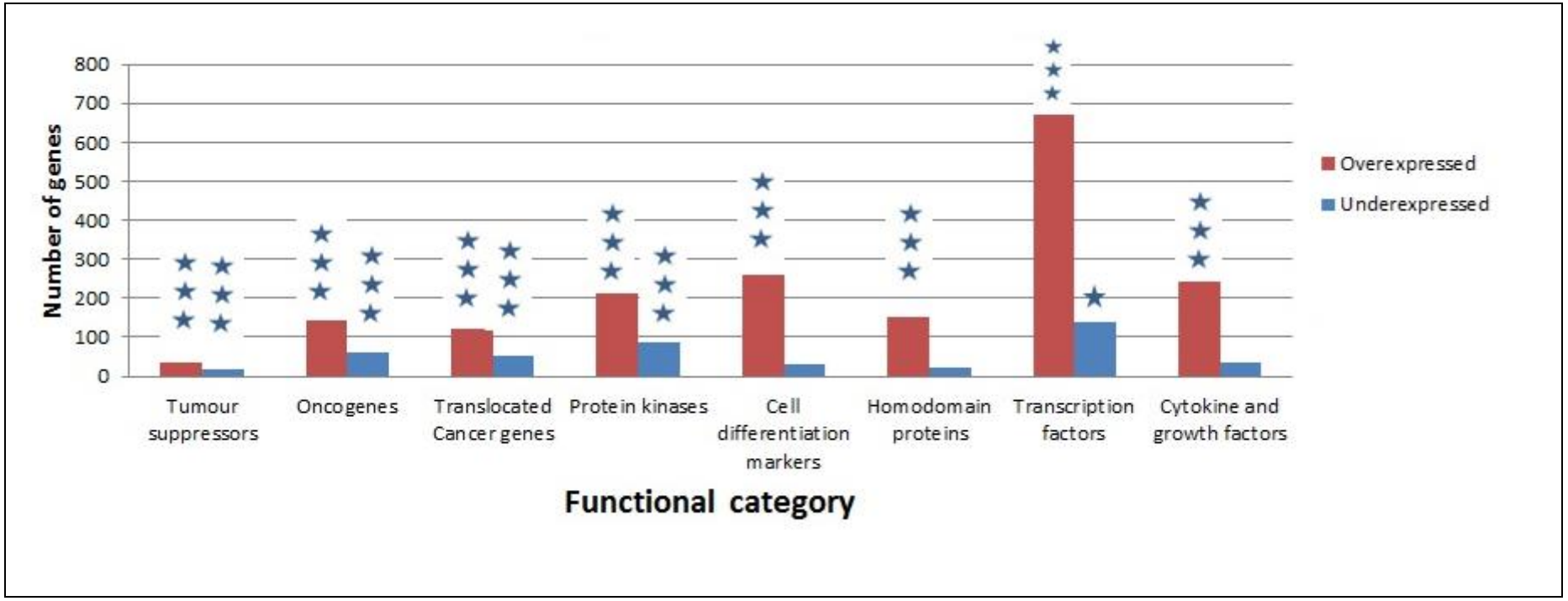

Figure 7. Enrichment of differentially expressed genes in astrocytoma in selected functional categories: tumor suppressor genes, oncogenes, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors and cytokine and growth factors (see Methods for details). Significant enrichment of genes in a category within a tumor type is represented by '*' ($p < 0.05$) and '***' ($p < 0.0001$) (Fisher's exact test).

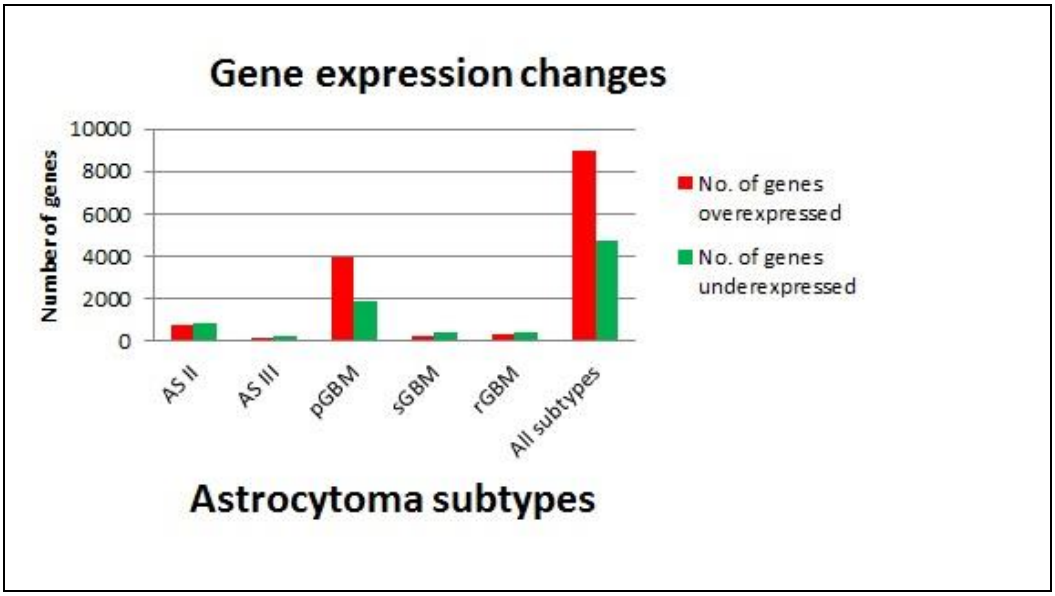

Figure 8. Differentially expressed genes in astrocytoma subtypes when compared to normal (log FC of 2, ($p \leq 0.001$ and FDR ≤ 0.002).

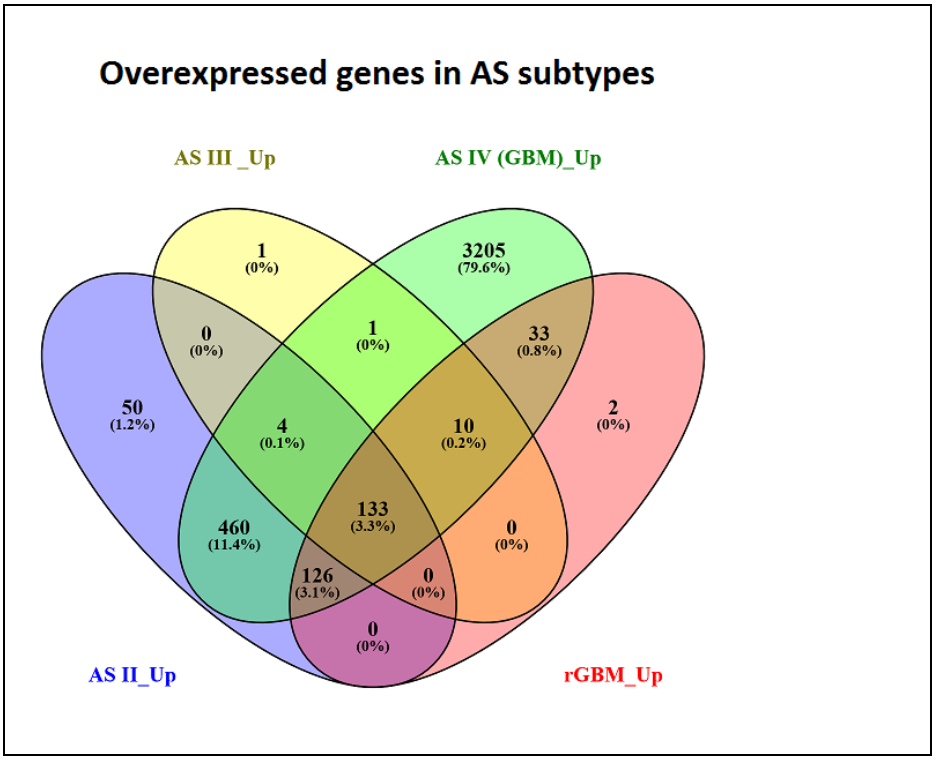

Figure 9. Overlap of upregulated genes between grades II-IV of Astrocytoma. A statistically significant overlap is seen across all subtypes (p < 8.28E-174 in all AS subtypes).

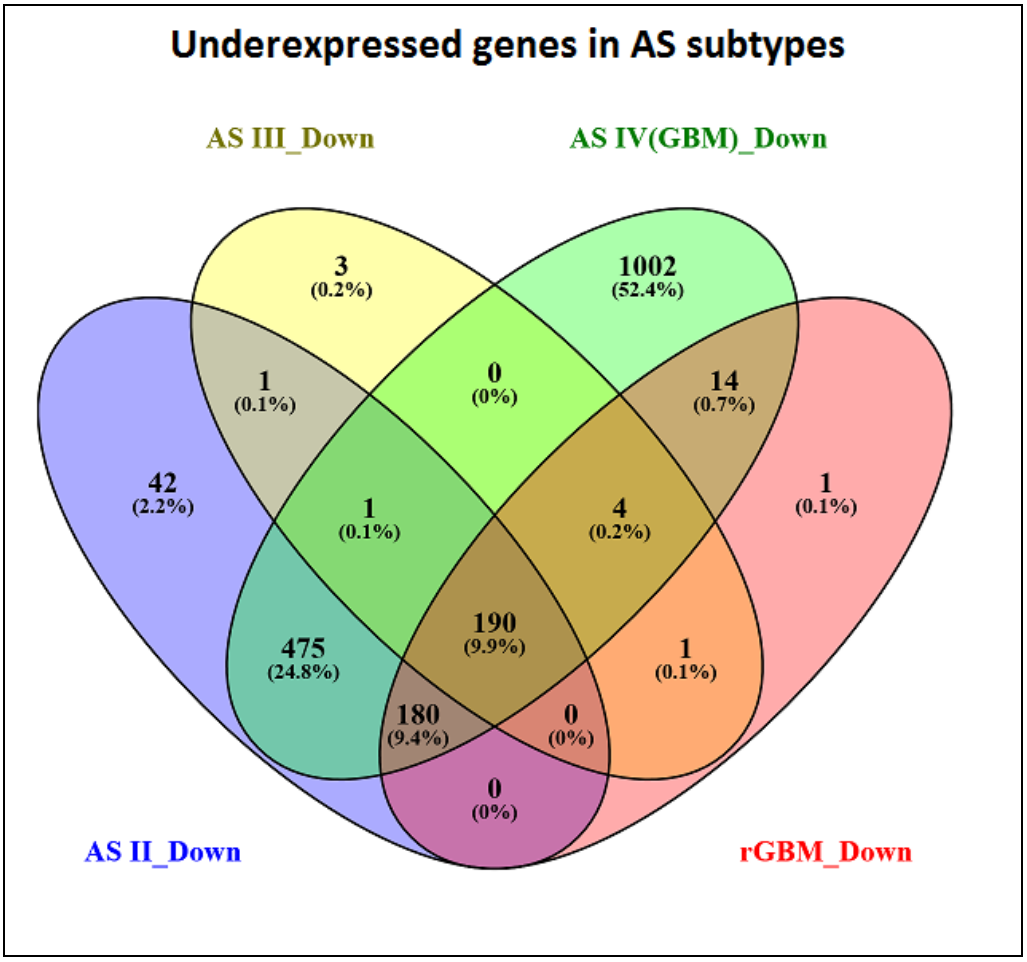

Figure 10. Overlap of downregulated genes between grades II-IV of Astrocytoma. A statistically significant overlap is seen across all subtypes ($p < 6.61E^{-288}$ in all AS subtypes).

The number of over-expressed and under-expressed genes shared between grade IV GBM subtypes were 247 and 330, respectively (Figure 11).

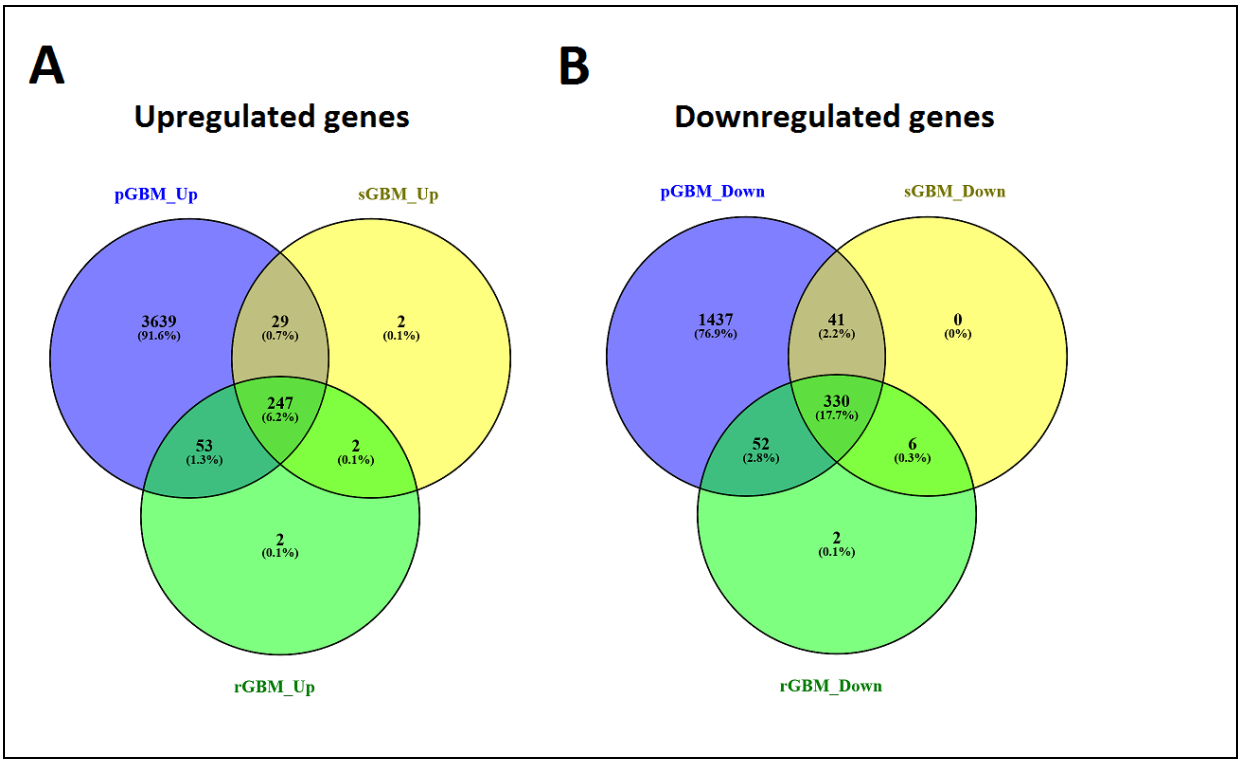

Figure 11. Overlap of differentially expressed genes in grade IV astrocytoma subtypes. A. Statistically significant overlap of upregulated genes was seen between pGBM, sGBM and rGBM. There is significant overlap between each of the GBM subtype (p < 1.853e-319 in all GBM subtype). B. Statistically significant overlap of downregulated genes between pGBM, sGBM and rGBM was observed (p < 0.00E+00 in all GBM subtype).

I next performed global gene expression analysis for grades II –IV astrocytoma using selected 2884 genes that were exclusively expressed in each of these subtypes. I observed a distinct gene expression pattern was observed between astrocytoma grade II and grade IV (Figure 12). Although a different expression pattern between primary GBM and other grade IV GBM subtypes can be seen, I observed a high overlap between grade IV subtypes.

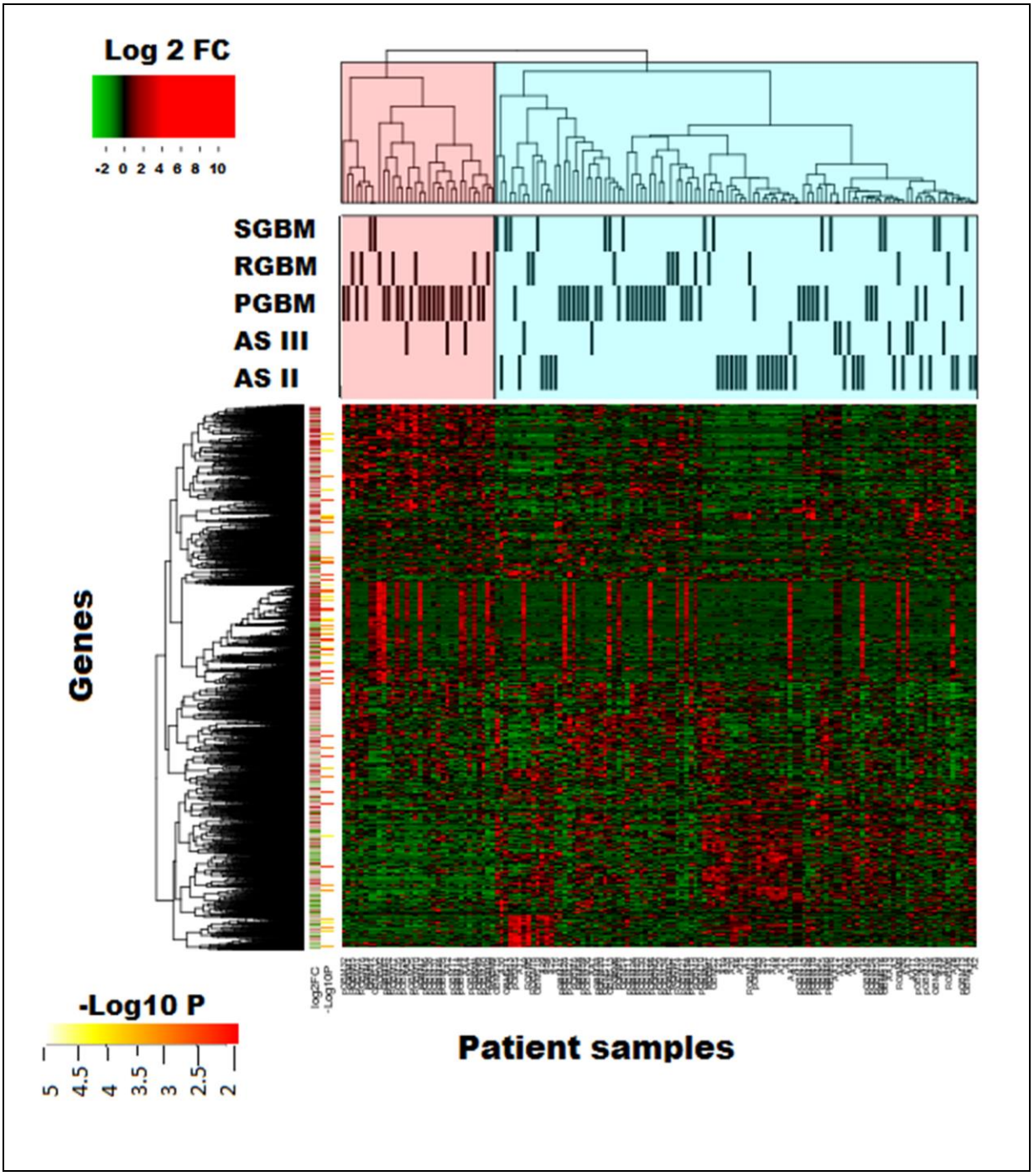

Figure 12. Heat map of differentially expressed genes in different Astrocytoma subtypes. Heat map for selected 2884 genes (at log Fold Change $\geq 2/\leq -2$, $p \leq 0.001$ and FDR ≤ 0.002) that were exclusively expressed across different subtypes showing significant differences between grade IV GBM and grades II-II of astrocytoma. Significant differences within the grade IV GBM, between primary and secondary GBM can also be seen in this heatmap.

Molecular functions enriched in astrocytoma grades II-IV

Among the selected functional categories of gene sets (tumor suppressor genes, oncogenes, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors/co-factors and cytokine and growth factors), oncogenes are significantly enriched ($p < 0.005$) in both overexpressed and under expressed genes across all astrocytoma subtypes and Homeodomain protein genes are the least represented genes among the selected gene sets used in the enrichment analysis across all subtypes (Figures 13-14).

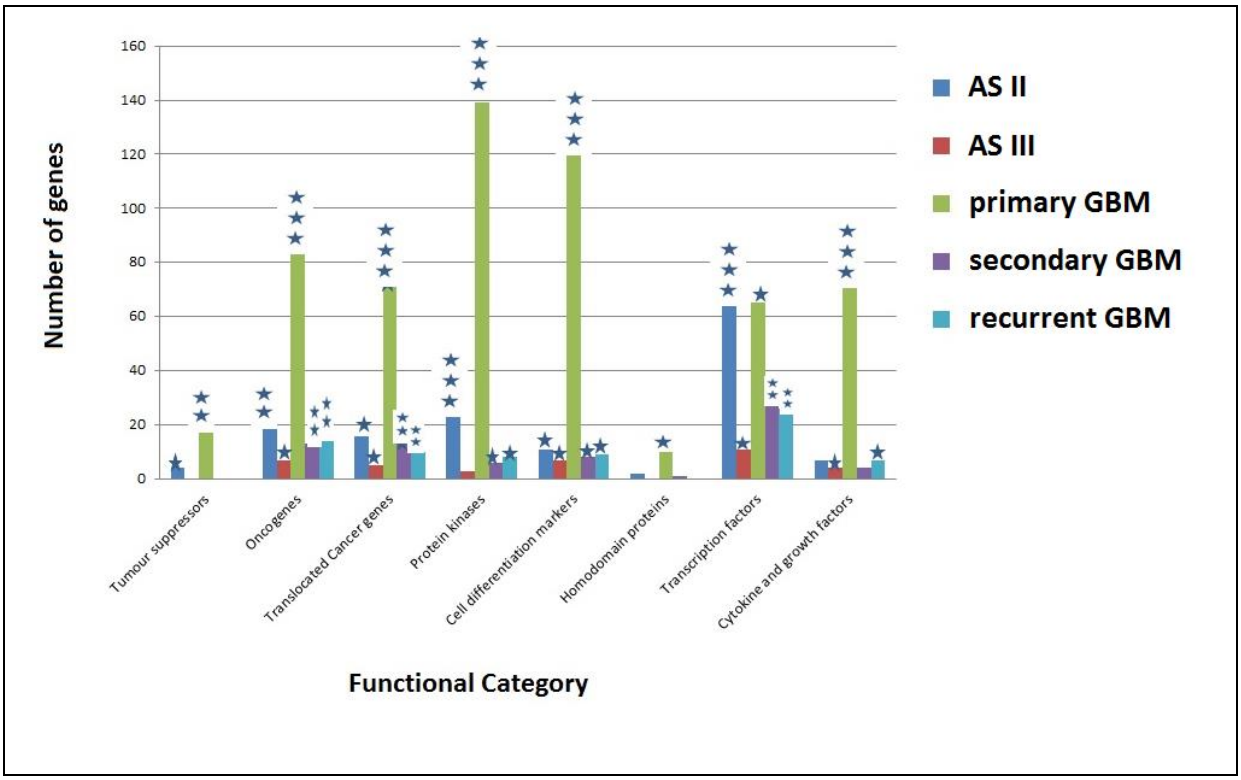

Figure 13.Gene set enrichment analysis of over-expressed genes in astrocytoma subtypes. Number of differentially expressed genes annotated in selected functional categories: tumor suppressor genes, oncogenes, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors and cytokine and growth factors (see Methods for details). Significant enrichment of genes in a category within a tumor type is represented by '*' ($p < 0.05$), '**' ($p < 0.001$), and '***' ($p < 0.0001$) (Fisher's exact test).

In AS II, tumor suppressor genes, translocated cancer genes, protein kinases, cell differentiation markers, transcription factors/co-factors are significantly enriched in both over expressed and under expressed genes (Figures 13-14).

In AS III, translocated cancer genes, cell differentiation markers, transcription factors/co-factors, and cytokine and growth factors are significantly enriched in over expressed genes (Figure 13), whereas, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors/co-factors and cytokine and growth factors are significantly enriched in the under expressed genes (Figure 14). Tumour suppressor genes are significantly enriched in down regulated genes in all astrocytoma subtypes, except AS III.

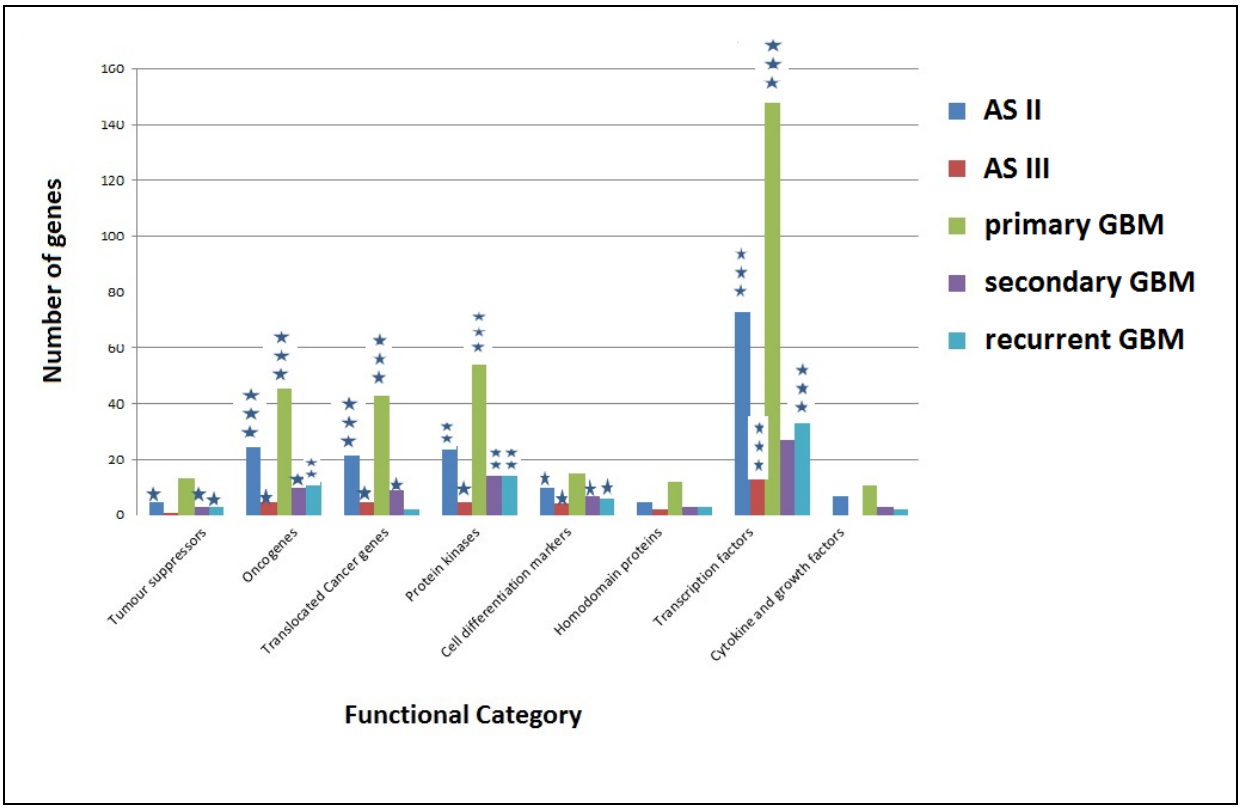

Figure 14. Gene set enrichment analysis of under-expressed genes in astrocytoma subtypes. Number of differentially expressed genes annotated in selected functional categories: tumor suppressor genes, oncogenes, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors and cytokine and growth factors (see Methods for details). Significant enrichment of genes in a category within a tumor type is represented by '*' ($p < 0.05$), '**' ($p < 0.001$), and '***' ($p < 0.0001$) (Fisher's exact test).

Significant enrichment of all of the selected gene sets was observed in over expressed genes in the WHO grade IV pGBM and all selected gene sets, except homeodomain proteins, and cytokine and growth factors, were significantly enriched in under expressed genes

(Figure 14). Collagens comprises largest family of genes that are significantly over expressed in pGBM (6/20, 7/50 and 10/100 of top 100 most significant genes).

In Secondary GBM (grade IV), translocated cancer genes, protein kinases, cell differentiation markers, transcription factors/co-factors are significantly enriched in both over expressed and under expressed genes. Tumour suppressor genes are also significantly enriched in the under expressed genes in sGBM (Figure 14).

Translocated cancer genes, protein kinases, cell differentiation markers, transcription factors/co-factors are significantly enriched in over expressed genes in recurrent GBM (grade IV), whereas, translocated cancer genes, protein kinases, cell differentiation markers, transcription factors/co-factors and cytokine and growth factors are significantly enriched in the under expressed genes (Figure 14).

Translocated cancer genes, protein kinases, cell differentiation markers, transcription factors/co-factors were significantly enriched in over expressed genes in recurrent GBM (grade IV), whereas, translocated cancer genes, protein kinases, cell differentiation markers, transcription factors/co-factors and cytokine and growth factors were significantly enriched in the under expressed genes (Figure 14). There are 133 genes that are commonly over expressed in all astrocytoma subtypes and these subtypes also share 199 genes that are under expressed. There was a significant overlap between genes that are differentially expressed across all astrocytoma subtypes ($p < 8.28E^{-174}$).

4.1.3 Genes previously reported in astrocytoma grades II-IV

To identify the genes previously reported to be implicated in astrocytoma, I did a literature review using PubMed. I have highlighted (in bold) some of the genes previously implicated in astrocytoma in the top 25 genes most differentially expressed in grades II-IV in this study (Table 7).

I found similar pattern of gene expression for genes in this study that were previously reported to characterize the GBM IV subtype, with an exception of MDM2, MDM4, AXL, FGFR3, and MERTK genes, which were reported to overexpress in glioma, were under expressed in this study. MDM2 and MDM4 are known to inhibit a p53-dependent growth control (Song et al., 2014, Matsuda et al., 2009), which were reported to be overexpressed in

GBM IV, was significantly under expressed in all GBM subtypes in this study (pGBM(-2.92 log FC), sGBM(-2.14 log FC), and rGBM(-2.57 log FC).

However, amplification of the CDK4 gene and overexpression of cyclin D1 suggested to have similar effects to p16 or pRb inactivation, these mechanisms may provide additional alternatives to subvert cell-cycle control and facilitate progression to GBM (Schmidt et al., 1994). CDK4 and CCND1 (CyclinD1) are overexpressed in our WHO grade IV primary GBM samples.

MALAT1 (Metastasis-associated lung adenocarcinoma transcript 1), is a type of long noncoding RNA. It is associated with metastasis and is a favorable prognostic factor, reported to play a vital role in reduction of extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signaling activity and expression of matrix metalloproteinase 2 (MMP2). MALAT1 gene is under expressed in all AS subtypes in this study (Han et al., 2016). Connexin (Cx) 30 gene gap junction beta-6 (GJB6) is reported to be deleted and or mutated in glioma (Artesi et al., 2015), is significantly under expressed in all AS subtypes in this study. Y-box binding protein-1 (YBX1) is a member of the cold shock protein family and functions in transcription and translation and reported to highly express in tumour cells and as a marker for tumour aggressiveness and clinical prognosis in various types of cancers, including glioblastoma (Takahashi et al., 2010). YBX1 gene is overexpressed in all AS subtypes in this study. WIF1 (Wnt inhibitory factor 1) is a candidate tumour suppressor gene in glioblastoma and was reported to be downregulated in most glioblastomas (Lambiv et al., 2011). WIF1 gene is under expressed in all AS subtypes in this study.

SLC1A2 (Solute Carrier Family 1 (Glial High Affinity Glutamate Transporter), Member 2) is downregulated in glioma and was suggested to assist in metastasis in glioma (Tong et al., 2015), is under expressed in all AS subtypes. PFN1 (phosphorylation of profilin-1) directs the angiocrine expression and glioblastoma progression through HIF-1α accumulation (Fan et al., 2014) and is over expressed in grade IV GBM subtypes in this study.

Table 7. 25 most significantly over-/ under-expressed genes in each astrocytoma subtype.

Upregulated genes				
AS II	**AS III**	**PGBM**	**SGBM**	**RGBM**
OLIG1	**COL1A1**	**COL3A1**	**COL1A1**	**COL1A1**
FLJ16779	MIR6087	**COL1A2**	MIR6087	MIR6087
MIR6087	**SOD2**	**COL6A2**	**RGMA**	FN1
RGMA	FKBP10	**IGFBP2**	FKBP10	FKBP10
SOX11	**GFAP**	**FCGBP**	ELN	**RGMA**
HES6	**HLA-B**	**TGFBI**	**YBX1**	**COL6A1**
APOE	**COL6A1**	**ABCC3**	ALYREF	**HLA-B**
LTBP3	**VEGFA**	**COL1A1**	SEPN1	**SOD2**
FAM181B	**APOE**	MRC2	**GFAP**	ELN
TSC22D4	FADS2	**F2R**	**PFN1**	SEPN1
ADGRB1	**GPC1**	**MMP14**	**KHSRP**	**PFN1**
GFAP	TSC22D4	MXRA5	**HLA-B**	**CHI3L1**
FADS2	TIMP2	CHI3L2	PRR11	RPS3
A2M	MYO9B	**PLAU**	EEF2	**VEGFA**
SLC12A4	**FN1**	**COL4A1**	TNRC18	SEC61A1
SCRIB	TTYH3	**COL5A1**	**NOTCH1**	HLA-H
ZNF428	SEC61A1	**CD163**	RAB5C	ALYREF
MTSS1L	**CHI3L1**	**MMP2**	**LAMP1**	**GPC1**
FLNA	**YBX1**	**ANXA1**	SEC61A1	**YBX1**
GNB2	**HLA-H**	**IFI30**	RPSAP58	IGFBP7
APC2	KCTD12	NMB	**APOE**	**APOE**
PLEKHG2	EEF2	SERPINE1	MYO9B	RAB5C
SEPN1	ADGRB1	IGFBP3	TIMP2	CTSD
EEF2	TNRC18	PLVAP	ADGRB1	SURF4
TMEM259	RPL27A	MDFI	PRKCSH	TTYH3
Downregulated genes				
AS II	**AS III**	**PGBM**	**SGBM**	**RGBM**
HIST1H4C	GPC5-AS1	IDI2-AS1	GPC5-AS1	GPC5-AS1
LOC101928590	LINC00499	**GJB6**	LINC00499	LINC00499
IDI2-AS1	LOC101928590	**WIF1**	LOC101928590	LOC101928590
CLDN10	**WIF1**	GPR22	CLDN10	HIST1H4C
MALAT1	HIST1H4C	CCDC144A	IDI2-AS1	CCDC144A
LOC100289473	**GJB6**	GABRA2	**WIF1**	IDI2-AS1
CCDC144A	CCDC144A	CLDN10	CCDC144A	**WIF1**
RYR3	IDI2-AS1	ETNPPL	ETNPPL	CLDN10
LRRC16A	SLC13A5	MGAT4C	SLC13A5	GPR22
GABRA2	CLDN10	PWARSN	**GJB6**	**GJB6**
SAT1	GABRA2	GABRB1	HIST1H4C	MGAT4C
SLC13A5	GPR22	**BMPR1B**	SLCO1C1	GABRA2
CPNE8	**MALAT1**	**MALAT1**	RYR3	SLC13A5
LGI1	ANKRD36C	SLCO1C1	MGAT4C	ETNPPL
GJB6	LRRC16A	RRN3P2	LRRC16A	GABRB1

EFNA5	CACNB2	AQP4-AS1	GABRA2	PWARSN
GPR22	PWARSN	SLC13A5	GPR22	**BMPR1B**
LOC101927692	GABRB1	ANKRD36C	**MALAT1**	**LGI1**
DIO2	ADAM20	**LGI1**	**LGI1**	SLCO1C1
FAS	MGAT4C	CACNB2	**BMPR1B**	**MALAT1**
LINC00632	SLC2A4	EFNA5	AQP4-AS1	AQP4-AS1
ANKRD36B	CPNE8	LRRC16A	SLC30A10	RYR3
SLCO1C1	**SLC1A2**	SLC2A4	SLC2A4	LRRC16A
GABRB1	LGI1	ADGRV1	EFNA5	ANKRD36C
KLF4	ANKRD36B	ANKRD36	GABRB1	CCDC144B

*Genes that are previously reported in astrocytoma were highlighted in the table.

** All Up-regulated genes in this table has log FC of > 3.7, ($p \leq 0.000002$ and FDR $\leq$ 0.00002). ***All downregulated genes in this table has log FC of < -1.5, ($p \leq 0.00002$and FDR ≤ 0.000009).

BMPR1B (bone morphogenetic protein receptor IB subunit) is under expressed in glioblastoma and is reported to reduces the malignancy of glioblastoma cells by upregulation of p21 and p27Kip1 (Liu et al., 2012). BMPR1B is under expressed in all AS subtypes in this study. Lysosomal-associated membrane protein-1 (LAMP-1) is may play a role in metastasis in astrocytoma (Jensen et al., 2013). LAMP1 is significantly over expressed in all AS subtypes.

Samples in AS II subtype in this study showed gene expression pattern specific to AS II subtype that was previously reported (Seifert et al., 2015). CDH4, which encodes for a cell adhesion protein involved in neural out growth and brain segmentation was under expressed (log FC -1.97) (Miotto, 2004). Notch ligand DLL3 known for its function in neurogenesis and glioma biology was over expressed (log FC 8.99) (Phillips et al., 2006). Apoptotic gene GAS2 (log FC -2.41) and SHROOM2 (log FC -2.21), involved in cell spreading, were under expressed. Gene NR2E1, involved in anterior brain differentiation, was under expressed (log FC -1.57). Under expression of NR2E1 gene is previously reported to be associated with cancer stem cell death and longer survival of G-CIMP glioma patients (Xie et al., 2014).

Similarly, AS III subtype samples in this study showed expression pattern in genes that are previously implicated in AS III subtype. To name some, CDC27 (which is a part of anaphase promoting complex and involved in timing of mitosis) (Park et al., 2005), PPM1D

(involved in p53 mediated cell cycle arrest and gain of function mutations in this gene reported for brain stem gliomas) (Zhang et al., 2014, Wang et al., 2011), and ZNF24 (maintains progenitor cell states and reported to have negative regulation of angiogenesis) (Harper et al., 2007), showed similar expression pattern (log FC -0.23, log FC 1.94, log FC 0.198, respectively), however, without statistical significance. Lack of statistical significance could be due to the fewer number (13) of samples used in this study.

Known cancer genes previously reported in brain cancer, such as BIRC3, BRCA1, EGFR, ERBB2, PDGFR1B, and VEGFA, were also over expressed in our grade IV GBM samples, pGBM, sGBM and rGBM, albeit, at varying degrees of significance. BIRC3 (CIAP2), which encodes a member of the IAP family of proteins that inhibit apoptosis by binding to tumor necrosis factor receptor-associated factors TRAF1 and TRAF2. This gene is reported to be a transcriptional target of NF-κB p65 and reduced levels of p65 renders glioma cells more sensitive to the cytotoxic effects of TNF-α, suggesting a good therapeutic target for the treatment of malignant gliomas (Gressot et al., 2017, Zhao et al., 2011).

EGFR is a receptor tyrosine kinase of the ErbB family, known to activate at least 4 major downstream signaling cascades including the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC and STATs modules. This gene is reported to be over expressed in GBM, was significantly over expressed in all GBM subtypes in this study (pGBM (4.06 log FC), sGBM (1.95 log FC), and rGBM (2.57 log FC). Also, SMARC4 (involved in controlling cell proliferation, migration, and invasion) has been reported to be over expressed in gliomas was significantly over expressed in this study, (pGBM (1.87 log FC), sGBM (2.46 log FC), and rGBM (2.05 log FC) (Bai et al., 2012). NTRK3, reported to be under expressed in high grade gliomas due to DNA methylation changes, is also significantly under expressed in this study (pGBM (-2.09 log FC), sGBM (-1.22 log FC), and rGBM (-1.71 log FC) (Palani et al., 2014).

TGFBI (transforming growth factor beta induced) gene is significantly overexpressed in our primary GBM samples. It encodes an RGD-containing protein that binds to type I, II and IV collagens. Collagens are also significantly overexpressed in our pGBM samples. In this study, pGBM samples are enriched in the genes involved in TGFBI module, namely, TGFBI, Collagens, MMP2 (matrix metalloproteinase 2), SPARC (secreted protein, acidic and rich in cysteine), and fibronectin (FN). TGFBI module may present as an alternative signaling pathway (to the canonical SMAD-mediated) TGF-beta signaling pathway in GBM

(Lin et al., 2010). All the genes that are associated with this alternative pathway are significantly overexpressed in our AS grade IV pGBM samples.

4.1.4 Genes exclusively expressed in different subtypes of astrocytoma

AS II

At log Fold Change of 2, ($p \leq 0.001$ and False Discovery Rate of ≤ 0.002, 50 genes are exclusively over expressed and 42 genes are exclusively under expressed in astrocytoma subtype AS II. This list of exclusively over expressed genes in AS II includes Transcription factors, THRA (Thyroid Hormone Receptor is nuclear hormone receptor that can act as a repressor or activator of transcription and play a role in development NOTCH1-mediated pathway for NF-KB activity modulation), KLF15(which is a negative regulator of TP53 acetylation and also inhibits NF-kappa-B activation through repression of EP300-dependent RELA acetylation), and FHL1 genes, Protein kinase gene, DCLK2, and Cytokine and growth factors, IL17D, CMTM4 and SEMA6A (Wolter et al., 2015). Interestingly, IL-17D gene expression was reported to be suppressed in more advanced, higher stage gliomas (WHO Grade III astrocytoma, Grade IV glioblastoma multiforme (GBM)) relative to less advanced, lower stage gliomas (WHO Grade II oligodendroglioma) . IL-17D expression was also suppressed in Grade IV GBM when compared to Grade III astrocytomas and that high expression of IL-17D in tumor biopsies correlated with a greater survival time for a subset of patients with Grade IV GBM (O'Sullivan et al., 2014).

Oncogenes TFRC, MDM2, ETV5, transcription factor ENO1, and cytokine and growth factor PTN are exclusively under expressed in AS II subtype, in this study. Interestingly, MDM2 is one of the key genes that is reported to be overexpressed in gliomas (Soni et al., 2005). TFRC is suggested to be expressed in normal brain and may be dysregulated in brain cancers (Hänninen et al., 2009). Interestingly, previous studies suggested ENO1 overexpression is associated with tumour progression in high grade gliomas (Song et al., 2014). However, ENO1 is exclusively under expressed in astrocytoma subtype AS II in this study.

AS III

ITM2C (BRI3), is the only gene that is exclusively overexpressed in AS III subtype. ITM2C is a negative regulator of beta amyloid peptide production, which inhibit the

processing of APP by blocking its access to alpha- and beta-secretase. ITM2C is suggested to play a role in TNF-induced cell death and neuronal (Matsuda et al., 2009).

Primary GBM IV

In primary GBM, there are 3639 genes that are exclusively overexpressed and 994 genes exclusively under expressed. There is a significant enrichment of over and under expressed genes in gene subsets of selected functional annotations (tumor suppressor genes, oncogenes, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors/co-factors and cytokine and growth factors) (Figure 15). BIRC3 and SMARCA4 are among the exclusively over expressed genes that were previously reported to be exclusive over expressed in GBM grade IV. Also, FGFR2 and NTKR3 genes are suggested to be exclusively under expressed in WHO grade IV GBM.

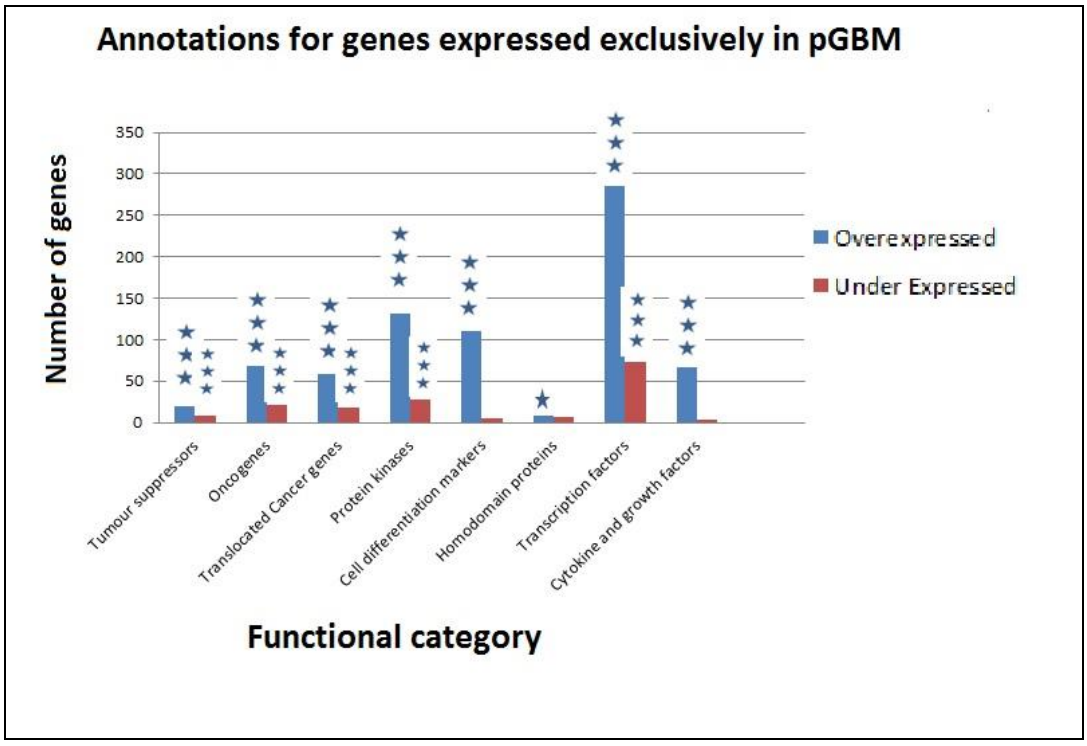

Figure 15. Enrichment of differentially expressed genes that are exclusive to pGBM in selected functional categories: tumor suppressor genes, oncogenes, translocated cancer genes, protein kinases, cell differentiation markers, homeodomain proteins, transcription factors and cytokine and growth factors (see Methods for details). Significant enrichment of genes in a category within a tumor type is represented by '*' ($p < 0.05$) and '***' ($p < 0.0001$) (Fisher's exact test).

Secondary GBM IV

SOX4 and SALL3 genes are exclusively overexpressed in WHO grade IV secondary Glioblastoma (sGBM), in this study. SOX4 (sex determining region Y-box 4) is reported to be overexpressed in GBM (Lin et al., 2010). SOX4 was shown to transcriptionally activate EGFR by binding to its promoter (Scharer et al., 2009). Other growth factors that are reported to be targets of SOX4 are FGFRL1 and IGF2R. MT1JP, a pseudogene, is the only gene that is under expressed exclusively in sGBM.

Recurrent GBM IV

CTSL and FTL genes are over expressed exclusively in AS grade IV recurrent GBM in this study. FTL is reported to be overexpressed in GBM (Gautam et al., 2012).

4.1.5 Pathways implicated in Astrocytoma subtypes

Genes differentially expressed in each subtype were investigated for overlap with the gene sets in various canonical pathways available on MsigDB. Results reported here are the extracts from the top 100 most significant pathways observed in each subtype with ($p \leq$ 0.0013 and FDR $\leq$ 0.0018. MsigDB uses various sources of GO for the gene set enrichment analysis and often results in redundant pathways. Hence, we manually selected the most significant pathways that were reported in the literature in this pathway analysis (Table 8).

Table 8. Extraction from top 100 significant pathways implicated in astrocytoma

Suggested function	Pathways	AS-II	AS-III	pGBM	sGBM	rGBM
Evasion of Apoptosis	APOPTOSIS			Y	Y	
	CLASS_I_MHC_PATHWAY	Y	Y	Y	Y	Y
	NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY			Y		
	P53_SIGNALING_PATHWAY	Y		Y		
	P73PATHWAY	Y	Y	Y	Y	
	TAP63_PATHWAY(LKB1)	Y			Y	
Growth and survival	NEUROTROPHIN_SIGNALING_PATHWAY		Y	Y		
	PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	Y	Y	Y		
	PI3KCI_AKT_PATHWAY			Y		
Homeostasis	TCR_PATHWAY	Y		Y		
	TOLL_RECEPTOR_CASCADES			Y		
Metastasis	RAC1_PATHWAY				Y	
	RHO_REG_PATHWAY	Y		Y		
	SEMAPHORIN_INTERACTIONS	Y		Y	Y	Y
	UPA_UPAR_PATHWAY			Y		
Proliferation	MTOR_4PATHWAY					
	MYC_ACTIV_PATHWAY	Y				
	NOTCH_SIGNALING_PATHWAY			Y		
	TXA2_PATHWAY	Y		Y		
Proliferation and invasion	CELL_ADHESION_MOLECULES_CAMS	Y	Y	Y	Y	Y
	CHEMOKINE_SIGNALING_PATHWAY	Y	Y	Y		Y
	COLLAGENS	Y	Y	Y	Y	Y
	CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	Y	Y	Y		
	ECM_PATHWAY		Y	Y	Y	Y
	ERBB_SIGNALING_PATHWAY	Y	Y	Y		
	ERK1_ERK2_MAPK_PATHWAY			Y		
	FOCAL_ADHESION	Y	Y	Y	Y	Y
	GAP_JUNCTION	Y	Y		Y	Y
	INTEGRIN_PATHWAY	Y	Y	Y	Y	Y
	LYSOPHOSPHOLID_PATHWAY	Y	Y			Y
	MAPK_SIGNALING_PATHWAY			Y		
	MATRISOME_ASSOCIATED	Y	Y	Y	Y	Y
	PDGF_PATHWAY			Y		
	PLATELET_ACTIVATION_SIGNALING	Y	Y	Y	Y	Y
	REGULATION_OF_ACTIN_CYTOSKELETON	Y	Y	Y	Y	Y
	REGULATION_OF_HYPOXIA	Y	Y		Y	Y
	SIGNALING_BY_EGFR_IN_CANCER	Y	Y	Y	Y	Y
	SIGNALING_BY_FGFR			Y		
	SYNDECAN_PATHWAY			Y		
	VEGF_SIGNALING_PATHWAY	Y		Y		
	WNT_BETA_CATENIN_PATHWAY		Y		Y	Y
	WNT_SIGNALING_PATHWAY		Y	Y	Y	Y
Tumourigenesis	GLIOMA	Y				
	GNRH_SIGNALING_PATHWAY	Y	Y			
	HDAC_CLASSI_PATHWAY	Y				

*Y- indicates significant enrichment of that pathway. All the pathways reported above have a ($p \leq 0.0013$ and FDR ≤ 0.0018).

Key glioma pathway genes that were differentially expressed in pGBM

Key genes in glioma pathways (de novo/secondary pathway), such as cytokine-cytokine receptor interaction genes EGF, TGFα, PDGF and their receptors EGFR (oncogene) and PDGFR were significantly overexpressed in pGBM. ErbB signaling pathway genes SHC (SHC (Src Homology 2 Domain Containing) Transforming Protein 1) were significantly overexpressed in pGBM whereas SOS2 (SOS Ras/Rho Guanine Nucleotide Exchange Factor 2) gene was downregulated in pGBM. Calcium signaling pathway genes PLCγ (phospholipase C gamma) were significantly overexpressed in pGBM and CAMK2G (Calcium/Calmodulin Dependent Protein Kinase II Gamma) gene was downregulated. Genes involved in The MAPK/ERK pathway (also known as the Ras-Raf-MEK-ERK pathway) such as oncogene Ras, Raf, MEK (MAPK/ERK Kinase), ERK, and AKT1/2 genes were upregulated in pGBM. MTOR (Mechanistic Target Of Rapamycin) signaling pathway genes such as PI3K (Phosphatidylinositol-4, 5-Bisphosphate 3-Kinase) genes, PKB/AKT, and MTOR genes were significantly upregulated in pGBM. Genes in p53 signaling pathway, such as TP14/TP16/ARF/CDKN2A (Cyclin-Dependent Kinase Inhibitor 2A), CCND1 (cyclinD1), CDK4/6, and E2F transcription factors 1-6 were significantly upregulated in pGBM and TP53 (Tumor protein 53) gene was significantly downregulated in pGBM. Tumor suppressor gene PTEN (Phosphatase and Tensin Homolog) gene was significantly downregulated in all astrocytoma grades (Figure 16).

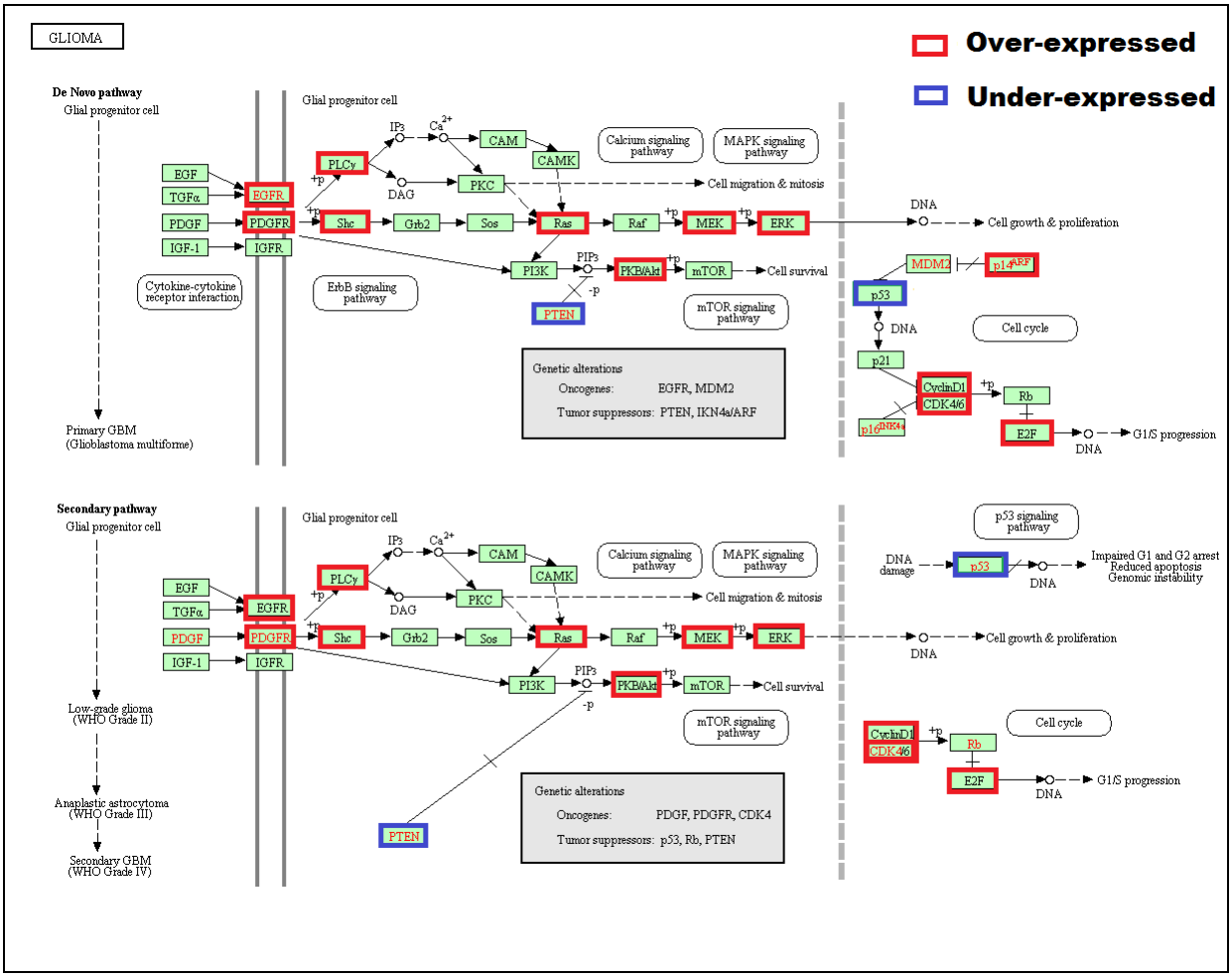

Figure 16. Gene expression changes in KEGG glioma pathway. KEGG glioma pathway map (hsa: 04060) was manually modified where genes were highlighted with colored rectangles according to their observed expression level and AS subtype(s) as shown in the color code. Genes that were not highlighted by colored rectangles were either absent or unchanged in this study.

4.2. Collagen and DDR1 expression in glioblastoma

In this section, I investigated the gene and protein expression of collagen and its protein tyrosine kinase receptor(s).

4.2.1 Gene expression analysis of collagen and its protein tyrosine kinase receptors

One of the striking observations I made in the differential gene expression analysis of astrocytoma grades II-IV in my study is the presence of collagen genes among the most upregulated genes (Table 9). There are 21 different collagens that were significantly overexpressed in primary GBM in my study. Among these, COL1A1 and COL6A1 are significantly overexpressed in all subtypes of AS in my study. COL1A1 is the most significantly expressed gene in AS-III, sGBM and rGBM and present in the top 10 overexpressed genes in pGBM (Table 7). I made similar observation in the heat map generated using the collagen gene sets, where the number and intensity of collagen expression has increased with increase in tumour aggressiveness in my study. There is a clear distinction in the expression profile of lower grade astrocytoma subtypes (grade II-III) and higher grade glioblastomas GBM IV) (Figure 17).

The most significantly over expressed gene in pGBM is COL3A1, an important paralog of COL1A1. This observation is significant as COL1A1 and COL3A1 genes encode pro-alpha1 chains of type I and III collagen and are previously reported to play a role in invasion and adhesion by activation of matrix metalloproteinase-2 (MMP-2) gene by binding to DDR1(Discoidin Domain Receptor Tyrosine Kinase 1) gene (Ram et al., 2006). Previous studies have reported that DDR1 is primarily expressed in epithelial cells in the brain, gastrointestinal tract, lung, and kidney, while DDR2 is expressed in brain, heart, and in muscle tissues. In the light of this evidence, I next wanted to identify the receptor tyrosine kinases enriched in the differentially expressed genes. Among the receptor tyrosine kinases family of genes, DDR1 is the only gene that is significantly overexpressed in all AS subtypes in this study (Table 9).

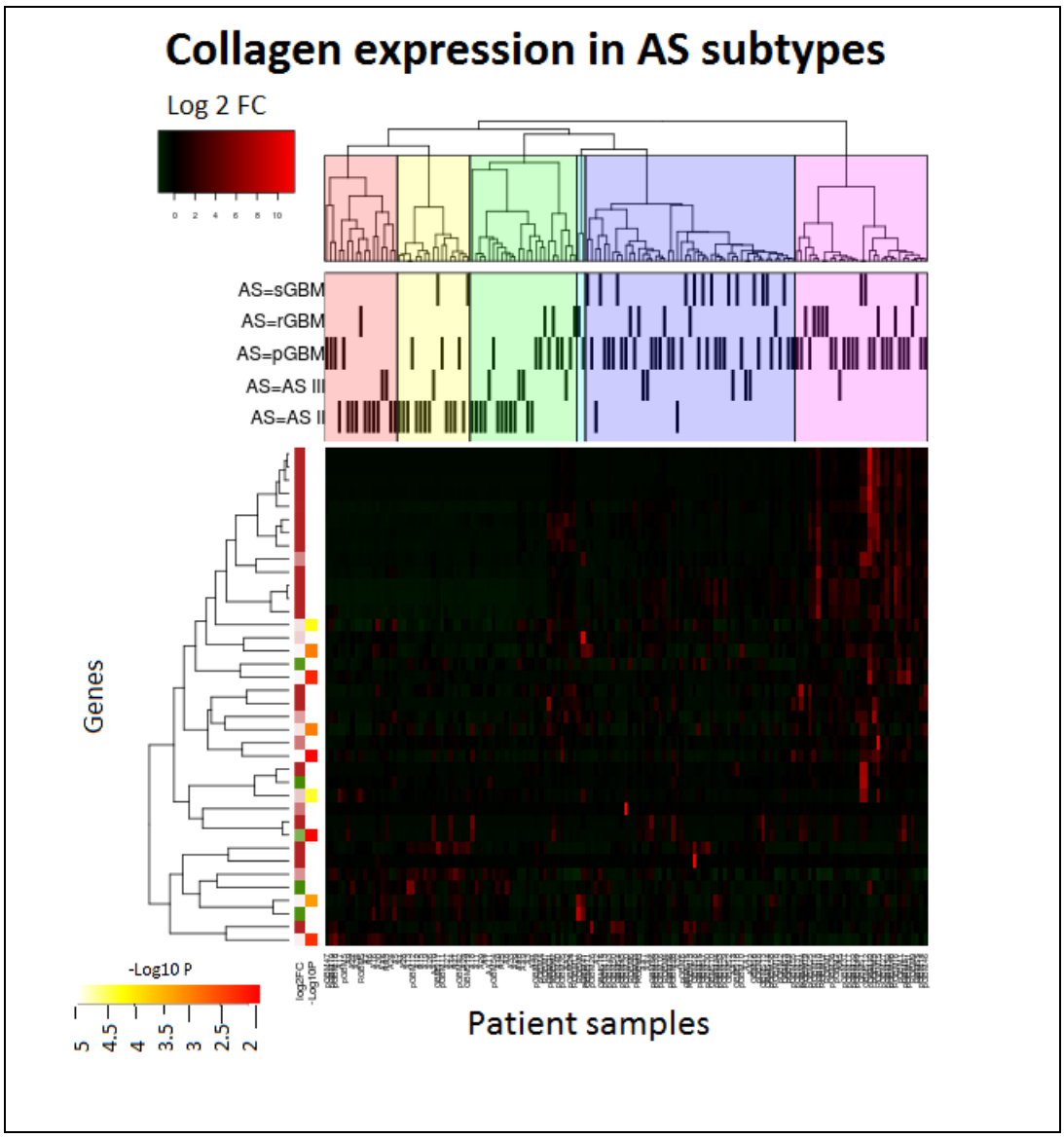

Figure 17. Expression profile of collagen family member genes in our AS subtypes. Collagen expression significantly varies between lower grade astrocytoma to that of higher grade glioblastomas ($p \leq 0.01$and FDR ≤ 0.015).

Table 9. Collagens and receptor tyrosine kinases expressed in astrocytoma subtypes.

RTK	Collagen	AS-II		AS-III		pGBM		sGBM		rGBM	
		RTK	Collagen	RTK	Collagen	RTK	Collagen	RTK	Collagen	RTK	Collagen
AATK	COL1A1	DDR1	COL1A1	DDR1	COL1A1	AATK	COL1A1	DDR1	COL1A1	DDR1	COL1A1
ALK	COL1A2		COL6A1	AXL	COL6A1	ALK	COL1A2	AXL	COL6A1	FGFR3	COL5A3
AXL	COL2A1		COL11A1			CSF1R	COL3A1	FGFR3		MERTK	COL6A1
CSF1R	COL3A1					DDR1	COL4A1	MERTK			
DDR1	COL4A1					FLT1	COL4A2	NTRK2			
DDR2	COL4A2					FLT4	COL5A1				
FGFR1	COL4A3					KDR	COL5A2				
FGFR2	COL4A4					LMTK3	COL5A3				
FGFR3	COL4A5					MET	COL6A1				
FGFR4	COL4A6					PDGFRA	COL6A2				
FLT1	COL5A1					PTK7	COL6A3				
FLT3	COL5A2					RET	COL7A1				
FLT4	COL5A3					TEK	COL8A2				
IGF1R	COL6A1					TIE1	COL9A3				
INSR	COL6A2					AXL	COL11A2				
INSRR	COL6A3					FGFR2	COL12A1				
KDR	COL6A4P1					FGFR3	COL14A1				
KIT	COL6A4P2					INSR	COL15A1				
LMTK2	COL6A5					MERTK	COL16A1				
LMTK3	COL6A6					NTRK2	COL18A1				
LTK	COL7A1					NTRK3	COL22A1				
MERTK	COL8A1						COL4A3				
MET	COL8A2						COL11A1				
MST1R	COL9A1						COL21A1				
MUSK	COL9A2										
NTRK1	COL9A3										
NTRK2	COL10A1										
NTRK3	COL11A1										
PDGFRA	COL11A2										
PDGFRB	COL12A1										
PTK7	COL13A1										
RET	COL14A1										
ROR1	COL15A1										
ROR2	COL16A1										
ROS1	COL17A1										
RYK	COL18A1										
STYK1	COL19A1										
TEK	COL20A1										
TIE1	COL21A1										
TYRO3	COL22A1										
	COL23A1										
	COL24A1										
	COL25A1										
	COL26A1										
	COL27A1										
	COL28A1										
		Over expressed									
		Under expressed									
BOLD LETTER		Common between subtypes									

4.2.2 DDR1 and collagen protein expression analysis

Having shown that collagen genes are among the most over-expressed genes in the grade IV GBM and DDR1 is the only receptor tyrosine kinase present in all grades in this study, I next wanted to perform a preliminary study of collagen and DDR1 expression in an independent cohort of GBM samples and also aimed to investigate their correlation with the clinicopathological factors and the overall survival of GBM patients from an independent cohort.

Gene expression analysis in different subtypes of grade IV astrocytoma (Table 9) showed over-expression of 21 different types of collagen. It is not feasible to perform staining using 21 different antibodies for collagen due to limitation with the available biopsy material. So, I opted to perform Van Gieson staining which will enable to me to visualize the presence and location of collagen in these patient biopsies. I stained for collagen in 48 GBM samples, of which 5 samples were non-evaluable and were excluded from the analysis.

Collagen expression was present in tumor areas near necrosis and in blood vessels. In tumor areas distant from necrosis malignant glial cells were surrounded by fine collagen fibrils. In some cases weak collagen positivity was also present in malignant glial cells. Cases with stromal reaction were all positive for collagen (Figure 18).

I next stained these 48 GBM samples for DDR1 expression by immunohistochemistry. Positive staining for DDR1 was defined as membranous and cytoplasmic staining in brown color. Strong expression was observed for DDR1 in glioma cells and stroma in GBM (Figure 18D). In normal brain white matter strong DDR1 positivity were present in neurons, whereas glial cells are moderate and weakly positive. (Figure18C).

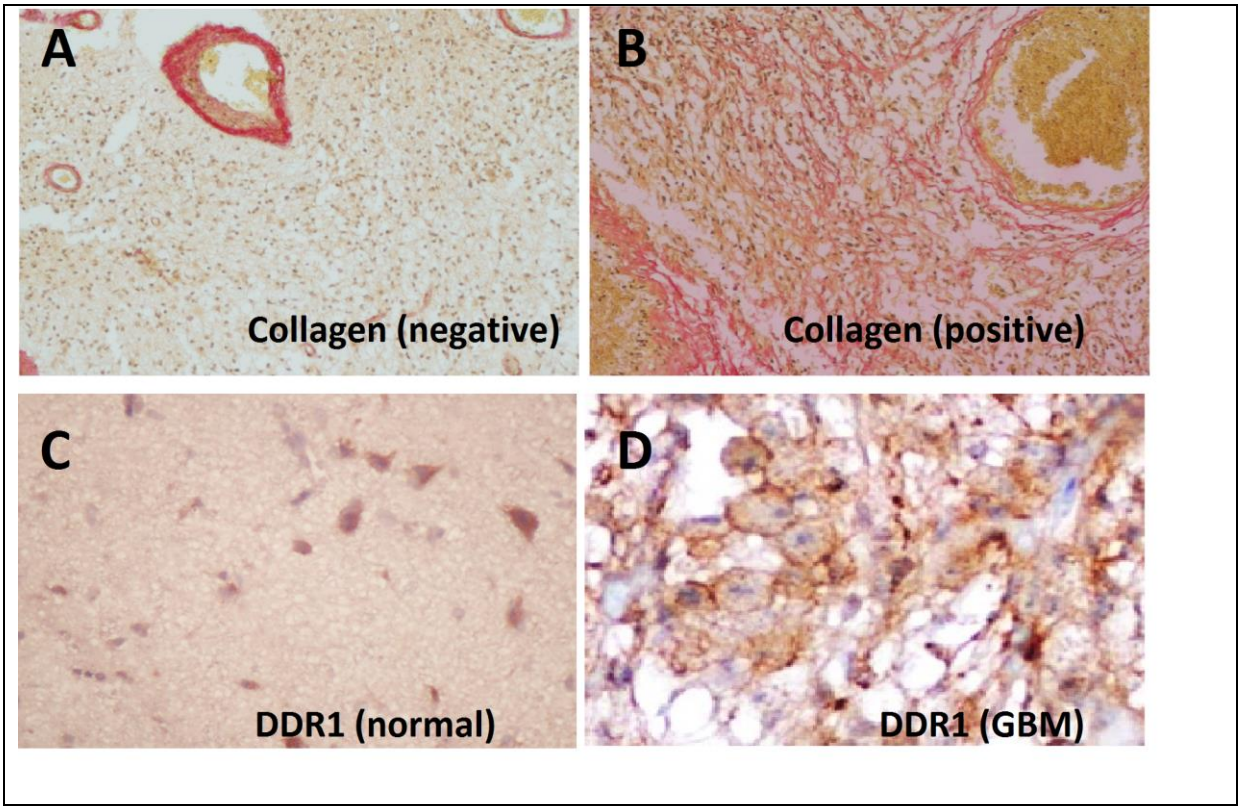

Figure 18. Collagen and DDR1 protein expression. Immunohistochemical analysis in GBM patients showing negative staining (10X) (A) and positive (20X) (B) staining for collagen and low/weak expression of DDR1 for glial cells and strong expression for neurons in normal brain sample (40X) (C) and strong expression for DDR1 in glioma cells and stroma in GBM (40X) (D).

Patient characteristics for DDR1 expression

For DDR1 expression I used median H-score value of 185 as cutoff value. High DDR1 expression (H-score ≥ 185) was detected in 24(50%) patients, and the other 24(50%) patients showed low expression of DDR1 (H-score < 185). Among these 48 patients included 20 (41.7%) were female and 28 (58.3%) were male with the mean age of 65.19 years and the median age of 66 years (range, 46 to 85 years). 41(85.4) had primary glioblastoma, 7(14.6) had secondary glioblastoma. The preoperative and postoperative KPS parameters were determined for each patient, where the median preoperative KPS of the patients was 80, with 35 of 48 patients (72.9%) presenting with a preoperative KPS ≥80. The postoperative KPS was ≥80 in 33 of the 48 patients (68.8%). 24/48 patients (50.0%) had total resection and 23/48 patients (47.9%) had subtotal resection, whereas 1 patient lack this information. Overall survival was defined as the time between date of surgery and date of death by any

cause or last known follow-up date. The mean and median survival time for the patient cohort was 8.75 and 4 months, respectively (range 1-63 months). Within this follow up time 44 patients (91.7%) were dead mostly from glioblastoma. Clinical data regarding recurrence was not included in the study as this information was missing for majority of cases. 3 normal brain tissues were obtained from archive to compare marker expression.

Table 10. Patient characteristics

Parameters	**DDR1 expression**		
	Number of samples (%)	**mean ± SD**	***p*-value**
Number of samples	48		
Age (Years)			
<66	23 (47.9)	189.1±52.9	0.690
≥66	25 (52.1)	182.4±63.1	
Gender			
Female	20 (41.7)	182.25±54.22	0.739
Male	28 (58.3)	188.03±61.36	
Grades			
GBM primary	41 (85.4)	189.51±57.77	0.265
GBM secondary	7 (14.6)	162.85±57.94	
KPS before surgery			
<80	13 (27.1)	155.38±60.36	**0.034**
≥80	35 (72.9)	196.85±53.66	
KPS after surgery			
<80	15 (31.3)	159.33±64.55	0.055
≥80	33 (68.8)	197.57±51.38	
Surgery			
Subtotal	23 (47.9)	181.74±63.13	0.652
Total resection	24 (50.0)	189.58±54.97	
NK	1 (2.1)		
Collagen			
Negative	30 (62.5)	182.83±55.09	0.156
Positive	13 (27.1)	209.62±57.71	
Missed	5 (10.4)		

Collagen was positive in malignant glial cells in 13 (27.1%) cases while 30 (62.5%) cases were negative and 5(10.4%) cases were missing. DDR1 expression was high in the collagen positive cases (mean rank 26.27 vs. 20.15). No significant association between DDR1 expression and age, gender, tumour grade, postoperative-KPS ≥80, and surgery was

observed in these 48 GBM cases (p=0.0034). Significant association between preoperative-KPS score ≥80 and DDR1 expression was observed (p=0.0034) (Table 10).

Correlation between DDR1 and overall survival in GBM

Overall survival analysis using Kaplan–Meier overall survival (OS) curve showed GBM patients with high DDR1 expression had better survival compared to those with low or no DDR1 expression (log–rank test, p = 0.022), as shown in Figure 19.

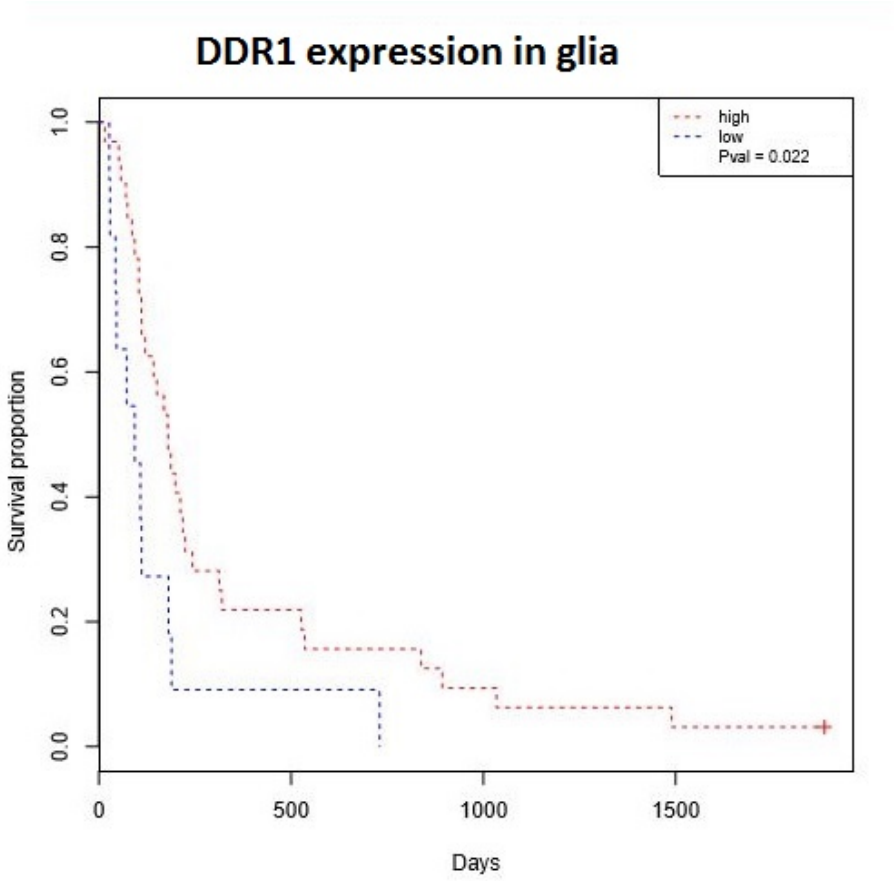

Figure 19. Overall survival plot for DDR1 expression in glia of GBM. Statistically significant association was observed between high expression of DDR1 and better survival in glioblastoma patients.

4.3. CD74 as a prognostic marker in glioblastoma

In this section, I investigated the gene and protein expression of MIF and its receptor CD74.

4.3.1 Gene expression analysis of CD74

I also made a striking discovery in the gene expression analysis, where the expression level of CD74 increased with increasing tumour grade in these samples (Figure 20). This is of interest because the overexpression of CD74 has thought to enhance tumor growth in several human neoplasms, such as esophageal squamous cell carcinoma (Ren et al., 2005), breast cancer (Xu et al., 2008), pancreatic cancer (Nagata et al., 2009), lung cancer (McClelland et al., 2009), cervical squamous cell carcinoma (Cheng et al., 2011), pleural mesothelioma (Otterstrom et al., 2014), and thyroid carcinoma (Cheng et al., 2015).

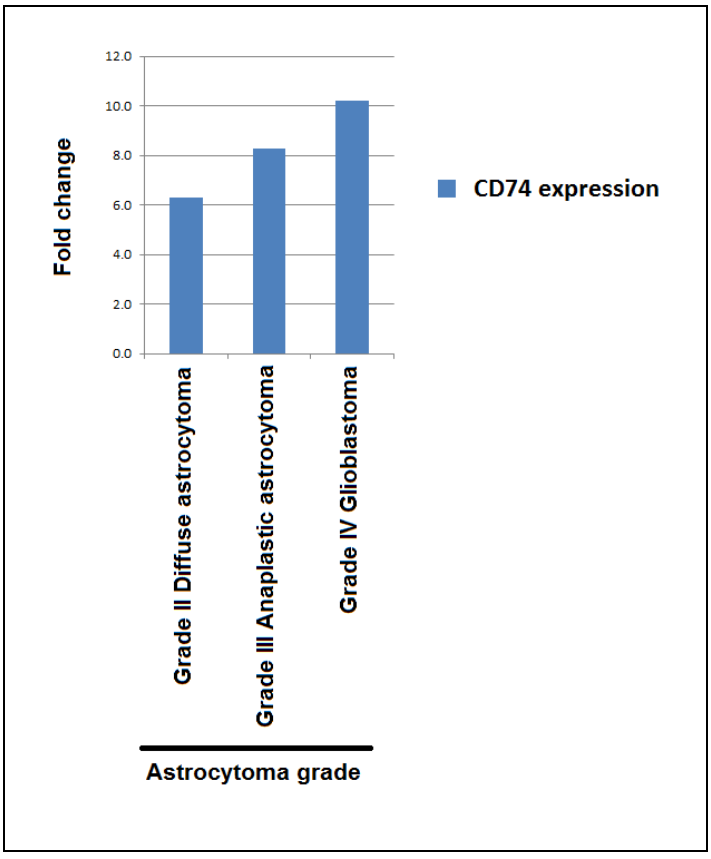

Figure 20. CD74 gene expression in astrocytoma grades II-IV. Increasing level of CD74 expression was observed for CD74 with increasing tumour grade.

4.3.2 CD74 protein expression in astrocytoma

Patient characteristics

Patients analyzed for prognostic study of CD74 expression included 29 (40.27%) female and 43 (59.73%) male with the mean age of 58.21 years and the median age of 62 years (range, 24 to 85 years). Among these 72 patients, 15 (20.83%) had grade II diffuse astrocytoma, 9 (12.5%) grade III anaplastic astrocytoma and 48 (66.67%) had grade IV glioblastoma. The preoperative and postoperative KPS parameters were determined for each patient, where the median preoperative KPS of the patients was 80, with 54 of 72 patients (75%) presenting with a preoperative KPS of ≥80. The postoperative KPS was ≥80 in 53 of the 72 patients (73.62%). 29/72 patients (40.28%) had total resection and 40/72 patients (55.55%) had subtotal resection, whereas 3 patients lack this information. Overall survival was defined as the time between date of surgery and date of death by any cause or last known follow-up date. The mean and median survival time for the patient cohort was 15.24 and 5 months, respectively (range 0-81 months). Within this follow up time 59 patients (81.95%) were dead mostly from glioblastoma. Clinical data regarding recurrence was not included in the study as this information was missing for majority of cases. 3 normal brain tissues were obtained from archive to compare marker expression.

Table 11. Patient characteristics

Parameters	**CD74 expression**		
	Number of samples (%)	**mean ± SD**	**p-Value**
Number of samples	72		
Age (Years)			
<62	35 (48.62)	7.2±3.50	**< 0.003**
≥62	37 (51.38)	9.62±3.05	
Gender			
Female	29 (40.27)	8.21±3.74	< 0.756
Male	43 (59.73)	8.60±3.32	
Grades			
DA	15 (20.83)	6.53±3.00	**< 0.004**
AA	9 (12.5)	6.67±3.61	
GBM	48 (66.67)	9.38±3.28	
KPS before surgery			
<80	18 (25.00)	7.83±3.80	< 0.429
≥80	54 (75.00)	8.65±3.37	
KPS after surgery			
<80	19 (26.39)	8.53±3.71	< 0.842
≥80	53 (73.61)	8.42±3.42	
Surgery			
Subtotal	40 (55.55)	7.9±3.28	< 0.068
Total resection	29 (40.28)	9.24±3.61	
NK	3 (4.17)		

CD74 expression increases with tumor grade progression in astrocytoma

CD74 expression in 72 astrocytoma samples was analyzed using the IHC. Positive staining for CD74 was defined as membranous and perinuclear staining in brown color. In normal human white matter very few CD74-positive cells with elongated cellular processes were present (Figure 21). In contrast to CD74-positive cells in normal tissue, cells expressing CD74 in WHO grades II–IV presented with a more round-shape morphology, mainly lacking fine cellular processes. The representative micrographs of IHC from different grades of astrocytoma can be viewed in figure 21.

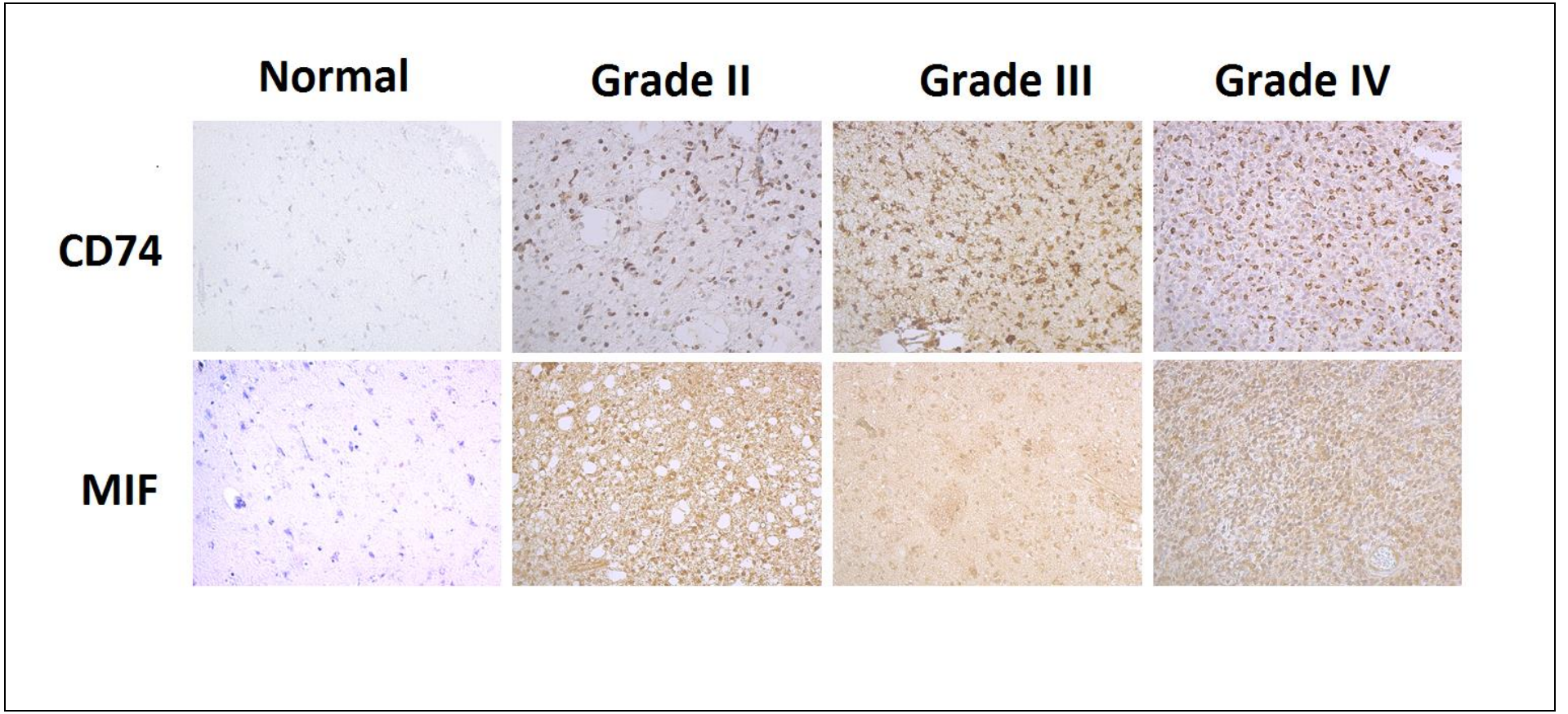

Figure 21. CD74 and MIF expression in grade II-IV astrocytoma. Very low expression of CD74 was observed in normal brain tissue whereas increased expression of CD74 was seen with increasing astrocytoma grade. MIF expression was absent in normal brain samples and overexpression MIF was seen in grade II-IV astrocytoma (200X magnification).

High CD74 expression (Hara IRS > 6) was detected in 47 patients, and the other 25 patients showed low expression of CD74 (Hara IRS ≤ 6). CD74 expression increased as the tumor grade progressed (Table 12). Diffuse astrocytoma (6/15; 40%) and anaplastic astrocytoma (5/9; 55.55%) patients had lower CD74 expression than the grade IV GBM (36/48; 75%). Statistically significant correlation between the CD74 expression levels and the progression of astrocytoma ($p < 0.004$; Table 12) was observed.

MIF expression was absent in normal brain samples and overexpression of MIF was seen in grades II-IV of astrocytoma (Figure 21). No significant correlation between MIF expression and clinicopathological factors was found in this study (data is not provided).

Uni-and multivariate analysis of CD74 expression and clinicopathological parameters of astrocytoma

According to the univariate analysis for different clinicopathological characteristics, including age, gender, MIF expression, CD74 expression, pre- and post-operative KPS score, we found that CD74 expression, age group, pre-operative KPS and post-operative KPS score were significantly associated with OS ($p = 0.032$, $p= 0.0001$, $p= 0.018$ and $p = 0.0001$ respectively; Table 13). Furthermore, multivariate logistic analysis showed that age (HR 0.412, 95% CI 0.223–0.762; $p= 0.005$; Table13) and post-operative KPS score (HR 3.130, 95% CI 1.681–5.829; $p = 0.0001$; Table 13) were statistically independent predictive factors of poorer prognosis in astrocytoma patients, whereas high CD74 expression had a less significant association with poor prognosis alone (CD74 high, HR 1.114, 95% CI 0.995–1.249; $p = 0.062$; Table 13). However, C74 expression is statistically independent predictive factor of poor prognosis in multivariable analysis with MIF expression (CD74 high, HR 1.097, 95% CI 1.005-1.197; $p= 0.039$).

Table 12. Univariate and multivariate logistic analysis of clinicopathological independent prognostic factors for survival of astrocytoma patients

		Univariate		**Multivariate**	
		HR (95% CI)	**p-Value**	**HR (95% CI)**	**p-Value**
CD74 expression	Low (<6)	1		1	
	High (≥6)	1.099 (1.008-1.198)	**0.032**	1.114 (0.995-1.249)	0.062
MIF expression	Low (<6)	1		1	
	High (≥6)	0.964(0.884-1.051)	0.402	0.962(0.878-1.054)	0.404
Age (Years)	< 62	1		1	
	≥ 62	1.062 (1.038 - 1.087)	**0.0001**	0.412 (0.223 - 0.762)	**0.005**
Gender	Female	1		1	
	Male	1.303 (0.769 - 2.206)	0.325	0.728 (0.419 - 1.267)	0.26217
KPS before surgery	<80	1		1	
	≥80	0.986 (0.974 - 0.998)	**0.018**	1.230 (0.544 - 2.783)	0.619
KPS after surgery	<80	1		1	
	≥80	0.950 (0.928 - 0.972)	**0.0001**	3.130 (1.681 - 5.829)	**0.0001**

Correlation between CD74 expression and survival in glioblastoma

Kaplan–Meier overall survival (OS) curve (Figure 22A) of all astrocytoma patients for CD74 expression levels showed patients with high CD74 expression had significantly poorer prognosis compared to those with low or no CD74 expression (log–rank test, p = 0.05). This study also found a significant correlation (Figure 22B) between high grade astrocytoma and overall survival rate (log–rank test, p = 0.001) confirming worst prognosis for high grade astrocytoma.

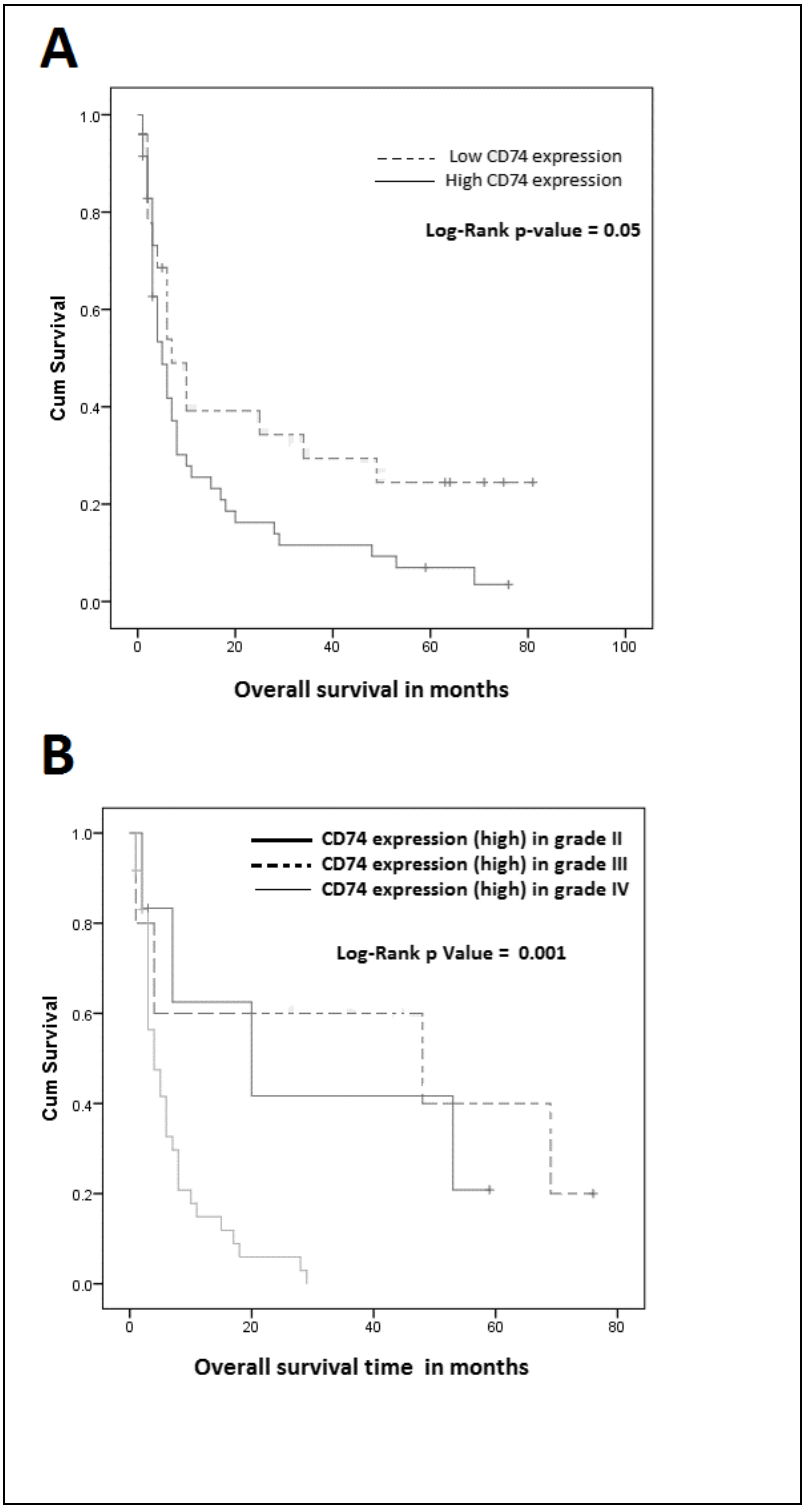

Figure 22. Kaplan–Meier overall survival curves for astrocytoma patients with different levels of CD74 expression. The maximum follow-up time was 60 months. B. Overall survival rates for grade II diffuse astrocytoma, grade II anaplastic astrocytoma, and grade IV glioblastoma patients with different CD74 expressions.

5. DISCUSSION

5.1. Gene expression profiling of astrocytoma grades II-IV

My study was focused on describing similarities and differences in gene expression in adult astrocytoma samples by transcriptome analysis. My investigation revealed a distinct gene expression pattern between grade II and astrocytoma grade IV. This observation was similar to previous studies which described that AS II grade is less proliferative and less aggressive compared to grade IV astrocytoma4. IL2-STAT5 pathway genes were significantly enriched in grade IV astrocytoma alone and previous studies have reported that STAT5 regulates glioma cell invasion by pathways dependent and independent of STAT5 DNA binding (Cao et al., 2011). The number of differentially expressed genes per sample in pGBM (n=58) and AS II (n=34) were higher than those of AS III (n=13), sGBM (n=19), and rGBM (n=19), however this difference could partly due to higher number of samples in these two subtypes.

My data has confirmed gene expression pattern for genes that were previously reported to characterize the GBM IV, with an exception of expression pattern in genes MDM2, MDM4, AXL, FGFR3, and MERTK, which were reported to be over-expressed in glioma and were significantly under-expressed in all GBM subtypes in my study (pGBM (-2.92 log FC), sGBM (-2.14 log FC), and rGBM (-2.57 log FC). MDM2 and MDM4 are known to inhibit a p53-dependent growth control (Pedeutour-Braccini et al., 2015, Riemenschneider et al., 1999, Riemenschneider et al., 2003). However, amplification of the CDK4 gene and over-expression of cyclin D1 are suggested to have similar effects to p16 or pRb inactivation and these mechanisms may provide additional alternatives to subvert cell-cycle control and facilitate progression to GBM (Schmidt et al., 1994). CDK4, CCND1 (CyclinD1) and E2F were over-expressed in primary GBM samples in my study, which may have contributed to the evasion of cell cycle control and facilitate G1/S progression (Figure 16).

BIRC3 (CIAP2) encodes a member of the IAP family of proteins that inhibit apoptosis by binding to tumor necrosis factor receptor-associated factors TRAF1 and TRAF2. This gene was reported to be a transcriptional target of NF-κB/p65 and reduced levels of p65 renders glioma cells more sensitive to the cytotoxic effects of TNF-α, suggesting a good

therapeutic target for the treatment of malignant gliomas (Gressot et al., 2014, Zhao et al., 2011).

NTRK3 was reported to be under-expressed in high-grade gliomas due to DNA methylation changes, and was also significantly under-expressed in my study (pGBM (-2.09 log FC), sGBM (-1.22 log FC), and rGBM (-1.71 log FC) (Palani et al., 2014). TGFBI (transforming growth factor beta induced) gene was significantly over-expressed in my primary GBM samples. It encodes an RGD-containing protein that binds to type I, II and IV collagens. Collagens were also significantly over-expressed in my pGBM samples. In my study, pGBM samples were enriched in the genes involved in TGFBI module, namely, TGFBI, Collagens, MMP2 (matrix metalloproteinase 2), SPARC (secreted protein, acidic and rich in cysteine), and fibronectin (FN). TGFBI module may present as an alternative signaling pathway (to the canonical SMAD-mediated) TGF-beta signaling pathway in GBM (Lin et al., 2010). All the genes that are associated with this alternative pathway were significantly over-expressed in my AS grade IV pGBM samples (see Table 7).

Genes exclusively over-expressed in AS II include the transcription factors such as THRA (Thyroid Hormone Receptor) gene which is a nuclear hormone receptor that can act as a repressor or activator of transcription and play a role in development (NOTCH1-mediated pathway for NF-KB activity modulation), KLF15 (which is a negative regulator of TP53 acetylation and also inhibits NF-kappa-B activation through repression of EP300-dependent RELA acetylation), and FHL1 genes, Protein kinase gene, DCLK2, and Cytokine and growth factors, IL17D, CMTM4 and SEMA6A (Wolter et al., 2015).

Interestingly, IL-17D gene expression was reported to be suppressed in more advanced, higher grade gliomas (grade III and grade IV) relative to less advanced, lower stage gliomas (grade II oligodendroglioma). IL-17D expression was also suppressed in Grade IV GBM when compared to Grade III astrocytoma, high expression of IL-17D in tumor biopsies correlated with a greater survival time for a subset of patients with grade IV GBM (O'Sullivan et al., 2014). Oncogenes TFRC, MDM2, ETV5, and Transcription factor ENO1, and cytokine and growth factor PTN are exclusively under-expressed in the AS II subtype in my study. Interestingly, MDM2 gene which was reported to be over-expressed in gliomas has under or un-altered expression in my study (Soni et al., 2005). TFRC was suggested to be expressed in the normal brain and may be dysregulated in brain cancers (Hänninen et al.,

2009). Previous studies suggested ENO1 over-expression is associated with tumor progression in high-grade gliomas (Song et al., 2014). ENO1 was exclusively under-expressed in astrocytoma subtype AS II and non-significant ($p > 0.05$) over-expression of ENO1 gene was observed in AS-III and grade IV in my study. ITM2C (BRI3) was the only gene that was exclusively over-expressed in AS III subtype. ITM2C is a negative regulator of beta-aourloid peptide production, which inhibits the processing of APP by blocking its access to alpha- and beta-secretase. ITM2C was suggested to play a role in TNF-induced cell death and neuronal differentiation (Matsuda et al., 2009). SOX4 and SALL3 genes were exclusively over-expressed in secondary GBM in my study. SOX4 (sex determining region Y-box 4) was reported to be over-expressed in GBM53. SOX4 was shown to transcriptionally activate EGFR by binding to its promoter (Scharer et al., 2009). Other growth factors that are reported to be targets of SOX4 are FGFRL1 and IGF2R.

Gene set enrichment analysis of differentially expressed genes in each AS subtype revealed important cancer-relevant signaling processes. Transcription factors/co-factors in astrocytoma grades were enriched in cell cycle control, DNA repair, TGF BETA signaling, hypoxia, proliferation and apoptosis, with the exception of grade III where enrichment of cell cycle (E2F) targets and chromatin binding was observed. Pathway analysis of each astrocytoma subtype revealed a significant enrichment of hallmark pathways in cancer such as evasion of apoptosis (e.g., Class I MHC pathway), growth and survival (e.g., Phosphatidylinositol signaling pathway), and proliferation and invasion (e.g., EGFR signaling, collagens, ECM, CAM, Chemokines, ERBB signaling) (see Table 8). Mapping of the genes that were differentially expressed genes in AS subtypes on KEGG glioma pathway revealed key genes involved in glioma-genesis (see Figures 16).

5.2. Collagen activated DDR1 as a potential biomarker for astrocytoma

One of the most striking observation I made in the gene expression analysis of astrocytoma grades II-IV in my study is over expression of majority (21/46) of the collagen types, especially in the primary GBM samples where these were among the most upregulated genes (Table 7). This observation is of some importance as collagen has been shown to play a vital role in tumour invasion and metastasis in various cancers, including brain tumours. Where collagen had been shown to function as scaffolding for adhesion for cells aiding glioma cell migration, and as a reservoir and facilitator of matricellular proteins,

proteoglycans and growth factors (Sweeney et al., 2008). Interaction between collagen and its receptors also shown to play a role in the activation of signal transduction networks required for tumor growth, differentiation and invasion (Leitinger, 2011). In line with this evidence, my data shows that the number and intensity of collagen expression is increased with increase in tumour aggressiveness in my study.

Other important observation that I made in the gene expression analysis is the overexpression of COL1A1 and COL6A1 in all subtypes of AS (II-IV) (Table 7). COL6A1 expression has been reported to be a marker of tumour progression in astrocytomas (Fujita et al., 2008). The pro-alpha1 chains of type I and III collagen have been shown to involve in tumour invasion and adhesion through the activation of matrix metalloproteinase-2 (MMP-2) gene by binding to DDR1(Discoidin Domain Receptor Tyrosine Kinase 1) gene (Ram et al., 2006). Furthermore, DDR1 was also found to be overexpressed in mammary, ovarian, and lung carcinomas, suggesting that this receptor may play a role in the tumorigenesis of epithelial carcinomas (Alves et al., 1995). Subsequent studies on glioma have reported that DDR1 is activated by collagens type I, II, III, IV, V and VIII, but in particular by collagen type I and type III. DDR1a reported to promote glioma cell migration and invasion and stimulated activation of MMP-2 (Ram et al., 2006). DDR1 is also reported to play a role in tumorigenesis via p53/DDR1-dependent activation of Notch1 (Kim et al., 2017). Several studies suggested potential therapeutic benefit of targeting DDR1 in metastatic tumours (Kothiwale et al., 2015, Rammal et al., 2016) (Figure 23).

Gene expression analysis of receptor tyrosine kinases in my study revealed that DDR1 is the only receptor tyrosine kinase that is significantly overexpressed in all astrocytoma subtypes (grades II-IV) (Table 9), suggesting a possible co-expression of collagen and DDR1 in astrocytoma. To strengthen this theory, I performed a preliminary immunohistochemical study to describe the expression of collagen and DDR1 in an independent cohort of GBM samples (n=48), which revealed a significant positive correlation between collagen and DDR1 expression (DDR1-COL6A1, p = 0.0002; DDR1-COL3A1, p = 0.022). Also, higher DDR1 expression in primary GBM compared to secondary GBM further supports the idea of its participation in primary GBM progression.

.

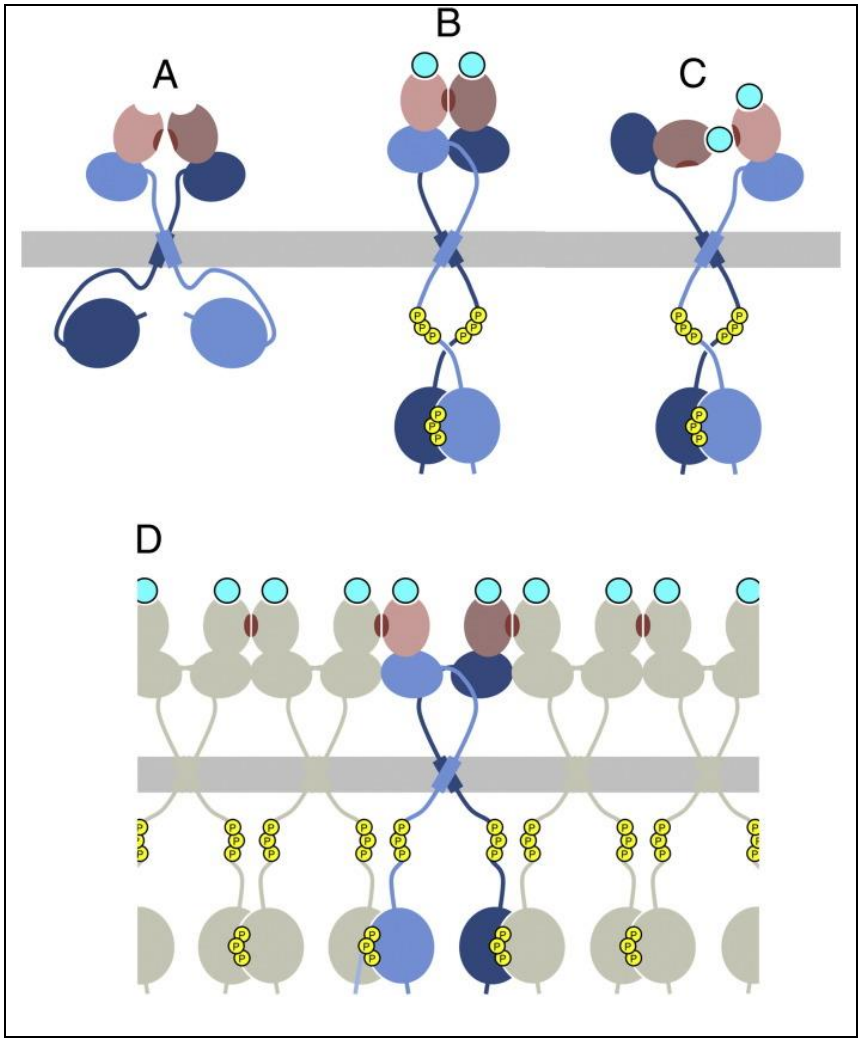

Figure 23. Possible mechanisms of DDR activation. (A) In the absence of collagen, inactive DDR is dimerised by interactions between the TM regions. The long cytoplasmic JM region may be involved in DDR auto inhibition. (B) A collagen-bound active DDR dimer, in which the conserved patch in the DS domain (shown in brown) makes dimer contacts. Collagen triple-helices are shown as cyan filled circles. (C) An alternative active DDR dimer, in which the conserved patch contributes to collagen binding. (D) A cluster of collagen-bound DDR dimers, in which the conserved patch makes contacts between dimers.

In protein expression analysis of collagen in independent cohort of GBM samples, although I observed collagen expression in 13/48 samples, DDR1 expression was found to be expressed at high level in these cases compared to collagen negative cases (mean rank 26.27 vs. 20.15), however not statistically significant (p=0.156). Also, DDR1 positivity was higher in primary glioblastoma compared to secondary GBM, however it was not statistically significant (p=0.265). Lack of statistical significance in these analyses could have been due small sample size.

5.3. CD74 as a potential biomarker for astrocytoma

To the extent of our knowledge, this current study is the first study to show CD74 expression as a bad prognostic marker in grade II-IV astrocytoma. Zeiner et. al., showed that CD74 expression is associated with good prognosis in high grade gliomas and is mostly correlated with M1 polarization, where anti-tumor interferon (IFN)- γ secretion leads to the death of tumor cells either by pharmacological reaction or through siRNA mediated knockdown (Zeiner et al., 2015). However, subsequent study by Ghoochani et al., showed that Macrophage migration inhibitory factor (MIF) activates CD74 and inhibits interferon (IFN)- γ secretion in microglia through phosphorylation of microglial ERK1/2 (extracellular signal-regulated protein kinases 1 and 2) (Ghoochani et al., 2016). Similar to this study, I demonstrated that overexpression of CD74 is associated with tumor progression in astrocytoma and also observed overexpression of MIF in grade II-IV astrocytoma. MIF exerts multimodal functions in glioblastoma including pro-proliferative, pro-migratory, pro-angiogenic as well as immune-evasive properties through CD74 (Bacher et al., 2003a, Baron et al., 2011, Mittelbronn et al., 2011). The binding of MIF to the extracellular domain of CD74 is necessary as an initial step for the MIF signaling cascade (Shi et al., 2006) resulting in tumor vascularization (Binsky et al., 2007, Liu et al., 2008, McClelland et al., 2009) and the promotion of neoplastic cell proliferation and survival via ERK1/2-MAPK pathway (McClelland et al., 2009, Otterstrom et al., 2014). In breast cancers, MIF was shown to be upregulated by HIF-1α and other potential hypoxia induced mechanisms leading to cell survival by activation of PI3K/AKT signaling pathway through interaction with its main receptor CD74 and CXCR4 (Figure 24). MIF has also been shown to promote angiogenesis by increased secretion levels of VEGF and IL-8 and inhibiting autophagy (Richard et al., 2015).

CD74, a cell membrane receptor for macrophage migration inhibitory factor (MIF) and a potential therapeutic target. CD74 is a non-polymorphic type II integral membrane protein (Shi et al., 2006). It functions as an MHC-II chaperone and participates in the trafficking of MHC-II molecules in antigen-presenting cells (Borghese and Clanchy, 2011) and also shown to express independently of MHC-II and can bind to non-MHC-II proteins (Ogrinc et al., 1993, Henne et al., 1995). The binding of MIF to the extracellular domain of CD74 is necessary as an initial step for the MIF signaling cascade (Shi et al., 2006). MIF signals upstream of cytokines via receptor binding in CD74-dependent pathways (Calandra and

Roger, 2003, Leng et al., 2003). The pro-inflammatory cytokine MIF and its receptor CD74 are associated with tumor vascularization (Binsky et al., 2007, Liu et al., 2008, McClelland et al., 2009) and the promotion of neoplastic cell proliferation and survival via ERK1/2-MAPK (extracellular signal-regulated protein kinases 1/2 and mitogen-activated protein kinase) pathway (McClelland et al., 2009, Otterstrom et al., 2014). MIF can activate the extracellular signal-regulated kinase phosphorylation via CD74 and CD44, where CD44 activates non-receptor tyrosine kinases. This is thought to enhance tumor growth in several human neoplasms. Furthermore, MIF and CD74 may also act as non-cognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment (Bernhagen et al., 2007). MIF and CD74 form complexes with the cysteine-X-cysteine (CXC)- chemokine receptors CXCR2 on myeloid cells and CXCR4 on T cells (Bernhagen et al., 2007).

CD74 expression increased as the tumor grade progressed in this study (Table 12). 40% of diffuse astrocytoma samples, 55% of anaplastic astrocytoma samples and 75% of grade IV GBM showed significant higher expression of CD74 ($p < 0.004$). Similar to previous association studies of CD74 expression and survival in other cancers, Kaplan–Meier overall survival analyses (Figure 22A) of all astrocytoma patients for CD74 expression levels revealed a significant association between high CD74 expression and poorer prognosis (log–rank test, $p = 0.05$) (Binsky et al., 2007, McClelland et al., 2009, Otterstrom et al., 2014, Nagata et al., 2009, Cheng et al., 2015). I also found a significant correlation (Figure 22B) between high grade astrocytoma and overall survival (log–rank test, $p = 0.001$) confirming worst prognosis for high grade astrocytoma.

Univariate analysis for CD74 expression along with other clinicopathological characteristics, including age, pre-operative KPS and post-operative KPS score, revealed a significant association with OS ($p = 0.032$, $p = 0.0001$, $p = 0.018$ and $p= 0.0001$ respectively; Table 13).

Multivariate analyses showed no significant association for CD74 expression with poor prognosis alone (HR 1.114, 95% CI 0.995–1.249; $p = 0.062$; Table 13). However, CD74 expression is statistically independent predictive factor of poor prognosis in multivariable analysis with MIF expression (CD74 high, HR 1.097, 95% CI 1.005-1.197; $p = 0.039$), suggesting targeting CD74 alone in astrocytoma patients will not result in a better outcome.

Post-operative KPS score was significantly independent predictive factor for poor prognosis (HR 3.130, 95% CI 1.681–5.829; p = 0.0001; Table 13). Age and Karnofsky performance status (KPS) were considered as biomarkers in preoperative decision making (Stupp et al., 2005). Postoperative KPS scores have superior predictive capabilities for overall survival in glioblastoma patients and should replace preoperative KPS scores when estimating prognosis (Chambless et al., 2014).

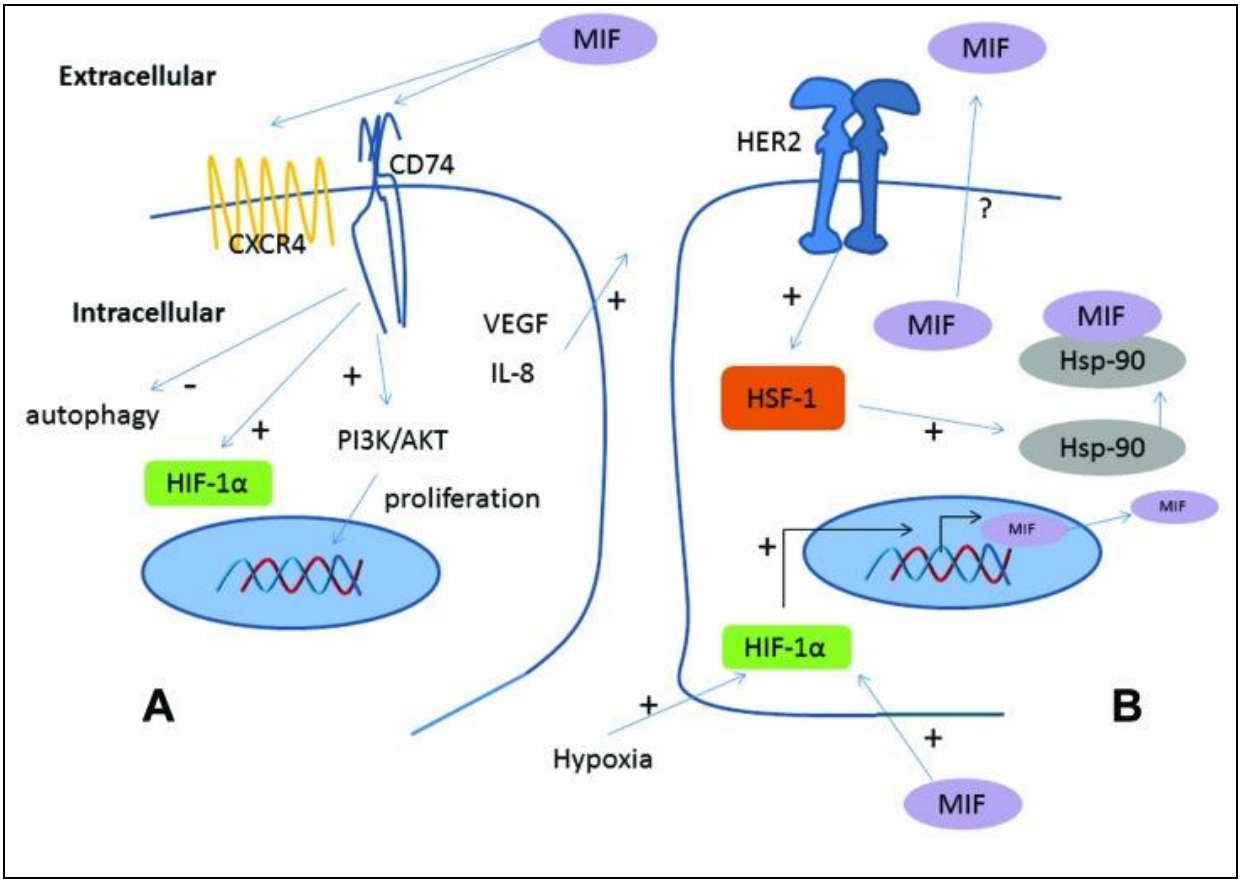

Figure 24. Schematic model of MIF pathways in breast cancer cells (BCC). (A) Auto- and paracrine MIF effects: MIF interacts with its main receptor CD74 and CXCR4, promoting cell survival by activation of PI3K/AKT signaling pathway, neo-angiogenesis by increased secretion levels of VEGF and IL-8 and inhibiting autophagy. (B) Regulation of MIF synthesis: MIF is upregulated in BCC by HIF-1α and other potential hypoxia induced mechanisms, stabilized by Hsp-90 particularly in HER2 overexpressed BCC and secreted by unconventional pathway. In turn it enhances HIF-1α activation.

6. SUMMARY

This study revealed similarities and differences in gene expression levels between grades II-IV AS subtypes by comparing their gene expression levels to those of normal brain samples, especially between grade II and grade IV astrocytoma. I have identified genes that were exclusively expressed in each of the AS subtype. Pathway analysis of differentially expressed genes in each AS subtype revealed important cancer-relevant signaling processes. Furthermore, I may have identified an alternative signaling pathway (to the canonical SMAD-mediated) TGF-beta signaling pathway in GBM. Literature review of the genes identified in this study revealed that many of these genes were implicated in glioma pathogenesis and other cancers. This study confirmed existing findings and shed a new light on some important genes and molecular processes that will improve our understanding of glioma biology. However, an integrated study involving much larger cohort augmented with methylation, whole genome and gene expression data from both tumor and normal brain samples will provide a greater understanding of glioma development and aid in personalized therapy. I believe these findings will provide new insights into mechanisms involving pathogenesis of glioma and provide a valuable resource for future studies.

One of the important observations that I made in the gene expression analysis of astrocytoma grades II-IV, is the overexpression of collagen types COL1A1 and COL6A1, and DDR1 in all astrocytoma subtypes. There has been an increasing evidence describing the importance of interaction between collagen and its receptors, in particular DDR1, in various cancers including brain tumours. This data suggests a possible co-expression of collagen and DDR1 in astrocytoma samples, especially in GBM, however an immunological study on co-expression of specific collagen type(s) and DDR1 using a large cohort of samples will confirm this observation.

In this preliminary study on collagen and DDR1 expression, I found higher expression of DDR1 in GBM cases that also had higher expression of collagen indicating that collagen may modulate the expression of DDR1 expression which supports to this proposed model of collagen mediated activation of DDR1. Also, higher DDR1 expression in primary GBM compared to secondary GBM further supports the idea of its participation in primary GBM progression. I consider that this model deserves to be a subject for further studies in patients with GBM. Currently I am collaborating with a group at the University of Birmingham to

investigate the expression of specific collagen and DDR1 protein expression in a larger sample cohort of tumour and normal cases which will enable us to understand the interaction between collagen and DDR1 and also the role of collagen and DDR1 in the pathogenesis of glioma.

CD74 is a cell membrane receptor for macrophage migration inhibitory factor (MIF) and a potential therapeutic target. CD74 is a non-polymorphic type II integral membrane protein (Shi et al., 2006). It functions as an MHC-II chaperone and participates in the trafficking of MHC-II molecules in antigen-presenting cells (Borghese and Clanchy, 2011) and also shown to express independently of MHC-II and can bind to non-MHC-II proteins (Ogrinc et al., 1993, Henne et al., 1995). The binding of MIF to the extracellular domain of CD74 is necessary as an initial step for the MIF signaling cascade (Shi et al., 2006). MIF have been shown to signal upstream of cytokines via receptor binding in CD74-dependent pathways (Calandra and Roger, 2003, Leng et al., 2003). The pro-inflammatory cytokine MIF and its receptor CD74 (Leng et al., 2003) are associated with tumor vascularization (Binsky et al., 2007, Liu et al., 2008, McClelland et al., 2009) and the promotion of neoplastic cell proliferation and survival via ERK1/2-MAPK (extracellular signal-regulated protein kinases 1/2 and mitogen-activated protein kinase) pathway (McClelland et al., 2009, Otterstrom et al., 2014). MIF can activate the extracellular signal-regulated kinase phosphorylation via CD74 and CD44, where CD44 activates non-receptor tyrosine kinases (Shi et al., 2006). This is thought to enhance tumor growth in several human neoplasms. Furthermore, MIF and CD74 may also act as non-cognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment (Bernhagen et al., 2007). MIF and CD74 form complexes with the cysteine-X-cysteine (CXC)- chemokine receptors CXCR2 on myeloid cells and CXCR4 on T cells (Bernhagen et al., 2007).

The overexpression of MIF and/or CD74 has thought to enhance tumor growth in several human neoplasms, such as esophageal squamous cell carcinoma (Ren et al., 2005), breast cancer (Xu et al., 2008), pancreatic cancer (Nagata et al., 2009), lung cancer(McClelland et al., 2009), cervical squamous cell carcinoma (Cheng et al., 2011), pleural mesothelioma(Otterstrom et al., 2014), and thyroid carcinoma (Cheng et al., 2015). Targeted therapies for CD74 that are currently under investigation includes Milatuzumab, a humanized MAB that recognizes CD74, that was able to stabilize advanced multiple myeloma (Kaufman et al., 2013). Milatuzumab is in phase I clinical trials as a cancer therapy, currently mainly

focusing on hematopoietic neoplasms (Berkova et al., 2009). In conclusion, my study highlighted that CD74 could be a potential biomarker for prognosis and treatment of astrocytoma patients. However, a major limitation of my retrospective study is relatively small sample cohort of each different grade of astrocytoma and larger studies would be necessary for clarification of the prognostic value of CD74 expression in astrocytoma.

My work has provided important insights and a platform for future studies on the biology and pathogenesis of astrocytoma.

7. SOUHRN

Dizertační práce se zaměřuje na podobnosti a rozdíly v expresi genů v astrocytomech grade II-IV a porovnává je s hladinou exprese genů ve zdravé mozkové tkáni. Tím byly identifikovány geny, které se exprimují pouze v určitých subtypech astrocytomů. Analýza signálních drah odhalila pro nádor důležité signální procesy, což odhalilo alternativní signální dráhu TGF-beta (ke kanonické dráze zprostředkované proteinem SMAD) v glioblastomech. Dostupná literatura popisuje roli genů identifikovaných v této studii v patogenezi gliomů a dalších typů nádorů. Předložená práce potvrzuje existující poznatky a objasňuje důležité geny a molekulární procesy, které pomohou zlepšit pochopení biologie gliomů. Věřím, že tyto poznatky přispějí k pochopení mechanizmu patogeneze gliomů a poskytnou základ pro další studie.

Jedním z hlavních zjištění v této práci je fakt, že u všech subtypů astrocytomů grade II-IV dochází ke zvýšené expresi kolagenů typu COL1A1 a COL1A6 a DDR1. Je známo, že interakce mezi kolagenem a jeho receptory, hlavně DDR1, je důležitá u řady nádorů, včetně nádorů mozku. Data popisují možnou koexpresi kolagenu a DDR1 v astrocytomech, hlavně v glioblastomech. Tento vztah je ovšem nutné potvrdit imunohistochemickou studií provedenou na rozsáhlé skupině vzorků.

Tato předběžná studie exprese kolagenu a DDR1 ukazuje vyšší expresi DDR1 spolu s vyšší expresí kolagenu v případech s GBM. Tyto výsledky indikují, že kolagen by mohl modulovat expresi DDR1. Vyšší exprese DDR1 v primárním GBM než v sekundárním GBM by mohla naznačovat jeho roli v progresi primárního GBM. Momentálně spolupracuji s Univerzitou v Birminghamu na studiu exprese kolagenu a DDR1 v rozsáhlé skupině vzorků, což by mělo pomoci objasnit jejich roli v patogenezi gliomů.

Tato dizertační práce se dále zabývá studiem receptoru CD74. CD74 je membránový receptor pro faktor inhibující migraci makrofágů (MIF) a také potenciální terapeutický cíl. CD74 je nepolymorfní integrální membránový protein typu II (Shi et al., 2006). Má funkci MHC-II chaperonu a účastní se transportu MHC-II molekul v antigen prezentujících buňkách (Borghese and Clancy, 2011). Jeho exprese je nezávislá na MHC-II a také se může vázat na jiné proteiny než MHC-II (Ogrinc et al., 1993, Henne et al., 1995). Vazba MIF na extracelulární doménu CD74 je prvním krokem nutným pro aktivaci MIF signální dráhy (Shi et al., 2006). MIF je faktor, který je v dráze závislé na CD74 nadřazený cytokinům (Calandra

and ROger, 2003, Leng et al., 2003). Prozánětlivý cytokin MIF a jeho receptor CD74 (Leng et al., 2003) hrají roli ve vaskularizaci nádorů (Binsky et al., 2007, Liu et al., 2008, McClelland et al., 2009) a podporuje proliferaci neoplastických buněk a jejich přežívání přes signální dráhu ERK1/2-MAPK (McClelland et al., 2009, Ottersotrom et al., 2014). MIF může regulovat fosforylaci přes CD74 a CD44, kdy CD44 aktivuje nereceptorové kinázy (Shi et al., 2006). To pravděpodobně podporuje růst nádoru. MIF a CD74 mohou fungovat jako ligandy CXC chemokinového receptoru u zánětlivých a atherogenních procesů. (Bernhagen et al., 2007). MIF a CD74 vytváří komplexy s CXC chemokinových receptorem CXCR2 na myeloidních buňkách a s CXCR4 na T-buňkách (Bernhagen et al., 2007).

Nadměrná exprese MIF a/nebo CD74 podporuje růst nádoru u řady neoplázií, např. u dlaždicobuněčného karcinomu jícnu (Ren et al., 2005), nádoru prsu (Xu et al., 2008), nádoru slinivky (Nagata et al., 2009), plic (McClelland et al., 2009), děložního krčku (Cheng et al., 2011), pleurálního mezoteliomu (Otterstrom et al, 2014) a karcinomu štítné žlázy (Cheng et al., 2015). Terapie cílená proti CD74 zahrnuje klinické zkoušky humanizované protilátky pro CD74 Milatuzumab, která je schopná také stabilizovat pokročilý myelom (Kaufman et al., 2013). Milatuzumab je v I. fázi klinických zkoušek hlavně pro hematopoetické neoplázie (Berkova et al., 2009). Závěrem moje studie zdůrazňuje, že CD74 by mohl být potenciálním markerem prognózy a léčby pacientů s astrocytomem. Hlavním limitujícím faktorem této retrospektivní studie je relativně malý vzorek pacientů pro různý grade astrocytomů. Pro objasnění prognostické validity CD74 je třeba větší studie.

Moje práce poskytuje důležitý podklad pro další studie biologie a patogeneze astrocytomů.

8. APPENDIX

8.1. Appendix 1: Samples used in gene expression analysis

Sample	Sample ID in GEO	AS subtype / Normal	Sample	Sample ID in GEO	AS subtype / Normal
A1	CGGA_252	Grade II	sGBM2	CGGA_374	Grade IV
A2	CGGA_256	Grade II	sGBM3	CGGA_773	Grade IV
A3	CGGA_261	Grade II	sGBM4	CGGA_899	Grade IV
A4	CGGA_267	Grade II	sGBM5	CGGA_1068	Grade IV
A5	CGGA_269	Grade II	sGBM6	CGGA_1136	Grade IV
A6	CGGA_300	Grade II	sGBM7	CGGA_1170	Grade IV
A7	CGGA_493	Grade II	sGBM8	CGGA_1175	Grade IV
A8	CGGA_502	Grade II	sGBM9	CGGA_1218	Grade IV
A9	CGGA_601	Grade II	sGBM10	CGGA_1227	Grade IV
A10	CGGA_607	Grade II	sGBM11	CGGA_1271	Grade IV
A11	CGGA_642	Grade II	sGBM12	CGGA_1272	Grade IV
A12	CGGA_685	Grade II	sGBM13	CGGA_1324	Grade IV
A13	CGGA_698	Grade II	sGBM14	CGGA_1375	Grade IV
A14	CGGA_738	Grade II	sGBM15	CGGA_1412	Grade IV
A15	CGGA_757	Grade II	sGBM16	CGGA_D34	Grade IV
A16	CGGA_796	Grade II	sGBM17	CGGA_D59	Grade IV
A17	CGGA_807	Grade II	sGBM18	CGGA_D63	Grade IV
A18	CGGA_818	Grade II	sGBM19	CGGA_D64	Grade IV
A19	CGGA_884	Grade II	rGBM1	CGGA_309	Grade IV
A20	CGGA_898	Grade II	rGBM2	CGGA_318	Grade IV
A21	CGGA_904	Grade II	rGBM3	CGGA_545	Grade IV
A22	CGGA_905	Grade II	rGBM4	CGGA_624	Grade IV
A23	CGGA_908	Grade II	rGBM5	CGGA_669	Grade IV
A24	CGGA_1004	Grade II	rGBM6	CGGA_681	Grade IV
A25	CGGA_1005	Grade II	rGBM7	CGGA_1060	Grade IV
A26	CGGA_1013	Grade II	rGBM8	CGGA_1078	Grade IV
A27	CGGA_1020	Grade II	rGBM9	CGGA_1124	Grade IV
A28	CGGA_1031	Grade II	rGBM10	CGGA_1343	Grade IV
A29	CGGA_1046-1	Grade II	rGBM11	CGGA_1381	Grade IV
A30	CGGA_1263	Grade II	rGBM12	CGGA_1394	Grade IV
A31	CGGA_1330	Grade II	rGBM13	CGGA_D02	Grade IV
A32	CGGA_1406	Grade II	rGBM14	CGGA_D26	Grade IV
A33	CGGA_D04	Grade II	rGBM15	CGGA_D32	Grade IV
A34	CGGA_D20	Grade II	rGBM16	CGGA_D36	Grade IV
AA1	CGGA_247	Grade III	rGBM17	CGGA_D38	Grade IV
AA2	CGGA_426	Grade III	rGBM18	CGGA_D51	Grade IV
AA3	CGGA_564	Grade III	rGBM19	CGGA_J100	Grade IV
AA4	CGGA_616	Grade III	SC1	HCC_astrocytes 15	Normal
AA5	CGGA_661	Grade III	SC2	HCC_astrocytes 62	Normal
AA6	CGGA_727	Grade III	SC3	HCC_astrocytes 68	Normal
AA7	CGGA_732	Grade III	SC4	HCC_astrocytes 95	Normal
AA8	CGGA_791	Grade III	SC5	HCC_astrocytes 99	Normal
AA9	CGGA_1034	Grade III	SC6	HCC_astrocytes 105	Normal
AA10	CGGA_1146	Grade III	SC7	HCC_astrocytes 109	Normal

AA11	CGGA_1280	Grade III	SC8	HCC_astrocytes 111	Normal
AA12	CGGA_1284	Grade III	SC9	HCC_astrocytes 128	Normal
AA13	CGGA_D21	Grade III	SC10	HCC_astrocytes 130	Normal
pGBM1	CGGA_274	Grade IV	SC11	HCC_astrocytes 136	Normal
pGBM2	CGGA_413	Grade IV	SC12	HCC_astrocytes 137	Normal
pGBM3	CGGA_483	Grade IV	SC13	HCC_astrocytes 140	Normal
pGBM4	CGGA_491	Grade IV	SC14	HCC_astrocytes 146	Normal
pGBM5	CGGA_494	Grade IV	SC15	HCC_astrocytes 147	Normal
pGBM6	CGGA_499	Grade IV	SC16	HCC_astrocytes 150	Normal
pGBM7	CGGA_604	Grade IV	SC17	HCC_astrocytes 151	Normal
pGBM8	CGGA_658	Grade IV	SC18	HCC_astrocytes 156	Normal
pGBM9	CGGA_676	Grade IV	SC19	HCC_astrocytes 157	Normal
pGBM10	CGGA_679	Grade IV	SC20	HCC_astrocytes 159	Normal
pGBM11	CGGA_680	Grade IV	SC21	HCC_astrocytes 161	Normal
pGBM12	CGGA_700	Grade IV	SC22	HCC_astrocytes 173	Normal
pGBM13	CGGA_710	Grade IV	SC23	HCC_astrocytes 175	Normal
pGBM14	CGGA_731	Grade IV	SC24	HCC_astrocytes 178	Normal
pGBM15	CGGA_761	Grade IV	SC25	HCC_astrocytes 179	Normal
pGBM16	CGGA_782	Grade IV	SC26	HCC_astrocytes 180	Normal
pGBM17	CGGA_802	Grade IV	SC27	HCC_astrocytes 181	Normal
pGBM18	CGGA_804	Grade IV	SC28	HCC_astrocytes 183	Normal
pGBM19	CGGA_808	Grade IV	SC29	HCC_astrocytes 184	Normal
pGBM20	CGGA_837	Grade IV	SC30	HCC_astrocytes 185	Normal
pGBM21	CGGA_842	Grade IV	SC31	HCC_astrocytes 186	Normal
pGBM22	CGGA_848	Grade IV	SC32	HCC_astrocytes 189	Normal
pGBM23	CGGA_850	Grade IV	SC33	HCC_astrocytes 190	Normal
pGBM24	CGGA_859	Grade IV	SC34	HCC_astrocytes 191	Normal
pGBM25	CGGA_876	Grade IV	SC35	HCC_astrocytes 192	Normal
pGBM26	CGGA_878	Grade IV	SC36	HCC_astrocytes 194	Normal
pGBM27	CGGA_902	Grade IV	SC37	HCC_astrocytes 195	Normal
pGBM28	CGGA_1006	Grade IV	SC38	HCC_astrocytes 196	Normal
pGBM29	CGGA_1007	Grade IV	SC39	HCC_astrocytes 197	Normal
pGBM30	CGGA_1008	Grade IV	SC40	HCC_astrocytes 198	Normal
pGBM31	CGGA_1011	Grade IV	SC41	HCC_astrocytes 199	Normal
pGBM32	CGGA_1015	Grade IV	SC42	HCC_astrocytes 201	Normal
pGBM33	CGGA_1024	Grade IV	SC43	HCC_astrocytes 202	Normal
pGBM34	CGGA_1026	Grade IV	SC44	HCC_astrocytes 203	Normal
pGBM35	CGGA_1035	Grade IV	SC45	HCC_astrocytes 208	Normal
pGBM36	CGGA_1039	Grade IV	SC46	HCC_astrocytes 209	Normal
pGBM37	CGGA_1045	Grade IV	SC47	HCC_astrocytes 211	Normal
pGBM38	CGGA_1049	Grade IV	SC48	HCC_astrocytes 212	Normal
pGBM39	CGGA_1072	Grade IV	SC49	HCC_astrocytes 272	Normal
pGBM40	CGGA_1077	Grade IV	SC50	HCC_astrocytes 298	Normal
pGBM41	CGGA_1109	Grade IV	SC51	HCC_astrocytes 304	Normal
pGBM42	CGGA_1116	Grade IV	SC52	HCC_astrocytes 314	Normal
pGBM43	CGGA_1171	Grade IV	SC53	HCC_astrocytes 315	Normal
pGBM44	CGGA_1234	Grade IV	SC54	HCC_astrocytes 316	Normal
pGBM45	CGGA_1237	Grade IV	SC55	HCC_astrocytes 317	Normal
pGBM46	CGGA_1240	Grade IV	SC56	HCC_astrocytes 320	Normal
pGBM47	CGGA_1287	Grade IV	SC57	HCC_astrocytes 321	Normal
pGBM48	CGGA_1301	Grade IV	SC58	HCC_astrocytes 323	Normal

pGBM49	CGGA_1313	Grade IV	SC59	HCC_astrocytes 329	Normal
pGBM50	CGGA_1314	Grade IV	SC60	HCC_astrocytes 331	Normal
pGBM51	CGGA_1338	Grade IV	SC61	HCC_astrocytes 332	Normal
pGBM52	CGGA_1342	Grade IV	N1	Control 16	Normal
pGBM53	CGGA_1384	Grade IV	N2	Control 34	Normal
pGBM54	CGGA_1409	Grade IV	N3	HBRR_Replicate1	Normal
pGBM55	CGGA_D03	Grade IV	N4	HBRR_Replicate2	Normal
pGBM56	CGGA_D09	Grade IV	N5	HBRR_Replicate3	Normal
pGBM57	CGGA_D37	Grade IV	N6	HBRR_Replicate4	Normal
pGBM58	CGGA_D57	Grade IV	N7	HBRR_Replicate5	Normal
sGBM1	CGGA_272	Grade IV			

8.2. Appendix 2: List of 100 most upregulated genes in grade II diffuse astrocytoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
OLIG1	59.27416	**KHSRP**	14.20773	**PTBP1**	11.3627
FLJ16779	41.96556	**POFUT1**	14.17482	**ANO8**	11.3294
MIR6087	40.41731	**DGCR2**	14.02181	**EML3**	11.29683
RGMA	37.95875	**RAB5C**	14.00576	**BAX**	11.22313
SOX11	32.14941	**RPS9**	13.7576	**SOX13**	11.19577
HES6	29.38315	**ARHGDIA**	13.52611	**TRAF4**	11.11985
APOE	28.95826	**SH3GL1**	13.37182	**RNASET2**	11.09062
LTBP3	26.7987	**TNK2**	13.34742	**RPSA**	10.94186
FAM181B	24.40859	**LRP10**	13.25763	**PRKCSH**	10.92818
TSC22D4	23.89341	**YBX1**	13.15423	**APH1A**	10.85038
ADGRB1	23.84622	**EXD3**	13.14022	**PFN1**	10.8341
GFAP	22.98267	**MID1IP1**	13.08237	**GMIP**	10.81888
FADS2	22.64067	**ZFAND3**	13.02708	**EMC10**	10.76906
A2M	21.10397	**NFIX**	12.8698	**H1F0**	10.69671
SLC12A4	19.46536	**NCOR2**	12.73074	**FKBP10**	10.60548
SCRIB	19.0546	**FJX1**	12.65043	**ALYREF**	10.5257
ZNF428	17.80326	**CHPF**	12.62539	**NTNG2**	10.51478
MTSS1L	17.33047	**LOC728392**	12.56631	**ZNF703**	10.49449
FLNA	17.14526	**REPIN1**	12.47562	**INPPL1**	10.29049
GNB2	17.10658	**CROCC**	12.32595	**USF2**	10.2866
APC2	16.81545	**TIMP2**	12.14777	**PPIF**	10.28218
PLEKHG2	16.77908	**CADM4**	12.14694	**SORBS3**	10.25142
SEPN1	16.31198	**SP100**	12.14011	**RPSAP58**	10.22418
EEF2	16.12559	**VCAN**	12.11783	**MAZ**	10.1364
TMEM259	15.82185	**CD151**	12.10159	**PEA15**	10.08307
RPS2	15.79377	**FBXO32**	12.04704	**TAF1C**	10.08008
MBD3	15.48024	**RALY**	11.9156	**ELN**	9.958861
SLC7A5	15.33193	**HLA-B**	11.76767	**RPS8**	9.956314
IGFBP5	15.19658	**ACAP3**	11.64669	**WWTR1**	9.935777
PLEC	15.12113	**LMF1**	11.64492	**MYO9B**	9.928524
NSMF	15.02247	**NOTCH1**	11.64158	**SEPT9**	9.923326
KCTD12	14.49113	**ZC3H3**	11.60188	**CUEDC1**	9.891511
TNRC18	14.38456	**RAB11B**	11.43331		
CAPS	14.35172	**SCAMP4**	11.41441		

8.3. Appendix 3: List of 100 most downregulated genes in grade II diffuse astrocytoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
HIST1H4C	-432.37	**SLC39A12**	-29.2157	**ANO5**	-19.1206
LOC101928590	-166.963	**SYCP3**	-29.1778	**KLF3-AS1**	-18.8815
IDI2-AS1	-111.61	**GNA14**	-28.0009	**ANKAR**	-18.797
CLDN10	-94.245	**WIF1**	-27.7918	**CAPZA1**	-18.7774
MALAT1	-72.8538	**SLC7A10**	-26.9257	**PREX2**	-18.6123
LOC100289473	-67.2121	**ADRB1**	-26.8017	**HSD17B6**	-18.4885
CCDC144A	-66.9775	**INTU**	-25.3993	**ZNF404**	-18.2551
RYR3	-59.1401	**IQCA1**	-25.156	**SMG1P1**	-18.1912
LRRC16A	-58.9605	**FAM76A**	-24.7983	**ADGRV1**	-18.0456
GABRA2	-56.6275	**CTH**	-24.7159	**SLC1A2**	-17.6817
SAT1	-53.4972	**NBPF14**	-24.612	**TFB1M**	-17.3418
SLC13A5	-52.4633	**LOC399715**	-24.5385	**ATP13A4**	-17.262
CPNE8	-46.8551	**RBMS3**	-24.3752	**RNF138P1**	-17.193
LGI1	-44.539	**CXCL2**	-24.3473	**GRAMD1C**	-17.0975
GJB6	-44.0616	**NBPF20**	-24.3306	**ADRB2**	-16.9398
EFNA5	-43.9584	**CMYA5**	-23.9855	**SLC2A4**	-16.8876
GPR22	-43.4696	**PWARSN**	-23.3939	**RORB**	-16.8521
LOC101927692	-42.7561	**TPD52L1**	-23.3902	**FBXO4**	-16.7705
DIO2	-42.0722	**SRPX2**	-23.0397	**ACOX2**	-16.6259
FAS	-38.2099	**MKLN1-AS**	-21.7675	**TLK1**	-16.5975
LINC00632	-37.0399	**ZNF460**	-21.0902	**MGAT4C**	-16.5813
ANKRD36B	-34.7806	**MT1G**	-20.9045	**NEAT1**	-16.1694
SLCO1C1	-33.5541	**GPR143**	-20.8389	**FAM184A**	-15.982
GABRB1	-33.3204	**SMG1P3**	-20.7076	**RNF43**	-15.9258
KLF4	-32.5544	**RGS20**	-20.6912	**MT1M**	-15.9063
ADORA2B	-32.0824	**HGF**	-20.5989	**RLBP1**	-15.723
ANKRD36C	-32.0791	**AASS**	-20.4115	**LINC00472**	-15.6268
ANKRD36	-31.7522	**AXL**	-20.3415	**ARRDC3**	-15.4278
PLIN5	-31.6412	**P2RY14**	-20.1506	**CCDC144B**	-15.3796
AQP4-AS1	-31.4895	**NPTN-IT1**	-20.0987	**RNPC3**	-15.3753
ZIC2	-30.801	**FGD6**	-19.8219	**SELENBP1**	-14.9357
F3	-29.7994	**PCDH9**	-19.6656	**KLHDC1**	-14.8585
BMPR1B	-29.4875	**SLC30A10**	-19.2461		
CACNB2	-29.4141	**LOC100505715**	-19.1729		

8.4. Appendix 4: List of 100 most upregulated genes in grade III anaplastic astrocytoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
COL1A1	73	**HLA-C**	12	**PDAP1**	9
MIR6087	54	**NECAP2**	12	**SSRP1**	9
SOD2	28	**IGFBP7**	12	**PRRC2A**	8
FKBP10	27	**INPP5D**	11	**RPL37**	8
GFAP	23	**RAB11B**	11	**HSPB1**	8
HLA-B	22	**TRIM47**	11	**GNS**	8
COL6A1	21	**NFIX**	11	**AHDC1**	8
VEGFA	19	**NOTCH1**	11	**NUCB1**	8
APOE	18	**JUNB**	11	**SDC3**	8
FADS2	18	**TNFRSF1A**	11	**RPL28**	8
GPC1	17	**MTSS1L**	11	**TUBA1C**	8
TSC22D4	16	**MYH9**	11	**RPL13**	8
TIMP2	16	**C7orf50**	11	**IRS2**	8
MYO9B	16	**GANAB**	11	**AP1B1**	7
FN1	16	**LAMP1**	10	**NPDC1**	7
TTYH3	16	**GNAI2**	10	**CFL1**	7
SEC61A1	16	**SLC38A10**	10	**B2M**	7
CHI3L1	16	**EGFR**	10	**RPLP0**	7
YBX1	15	**SAMD9**	10	**RPL35**	7
HLA-H	15	**S100A6**	10	**PEA15**	7
KCTD12	15	**TMSB4X**	10	**ECE1**	7
EEF2	13	**DDR1**	10	**SART1**	7
ADGRB1	13	**MSN**	10	**CSPG5**	7
TNRC18	13	**MTA2**	10	**RPLP2**	7
RPL27A	13	**VOPP1**	9	**NAMPT**	7
CTSD	13	**HLA-E**	9	**RPS15A**	7
CLIC4	13	**RPS8**	9	**NDRG1**	7
PLEC	13	**SNRNP70**	9	**EPAS1**	7
OLFM2	13	**GALNT2**	9	**LRP1**	7
CD99	13	**HNRNPUL1**	9	**RPL36**	7
SURF4	13	**RPS18**	9	**RPS24**	7
VIM	12	**PLCB3**	9	**RPL30**	6
TMEM132A	12	**TAOK2**	9		
IFI6	12	**CD81**	9		

8.5. Appendix 5: List of 100 most downregulated genes in grade III anaplastic astrocytoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
GPC5-AS1	-3368.49	**CCDC144B**	-28.5559	**NHSL1**	-14.9648
LINC00499	-1118.8	**GRM3**	-27.7522	**TCF7L2**	-14.6839
LOC101928590	-599.228	**NBPF20**	-27.5639	**ANKRD26**	-14.0659
WIF1	-497.288	**SLCO1C1**	-26.8439	**GARNL3**	-13.6195
HIST1H4C	-296.714	**AQP4-AS1**	-26.4184	**ASB3**	-13.1655
GJB6	-202.132	**RYR3**	-24.8997	**ADHFE1**	-13.1247
CCDC144A	-173.692	**GABRG1**	-24.2777	**ALDOC**	-13.0701
IDI2-AS1	-123.415	**RBMS3**	-23.9739	**FAM133CP**	-12.9295
SLC13A5	-117.126	**SAT1**	-23.5948	**CACUL1**	-12.9
CLDN10	-79.9957	**ETNPPL**	-23.1449	**TLK1**	-12.7341
GABRA2	-65.6631	**AXL**	-23.078	**SLC7A11**	-12.6786
GPR22	-61.9983	**ATP13A4**	-22.2379	**FAM184A**	-12.6248
MALAT1	-57.9362	**DIO2**	-21.7513	**PTBP2**	-12.6154
ANKRD36C	-46.2164	**PREX2**	-21.1694	**SLC25A18**	-12.4726
LRRC16A	-45.435	**FAM76A**	-20.9905	**EMX2OS**	-12.305
CACNB2	-45.3836	**SNORA109**	-20.2033	**SLC48A1**	-12.0399
PWARSN	-44.4055	**ZNF460**	-19.6073	**TOB1**	-11.9464
GABRB1	-40.5713	**RANBP3L**	-19.5979	**RNPC3**	-11.9377
ADAM20	-38.8202	**ZIC2**	-19.3077	**AMER2**	-11.9237
MGAT4C	-38.55	**MT1G**	-18.8073	**TNRC6B**	-11.4589
SLC2A4	-36.7462	**CTH**	-18.4523	**GPAM**	-11.4222
CPNE8	-36.6826	**VIP**	-18.1848	**GRAMD1C**	-11.3768
SLC1A2	-36.2919	**HIF3A**	-17.9669	**PER2**	-11.3616
LGI1	-35.366	**KIAA0319**	-17.8368	**DDAH1**	-11.1858
ANKRD36B	-34.3687	**STON2**	-17.5682	**NRXN1**	-11.0836
TPD52L1	-33.0314	**SMG1P3**	-17.3815	**CAPZA1**	-11.0715
SLC39A12	-32.5532	**PLIN5**	-17.1925	**AHI1**	-11.0675
ANKRD36	-32.3413	**OMG**	-16.8776	**ACSL6**	-11.0573
ADRB1	-31.9679	**SMG1P1**	-16.7731	**MRVI1**	-10.8424
RGS20	-31.1196	**SLITRK2**	-16.1451	**ZFHX4**	-10.8205
ADGRV1	-30.7154	**IQCA1**	-15.8715	**NFIB**	-10.716
BMPR1B	-29.9401	**SLC39A11**	-15.8698	**NBPF10**	-10.7017
PCDH9	-29.2196	**AASS**	-15.6552		
NBPF14	-29.0738	**INTU**	-15.025		

8.6. Appendix 6: List of 100 most upregulated genes in grade IV primary glioblastoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
COL3A1	1562.727	**SIGLEC1**	93.14211	**S100A10**	71.06089
COL1A2	502.048	**HSPG2**	92.729	**HELZ2**	70.87974
COL6A2	344.4161	**LAPTM5**	92.67361	**CD44**	70.75403
IGFBP2	287.8228	**VSIG4**	91.97493	**TYROBP**	70.68969
FCGBP	277.6274	**HLA-DRB1**	91.29291	**CMTM3**	70.50907
TGFBI	273.819	**C3**	89.59428	**LILRB4**	70.31511
ABCC3	272.8876	**LOX**	89.4771	**HLA-DMB**	70.15866
COL1A1	245.1593	**VCAM1**	89.28191	**HLA-DPA1**	69.20167
MRC2	225.7456	**FOXJ1**	88.90136	**ALOX5AP**	69.07543
F2R	199.7209	**ITGB2**	88.86298	**THBS1**	69.03221
MMP14	194.1601	**FCGR3A**	86.9631	**MSR1**	68.32442
MXRA5	182.9241	**TYMP**	86.12664	**CD74**	66.65066
CHI3L2	179.3448	**CD14**	85.20805	**TAGLN2**	66.63782
PLAU	174.006	**MS4A6A**	84.40547	**RGS1**	66.5121
COL4A1	171.8057	**FOXM1**	83.97945	**COL18A1**	65.60555
COL5A1	171.3157	**C1QC**	83.43079	**ADAMTS15**	64.98485
MMP2	152.7387	**HLA-DQA1**	83.42423	**TNFRSF19**	64.97415
CD163	152.4363	**HLA-DRB5**	81.61281	**STEAP3**	64.95742
ANXA1	143.5054	**SLC16A3**	80.66654	**TGFB1**	64.54718
IFI30	136.9448	**C1QA**	80.57751	**ARHGDIB**	63.2508
NMB	130.507	**COL6A3**	80.0987	**AGRN**	61.68061
SERPINE1	115.1537	**GPNMB**	79.86016	**SULF1**	61.42272
IGFBP3	111.1884	**OLFML2B**	78.88382	**CTSS**	61.01441
PLVAP	110.9019	**TREM2**	77.23334	**GAL3ST4**	60.86989
MDFI	107.0202	**CTGF**	76.93684	**OAS1**	60.79683
C1QL1	105.3336	**C1QB**	76.48459	**PCOLCE**	60.76841
S100A11	101.8102	**CD93**	74.63651	**COL22A1**	60.52599
COL4A2	99.03796	**ITGA5**	73.87044	**HLA-DPB1**	60.13876
CNGA3	98.993	**CAV1**	73.57592	**SPARC**	58.74106
TNFRSF12A	98.7045	**HLA-DRA**	73.53501	**SERPINA1**	57.84761
F13A1	97.93235	**STAB1**	73.48663	**TPX2**	57.06609
LOXL2	97.91691	**FMOD**	73.21972	**TACC3**	56.63418
EMILIN1	95.20179	**CYBA**	72.3215		
CD68	94.77698	**CD248**	72.08764		

8.7. Appendix 7: List of 100 most downregulated genes in grade IV primary glioblastoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
IDI2-AS1	-166	**GABRG1**	-35	**LOC101929372**	-23
GJB6	-160	**RANBP3L**	-35	**INTU**	-23
WIF1	-148	**LOC399715**	-34	**ANO5**	-23
GPR22	-115	**CCDC144B**	-33	**AXL**	-22
CCDC144A	-113	**DNM3OS**	-33	**HIF3A**	-22
GABRA2	-107	**NBPF20**	-32	**STOX1**	-22
CLDN10	-94	**SYCP3**	-32	**KLF3-AS1**	-22
ETNPPL	-84	**RGS20**	-31	**ZIC2**	-22
MGAT4C	-77	**RBMS3**	-31	**LINC01354**	-22
PWARSN	-59	**ATP13A4**	-31	**P2RY14**	-21
GABRB1	-56	**IQCA1**	-31	**ZNF460**	-21
BMPR1B	-56	**ADRB2**	-30	**OMG**	-21
MALAT1	-55	**PCDH9**	-30	**GABRA4**	-21
SLCO1C1	-55	**GNA14**	-30	**SEMA4A**	-21
RRN3P2	-54	**CPNE8**	-30	**HPR**	-21
AQP4-AS1	-53	**MKLN1-AS**	-29	**LINC00982**	-20
SLC13A5	-53	**CTH**	-29	**FGD6**	-20
ANKRD36C	-52	**NPTN-IT1**	-28	**KIAA0319**	-20
LGI1	-51	**LOC100505715**	-27	**PREX2**	-20
CACNB2	-50	**SMG1P3**	-26	**TET1**	-20
EFNA5	-50	**AASS**	-26	**SLC7A10**	-20
LRRC16A	-49	**KLF4**	-26	**NME9**	-20
SLC2A4	-45	**ZNF165**	-26	**CLUHP3**	-20
ADGRV1	-45	**ZNF404**	-26	**NKAIN3**	-20
ANKRD36	-43	**DIO2**	-26	**ANKRD26**	-19
RYR3	-43	**HSD17B6**	-25	**NR6A1**	-19
SLC1A2	-40	**CCDC144CP**	-25	**ZNF540**	-19
PLIN5	-39	**KLHDC1**	-25	**SAT1**	-19
TPD52L1	-38	**GRM3**	-25	**ZNF280B**	-19
ANKRD36B	-38	**STON2**	-24	**RNPC3**	-19
SLC39A12	-38	**FAM76A**	-24	**ADORA2B**	-19
SLC30A10	-37	**ADHFE1**	-24	**TTPA**	-19
NBPF14	-36	**SNORA109**	-24		
ADRB1	-36	**KLKB1**	-23		

8.8. Appendix 8: List of 100 most upregulated genes in grade IV secondary glioblastoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
COL1A1	171	**FN1**	12	**NUSAP1**	10
MIR6087	78	**FADS2**	12	**RPLP0**	9
RGMA	31	**HNRNPUL1**	12	**RPL11**	9
FKBP10	28	**RAB11B**	12	**RPL13**	9
ELN	24	**RPL6**	12	**RPL35**	9
YBX1	23	**SURF4**	12	**RPL28**	9
ALYREF	23	**GANAB**	12	**CLIC4**	9
SEPN1	19	**EMC10**	12	**MYH9**	9
GFAP	19	**HLA-H**	12	**TMOD1**	9
PFN1	17	**EEF1D**	11	**HLA-C**	9
KHSRP	17	**COL6A1**	11	**GNAI2**	9
HLA-B	17	**PLXNA1**	11	**RPS16**	9
PRR11	17	**MTSS1L**	11	**RPL30**	9
EEF2	17	**KCTD12**	11	**RPS15A**	9
TNRC18	16	**VEGFA**	11	**SART1**	9
NOTCH1	16	**SSRP1**	11	**GNA11**	9
RAB5C	15	**RPS21**	11	**TUBB3**	9
LAMP1	15	**RPLP2**	11	**VIM**	9
SEC61A1	15	**C7orf50**	11	**MZT2A**	9
RPSAP58	15	**RPL26**	10	**SNRNP70**	9
APOE	14	**GPC1**	10	**TRIM47**	9
MYO9B	14	**NFIX**	10	**LMF1**	9
TIMP2	14	**IGFBP7**	10	**SOD2**	9
ADGRB1	14	**SLC38A10**	10	**RPL27A**	8
PRKCSH	14	**RPL32**	10	**MEX3D**	8
APC2	13	**MARCKSL1**	10	**AKT2**	8
TMEM132A	13	**CADM4**	10	**NPDC1**	8
TSC22D4	13	**TUBB**	10	**PLCB3**	8
GMIP	13	**RPS4X**	10	**NME4**	8
RPS18	13	**RASD1**	10	**RPL7A**	8
MTA2	12	**ANP32A**	10	**JUNB**	8
TTYH3	12	**PDAP1**	10	**RPL8**	8
RPL37	12	**CORO1C**	10		
RPS8	12	**PRRC2A**	10		

8.9. Appendix 9: List of 100 most downregulated genes in grade IV secondary glioblastoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
GPC5-AS1	-4424	**CACNB2**	-31	**KIAA0319**	-21
LINC00499	-377	**ANKRD36C**	-31	**SNORA109**	-21
LOC101928590	-228	**AXL**	-30	**MT1G**	-21
CLDN10	-121	**SAT1**	-30	**INTU**	-20
IDI2-AS1	-108	**DIO2**	-30	**SEMA4A**	-20
WIF1	-82	**ZIC2**	-29	**LOC100505715**	-19
CCDC144A	-80	**LOC101929372**	-29	**ZNF460**	-19
ETNPPL	-71	**RNF219-AS1**	-28	**PRSS35**	-18
SLC13A5	-71	**RANBP3L**	-28	**GRM3**	-18
GJB6	-70	**SLC1A2**	-27	**F3**	-18
HIST1H4C	-70	**NBPF20**	-27	**NHSL1**	-18
SLCO1C1	-65	**ADGRV1**	-27	**PON2**	-18
RYR3	-61	**RGS20**	-27	**SMG1P3**	-17
MGAT4C	-61	**CTH**	-27	**GRAMD1C**	-17
LRRC16A	-61	**FAM76A**	-27	**STOX1**	-17
GABRA2	-61	**DNAH7**	-25	**SMG1P1**	-17
GPR22	-55	**HSD17B6**	-25	**TRPM3**	-17
MALAT1	-51	**CCDC144B**	-25	**ADHFE1**	-17
LGI1	-50	**LOC399715**	-24	**STON2**	-17
BMPR1B	-49	**CPNE8**	-24	**TTPA**	-17
AQP4-AS1	-44	**ANKRD36**	-24	**ANKAR**	-17
SLC30A10	-42	**ANKRD36B**	-24	**TOB1**	-17
SLC2A4	-42	**GNA14**	-23	**SELENBP1**	-16
EFNA5	-40	**AASS**	-23	**LINC00472**	-16
GABRB1	-39	**RBMS3**	-23	**FGD6**	-16
KLF4	-38	**SYCP2**	-23	**SLC39A11**	-16
PCDH9	-37	**HIF3A**	-22	**NEAT1**	-16
NBPF14	-37	**HPR**	-22	**MT1M**	-16
ATP13A4	-35	**PLIN5**	-22	**GPC5**	-15
SYCP3	-34	**GABRG1**	-22	**SLC7A11**	-15
IQCA1	-33	**PREX2**	-22	**OMG**	-15
ADRB1	-33	**SLC39A12**	-21	**FAS**	-15
MKLN1-AS	-32	**ANO5**	-21		
PWARSN	-32	**TPD52L1**	-21		

8.10. Appendix 10: List of 100 most upregulated genes in grade IV recurrent glioblastoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
COL1A1	373	**TNRC18**	14	**SSRP1**	10
MIR6087	42	**FADS2**	14	**LAMP1**	10
FN1	39	**GFAP**	14	**NOTCH1**	10
FKBP10	38	**MYH9**	14	**ADGRB1**	10
RGMA	38	**P4HB**	14	**APC2**	10
COL6A1	38	**GANAB**	14	**PLEC**	10
HLA-B	31	**EEF2**	13	**CD99**	10
SOD2	30	**VIM**	13	**PDAP1**	10
ELN	28	**EMC10**	13	**CEBPB**	10
SEPN1	28	**C7orf50**	13	**DRAP1**	10
PFN1	26	**SAMD9**	13	**RPL6**	10
CHI3L1	25	**PRR11**	12	**RPL37**	10
RPS3	24	**IFI6**	12	**CADM4**	10
VEGFA	23	**TNFRSF1A**	12	**TRIM47**	10
SEC61A1	20	**RAB11B**	12	**MSN**	9
HLA-H	19	**TIMP2**	12	**GNS**	9
ALYREF	19	**JUNB**	12	**TMOD1**	9
GPC1	19	**FTH1**	12	**GNA11**	9
YBX1	18	**SLC38A10**	12	**NUCB1**	9
IGFBP7	18	**MTA2**	11	**NFIX**	9
APOE	18	**GNAI2**	11	**RPL11**	9
RAB5C	17	**KCTD12**	11	**PLXNB2**	9
CTSD	17	**CLIC4**	11	**NAMPT**	9
SURF4	17	**HSPB1**	11	**GALNT2**	9
TTYH3	17	**RPS18**	11	**EEF1D**	9
PRKCSH	16	**SEC61G**	11	**MZT2A**	9
TSC22D4	16	**S100A6**	11	**PRRC2A**	9
KHSRP	16	**HNRNPUL1**	11	**RPSAP58**	9
GMIP	16	**TUBA1C**	11	**CFL1**	9
TMEM132A	16	**TMSB4X**	11	**SNRNP70**	9
HLA-C	16	**HLA-E**	10	**HLA-A**	9
MYO9B	15	**NECAP2**	10	**B4GALT5**	9
PLXNA1	15	**RPL28**	10		
TMSB10	15	**RPS8**	10		

8.11. Appendix 11: List of 100 most downregulated genes in grade IV recurrent glioblastoma

Gene	Fold change	Gene	Fold change	Gene	Fold change
GPC5-AS1	-4472	**NBPF20**	-32	**GABRA4**	-19
LINC00499	-685	**ANKRD36B**	-32	**SMG1P3**	-19
LOC101928590	-506	**RGS20**	-31	**ZNF460**	-19
HIST1H4C	-362	**SLC1A2**	-31	**KIAA0319**	-18
CCDC144A	-147	**SLC39A12**	-31	**SYCP2**	-18
IDI2-AS1	-100	**ZIC2**	-29	**SMG1P1**	-18
WIF1	-97	**CTH**	-27	**FAM184A**	-17
CLDN10	-92	**SNORA109**	-27	**NHSL1**	-17
GPR22	-85	**GNA14**	-27	**NKAIN3**	-17
GJB6	-83	**PCDH9**	-27	**SEMA4A**	-17
MGAT4C	-73	**RANBP3L**	-27	**HSD17B6**	-17
GABRA2	-70	**RBMS3**	-26	**LINC00472**	-16
SLC13A5	-57	**MKLN1-AS**	-26	**TCF7L2**	-16
ETNPPL	-55	**DNM3OS**	-26	**IQCA1**	-16
GABRB1	-55	**HPR**	-26	**HIF3A**	-16
PWARSN	-54	**DIO2**	-26	**RNPC3**	-16
BMPR1B	-52	**AASS**	-25	**PLIN5**	-16
LGI1	-52	**RNF219-AS1**	-25	**ADORA2B**	-16
SLCO1C1	-50	**TPD52L1**	-24	**DNAH7**	-16
MALAT1	-48	**SYCP3**	-24	**UBE2Q2P1**	-16
AQP4-AS1	-47	**KLF4**	-23	**STOX1**	-16
RYR3	-47	**CPNE8**	-23	**GRAMD1C**	-16
LRRC16A	-45	**ANO5**	-22	**ADHFE1**	-15
ANKRD36C	-44	**FAM76A**	-22	**FGD6**	-15
CCDC144B	-43	**PREX2**	-22	**NTM**	-15
ADGRV1	-43	**GRM3**	-21	**ZFHX4**	-15
ATP13A4	-41	**CCDC144CP**	-21	**SYNE1**	-15
EFNA5	-40	**STON2**	-21	**ASB3**	-15
CACNB2	-36	**LOC399715**	-21	**ANKAR**	-15
SLC30A10	-34	**AXL**	-21	**OMG**	-15
SLC2A4	-34	**ZNF404**	-21	**ANKRD26**	-15
ANKRD36	-34	**LOC100505715**	-20	**TTPA**	-15
NBPF14	-34	**LOC101929372**	-20		
ADRB1	-33	**INTU**	-19		

9. ABBREVIATIONS

AS	astrocytoma
CAMK2G	Calcium/Calmodulin Dependent Protein Kinase II Gamma
COL	collagenous
CXCR4	cysteine-X-cysteine (CXC) - chemokine receptors
DDRs	discoidin domain receptors
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EGFRvIII	epidermal growth factor receptor (EGFR) variant III
FACITs	fibril-associated collagens with interrupted triple helices),
FFPE	formalin-fixed paraffin embedded
GBM	glioblastoma
GDFs	growth and differentiation factors
GEO	gene Expression Omnibus
GSEA	gene set enrichment analysis
ICGC	International Cancer Genome Consortium
IDH	isocitrate dehydrogenase
LOH	loss of heterozygosity
KPS	Karnofsky performance score
MAPK/ERK	extracellular signal-regulated protein kinases 1/2 and mitogen-activated protein kinase
MGMT	O-6-methylguanine-DNA methyltransferase
MIF	macrophage migration inhibitory factor

MSigDB	molecular Signatures Database
MULTIPLEXINs	multiple triple-helix domains and interruptions
NC	non-collagenous
pGBM	primary glioblastoma
PI3K	phosphatidylinositol-4, 5-Bisphosphate 3-Kinase
PLCγ	phospholipase C gamma
rGBM	recurrent glioblastoma
RTKs	receptor tyrosine kinases
sGBM	secondary glioblastoma multiforme
SHC	Src Homology 2 Domain Containing
SOS2	SOS Ras/Rho Guanine Nucleotide Exchange Factor 2
TCGA	The Cancer Genome Atlas
TMM	trimmed mean of M values)
TMZ	temozolomide
TNF-α	tumor necrosis factor-α
WHO	World Health Organization

10. LIST OF PUBLICATIONS

Reviews and original papers

1. Gvantsa Kharaishvili, Dana Simkova1, Katerina Bouchalova, Mariam Gachechiladze, **Nato Narsia** and Jan Bouchal „The role of cancer-associated fibroblasts, solid stress and other microenvironmental factors in tumor progression and therapy resistance.“ Cancer Cell International 2014, 14:41

2. Nato Narsia, Pradeep Ramagiri, Jiri Ehrmann, Zdenek Kolar „Transcriptome analysis reveals distinct gene expression profiles in astrocytoma grades II-IV.“ Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2017; 161:XX

3. Nato Narsia, Pradeep Ramagiri, Nino Lomtadze, Manana Arabuli, Jiri Ehrman, Zdenek Kolar. „CD74 as a Potential Prognostic Marker in Glioblastoma.“ International Annals of Medicine. 2017;1

11. REFERENCES

ALVES, F., VOGEL, W., MOSSIE, K., MILLAUER, B., HOFLER, H. & ULLRICH, A. 1995. Distinct structural characteristics of discoidin I subfamily receptor tyrosine kinases and complementary expression in human cancer. *Oncogene,* 10**,** 609-18.

ANDERS, S. & HUBER, W. 2010. Differential expression analysis for sequence count data. *Genome Biol,* 11**,** R106.

ARNVIG, K. B., COMAS, I., THOMSON, N. R., HOUGHTON, J., BOSHOFF, H. I., CROUCHER, N. J., ROSE, G., PERKINS, T. T., PARKHILL, J., DOUGAN, G. & YOUNG, D. B. 2011. Sequence-based analysis uncovers an abundance of non-coding RNA in the total transcriptome of Mycobacterium tuberculosis. *PLoS Pathog,* 7**,** e1002342.

ARTESI, M., KROONEN, J., BREDEL, M., NGUYEN-KHAC, M., DEPREZ, M., SCHOYSMAN, L., POULET, C., CHAKRAVARTI, A., KIM, H., SCHOLTENS, D., SEUTE, T., ROGISTER, B., BOURS, V. & ROBE, P. A. 2015. Connexin 30 expression inhibits growth of human malignant gliomas but protects them against radiation therapy. *Neuro Oncol,* 17**,** 392-406.

BACHER, M., SCHRADER, J., THOMPSON, N., KUSCHELA, K., GEMSA, D., WAEBER, G. & SCHLEGEL, J. 2003a. Up-Regulation of Macrophage Migration Inhibitory Factor Gene and Protein Expression in Glial Tumor Cells during Hypoxic and Hypoglycemic Stress Indicates a Critical Role for Angiogenesis in Glioblastoma Multiforme. *The American Journal of Pathology,* 162**,** 11-17.

BACHER, M., SCHRADER, J., THOMPSON, N., KUSCHELA, K., GEMSA, D., WAEBER, G. & SCHLEGEL, J. 2003b. Up-regulation of macrophage migration inhibitory factor gene and protein expression in glial tumor cells during hypoxic and hypoglycemic stress indicates a critical role for angiogenesis in glioblastoma multiforme. *Am J Pathol,* 162**,** 11-7.

BAERISWYL, V. & CHRISTOFORI, G. 2009. The angiogenic switch in carcinogenesis. *Semin Cancer Biol,* 19**,** 329-37.

BAI, J., MEI, P.-J., LIU, H., LI, C., LI, W., WU, Y.-P., YU, Z.-Q. & ZHENG, J.-N. 2012. BRG1 expression is increased in human glioma and controls glioma cell proliferation, migration and invasion in vitro. *Journal of Cancer Research and Clinical Oncology,* 138**,** 991-998.

BAI, R. Y., STAEDTKE, V. & RIGGINS, G. J. 2011. Molecular targeting of glioblastoma: Drug discovery and therapies. *Trends Mol Med,* 17**,** 301-12.

BARON, N., DEUSTER, O., NOELKER, C., STUER, C., STRIK, H., SCHALLER, C., DODEL, R., MEYER, B. & BACHER, M. 2011. Role of macrophage migration inhibitory factor in primary glioblastoma multiforme cells. *J Neurosci Res,* 89**,** 711-7.

BELLAIL, A. C., HUNTER, S. B., BRAT, D. J., TAN, C. & VAN MEIR, E. G. 2004. Microregional extracellular matrix heterogeneity in brain modulates glioma cell invasion. *Int J Biochem Cell Biol,* 36**,** 1046-69.

BERKOVA, Z., TAO, R.-H. & SAMANIEGO, F. 2009. Milatuzumab – a promising new immunotherapeutic agent. *Expert Opinion on Investigational Drugs,* 19**,** 141-149.

BERNHAGEN, J., KROHN, R., LUE, H., GREGORY, J. L., ZERNECKE, A., KOENEN, R. R., DEWOR, M., GEORGIEV, I., SCHOBER, A., LENG, L., KOOISTRA, T., FINGERLE-ROWSON, G., GHEZZI, P., KLEEMANN, R., MCCOLL, S. R., BUCALA, R., HICKEY, M. J. & WEBER, C. 2007. MIF is a noncognate ligand of

CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Natrure Medicine,* 13**,** 587-596.

BINSKY, I., HARAN, M., STARLETS, D., GORE, Y., LANTNER, F., HARPAZ, N., LENG, L., GOLDENBERG, D. M., SHVIDEL, L., BERREBI, A., BUCALA, R. & SHACHAR, I. 2007. IL-8 secreted in a macrophage migration-inhibitory factor- and CD74-dependent manner regulates B cell chronic lymphocytic leukemia survival. *Proceedings of the National Academy of Sciences,* 104**,** 13408-13413.

BLEEHEN, N. M. 1979. Cancer trials. *Lancet,* 2**,** 746.

BLOOM, B. R. & BENNETT, B. 1966. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science,* 153**,** 80-2.

BORGHESE, F. & CLANCHY, F. I. L. 2011. CD74: an emerging opportunity as a therapeutic target in cancer and autoimmune disease. *Expert Opinion on Therapeutic Targets,* 15**,** 237-251.

BRENNAN, CAMERON W., VERHAAK, ROEL G. W., MCKENNA, A., CAMPOS, B., NOUSHMEHR, H., SALAMA, SOFIE R., ZHENG, S., CHAKRAVARTY, D., SANBORN, J. Z., BERMAN, SAMUEL H., BEROUKHIM, R., BERNARD, B., WU, C.-J., GENOVESE, G., SHMULEVICH, I., BARNHOLTZ-SLOAN, J., ZOU, L., VEGESNA, R., SHUKLA, SACHET A., CIRIELLO, G., YUNG, W. K., ZHANG, W., SOUGNEZ, C., MIKKELSEN, T., ALDAPE, K., BIGNER, DARELL D., VAN MEIR, ERWIN G., PRADOS, M., SLOAN, A., BLACK, KEITH L., ESCHBACHER, J., FINOCCHIARO, G., FRIEDMAN, W., ANDREWS, DAVID W., GUHA, A., IACOCCA, M., O'NEILL, BRIAN P., FOLTZ, G., MYERS, J., WEISENBERGER, DANIEL J., PENNY, R., KUCHERLAPATI, R., PEROU, CHARLES M., HAYES, D. N., GIBBS, R., MARRA, M., MILLS, GORDON B., LANDER, E., SPELLMAN, P., WILSON, R., SANDER, C., WEINSTEIN, J., MEYERSON, M., GABRIEL, S., LAIRD, PETER W., HAUSSLER, D., GETZ, G. & CHIN, L. 2013. The Somatic Genomic Landscape of Glioblastoma. *Cell,* 155**,** 462-477.

BRISTOW, R. G. & HILL, R. P. 2008. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer,* 8**,** 180-92.

BRODSKY, B. & PERSIKOV, A. V. 2005. Molecular structure of the collagen triple helix. *Adv Protein Chem,* 70**,** 301-39.

BURTON, J. D., ELY, S., REDDY, P. K., STEIN, R., GOLD, D. V., CARDILLO, T. M. & GOLDENBERG, D. M. 2004. CD74 is expressed by multiple myeloma and is a promising target for therapy. *Clin Cancer Res,* 10**,** 6606-11.

CALANDRA, T. & ROGER, T. 2003. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nature Reviews Immunology,* 3**,** 791-800.

CANCER GENOME ATLAS RESEARCH, N. 2008. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature,* 455**,** 1061-8.

CAO, S., WANG, C., ZHENG, Q., QIAO, Y., XU, K., JIANG, T. & WU, A. 2011. STAT5 regulates glioma cell invasion by pathways dependent and independent of STAT5 DNA binding. *Neurosci Lett,* 487**,** 228-33.

CARRO, M. S., LIM, W. K., ALVAREZ, M. J., BOLLO, R. J., ZHAO, X., SNYDER, E. Y., SULMAN, E. P., ANNE, S. L., DOETSCH, F., COLMAN, H., LASORELLA, A., ALDAPE, K., CALIFANO, A. & IAVARONE, A. 2009. The transcriptional network for mesenchymal transformation of brain tumours. *Nature,* 463**,** 318-325.

CASTELLSAGUE, X., BOSCH, F. X. & MUNOZ, N. 2002. Environmental co-factors in HPV carcinogenesis. *Virus Res,* 89**,** 191-9.

CHAMBLESS, L. B., KISTKA, H. M., PARKER, S. L., HASSAM-MALANI, L., MCGIRT, M. J. & THOMPSON, R. C. 2014. The relative value of postoperative versus preoperative Karnofsky Performance Scale scores as a predictor of survival after surgical resection of glioblastoma multiforme. *Journal of Neuro-Oncology,* 121**,** 359-364.

CHANG, C. H., HORTON, J., SCHOENFELD, D., SALAZER, O., PEREZ-TAMAYO, R., KRAMER, S., WEINSTEIN, A., NELSON, J. S. & TSUKADA, Y. 1983. Comparison of postoperative radiotherapy and combined postoperative radiotherapy and chemotherapy in the multidisciplinary management of malignant gliomas. A joint Radiation Therapy Oncology Group and Eastern Cooperative Oncology Group study. *Cancer,* 52**,** 997-1007.

CHANG, C. H., SAPRA, P., VANAMA, S. S., HANSEN, H. J., HORAK, I. D. & GOLDENBERG, D. M. 2005. Effective therapy of human lymphoma xenografts with a novel recombinant ribonuclease/anti-CD74 humanized IgG4 antibody immunotoxin. *Blood,* 106**,** 4308-14.

CHENG, R. J., DENG, W. G., NIU, C. B., LI, Y. Y. & FU, Y. 2011. Expression of macrophage migration inhibitory factor and CD74 in cervical squamous cell carcinoma. *Int J Gynecol Cancer,* 21**,** 1004-12.

CHENG, S. P., LIU, C. L., CHEN, M. J., CHIEN, M. N., LEUNG, C. H., LIN, C. H., HSU, Y. C. & LEE, J. J. 2015. CD74 expression and its therapeutic potential in thyroid carcinoma. *Endocr Relat Cancer,* 22**,** 179-90.

CHINTALA, S. K., SAWAYA, R., GOKASLAN, Z. L., FULLER, G. & RAO, J. S. 1996. Immunohistochemical localization of extracellular matrix proteins in human glioma, both in vivo and in vitro. *Cancer Lett,* 101**,** 107-14.

COOPER, L. A. D., GUTMAN, D. A., LONG, Q., JOHNSON, B. A., CHOLLETI, S. R., KURC, T., SALTZ, J. H., BRAT, D. J. & MORENO, C. S. 2010. The Proneural Molecular Signature Is Enriched in Oligodendrogliomas and Predicts Improved Survival among Diffuse Gliomas. *PLoS ONE,* 5**,** e12548.

COUGHLIN, C., SCOTT, C., LANGER, C., COIA, L., CURRAN, W. & RUBIN, P. 2000. Phase II, two-arm RTOG trial (94-11) of bischloroethyl-nitrosourea plus accelerated hyperfractionated radiotherapy (64.0 or 70.4 Gy) based on tumor volume (> 20 or < or = 20 cm(2), respectively) in the treatment of newly-diagnosed radiosurgery-ineligible glioblastoma multiforme patients. *Int J Radiat Oncol Biol Phys,* 48**,** 1351-8.

DATTA, M. W., SHAHSAFAEI, A., NADLER, L. M., FREEMAN, G. J. & DORFMAN, D. M. 2000. Expression of MHC class II-associated invariant chain (Ii;CD74) in thymic epithelial neoplasms. *Appl Immunohistochem Mol Morphol,* 8**,** 210-5.

DE WEVER, O., NGUYEN, Q. D., VAN HOORDE, L., BRACKE, M., BRUYNEEL, E., GESPACH, C. & MAREEL, M. 2004. Tenascin-C and SF/HGF produced by myofibroblasts in vitro provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. *FASEB J,* 18**,** 1016-8.

DESHMUKH, H., YU, J., SHAIK, J., MACDONALD, T. J., PERRY, A., PAYTON, J. E., GUTMANN, D. H., WATSON, M. A. & NAGARAJAN, R. 2011. Identification of transcriptional regulatory networks specific to pilocytic astrocytoma. *BMC Med Genomics,* 4.

DONN, R. P. & RAY, D. W. 2004. Macrophage migration inhibitory factor: molecular, cellular and genetic aspects of a key neuroendocrine molecule. *J Endocrinol,* 182**,** 1-9.

DREWE, P., STEGLE, O., HARTMANN, L., KAHLES, A., BOHNERT, R., WACHTER, A., BORGWARDT, K. & RATSCH, G. 2013. Accurate detection of differential RNA processing. *Nucleic Acids Res,* 41**,** 5189-98.

DUDLEY, A. C., SHIH, S. C., CLIFFE, A. R., HIDA, K. & KLAGSBRUN, M. 2008. Attenuated p53 activation in tumour-associated stromal cells accompanies decreased sensitivity to etoposide and vincristine. *Br J Cancer,* 99**,** 118-25.

FAN, Y., POTDAR, A. A., GONG, Y., ESWARAPPA, S. M., DONNOLA, S., LATHIA, J. D., HAMBARDZUMYAN, D., RICH, J. N. & FOX, P. L. 2014. Profilin-1 phosphorylation directs angiocrine expression and glioblastoma progression through HIF-1α accumulation. *Nature Cell Biology,* 16**,** 445-456.

FREIJE, W. A., CASTRO-VARGAS, F. E., FANG, Z., HORVATH, S., CLOUGHESY, T., LIAU, L. M., MISCHEL, P. S. & NELSON, S. F. 2004. Gene expression profiling of gliomas strongly predicts survival. *Cancer Res,* 64**,** 6503-10.

FRIEDMAN, H. S., PRADOS, M. D., WEN, P. Y., MIKKELSEN, T., SCHIFF, D., ABREY, L. E., YUNG, W. K., PALEOLOGOS, N., NICHOLAS, M. K., JENSEN, R., VREDENBURGH, J., HUANG, J., ZHENG, M. & CLOUGHESY, T. 2009. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol,* 27**,** 4733-40.

FU, H. L., VALIATHAN, R. R., ARKWRIGHT, R., SOHAIL, A., MIHAI, C., KUMARASIRI, M., MAHASENAN, K. V., MOBASHERY, S., HUANG, P., AGARWAL, G. & FRIDMAN, R. 2013. Discoidin domain receptors: unique receptor tyrosine kinases in collagen-mediated signaling. *J Biol Chem,* 288**,** 7430-7.

FUJITA, A., SATO, J. R., FESTA, F., GOMES, L. R., OBA-SHINJO, S. M., MARIE, S. K. N., FERREIRA, C. E. & SOGAYAR, M. C. 2008. Identiication of COL6A1 as a differentially expressed gene in human astrocytomas. *Genetics and Molecular Research,* 7**,** 371-378.

FURNARI, F. B., FENTON, T., BACHOO, R. M., MUKASA, A., STOMMEL, J. M., STEGH, A., HAHN, W. C., LIGON, K. L., LOUIS, D. N., BRENNAN, C., CHIN, L., DEPINHO, R. A. & CAVENEE, W. K. 2007. Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev,* 21**,** 2683-710.

FUTREAL, P. A., COIN, L., MARSHALL, M., DOWN, T., HUBBARD, T., WOOSTER, R., RAHMAN, N. & STRATTON, M. R. 2004. A census of human cancer genes. *Nature Reviews Cancer,* 4**,** 177-183.

GAN, H. K., CVRLJEVIC, A. N. & JOHNS, T. G. 2013. The epidermal growth factor receptor variant III (EGFRvIII): where wild things are altered. *FEBS J,* 280**,** 5350-70.

GAUTAM, P., NAIR, S. C., GUPTA, M. K., SHARMA, R., POLISETTY, R. V., UPPIN, M. S., SUNDARAM, C., PULIGOPU, A. K., ANKATHI, P., PUROHIT, A. K., CHANDAK, G. R., HARSHA, H. C. & SIRDESHMUKH, R. 2012. Proteins with Altered Levels in Plasma from Glioblastoma Patients as Revealed by iTRAQ-Based Quantitative Proteomic Analysis. *PLoS ONE,* 7**,** e46153.

GHOOCHANI, A., SCHWARZ, M. A., YAKUBOV, E., ENGELHORN, T., DOERFLER, A., BUCHFELDER, M., BUCALA, R., SAVASKAN, N. E. & EYUPOGLU, I. Y. 2016. MIF-CD74 signaling impedes microglial M1 polarization and facilitates brain tumorigenesis. *Oncogene*.

GODARD, S., GETZ, G., DELORENZI, M., FARMER, P., KOBAYASHI, H., DESBAILLETS, I., NOZAKI, M., DISERENS, A. C., HAMOU, M. F., DIETRICH, P. Y., REGLI, L., JANZER, R. C., BUCHER, P., STUPP, R., DE TRIBOLET, N., DOMANY, E. & HEGI, M. E. 2003. 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 Res,* 63**,** 6613-25.

GRESSOT, L. V., DOUCETTE, T., YANG, Y., FULLER, G. N., MANYAM, G., RAO, A., LATHA, K. & RAO, G. 2014. Analysis of the inhibitors of apoptosis identifies BIRC3 as a facilitator of malignant progression in glioma. *Oncotarget*.

GRESSOT, L. V., DOUCETTE, T., YANG, Y., FULLER, G. N., MANYAM, G., RAO, A., LATHA, K. & RAO, G. 2017. Analysis of the inhibitors of apoptosis identifies BIRC3 as a facilitator of malignant progression in glioma. *Oncotarget*.

HAN, Y., WU, Z., WU, T., HUANG, Y., CHENG, Z., LI, X., SUN, T., XIE, X., ZHOU, Y. & DU, Z. 2016. Tumor-suppressive function of long noncoding RNA MALAT1 in glioma cells by downregulation of MMP2 and inactivation of ERK/MAPK signaling. *Cell Death Dis,* 7**,** e2123.

HÄNNINEN, M. M., HAAPASALO, J., HAAPASALO, H., FLEMING, R. E., BRITTON, R. S., BACON, B. R. & PARKKILA, S. 2009. Expression of iron-related genes in human brain and brain tumors. *BMC Neuroscience,* 10**,** 36.

HARA, A. & OKAYASU, I. 2004. Cyclooxygenase-2 and inducible nitric oxide synthase expression in human astrocytic gliomas: correlation with angiogenesis and prognostic significance. *Acta Neuropathol,* 108**,** 43-8.

HARPER, J., YAN, L., LOUREIRO, R. M., WU, I., FANG, J., D'AMORE, P. A. & MOSES, M. A. 2007. Repression of Vascular Endothelial Growth Factor Expression by the Zinc Finger Transcription Factor ZNF24. *Cancer Research,* 67**,** 8736-8741.

HEGI, M. E., DISERENS, A. C., GORLIA, T., HAMOU, M. F., DE TRIBOLET, N., WELLER, M., KROS, J. M., HAINFELLNER, J. A., MASON, W., MARIANI, L., BROMBERG, J. E., HAU, P., MIRIMANOFF, R. O., CAIRNCROSS, J. G., JANZER, R. C. & STUPP, R. 2005. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med,* 352**,** 997-1003.

HENDRIKSEN, E. M., SPAN, P. N., SCHUURING, J., PETERS, J. P., SWEEP, F. C., VAN DER KOGEL, A. J. & BUSSINK, J. 2009. Angiogenesis, hypoxia and VEGF expression during tumour growth in a human xenograft tumour model. *Microvasc Res,* 77**,** 96-103.

HENNE, C., SCHWENK, F., KOCH, N. & MOLLER, P. 1995. Surface expression of the invariant chain (CD74) is independent of concomitant expression of major histocompatibility complex class II antigens. *Immunology,* 84**,** 177-82.

HOFER, S. & LASSMAN, A. B. 2010. Molecular markers in gliomas: impact for the clinician. *Target Oncol,* 5**,** 201-10.

HOFER, S., RUSHING, E., PREUSSER, M. & MAROSI, C. 2014. Molecular biology of high-grade gliomas: what should the clinician know? *Chin J Cancer,* 33**,** 4-7.

HU, M., YAO, J., CAI, L., BACHMAN, K. E., VAN DEN BRULE, F., VELCULESCU, V. & POLYAK, K. 2005. Distinct epigenetic changes in the stromal cells of breast cancers. *Nat Genet,* 37**,** 899-905.

HUIJBERS, I. J., IRAVANI, M., POPOV, S., ROBERTSON, D., AL-SARRAJ, S., JONES, C. & ISACKE, C. M. 2010. A role for fibrillar collagen deposition and the collagen internalization receptor endo180 in glioma invasion. *PLoS One,* 5**,** e9808.

HUNTER, S., YOUNG, A., OLSON, J., BRAT, D. J., BOWERS, G., WILCOX, J. N., JAYE, D., MENDRINOS, S. & NEISH, A. 2002. Differential Expression between Pilocytic and Anaplastic Astrocytomas: Identification of Apolipoprotein D as a Marker for Low-Grade, Non-Infiltrating Primary CNS Neoplasms. *J Neuropathol Exp Neurol,* 61**,** 275-281.

IOACHIM, H. L., PAMBUCCIAN, S. E., HEKIMGIL, M., GIANCOTTI, F. R. & DORSETT, B. H. 1996. Lymphoid monoclonal antibodies reactive with lung tumors. Diagnostic applications. *Am J Surg Pathol,* 20**,** 64-71.

ISHIGAMI, S., NATSUGOE, S., TOKUDA, K., NAKAJO, A., IWASHIGE, H., ARIDOME, K., HOKITA, S. & AIKOU, T. 2001. Invariant chain expression in gastric cancer. *Cancer Lett,* 168**,** 87-91.

IWAI, L. K., CHANG, F. & HUANG, P. H. 2013. Phosphoproteomic analysis identifies insulin enhancement of discoidin domain receptor 2 phosphorylation. *Cell Adh Migr,* 7**,** 161-4.

JAIN, R. K. 2003. Molecular regulation of vessel maturation. *Nat Med,* 9**,** 685-93.

JENSEN, S. S., AABERG-JESSEN, C., CHRISTENSEN, K. G. & KRISTENSEN, B. 2013. Expression of the lysosomal-associated membrane protein-1 (LAMP-1) in astrocytomas. *Int J Clin Exp Pathol,* 6**,** 1294-305.

JOHNSON, B. E., MAZOR, T., HONG, C., BARNES, M., AIHARA, K., MCLEAN, C. Y., FOUSE, S. D., YAMAMOTO, S., UEDA, H., TATSUNO, K., ASTHANA, S., JALBERT, L. E., NELSON, S. J., BOLLEN, A. W., GUSTAFSON, W. C., CHARRON, E., WEISS, W. A., SMIRNOV, I. V., SONG, J. S., OLSHEN, A. B., CHA, S., ZHAO, Y., MOORE, R. A., MUNGALL, A. J., JONES, S. J. M., HIRST, M., MARRA, M. A., SAITO, N., ABURATANI, H., MUKASA, A., BERGER, M. S., CHANG, S. M., TAYLOR, B. S. & COSTELLO, J. F. 2013. Mutational Analysis Reveals the Origin and Therapy-Driven Evolution of Recurrent Glioma. *Science,* 343**,** 189-193.

JONES, D. T. W., GRONYCH, J., LICHTER, P., WITT, O. & PFISTER, S. M. 2011. MAPK pathway activation in pilocytic astrocytoma. *Cellular and Molecular Life Sciences,* 69**,** 1799-1811.

JORNSTEN, R., ABENIUS, T., KLING, T., SCHMIDT, L., JOHANSSON, E., NORDLING, T. E., NORDLANDER, B., SANDER, C., GENNEMARK, P., FUNA, K., NILSSON, B., LINDAHL, L. & NELANDER, S. 2011. Network modeling of the transcriptional effects of copy number aberrations in glioblastoma. *Mol Syst Biol,* 7**,** 486.

KAUFMAN, J. L., NIESVIZKY, R., STADTMAUER, E. A., CHANAN-KHAN, A., SIEGEL, D., HORNE, H., WEGENER, W. A. & GOLDENBERG, D. M. 2013. Phase I, multicentre, dose-escalation trial of monotherapy with milatuzumab (humanized anti-CD74 monoclonal antibody) in relapsed or refractory multiple myeloma. *British Journal of Haematology,* 163**,** 478-486.

KELLY, P. 2010. Gliomas: Survival, origin and early detection. *Surgical Neurology International,* 1**,** 96.

KIM, H.-G., HWANG, S. Y., AARONSON, S. A., MANDINOVA, A. & LEE, S. W. 2017. DDR1 receptor tyrosine kinase promotes prosurvival pathway through Notch1 activation. *Journal of Biological Chemistry,* 292**,** 7162-7162.

KITANGE, G. J., CARLSON, B. L., SCHROEDER, M. A., DECKER, P. A., MORLAN, B. W., WU, W., BALLMAN, K. V., GIANNINI, C. & SARKARIA, J. N. 2010. Expression of CD74 in high grade gliomas: a potential role in temozolomide resistance. *J Neurooncol,* 100**,** 177-86.

KLOOSTERHOF, N. K., BRALTEN, L. B., DUBBINK, H. J., FRENCH, P. J. & VAN DEN BENT, M. J. 2011. Isocitrate dehydrogenase-1 mutations: a fundamentally new understanding of diffuse glioma? *Lancet Oncol,* 12**,** 83-91.

KOPEREK, O., ASARI, R., NIEDERLE, B. & KASERER, K. 2011. Desmoplastic stromal reaction in papillary thyroid microcarcinoma. *Histopathology,* 58**,** 919-24.

KOTHIWALE, S., BORZA, C. M., LOWE, E. W., POZZI, A. & MEILER, J. 2015. Discoidin domain receptor 1 (DDR1) kinase as target for structure-based drug discovery. *Drug Discovery Today,* 20**,** 255-261.

KREISL, T. N., KIM, L., MOORE, K., DUIC, P., ROYCE, C., STROUD, I., GARREN, N., MACKEY, M., BUTMAN, J. A., CAMPHAUSEN, K., PARK, J., ALBERT, P. S. & FINE, H. A. 2009. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J Clin Oncol,* 27**,** 740-5.

KRISTIANSEN, K., HAGEN, S., KOLLEVOLD, T., TORVIK, A., HOLME, I., NESBAKKEN, R., HATLEVOLL, R., LINDGREN, M., BRUN, A., LINDGREN, S., NOTTER, G., ANDERSEN, A. P. & ELGEN, K. 1981. Combined modality therapy of operated astrocytomas grade III and IV. Confirmation of the value of postoperative irradiation and lack of potentiation of bleomycin on survival time: a prospective multicenter trial of the Scandinavian Glioblastoma Study Group. *Cancer,* 47**,** 649-52.

LACROIX, M., ABI-SAID, D., FOURNEY, D. R., GOKASLAN, Z. L., SHI, W., DEMONTE, F., LANG, F. F., MCCUTCHEON, I. E., HASSENBUSCH, S. J., HOLLAND, E., HESS, K., MICHAEL, C., MILLER, D. & SAWAYA, R. 2001. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg,* 95**,** 190-8.

LAMBIV, W. L., VASSALLO, I., DELORENZI, M., SHAY, T., DISERENS, A.-C., MISRA, A., FEUERSTEIN, B., MURAT, A., MIGLIAVACCA, E., HAMOU, M.-F., SCIUSCIO, D., BURGER, R., DOMANY, E., STUPP, R. & HEGI, M. E. 2011. The Wnt inhibitory factor 1 (WIF1) is targeted in glioblastoma and has a tumor suppressing function potentially by induction of senescence. *Neuro-Oncology,* 13**,** 736-747.

LAMBORN, K. R., YUNG, W. K., CHANG, S. M., WEN, P. Y., CLOUGHESY, T. F., DEANGELIS, L. M., ROBINS, H. I., LIEBERMAN, F. S., FINE, H. A., FINK, K. L., JUNCK, L., ABREY, L., GILBERT, M. R., MEHTA, M., KUHN, J. G., ALDAPE, K. D., HIBBERTS, J., PETERSON, P. M., PRADOS, M. D. & NORTH AMERICAN BRAIN TUMOR, C. 2008. Progression-free survival: an important end point in evaluating therapy for recurrent high-grade gliomas. *Neuro Oncol,* 10**,** 162-70.

LASSMAN, A. B., IWAMOTO, F. M., CLOUGHESY, T. F., ALDAPE, K. D., RIVERA, A. L., EICHLER, A. F., LOUIS, D. N., PALEOLOGOS, N. A., FISHER, B. J., ASHBY, L. S., CAIRNCROSS, J. G., ROLDAN, G. B., WEN, P. Y., LIGON, K. L., SCHIFF, D., ROBINS, H. I., ROCQUE, B. G., CHAMBERLAIN, M. C., MASON, W. P., WEAVER, S. A., GREEN, R. M., KAMAR, F. G., ABREY, L. E., DEANGELIS, L. M., JHANWAR, S. C., ROSENBLUM, M. K. & PANAGEAS, K. S. 2011. International retrospective study of over 1000 adults with anaplastic oligodendroglial tumors. *Neuro Oncol,* 13**,** 649-59.

LEFRANC, F., BROTCHI, J. & KISS, R. 2005. Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. *J Clin Oncol,* 23**,** 2411-22.

LEITINGER, B. 2011. Transmembrane collagen receptors. *Annu Rev Cell Dev Biol,* 27**,** 265-90.

LENG, L., METZ, C. N., FANG, Y., XU, J., DONNELLY, S., BAUGH, J., DELOHERY, T., CHEN, Y., MITCHELL, R. A. & BUCALA, R. 2003. MIF signal transduction initiated by binding to CD74. *J Exp Med,* 197**,** 1467-76.

LIAO, B., ZHONG, B. L., LI, Z., TIAN, X. Y., LI, Y. & LI, B. 2010. Macrophage migration inhibitory factor contributes angiogenesis by up-regulating IL-8 and correlates with poor prognosis of patients with primary nasopharyngeal carcinoma. *J Surg Oncol,* 102**,** 844-51.

LIAO, Y., SMYTH, G. K. & SHI, W. 2013. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Research,* 41**,** e108-e108.

LIEW, L. C., SINGH, M. B. & BHALLA, P. L. 2013. An RNA-seq transcriptome analysis of histone modifiers and RNA silencing genes in soybean during floral initiation process. *PLoS One,* 8**,** e77502.

LIN, B., MADAN, A., YOON, J.-G., FANG, X., YAN, X., KIM, T.-K., HWANG, D., HOOD, L. & FOLTZ, G. 2010. Massively Parallel Signature Sequencing and Bioinformatics Analysis Identifies Up-Regulation of TGFBI and SOX4 in Human Glioblastoma. *PLoS ONE,* 5**,** e10210.

LIND-LANDSTROM, T., HABBERSTAD, A. H., SUNDSTROM, S. & TORP, S. H. 2012. Prognostic value of histological features in diffuse astrocytomas WHO grade II. *Int J Clin Exp Pathol,* 5**,** 152-8.

LIOTTA, L. A. & KOHN, E. C. 2001. The microenvironment of the tumour-host interface. *Nature,* 411**,** 375-9.

LIU, S., YIN, F., FAN, W., WANG, S., GUO, X.-R., ZHANG, J.-N., TIAN, Z.-M. & FAN, M. 2012. Over-expression of BMPR-IB reduces the malignancy of glioblastoma cells by upregulation of p21 and p27Kip1. *J Exp Clin Cancer Res,* 31**,** 52.

LIU, Y. H., LIN, C. Y., LIN, W. C., TANG, S. W., LAI, M. K. & LIN, J. Y. 2008. Up-Regulation of Vascular Endothelial Growth Factor-D Expression in Clear Cell Renal Cell Carcinoma by CD74: A Critical Role in Cancer Cell Tumorigenesis. *The Journal of Immunology,* 181**,** 6584-6594.

LOUIS, D. N., OHGAKI, H., WIESTLER, O. D., CAVENEE, W. K., BURGER, P. C., JOUVET, A., SCHEITHAUER, B. W. & KLEIHUES, P. 2007. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol,* 114**,** 97-109.

LUE, H., KAPURNIOTU, A., FINGERLE-ROWSON, G., ROGER, T., LENG, L., THIELE, M., CALANDRA, T., BUCALA, R. & BERNHAGEN, J. 2006. Rapid and transient activation of the ERK MAPK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on JAB1/CSN5 and Src kinase activity. *Cell Signal,* 18**,** 688-703.

LUE, H., THIELE, M., FRANZ, J., DAHL, E., SPECKGENS, S., LENG, L., FINGERLE-ROWSON, G., BUCALA, R., LUSCHER, B. & BERNHAGEN, J. 2007. Macrophage migration inhibitory factor (MIF) promotes cell survival by activation of the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity. *Oncogene,* 26**,** 5046-59.

MAHER, C. A., KUMAR-SINHA, C., CAO, X., KALYANA-SUNDARAM, S., HAN, B., JING, X., SAM, L., BARRETTE, T., PALANISAMY, N. & CHINNAIYAN, A. M. 2009. Transcriptome sequencing to detect gene fusions in cancer. *Nature,* 458**,** 97-101.

MALMSTROM, A., GRONBERG, B. H., MAROSI, C., STUPP, R., FRAPPAZ, D., SCHULTZ, H., ABACIOGLU, U., TAVELIN, B., LHERMITTE, B., HEGI, M. E., ROSELL, J. & HENRIKSSON, R. 2012. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. *Lancet Oncol,* 13**,** 916-26.

MALZKORN, B., WOLTER, M., RIEMENSCHNEIDER, M. J. & REIFENBERGER, G. 2011. Unraveling the glioma epigenome: from molecular mechanisms to novel biomarkers and therapeutic targets. *Brain Pathol,* 21**,** 619-32.

MANDAL, S., CURTIS, L., PIND, M., MURPHY, L. C. & WATSON, P. H. 2007. S100A7 (psoriasin) influences immune response genes in human breast cancer. *Exp Cell Res,* 313**,** 3016-25.

MARGUERAT, S. & BAHLER, J. 2010. RNA-seq: from technology to biology. *Cell Mol Life Sci,* 67**,** 569-79.

MARK, T., MARTIN, P., LEONARD, J. P. & NIESVIZKY, R. 2009. Milatuzumab: a promising new agent for the treatment of lymphoid malignancies. *Expert Opin Investig Drugs,* 18**,** 99-104.

MARTIN-VILLALBA, A., OKUDUCU, A. F. & VON DEIMLING, A. 2008. The Evolution of Our Understanding on Glioma. *Brain Pathology,* 18**,** 455-463.

MATSUDA, S., MATSUDA, Y. & D'ADAMIO, L. 2009. BRI3 Inhibits Amyloid Precursor Protein Processing in a Mechanistically Distinct Manner from Its Homologue Dementia Gene BRI2. *Journal of Biological Chemistry,* 284**,** 15815-15825.

MAXWELL, P. J., GALLAGHER, R., SEATON, A., WILSON, C., SCULLIN, P., PETTIGREW, J., STRATFORD, I. J., WILLIAMS, K. J., JOHNSTON, P. G. & WAUGH, D. J. 2007. HIF-1 and NF-kappaB-mediated upregulation of CXCR1 and CXCR2 expression promotes cell survival in hypoxic prostate cancer cells. *Oncogene,* 26**,** 7333-45.

MCCARTHY, D. J., CHEN, Y. & SMYTH, G. K. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research,* 40**,** 4288-4297.

MCCLELLAND, M., ZHAO, L., CARSKADON, S. & ARENBERG, D. 2009. Expression of CD74, the Receptor for Macrophage Migration Inhibitory Factor, in Non-Small Cell Lung Cancer. *The American Journal of Pathology,* 174**,** 638-646.

MIOTTO, E. 2004. Frequent Aberrant Methylation of the CDH4 Gene Promoter in Human Colorectal and Gastric Cancer. *Cancer Research,* 64**,** 8156-8159.

MITTELBRONN, M., PLATTEN, M., ZEINER, P., DOMBROWSKI, Y., FRANK, B., ZACHSKORN, C., HARTER, P. N., WELLER, M. & WISCHHUSEN, J. 2011. Macrophage migration inhibitory factor (MIF) expression in human malignant gliomas contributes to immune escape and tumour progression. *Acta Neuropathol,* 122**,** 353-65.

MUELLER, M. M. & FUSENIG, N. E. 2004. Friends or foes - bipolar effects of the tumour stroma in cancer. *Nat Rev Cancer,* 4**,** 839-49.

MUNAUT, C., BONIVER, J., FOIDART, J. M. & DEPREZ, M. 2002. Macrophage migration inhibitory factor (MIF) expression in human glioblastomas correlates with vascular endothelial growth factor (VEGF) expression. *Neuropathol Appl Neurobiol,* 28**,** 452-60.

NAGATA, S., JIN, Y.-F., YOSHIZATO, K., TOMOEDA, M., SONG, M., IIZUKA, N., KITAMURA, M., TAKAHASHI, H., EGUCHI, H., OHIGASHI, H., ISHIKAWA, O. & TOMITA, Y. 2009. CD74 Is a Novel Prognostic Factor for Patients with Pancreatic Cancer Receiving Multimodal Therapy. *Annals of Surgical Oncology,* 16**,** 2531-2538.

NELSON, W. G., DEWEESE, T. L. & DEMARZO, A. M. 2002. The diet, prostate inflammation, and the development of prostate cancer. *Cancer Metastasis Rev,* 21**,** 3-16.

NOUSHMEHR, H., WEISENBERGER, D. J., DIEFES, K., PHILLIPS, H. S., PUJARA, K., BERMAN, B. P., PAN, F., PELLOSKI, C. E., SULMAN, E. P., BHAT, K. P., VERHAAK, R. G. W., HOADLEY, K. A., HAYES, D. N., PEROU, C. M., SCHMIDT, H. K., DING, L., WILSON, R. K., VAN DEN BERG, D., SHEN, H., BENGTSSON, H., NEUVIAL, P., COPE, L. M., BUCKLEY, J., HERMAN, J. G.,

BAYLIN, S. B., LAIRD, P. W. & ALDAPE, K. 2010. Identification of a CpG Island Methylator Phenotype that Defines a Distinct Subgroup of Glioma. *Cancer Cell,* 17**,** 510-522.

NUÑO, M., BIRCH, K., MUKHERJEE, D., SARMIENTO, J. M., BLACK, K. L. & PATIL, C. G. 2013. Survival and Prognostic Factors of Anaplastic Gliomas. *Neurosurgery,* 73**,** 458-465.

NUTT, C. L., MANI, D. R., BETENSKY, R. A., TAMAYO, P., CAIRNCROSS, J. G., LADD, C., POHL, U., HARTMANN, C., MCLAUGHLIN, M. E., BATCHELOR, T. T., BLACK, P. M., VON DEIMLING, A., POMEROY, S. L., GOLUB, T. R. & LOUIS, D. N. 2003. Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res,* 63**,** 1602-7.

O'SULLIVAN, T., SADDAWI-KONEFKA, R., GROSS, E., TRAN, M., MAYFIELD, STEPHEN P., IKEDA, H. & BUI, JACK D. 2014. Interleukin-17D Mediates Tumor Rejection through Recruitment of Natural Killer Cells. *Cell Reports,* 7**,** 989-998.

OGRINC, T., DOLENC, I., RITONJA, A. & TURK, V. 1993. Purification of the complex of cathepsin L and the MHC class II-associated invariant chain fragment from human kidney. *FEBS Letters,* 336**,** 555-559.

OHGAKI, H. & KLEIHUES, P. 2005. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. *J Neuropathol Exp Neurol,* 64**,** 479-89.

OHGAKI, H. & KLEIHUES, P. 2009. Genetic alterations and signaling pathways in the evolution of gliomas. *Cancer Sci,* 100**,** 2235-41.

OHGAKI, H. & KLEIHUES, P. 2013. The definition of primary and secondary glioblastoma. *Clin Cancer Res,* 19**,** 764-72.

OTTERSTROM, C., SOLTERMANN, A., OPITZ, I., FELLEY-BOSCO, E., WEDER, W., STAHEL, R. A., TRIPONEZ, F., ROBERT, J. H. & SERRE-BEINIER, V. 2014. CD74: a new prognostic factor for patients with malignant pleural mesothelioma. *British Journal of Cancer,* 110**,** 2040-2046.

OZSOLAK, F. & MILOS, P. M. 2011. RNA sequencing: advances, challenges and opportunities. *Nat Rev Genet,* 12**,** 87-98.

PALANI, M., ARUNKUMAR, R. & VANISREE, A. J. 2014. Methylation and Expression Patterns of Tropomyosin-Related Kinase Genes in Different Grades of Glioma. *Neuromol Med,* 16**,** 529-539.

PARK, K. H., CHOI, S. E., EOM, M. & KANG, Y. 2005. Downregulation of the anaphase-promoting complex (APC)7 in invasive ductal carcinomas of the breast and its clinicopathologic relationships. *Breast Cancer Res,* 7**,** R238-47.

PARSONS, D. W., JONES, S., ZHANG, X., LIN, J. C. H., LEARY, R. J., ANGENENDT, P., MANKOO, P., CARTER, H., SIU, I. M., GALLIA, G. L., OLIVI, A., MCLENDON, R., RASHEED, B. A., KEIR, S., NIKOLSKAYA, T., NIKOLSKY, Y., BUSAM, D. A., TEKLEAB, H., DIAZ, L. A., HARTIGAN, J., SMITH, D. R., STRAUSBERG, R. L., MARIE, S. K. N., SHINJO, S. M. O., YAN, H., RIGGINS, G. J., BIGNER, D. D., KARCHIN, R., PAPADOPOULOS, N., PARMIGIANI, G., VOGELSTEIN, B., VELCULESCU, V. E. & KINZLER, K. W. 2008. An Integrated Genomic Analysis of Human Glioblastoma Multiforme. *Science,* 321**,** 1807-1812.

PEDEUTOUR-BRACCINI, Z., BUREL-VANDENBOS, F., GOZÉ, C., ROGER, C., BAZIN, A., COSTES-MARTINEAU, V., DUFFAU, H. & RIGAU, V. 2015. Microfoci of malignant progression in diffuse low-grade gliomas: towards the creation of an intermediate grade in glioma classification? *Virchows Archiv,* 466**,** 433-444.

Phase III study of rindopepimut/GM-CSF in patients with newly diagnosed glioblastoma. ClinicalTrials.gov identifier: NCT01480479. Verified November 2013 by Celldex Therapeutics. ClinicalTrials.gov processed this record on December 01, 2013.

PHILLIPS, H. S., KHARBANDA, S., CHEN, R., FORREST, W. F., SORIANO, R. H., WU, T. D., MISRA, A., NIGRO, J. M., COLMAN, H., SOROCEANU, L., WILLIAMS, P. M., MODRUSAN, Z., FEUERSTEIN, B. G. & ALDAPE, K. 2006. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell,* 9**,** 157-173.

PORTER, D., LAHTI-DOMENICI, J., KESHAVIAH, A., BAE, Y. K., ARGANI, P., MARKS, J., RICHARDSON, A., COOPER, A., STRAUSBERG, R., RIGGINS, G. J., SCHNITT, S., GABRIELSON, E., GELMAN, R. & POLYAK, K. 2003. Molecular markers in ductal carcinoma in situ of the breast. *Mol Cancer Res,* 1**,** 362-75.

Radiation therapy with or without temozolomide in treating patients with anaplastic glioma. ClinicalTrials.gov Identifier:NCT00626990. Verified May 2011 by National Cancer Institute (NCI). ClinicalTrials.gov processed this record on December 01, 2013

Radiation therapy with concomitant and adjuvant temozolomide or radiation therapy with adjuvant PCV or temozolomide alone in treating patients with anaplastic glioma. ClinicalTrials.gov identifier: NCT00887146. Verified November 2013 by Alliance for Clinical Trials in Oncology. ClinicalTrials.gov processed this record on December 01, 2013

RAM, R., LORENTE, G., NIKOLICH, K., URFER, R., FOEHR, E. & NAGAVARAPU, U. 2006. Discoidin domain receptor-1a (DDR1a) promotes glioma cell invasion and adhesion in association with matrix metalloproteinase-2. *J Neurooncol,* 76**,** 239-48.

RAMMAL, H., SABY, C., MAGNIEN, K., VAN-GULICK, L., GARNOTEL, R., BUACHE, E., EL BTAOURI, H., JEANNESSON, P. & MORJANI, H. 2016. Corrigendum: Discoidin Domain Receptors: Potential Actors and Targets in Cancer. *Frontiers in Pharmacology,* 7.

REIFENBERGER, G. & COLLINS, V. P. 2004. Pathology and molecular genetics of astrocytic gliomas. *J Mol Med (Berl),* 82**,** 656-70.

REN, Y., LAW, S., HUANG, X., LEE, P. Y., BACHER, M., SRIVASTAVA, G. & WONG, J. 2005. Macrophage Migration Inhibitory Factor Stimulates Angiogenic Factor Expression and Correlates With Differentiation and Lymph Node Status in Patients With Esophageal Squamous Cell Carcinoma. *Annals of Surgery,* 242**,** 55-63.

RICHARD, V., KINDT, N. & SAUSSEZ, S. 2015. Macrophage migration inhibitory factor involvement in breast cancer (Review). *Int J Oncol,* 47**,** 1627-33.

RICKMAN, D. S., BOBEK, M. P., MISEK, D. E., KUICK, R., BLAIVAS, M., KURNIT, D. M., TAYLOR, J. & HANASH, S. M. 2001. Distinctive molecular profiles of high-grade and low-grade gliomas based on oligonucleotide microarray analysis. *Cancer Res,* 61**,** 6885-91.

RIEMENSCHNEIDER, M. J., BUSCHGES, R., WOLTER, M., REIFENBERGER, J., BOSTROM, J., KRAUS, J. A., SCHLEGEL, U. & REIFENBERGER, G. 1999. Amplification and overexpression of the MDM4 (MDMX) gene from 1q32 in a subset of malignant gliomas without TP53 mutation or MDM2 amplification. *Cancer Res,* 59**,** 6091-6.

RIEMENSCHNEIDER, M. J., KNOBBE, C. B. & REIFENBERGER, G. 2003. Refined mapping of 1q32 amplicons in malignant gliomas confirmsMDM4 as the main amplification target. *International Journal of Cancer,* 104**,** 752-757.

RITCHIE, M. E., PHIPSON, B., WU, D., HU, Y., LAW, C. W., SHI, W. & SMYTH, G. K. 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res,* 43**,** e47.

ROBERTS, A., SCHAEFFER, L. & PACHTER, L. 2013. Updating RNA-Seq analyses after re-annotation. *Bioinformatics,* 29**,** 1631-7.

SAMPSON, J. H., HEIMBERGER, A. B., ARCHER, G. E., ALDAPE, K. D., FRIEDMAN, A. H., FRIEDMAN, H. S., GILBERT, M. R., HERNDON, J. E., 2ND, MCLENDON, R. E., MITCHELL, D. A., REARDON, D. A., SAWAYA, R., SCHMITTLING, R. J., SHI, W., VREDENBURGH, J. J. & BIGNER, D. D. 2010. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *J Clin Oncol,* 28**,** 4722-9.

SANAI, N., POLLEY, M. Y., MCDERMOTT, M. W., PARSA, A. T. & BERGER, M. S. 2011. An extent of resection threshold for newly diagnosed glioblastomas. *J Neurosurg,* 115**,** 3-8.

SCHARER, C. D., MCCABE, C. D., ALI-SEYED, M., BERGER, M. F., BULYK, M. L. & MORENO, C. S. 2009. Genome-Wide Promoter Analysis of the SOX4 Transcriptional Network in Prostate Cancer Cells. *Cancer Research,* 69**,** 709-717.

SCHEITHAUER, B. W., FULLER, G. N. & VANDENBERG, S. R. 2008. The 2007 WHO classification of tumors of the nervous system: controversies in surgical neuropathology. *Brain Pathol,* 18**,** 307-16.

SCHMIDT, E. E., ICHIMURA, K., REIFENBERGER, G. & COLLINS, V. P. 1994. CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. *Cancer Res,* 54**,** 6321-4.

SEIFERT, M., GARBE, M., FRIEDRICH, B., MITTELBRONN, M. & KLINK, B. 2015. Comparative transcriptomics reveals similarities and differences between astrocytoma grades. *BMC Cancer,* 15.

SETTY, M., HELMY, K., KHAN, A. A., SILBER, J., ARVEY, A., NEEZEN, F., AGIUS, P., HUSE, J. T., HOLLAND, E. C. & LESLIE, C. S. 2012. Inferring transcriptional and microRNA-mediated regulatory programs in glioblastoma. *Molecular Systems Biology,* 8.

SHAPIRO, W. R., GREEN, S. B., BURGER, P. C., MAHALEY, M. S., JR., SELKER, R. G., VANGILDER, J. C., ROBERTSON, J. T., RANSOHOFF, J., MEALEY, J., JR., STRIKE, T. A. & ET AL. 1989. Randomized trial of three chemotherapy regimens and two radiotherapy regimens and two radiotherapy regimens in postoperative treatment of malignant glioma. Brain Tumor Cooperative Group Trial 8001. *J Neurosurg,* 71**,** 1-9.

SHI, X., LENG, L., WANG, T., WANG, W., DU, X., LI, J., MCDONALD, C., CHEN, Z., MURPHY, J. W., LOLIS, E., NOBLE, P., KNUDSON, W. & BUCALA, R. 2006. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. *Immunity,* 25**,** 595-606.

SHIH, L., ONG, G. L., BURTON, J., MISHINA, D., GOLDENBERG, D. M. & MATTES, M. J. 2000. Localization of an antibody to CD74 (MHC class II invariant chain) to human B cell lymphoma xenografts in nude mice. *Cancer Immunol Immunother,* 49**,** 208-16.

SHOULDERS, M. D. & RAINES, R. T. 2009. Collagen structure and stability. *Annu Rev Biochem,* 78**,** 929-58.

SMOLL, N. R. & HAMILTON, B. 2014. Incidence and relative survival of anaplastic astrocytomas. *Neuro-Oncology,* 16**,** 1400-1407.

SONG, Y., LUO, Q., LONG, H., HU, Z., QUE, T., ZHANG, X. A., LI, Z., WANG, G., YI, L., LIU, Z., FANG, W. & QI, S. 2014. Alpha-enolase as a potential cancer prognostic marker promotes cell growth, migration, and invasion in glioma. *Molecular Cancer,* 13**,** 65.

SONI, D., KING, J. A. J., KAYE, A. H. & HOVENS, C. M. 2005. Genetics of glioblastoma multiforme: mitogenic signaling and cell cycle pathways converge. *Journal of Clinical Neuroscience,* 12**,** 1-5.

STEIN, R., MATTES, M. J., CARDILLO, T. M., HANSEN, H. J., CHANG, C. H., BURTON, J., GOVINDAN, S. & GOLDENBERG, D. M. 2007. CD74: a new candidate target for the immunotherapy of B-cell neoplasms. *Clin Cancer Res,* 13**,** 5556s-5563s.

STEIN, R., QU, Z., CARDILLO, T. M., CHEN, S., ROSARIO, A., HORAK, I. D., HANSEN, H. J. & GOLDENBERG, D. M. 2004. Antiproliferative activity of a humanized anti-CD74 monoclonal antibody, hLL1, on B-cell malignancies. *Blood,* 104**,** 3705-11.

STUPP, R., HEGI, M. E., MASON, W. P., VAN DEN BENT, M. J., TAPHOORN, M. J., JANZER, R. C., LUDWIN, S. K., ALLGEIER, A., FISHER, B., BELANGER, K., HAU, P., BRANDES, A. A., GIJTENBEEK, J., MAROSI, C., VECHT, C. J., MOKHTARI, K., WESSELING, P., VILLA, S., EISENHAUER, E., GORLIA, T., WELLER, M., LACOMBE, D., CAIRNCROSS, J. G., MIRIMANOFF, R. O., EUROPEAN ORGANISATION FOR, R., TREATMENT OF CANCER BRAIN, T., RADIATION ONCOLOGY, G. & NATIONAL CANCER INSTITUTE OF CANADA CLINICAL TRIALS, G. 2009. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol,* 10**,** 459-66.

STUPP, R., MASON, W. P. & VAN DEN BENT, M. J. 2005. "Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma". *Oncology Times,* 27**,** 15-16.

STURM, D., BENDER, S., JONES, D. T. W., LICHTER, P., GRILL, J., BECHER, O., HAWKINS, C., MAJEWSKI, J., JONES, C., COSTELLO, J. F., IAVARONE, A., ALDAPE, K., BRENNAN, C. W., JABADO, N. & PFISTER, S. M. 2014. Paediatric and adult glioblastoma: multiform (epi)genomic culprits emerge. *Nature Reviews Cancer,* 14**,** 92-107.

SWEENEY, S. M., ORGEL, J. P., FERTALA, A., MCAULIFFE, J. D., TURNER, K. R., DI LULLO, G. A., CHEN, S., ANTIPOVA, O., PERUMAL, S., ALA-KOKKO, L., FORLINO, A., CABRAL, W. A., BARNES, A. M., MARINI, J. C. & SAN ANTONIO, J. D. 2008. Candidate cell and matrix interaction domains on the collagen fibril, the predominant protein of vertebrates. *J Biol Chem,* 283**,** 21187-97.

TAKAHASHI, M., SHIMAJIRI, S., IZUMI, H., HIRANO, G., KASHIWAGI, E., YASUNIWA, Y., WU, Y., HAN, B., AKIYAMA, M., NISHIZAWA, S., SASAGURI, Y. & KOHNO, K. 2010. Y-box binding protein-1 is a novel molecular target for tumor vessels. *Cancer Science,* 101**,** 1367-1373.

TAVERAS, J. M., THOMPSON, H. G., JR. & POOL, J. L. 1962. Should we treat glioblastoma multiforme? A study of survival in 425 cases. *Am J Roentgenol Radium Ther Nucl Med,* 87**,** 473-9.

THELEN, A., SCHOLZ, A., BENCKERT, C., SCHRODER, M., WEICHERT, W., WIEDENMANN, B., NEUHAUS, P. & JONAS, S. 2008. Microvessel density

correlates with lymph node metastases and prognosis in hilar cholangiocarcinoma. *J Gastroenterol,* 43**,** 959-66.

TONG, H., YU, X., LU, X. & WANG, P. 2015. Downregulation of solute carriers of glutamate in gliosomes and synaptosomes may explain local brain metastasis in anaplastic glioblastoma. *IUBMB Life,* 67**,** 306-311.

TONN JC, W. M., RUTKA JT, GROSSMAN SA. 2006. Neuro-Oncology of CNS Tumors. Springer Science + Business Media.

TRAPNELL, C., ROBERTS, A., GOFF, L., PERTEA, G., KIM, D., KELLEY, D. R., PIMENTEL, H., SALZBERG, S. L., RINN, J. L. & PACHTER, L. 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc,* 7**,** 562-78.

TRAPNELL, C., WILLIAMS, B. A., PERTEA, G., MORTAZAVI, A., KWAN, G., VAN BAREN, M. J., SALZBERG, S. L., WOLD, B. J. & PACHTER, L. 2010. Transcript assembly and abundance estimation from RNA-Seq reveals thousands of new transcripts and switching among isoforms. *Nature biotechnology,* 28**,** 511.

VARTANIAN, A., SINGH, S. K., AGNIHOTRI, S., JALALI, S., BURRELL, K., ALDAPE, K. D. & ZADEH, G. 2014. GBM's multifaceted landscape: highlighting regional and microenvironmental heterogeneity. *Neuro Oncol,* 16**,** 1167-75.

VERHAAK, R. G. W., HOADLEY, K. A., PURDOM, E., WANG, V., QI, Y., WILKERSON, M. D., MILLER, C. R., DING, L., GOLUB, T., MESIROV, J. P., ALEXE, G., LAWRENCE, M., O'KELLY, M., TAMAYO, P., WEIR, B. A., GABRIEL, S., WINCKLER, W., GUPTA, S., JAKKULA, L., FEILER, H. S., HODGSON, J. G., JAMES, C. D., SARKARIA, J. N., BRENNAN, C., KAHN, A., SPELLMAN, P. T., WILSON, R. K., SPEED, T. P., GRAY, J. W., MEYERSON, M., GETZ, G., PEROU, C. M. & HAYES, D. N. 2010. Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell,* 17**,** 98-110.

WALKER, C., BABORIE, A., CROOKS, D., WILKINS, S. & JENKINSON, M. D. 2011. Biology, genetics and imaging of glial cell tumours. *Br J Radiol,* 84 Spec No 2**,** S90-106.

WALKER, M. D., ALEXANDER, E., JR., HUNT, W. E., MACCARTY, C. S., MAHALEY, M. S., JR., MEALEY, J., JR., NORRELL, H. A., OWENS, G., RANSOHOFF, J., WILSON, C. B., GEHAN, E. A. & STRIKE, T. A. 1978. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J Neurosurg,* 49**,** 333-43.

WALKER, M. D., GREEN, S. B., BYAR, D. P., ALEXANDER, E., JR., BATZDORF, U., BROOKS, W. H., HUNT, W. E., MACCARTY, C. S., MAHALEY, M. S., JR., MEALEY, J., JR., OWENS, G., RANSOHOFF, J., 2ND, ROBERTSON, J. T., SHAPIRO, W. R., SMITH, K. R., JR., WILSON, C. B. & STRIKE, T. A. 1980. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N Engl J Med,* 303**,** 1323-9.

WANG, C., FUNK, C. C., EDDY, J. A. & PRICE, N. D. 2013. Transcriptional Analysis of Aggressiveness and Heterogeneity across Grades of Astrocytomas. *PLoS ONE,* 8**,** e76694.

WANG, P., RAO, J., YANG, H., ZHAO, H. & YANG, L. 2011. PPM1D silencing by lentiviral-mediated RNA interference inhibits proliferation and invasion of human glioma cells. *Journal of Huazhong University of Science and Technology [Medical Sciences],* 31**,** 94-99.

WANG, Z., GERSTEIN, M. & SNYDER, M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet,* 10**,** 57-63.

WAUGH, D. J. & WILSON, C. 2008. The interleukin-8 pathway in cancer. *Clin Cancer Res,* 14**,** 6735-41.

WEN, P. Y. & KESARI, S. 2008. Malignant gliomas in adults. *N Engl J Med,* 359**,** 492-507.

WICK, W., PLATTEN, M., MEISNER, C., FELSBERG, J., TABATABAI, G., SIMON, M., NIKKHAH, G., PAPSDORF, K., STEINBACH, J. P., SABEL, M., COMBS, S. E., VESPER, J., BRAUN, C., MEIXENSBERGER, J., KETTER, R., MAYER-STEINACKER, R., REIFENBERGER, G., WELLER, M. & SOCIETY, N. O. A. S. G. O. N.-O. W. G. O. G. C. 2012. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol,* 13**,** 707-15.

WOLTER, M., WERNER, T., MALZKORN, B. & REIFENBERGER, G. 2015. Role of microRNAs Located on Chromosome Arm 10q in Malignant Gliomas. *Brain Pathology,* 26**,** 344-358.

WONG, E. T., HESS, K. R., GLEASON, M. J., JAECKLE, K. A., KYRITSIS, A. P., PRADOS, M. D., LEVIN, V. A. & YUNG, W. K. 1999. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol,* 17**,** 2572-8.

XIE, Q., FLAVAHAN, WILLIAM A., BAO, S. & RICH, J. 2014. The Tailless Root of Glioma: Cancer Stem Cells. *Cell Stem Cell,* 15**,** 114-116.

XU, L., PATHAK, P. S. & FUKUMURA, D. 2004. Hypoxia-induced activation of p38 mitogen-activated protein kinase and phosphatidylinositol 3'-kinase signaling pathways contributes to expression of interleukin 8 in human ovarian carcinoma cells. *Clin Cancer Res,* 10**,** 701-7.

XU, X., WANG, B., YE, C., YAO, C., LIN, Y., HUANG, X., ZHANG, Y. & WANG, S. 2008. Overexpression of macrophage migration inhibitory factor induces angiogenesis in human breast cancer. *Cancer Letters,* 261**,** 147-157.

YAMANAKA, R., ARAO, T., YAJIMA, N., TSUCHIYA, N., HOMMA, J., TANAKA, R., SANO, M., OIDE, A., SEKIJIMA, M. & NISHIO, K. 2006. Identification of expressed genes characterizing long-term survival in malignant glioma patients. *Oncogene,* 25**,** 5994-6002.

ZADEH, G. & GUHA, A. 2003. Angiogenesis in nervous system disorders. *Neurosurgery,* 53**,** 1362-74; discussion 1374-6.

ZEINER, P. S., PREUSSE, C., BLANK, A. E., ZACHSKORN, C., BAUMGARTEN, P., CASPARY, L., BRACZYNSKI, A. K., WEISSENBERGER, J., BRATZKE, H., REISS, S., PENNARTZ, S., WINKELMANN, R., SENFT, C., PLATE, K. H., WISCHHUSEN, J., STENZEL, W., HARTER, P. N. & MITTELBRONN, M. 2015. MIF Receptor CD74 is Restricted to Microglia/Macrophages, Associated with a M1-Polarized Immune Milieu and Prolonged Patient Survival in Gliomas. *Brain Pathol,* 25**,** 491-504.

ZHANG, L., CHEN, L. H., WAN, H., YANG, R., WANG, Z., FENG, J., YANG, S., JONES, S., WANG, S., ZHOU, W., ZHU, H., KILLELA, P. J., ZHANG, J., WU, Z., LI, G., HAO, S., WANG, Y., WEBB, J. B., FRIEDMAN, H. S., FRIEDMAN, A. H., MCLENDON, R. E., HE, Y., REITMAN, Z. J., BIGNER, D. D. & YAN, H. 2014. Exome sequencing identifies somatic gain-of-function PPM1D mutations in brainstem gliomas. *Nature Genetics,* 46**,** 726-730.

ZHAO, S., GUO, Y., SHENG, Q. & SHYR, Y. 2014a. Advanced Heat Map and Clustering Analysis Using Heatmap3. *BioMed Research International,* 2014**,** 1-6.

ZHAO, W., HE, X., HOADLEY, K. A., PARKER, J. S., HAYES, D. N. & PEROU, C. M. 2014b. Comparison of RNA-Seq by poly (A) capture, ribosomal RNA depletion, and DNA microarray for expression profiling. *BMC Genomics,* 15**,** 419.

ZHAO, X., LAVER, T., HONG, S. W., TWITTY, G. B., DEVOS, A., DEVOS, M., BENVENISTE, E. N. & NOZELL, S. E. 2011. An NF-κB p65-cIAP2 link is necessary for mediating resistance to TNF-α induced cell death in gliomas. *Journal of Neuro-Oncology,* 102**,** 367-381.

ZOU, P., XU, H., CHEN, P., YAN, Q., ZHAO, L., ZHAO, P. & GU, A. 2013. IDH1/IDH2 mutations define the prognosis and molecular profiles of patients with gliomas: a meta-analysis. *PLoS One,* 8**,** e68782.

ZUMSTEG, A. & CHRISTOFORI, G. 2009. Corrupt policemen: inflammatory cells promote tumor angiogenesis. *Curr Opin Oncol,* 21**,** 60-70.