Review of literature
**General perspective**

Glioma is a global health problem for human being and over the past decade the incidence of primary brain tumor has increased. Approximately 128,000 cases of annual mortality from central nervous system (CNS) cancer are diagnosed in worldwide [Parkin et al., 2001] and one of the most common cancers for children under 15 years of age [Slikker et al., 2004]. Malignant gliomas are a class of primary brain tumors occurring from the astrocytes or oligodendroglial cells or ependymal cells of the brain. In adults, gliomas account for about 2% of the primary malignant tumors which are characterized by invasive growth and neovasularisation potential surrounding normal brain tissue. These tumors generally do not have distinct borders and may spread to other areas in the brain or spine. The development of gliomas, like many other tumors, is a multistep process involving the genetic changes, growth factors, their receptors and also the loss of tumor suppressor gene by regulating cell growth and differentiation.

**Origin of Glioma**

The cells of origin for malignant gliomas remain enigmatic. Most glioma classifications made over past 80 years have postulated that astrocytomas arise from astrocytes and oligodendrogliomas from oligodendrocytes [Lousis et al., 2001]. However, there is no evidence that most brain cells undergo division normally during adult life, which would be a requirement if neoplasms arose from mature brain cells. Glial cells could undergo neoplastic events during reactive proliferation, such as trauma, with the development of glial brain tumors [Davis et al., 1998]. Recent work in both animal models and primary gliomas suggests the more likely possibility that malignant gliomas arise from neural progenitor cells. Neuroectodermal stem cells that reside in adult mammalian brains have proliferative potentials, are migratory, and can pursue diverse paths of differentiation- all these features are intrinsic to glioma cells and likely characteristics of neoplastic cells of origin. Thus, there is a huge possibility that the endogenous adult neuroectodermal stem cells are cells of origin for primary brain tumors [Holland et al., 2000]. Furthermore, malignant gliomas likely contains tumor stem cells, a relatively primitive population responsible for populating and repopulating the tumors as they develop and progress [Galli et al.,2004; Singh et al.,2004;]. Such tumor stem cells may be transformed variants of normal neural progenitor cells. The existence of these tumor stem cells may have major therapeutic implications as well because therapies that do not ablate the tumor stem cells will ultimately prove ineffective in eradicating the tumor [Lousis et al .,2006]. The grading (classification) of a tumor is determined by the evaluation of tumor characteristics by a pathologist by their phenotypic marker. On the basis of their increasing malignancy and cellular activity these tumors are graded into:
Pilocytic astrocytomas (WHO grade I):
Pilocytic astrocytomas are a group of Astrocytomas (AS) that are distinguishable from other astrocytomas by their distinctive pathologic appearance and almost invariably benign behaviour. Pilocytic astrocytomas are considered benign because they do not invade the surrounding normal brain tissue. These are slow growing tumors, but can become very large. It typically occurs in children and young adults and are usually located in the cerebellum. Pilocytic astrocytomas frequently have cystic portions filled with fluid and anoudle, which is the more solid portion [Riemenschneider et al., 2009; Collins et al., 2004].

Astrocytomas (WHO grade II):
Astrocytomas (AS) are most common in late middle age. These tumors invade surrounding healthy tissue but grow relatively slowly. Astrocytomas are poorly defined, grey-white tumors, and range from a few centimetres in diameter to enormous lesions that replace substantial parts of the cerebral hemisphere which infiltrate and distort the underlying brain. These tumors are often controllable by surgery, although recurrence is frequent. Recurrent tumors are often found to be less differentiated and of higher grade, suggesting their progressive nature, and, in spite of improved management protocols, the mean survival time is about six years5 and hence these tumors are by no means benign. Astrocytomas comprise 8% of primary brain tumors [Riemenshneider et al., 2009].

Anaplastic astrocytomas (WHO grade III):
Anaplastic astrocytomas comprise 4% of primary brain tumors and these are not grossly distinguishable from AS. Microscopically, however, they have anaplastic features such as increased hypercellularity, nuclear and cytoplasmic pleomorphism, and irregularity and nuclear hyperchromatism. Some patients in this group respond well to chemotherapy and/or radiotherapy, while many do not. However, the rate of survival drops considerably compared to AS, the mean survival time being about two years An anaplastic astrocytoma can be reoccurrence of lower grade , Previously treated astrocytoma [Riemenschneider et al., 2009; Angelis et al., 2001; Louis et al., 2006; Aoki et al. 2007].

Glioblastoma multiforme (WHO grade IV):
This is the most malignant type of tumor and has the poor prognosis. Glioblastoma multiforme (GBM) is distinguished from the other types of AS by its variegated appearance, hence the term multiforme. Approximately 50% of astrocytomas are glioblastomas typically contain more than one cell type. While one cell type may die off in response to a particular treatment, the other cell types may continue to multiply. Foci of necrosis, cysts and haemorrhages are common. The nuclei of the tumor cells are extremely variable in size and shape. This characteristic may makes Glioblastoma very difficult to
Glioblastomas comprise 23% of primary brain tumors in the U.S. and are most commonly diagnosed brain adults aged 45-74. The mean survival time is about one year [Nagarajan et al., 2009].

**THERAPIES OF GLIOMA**

Treatment of GBM includes surgery, radiotherapy (RT) and chemotherapy. Currently, surgery followed by standard RT with concomitant and adjuvant chemotherapy with temozolomide is the standard of care in patients with GBM aged <70 years in USA, however the prognosis remains poor, with a median survival of 12 to 15 months. Clearly, there is an urgent need to explore more effective therapies for patients with GBM. Truly speaking, at present the management of malignant of glioma is based on multimodality treatment. First, most of the treatment of the tumor tissue is commonly reduced by surgical resection in order to diminish the tumor burden and to prevent vital complications due to compression and elevated intracranial pressure. Moreover, cytoreduction is thought to improve the efficacy of the subsequent therapeutic interventions. However, gliomas often grow in neurologically important sites, thereby precluding a microscopic complete resection.

**I. SURGERY:**
The first-line treatment for malignant gliomas is surgery. It involves removing as much of the tumor as possible, while trying to reduce damage to healthy brain tissue. It has been suggested that the survival benefit for patients for whom surgery is effective relieves mass effect, thereby reducing tumor volume remaining to be treated with other modalities and removing the necrotic tumor core, which may be poorly accessible to circulating chemotherapy. Surgical resection may also be used to reduce mass effect, in order to buy time for additional therapeutic options. It has also been shown that maximizing surgical debulking may improve response to chemotherapy. The following are some examples of brain tumor surgeries: diagnostic surgery, staging surgery, debulking (or cytoreductive surgery, palliative surgery and restorative (or reconstructive) surgery. Chemo or radio therapy may combine with surgery during treatment.

**II. RADIATION THERAPY:**
This is one of the primary forms of conventional brain tumor treatment. Radiation therapy may be used alone, or in combination with surgery and or chemotherapy. Survival benefit for postoperative whole brain radiotherapy (WBRT) for malignant glioma was demonstrated in studies going back three decades ago. Median survival increased from 17 weeks in patients treated with conventional measures to 37.5 weeks in patients treated with postoperative WBRT. Subsequent advances in radiotherapy (RT) techniques have used improved imaging of the tumor and focused on RT techniques that maximize treatment to the tumor, while minimizing radiation to normal brain tissue. There is great interest in maximizing the radiation dose to the tumor bed without increasing radiation exposure to the
surrounding brain. Stereo tactic radio surgery (SRS) is a technique of external irradiation that uses multiple convergent beams to deliver a high single dose of radiation to a small (< 4 cm), discrete treatment volume. Radio surgery can be performed with high energy x-rays produced by a linear accelerator (multiple devices), with gamma radiation from Co$^{60}$ sources (Gamma Knife®) and with charged particles such as protons produced by cyclotrons, which are used less frequently. Stereo tactic radiation techniques produce a rapid falloff of dose at the edge of the target volume. Initial, small, single institution studies have revealed positive effects of SRS for newly diagnosed gliomas, but a prospective, multicenter trial showed no survival benefit after the addition of SRS to the standard therapy for GBM, SRS has also been used in the setting of recurrence; however, no randomized studies have been performed to date. However, Glioma patients with long remissions often suffer from late side effects of radiotherapy, diffuse leukoencephalopathy being the most feared complication that may appear several months or year after completion of radiotherapy [Noda et al., 2009].

III. CHEMOTHERAPY
Chemotherapy often called as “anticancer” drugs; aim to destroy brain cancer cells by impeding the cells’ growth and reproduction. Chemotherapy may be given intravenously (through a vein), by mouth, or by injection. Chemotherapy may be used alone, or in combination with other brain cancer treatments, such as radiation therapy and/or surgery but like radiotherapy, toxicity is also a major problem of chemotherapy for malignant glioma, limiting the antitumor efficacy and therapeutic success of cytotoxic drugs. Besides local side effects such as necrotizing leukoencephalopathy after radiochemotherapy, the most important complications include myelosuppression, eg. caused by nitrosoureas, or periphereral neurotoxicity, especially due to vinca alkaloids. If chemotherapy is initiated, several courses of the respective protocol are usually carried out during or after radiotherapy. Among them such a standard protocols includes monotherapy with nitrosoureas, mostly 1, 3-bis-(chloroethyl)-1nitrosourea or the PCV protocol [procarbazine, N-(2-chloroethyl)-N’-cyclohexyl-N-nitrosourea(CCNU), which has proven highly active against anaplastic oligodendroglioma [kim et al., 1996; Cairncross et al., 1994]. The impact of these therapies on natural course of GBMs is rather limited. Response rates to chemotherapy range between 20-30% and median survival rate is hardly 2-3months when chemotherapy is given after surgery and radiation therapy.

A. Temozolomide:
Temozolomide, an orally administered cytotoxic agent is most successful among all chemotherapeutic drugs. This drug crosslinks with the DNA of cancer cells at o$^6$ position so that they can no longer replicate. In clinical studies, TMZ has 100% oral bioavailability and readily penetrates blood-brain barrier. Based on the encouraging results of Phase I and Phase II [Yung et al., 2000], a randomized trial was conducted to compare the efficacy of TMZ with Procarbazine in patients with GBM at first
relapse [Yung et al., 2000]. TMZ performed the better then Procarbazine in terms of overall survival and objective response to treatment, although the differences were modest. Over the past several years, TMZ has become treatment of choice for patients with recurrent malignant glioma. However, the responses tend to be modest and short lived. TMZ has also been evaluated in combination with a number of chemotherapeutic and biological agents to improve the therapeutic benefit [Wen et al., 2004]. Phase I studies of the drug in combination with Procarbazine [Newlands et al., 2003], etoposide [Korones et al., 2003], and irinotecan [Gilbert et al., 2003; Gruber et al., 2004] have been completed and Phase II studies are in progress. TMZ chemotherapy is effective for the treatment of high-grade glioma in some patients without serious toxicity. Assessing the true efficacy of TMZ will require larger studies with comparison of long-term outcomes between other agents or combined therapeutic modalities [Oshiro et al., 2009]. The overall and progression–free survival of patients given concomitant and adjuvant Temozolomide are greater than in those given radiotherapy alone; however, this regimen incurs a greater deterioration in mental status. However, other cytotoxic chemotherapy options for patients with GBM include irinotecan (Camptosar), combination procarbazine (Matulane), lomustine, and vincristine, or carmustine alone.

IV. IMMUNOTHERAPY

Significant advances in the field of immunology have paved the way for the development of immune therapeutic strategies to combat primary intracranial neoplasms. A typical characteristic of malignant gliomas are notoriously evading the host immune system as well as systemic immunosuppression induced by several immunodepressive mechanism. Thus, glioma patients may exhibit lymphopenia, impaired antibody production, reduced lymphocyte protein synthesis, and diminished lymphocyte responsiveness. Therefore, a therapeutic approach counteracting glioma-induced immunosuppression could be an effective tool to restore the physiological ability of cytotoxic immune cells to attack malignant cells. TGF-β signaling and modulation of regulatory T-cells two specific ways to combat immunosuppression in glioma [Vega et al., 2008]. The various immunotherapeutic regimens tested thus far have resulted in mixed responses.

IV. A. Passive Immunotherapy

Passive immunotherapy involves the utilization of immune effector cells or a variety of molecules including monoclonal antibody (MoAbs) and cytokines under therapeutic modalities. The efficient use of MoAbs against brain tumors presents unique challenges. BBB favors small, uncharged, lipid soluble molecules. Thus, large size of antibodies as macromolecules requires novel delivery strategies to administer antibodies directly to the brain tumors. Convection Enhanced Delivery (CED) has boosted the mechanism of drug-delivery using physical pressure process. By this technique, either large or small molecules are infused at high pressure through an intracranial catheter.
IV. A.1. Antibody based Immunotherapy:
In 1992 Hall et al, first time approach the antibody-based immunotherapy which causes to kill the glioma cells directly after implementation. Various toxins or radioactive isotopes can be specifically targeted to glioma cells by conjugation to antibodies. However, this strategy depends on the recognition of specific antigens on tumor cells by the antibodies. As with vaccination therapies, the main obstacle to antibody-targeted immunotherapy is the lack of identified specific tumor antigens on human malignant glioma cells. Nevertheless, antibody conjugates have been used to treat glioma by employing antigens which are not exclusively but preferentially expressed on malignant brain tumors, including epidermal growth factor (EGF)-R-specific antibodies [Wikstrand et al., 1998]. EGFR, a transmembrane receptor tyrosine kinase, is associated with increased tumor growth rate and shorter survival. A mutation of EGFR, termed EGFR variant III (EGFRvIII), is frequently expressed in glioblastoma and enhances tumorigenicity. Cetuximab (Imclone, Bristol Meyers Squibb, New York, NY) is another agent has been shown to enhance the antitumor effects of chemotherapy and radiotherapy, inhibiting the EGFR pathway and also extracellular domain. While Nimotuzumab (h-R3, YM Biosciences, Mississauga, ON, Canada), which similarly targets the ECD of EGFR has been used in Phase II trials.

IV.A.2 Cytokine Immunotherapy:
The designation “single tumor necrosis factor” discloses that great expectations were once placed on the direct tumoricidal effects of cytokines on tumor cells. However, it soon became clear that the prevailing action of cytokines consist in the complex modulation of immunologic mechanisms rather than in direct induction of target cell death. Provided that these immune modifiers are prudently employed for therapeutic purposes, they could turn out be powerful tools to fight various neoplastic disorders.

Interleukin-2:
IL-2 has been shown that it possess immunoactivating properties [Yamasaki et al., 1988] when it used in combination with lymphokine activated killer (LAK) cells. While, the potential use of IL-2 as a single agent to treat glioma is complicated because local application of IL-2 into the CNS results in strong inflammatory changes at the injection site, impeding a possible therapeutic administration [Hanisch et al., 1997] and on the other hand glioma-infiltrating T cells frequently appear to express low levels of IL-2 receptor or a defective form of the receptor. However, it has also been demonstrated that gene transfer for IL-2 into malignant tumor cells can also lead to an enhanced specific immune response and the prevention or even regression of tumors [Gansbacher et al., 1990]. Unfortunately, this approach was not successful with malignant glioma [Fakhrai et al., 1996]. IL-2 could nevertheless gain
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further attention because of its ability to partially overcome transforming growth factor (TGF)-β-induced immunosuppression associated with malignant glioma [Inge et al., 1992].

**Interleukin-4:**

IL-4 has been employed against malignant glioma cells because it is able to induce a strong local immune response if administered intracerebrally. In the study of Yu et al. [Yu et al., 1993], glioma growth could be retarded after simultaneous intracranial application of glioma cells and IL-4 transfected plasmocytoma cells. Interestingly, in this mouse model, a T-cell-independent prominent activation of eosinophils occurred which was presumably responsible for the antiglioma effect.

**Tumor necrosis factor (TNF)-α:**
The pleiotropic cytokine TNF-α induces the death of only a few tumor cell types, whereas its main function appears to be the regulation of a broad range of immune reactions. TNF-α stimulates the growth of T cells, enhances the cytotoxicity of monocytes, granulocytes and natural killer (NK) cells, induces the secretion of other cytokines such as interleukin (IL)-1, IL-6, colony-stimulating factor (CSF) or platelet-derived growth factor (PDGF) by immune cells in vitro, induces the expression of adhesion molecules such as ICAM-1 and enhances the expression of IL-1β. Moreover, MHC class I and II molecules on immune cells are upregulated by TNF-α [Fabry et al., 1994]. However, cultured glioma cells are usually resistant to TNF-α. Not surprisingly, clinical trials studying the therapeutic efficacy of TNF-α on glioma have been rather disappointing. Other studies tried to achieve an enhancing effect on the activity of LAK cells by TNF-α gene transfer. Regarding a death-receptor-based immunotherapy, it may be important to take into consideration TNF-α-induced expression of CD-95 on glioma cells [Yoshida et al., 1992].

**Interferons (IFNs-) γ:**

IFN-γ, produced by T cells, is of crucial importance for the interaction of T cells with antigen-presenting cells such as macrophages. IFN-γ enhances the expression of MHC class I and II antigens on immune cells, thereby enabling more efficient antigen recognition by CD4+ and CD8+ T cells [Fabry et al., 1994]. Further improvement of antigen recognition could be achieved by the IFN-γ-induced upregulation of MHC I molecules on glioma cells and the inhibition of proliferation correlates with elevated levels of the cyclin-dependent kinase inhibitor p21 (waf1/cip1) [Kominsky et al., 1998]. There are several clinical studies on the effects of IFN-γ on patients with malignant glioma. IFN-γ, which is clinically well tolerated, has been administrated locally-intra-tumorally as well as systematically [Wild et al., 1991]. Moreover, IFN-γ potentiates LAK-induced glioma cell killing [Wild et al., 1991]. However, none of the clinical trials with IFN-γ for glioma patients was clearly successful. On the other hand, that IFN-γ induces the expression of the proapoptotic receptor CD95 and that IFN-
α sensitizes glioma cells to the cytotoxic actions of the CD95 ligand [Roth et al., 1998] could become meaningful for a death receptor/ligand-based immunotherapy of malignant glioma.

V. GENE THERAPY
Another proven biological treatment strategy for high-grade gliomas is the genetic therapy. Herpes simplex virus-thymidine kinase (HSV-tk) gene therapy has been the pioneering and most commonly used application. Gene delivery has been most commonly performed using direct delivery methods, involving either stereotactic intratumoral injection or intraoperative injections into the margins of the tumor cavity. The most commonly used strategy of gene transfer has been through the use of adenoviral particles as vehicles. Some studies have shown efficacy in adenovirus-mediated HSV-tk gene therapy against glioblastoma. The disadvantages of gene therapy are low tumoricidal effect in situ and a limited distribution of the transgenes and / or vectors to tumor cells, localized peripherally from the main tumor mass. In addition virus-derived vectors may have the potential to create damaging immunological reactions by immune-mediated toxicity, especially in the presence of circulating antibodies, to the virus vectors or by triggering immune reactions to self or transgene antigens.
## SUMMARY OF THERAPIES FOR GLIOMA

### Therapy Types

<table>
<thead>
<tr>
<th>Therapy Types</th>
<th>Subtypes</th>
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<tbody>
<tr>
<td><strong>Radiation therapy</strong></td>
<td>Stereotactic radiosurgery, Gliasite, Stereotactic Radiotherapy</td>
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| **Chemotherapy** | Temozolomide, carmustine, lomustine  
Procarbazine+ lomustine+ vincrisitine; temozolomide+cisapatin, carboplatin+etoposide,lomustine |
| **Gene therapy** | Tumor suppressor genes, Immunostimulatory genes, Chemosensitization genes, Oncolytic viruses. |
| **Targeted Molecular Therapy** | |
| VEGFR Inhibitor | PTK787 (ZK222584:PDGFR and VEGFR inhibitor);  
Sorafenib (VEGFR, PDGFR and RAF kinase inhibitor)  
SUO11248 (PDGFR, c-kit, and VEGFR inhibitor)  
ZD6474 (VEGFR and EGFR) |
| EGFR Inhibitor | Erlotinib (OSI-774, Tarceva)  
Lapatinib (GW-572016: EGFR and ErbB-2 inhibitor)  
ZD6474 (VEGFR and EGFR)  
Gefitinib (ZD1839, Iressa) |
| PDGFR Inhibitor | SUO11248 (PDGFR, c-kit, and VEGFR inhibitor),  
Imatinib mesylate (ST1571, Gleevec) |
| Integrin Inhibitor | Cilengitide (EMD121974) |
| Proteasome Inhibitors | Bortezomib (PS-341, Velcade) |
| Intratumoral Therapy | PE-Pseudomonas exotoxin, 131I-TM-601 |
| Gene Therapy | Chemosensitization genes, immunostimulatory genes, tumor suppressor genes, Oncolytic viruses. |
| Immunotherapy | Monoclonal Antibodies, Cytokines, Adoptive Immunotherapy, Vaccines (dendritic cells and Peptide) |
THE ROLE OF ANGIOGENESIS IN GLIOMA DEVELOPMENT

One of the earliest events during the transition of a tumor from the tumorigenic to the neoplastic phenotype is the acquisition of the angiogenic phenotype. Angiogenesis is a term that describes the growth of new blood vessels from preexisting vessels. This is a complex process characterized by the invasion of single tumor cells into the neighboring tissue, intravasate into lymph vessels or blood, followed by endothelial cell (EC) proliferation, migration, and finally tubule formation. Previous experiments have demonstrated that tumor angiogenesis is indispensable for the growth of solid tumors [Shubik et al., 1982; Reinhold et al., 1984]. Thus, the presence of angiogenesis in a glioblastoma could promote its rapid growth and clinical progression. Indeed, recent studies have indicated that of all clinical and pathologic characteristics of glioma, angiogenesis have the greatest prognostic value. When angiogenesis is extensively present in a glioblastoma, the prognosis is consistently poor [Wong et al., 2009; Machein et al., 2009; Jain et al., 2007]. Based on the clinical implications of and potential for therapeutic interventions for glioblastoma, the mechanisms leading to angiogenesis in this tumor must be identified.

Previous reports have shown that during the cancerous growth unregulated angiogenesis causes the generation of network of blood vessels that penetrates into cancerous growths supplying oxygen and removing metabolic waste products from tumor region. This phenomenon induces the expression of proangiogenic factors in tumor associated endothelial cells and if proangiogenic factors are in excess of antiangiogenic factors, it may lead to the switch to an angiogenic phenotype [Liotta and Stetler-Stevenson et al., 1991]. In glioma microenvironment various pro-angiogenic factors released by the tumor cells, inflammatory cells, and/or stromal cells; which bind to respective receptors of endothelial cell and initiate the progression of angiogenesis by stimulating the vascular endothelial cells to split rapidly [Goh et al., 2007; Kos et al., 2002] and trigger them. When the endothelial cells are stimulated to grow, they secrete proteases and other digestive enzymes that digest the basement membrane surrounding the vessel. Degradation of basement membrane and the extracellular matrix surrounding pre-existing capillaries, usually postcapillary venules, is a mechanism allowed by matrix metalloproteinases (MMPs), a family of metallo-endopeptidases. The degradation of extracellular matrix component also allows the release of proangiogenic factors (VEGF, TGFβ,) from the matrix. The junctions between endothelial cells become altered, cell projections pass through the space created, and the newly formed sprout grows toward the source of the stimulus. Endothelial cells invade the matrix and begin to migrate and proliferate into the tumor mass. In this location, newly formed endothelial cells organize into hollow tubes (canalization) and create new basement membrane for
vascular stability. Fused blood vessels newly established form the blood flow within the tumor. The formation of the lumen during canalization is driven by important interactions between cell-associated surface proteins and the extracellular matrix (ECM). Some of the surface proteins identified in this interaction are Integrin αvβ3, αvβ5, PECAM-1 (CD31), and VE-cadherin [Yang et al., 1999]. The anti-angiogenic factors that mediate regression can do so either by inhibition of migration, proliferations and inducing apoptosis of ECs. Thus, the switch to the angiogenic phenotype is regulated by a change in the local equilibrium between positive and negative regulators of the growth of microvessels.

The English surgeon Stephen Paget in 1889, first time published his ‘seed and soil’ explanation on nonrandomly patterns of metastasis, and suggest that in the tumor microenvironment interactions between tumor cells and host cells are critical to regulating tumorigenesis [Paget et al., 1989] that favored tumor cells (the ‘seed’), had a specific affinity for the growth-enhancing milieu within specific organs (the ‘soil’), and hence metastasis only occurred when ‘seed’ and ‘soil’ were compatible [Ribatti et al., 2006]. So, for the tumor growth there were several components of the ‘soil’ which regulating the growth mechanism has since been emphasized:

1. Stromal cells and their growth factors and inhibitors;
2. The extracellular matrix;
3. Microvessels and angiogenic factors;
4. Cytokines and inflammatory cells

THE FACTORS INVOLVED IN TUMOR ANGIOGENESIS

In the past decades, as angiogenesis required for the growth and metastasis of various types of cancers including glioma, positive tumor angiogenic growth factors has become an important tool in cancer biology and for the clinical oncology. In tumor microenvironment tumor cells and their neighbouring cells produce several growth factor /angiogenesis inducers, which play a crucial role in endothelial survival, proliferation and differentiation, as well as new vessel sprouting. However, their role in GBM progression is not always fixed. So regulators of tumor angiogenesis dived into several sub types:

A. Vascular endothelial growth factor and its receptor:
Vascular endothelial growth factor-A (VEGF-A) is known as positive regulators in tumor angiogenesis and plays a key role in glioma development [Velasco et al., 2002]. VEGF-A is upregulated in GBM tumors in which it is produced by tumor cells, inflammatory cells and endothelial cells. VEGF is a potent mitogen and chemoattractant for EC by binding to its high affinity receptors (Flt-1/VEGFR-1,
Flk-1/KDR/VEGFR-2), promotes the formation of the second messenger via hydrolysis of inositol, thus induces the autophosphorylation of the receptors in the presence of heparin-like molecules, that open phosphatidylinositol metabolic signal transduction pathways, activates MAP kinases in EC, as well as phosphatidylinositol-3-hydroxyI kinase (PI3K)/protein kinase B (Akt) pathway and thereby VEGF exerts its mitogenic effect by promoting EC proliferation. The expression of the receptors for VEGF-A (VEGFR1 and VEGFR2) is upregulated on the endothelial cells (ECs) in GBM tumors as compared to its expression on the ECs of normal brain [Kukk et al., 1996; Stratmann et al., 1997; Witmer et al., 2002]. The expression of VEGF is regulated by multiple tumor suppressor genes (such as SRC, RAs), cytokine and various signaling molecules (nitric oxide and mitogen activated protein kinase) and oncogenes [Dvorak et al., 2002; Ferrara et al., 2004]. Moreover, some other studies have revealed that VEGF has been shown to exhibit its angiogenic effect by inducing expression of the αvβ3-integrins that promote cell migration, proliferation, and reorganization of matrix. Simultaneously VEGF also induces a balanced system of proteolysis that can remodel ECM (Fig.1) components necessary for angiogenesis through EC production of MMP-2, MMP-9 [Beck et al., 1997; Velasco et al., 2002,] urokinase-like plasminogen activator (uPA), tissue type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) [Mandriota et al., 1995; Pepper et al., 1991]. So angiogenesis can be initiated by the release of positive or proangiogenic factors (e.g., VEGF, bFGF, & TNF-α) from tumor cells, inflammatory cells, mast cells, macrophages, [Velasco et al., 2002].

B. Fibroblast Growth Factor (FGF):

Another pro-angiogenic growth factor that is upregulated in various types of cancers is fibroblast growth factor (FGF). In tumors like glioma, bFGF is expressed by vascular cells and also by the tumor cells [Karcher et al., 2006] which induces the VEGF mRNA Expression. The receptors for bFGF is expressed by both the tumor cells and the tumor associated ECs are FGFR1. However, bFGF does not have a conventional signal peptide for secretion, but it can be found sequestered in the ECM bound to heparin-sulfate-containing proteoglycans, where it is released by enzymes degradation of ECM. Several studies have indicated that bFGF also induces proliferation of cell, protease production, and causes the modulation of integrin expression in ECs [Bussolino et al., 1996]. Binding of FGFs to their receptors causes the activation of the intrinsic tyrosine kinase and a cascade of events, leading eventually to the induction of immediate early gene transcription, and to cell proliferation.
Fig.1: Mechanism of angiogenesis by angiogenic factors [Gupta et al., 2003].
A. Angiopoietin and its Tie2 receptors:

It has been demonstrated that angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are critically involved in angiogenesis, by developing of the immature vessels, and maintenance of the vascular plexus [Lobov et al., 2002]. Ang-1 & Ang-2 proteins are ligands for the tyrosine kinase receptor and epidermal growth factor with privileged expression in endothelial cells. It was reported that Ang1 induced Tie2, p85 subunit of PI 3’-kinase phosphorylation increased PI3’-k activity in a dose-dependent manner, suggesting that angiopoietin and its receptors are crucial elements in signal transduction pathway leading to EC survival [Kim et al., 2000] (Fig. 1). Moreover, it has been found that Ang1-induced migration of EC might be mediated through PI3k which causes the tyrosine phosphorylation of p125FAK [Kim et al., 2000] by upregulation of MMP-2 secretion and suppressed TIMP-2 expression from ECs are also important determinants for inducing ECs sprouting [Kim et al., 2000] (Fig. 1). Several pre-clinical studies have shown that modulation of angiopoietin signalling leads to alterations in vascular morphology and inhibition of tumor growth. Several types of angiopoietin inhibitors are now in phase I–III clinical trials. Similar to VEGF receptors, Tie2 is critical for normal vascular development [Dumont et al., 1994]; however, in contrast to VEGF receptors that are down-regulated after embryonic angiogenesis has ceased, Tie2 appears to be constitutively expressed and phosphorylated in the adult vasculature [Wong et al., 1997]. This constitutive expression and activation suggests that signaling via Tie2 is important for the homeostasis of the mature vasculature. Indeed, Tie2 signaling promotes stable vessels that are covered by pericytes. Tie2 is bound by three different ligands that engage the same binding site on the receptor. Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) are the first ligands to be discovered and are the best studied, whereas the function of Ang-4 is less understood. These ligands are thought to bind to Tie2 with roughly similar affinities and cause receptor activation [Valenzuela et al., 1999]. A major observation has been that Ang-1 acts as a stimulating ligand for the Tie2 RTK that leads to receptor phosphorylation, whereas Ang-2 inhibits Tie2 phosphorylation, even in the presence of Ang-1 [Jones et al., 200, Maisonpierre et al., 1997]. Ang-1 can “seal” vessels in vivo and decrease vascular permeability [Jain et al., 2000]. Interestingly, the major regulator of the entire Tie2/Angiopoietin signaling pathway appears to be Ang-2. TCGA data for glioblastoma reveal that Ang-2 is up-regulated to a much higher extent than Ang-1, suggesting that Tie2 signaling is blocked in glioblastoma. The consequences of blocking Tie2 signaling in vivo, as evidenced by several pre-clinical studies, appears to be a shift towards an immature vasculature, whereas Ang-1 appears to “normalize” the vasculature. In addition to an impact on angiogenesis, Ang-2 was shown to mediate a pro-inflammatory phenotype. In Ang-2 null mice the inflammatory response to stimuli such as TNF-α was greatly diminished when compared to
controls [Fiedler et al., 2006]. Vice versa, in mice engineered to express an inducible form of Ang-2 in the vasculature, myeloid cells increased significantly over time in almost all organs, even without any pathological stimulus [Scholz et al., 2011]. These findings suggest that Ang-2 on its own is able to orchestrate an inflammatory response by specifically recruiting myeloid cells—and leaving lymphocytes behind [Kim et al., 2011].

B. Epidermal Growth Factor Receptor:

Among the receptor tyrosine kinase epidermal growth factor receptor (EGFR) was the first receptor tyrosine kinase to be discovered and remains the most investigated. In mammals EGFR consist of four members (known as ErbB1, ErbB2, ErbB3, ErbB4). Depending on the particular ligand and the receptor to which it binds, members of the ErbB/EGFR family mediate various cellular processes, including adhesion, cell division, migration, differentiation, and apoptosis [Yarden et al., 2001]. The EGFR family shares a general domain organization in which an extracellular ligand-binding region is linked through a hydrophobic transmembrane domain to a cytoplasmic region that contains both a tyrosine kinase domain and C-terminal tail. Upon ligand binding, EGFR undergoes receptor dimerization [Ferguson et al., 2003], tyrosine kinase activation, and trans-phosphorylation across receptor dimers on multiple tyrosine residues in the cytoplasmic tail [Hynes et al., 2005]. It is a single pass transmembrane receptor with two extracellular and cysteine-rich regions involved in ligand binding trigirng the autophosphorylation and formation of signaling complex. The formation of this signaling complex results in the initiation of various downstream signaling cascades, including the phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and signal transducer and activator of transcription 3 (STAT3) pathways, which regulate a multitude of cellular responses.
### Table 1. Major Angiogenic factors in malignant gliomas

<table>
<thead>
<tr>
<th>Angiogenic Factors</th>
<th>Function</th>
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<tbody>
<tr>
<td>VEGF-A</td>
<td>Angiogenesis, promotes endothelial proliferation and migration, and creation of blood vessel lumen</td>
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<tr>
<td>VEGFR1</td>
<td>Promotes tumor angiogenesis, &amp; in the activation of MMP</td>
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<tr>
<td>Angiopoietin</td>
<td>Induces stabilization, remodeling and maturation of blood vessels</td>
</tr>
<tr>
<td>Acidic &amp; basic FGF</td>
<td>Resists apoptosis of endothelial cells, induces endothelial cell proliferation and migration</td>
</tr>
<tr>
<td>EGF &amp; EGFR</td>
<td>Stimulates VEGF production in glioma cells &amp; Promotes tumor growth and angiogenesis <em>in vivo</em></td>
</tr>
<tr>
<td>HIF</td>
<td>Induce glioma angiogenesis through upregulation of VEGF in tumor cells as well as ECs</td>
</tr>
<tr>
<td>MMP-2 &amp; MMP-9</td>
<td>Involved in the proteolytic degradation of ECM components and facilitate cell motility during invasion and angiogenesis</td>
</tr>
<tr>
<td>Integrins</td>
<td>Responsible for the interaction of endothelial and tumor cell with ECM</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Participates in regulator of proliferation, migration, differentiate and ECM synthesis in endothelial cells</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Involved in systemic inflammation and acute phase reaction, induces tubular morphogenesis <em>in vitro</em></td>
</tr>
</tbody>
</table>

Velasco et al., 2002, Kukk et al., 1996, Vartio et al., 1982, Timpl et al., 1996,
THE ROLE OF EXTRACELLULAR MATRIX AND MMPs IN GLIOMA ANGIOGENESIS

The transition of a tumor from the tumorigenic to the neoplastic phenotype is marked by changes in the remodeling of both the pericapillary membrane and of its surrounding extracellular matrix (ECM), by endothelial cell proliferation, and by capillary tube formation. The extracellular matrix (ECM) provides a supportive and nutritious environment for the development of tissues. It is now evident that ECM turnover is a critical step in tissue remodeling that accompanies many physiological as well as pathological processes. The best characterized ECM glycoproteins are laminin and fibronectin. Laminin and fibronectin contribute to the molecular architecture of ECM by binding to ECM components (collagens and proteoglycans) (Fig.2) and mediating their attachment to cells. Laminin and fibronectin also bind to receptor proteins (integrins) present on the cell surface and play important roles in determining cell morphology, differentiation and division and in permitting cell locomotion [Lodish et al., 2000]. However, for ECs to form a neovasculature the basement membrane that forms a “cage” around the existing vasculature must be degraded. The proteolytic degradation of the basement membrane is mediated by proteases, such as the matrix metalloproteinases (MMPs), that are secreted by tumor as well as ECs.

The invasiveness of endothelial cells at foreign sites in angiogenesis is due to the activity of proteinases. There are four major classes of proteinases involved in ECM degradation and they are serine (eg: plasminogen activators), cysteine (eg: cathepsin B), aspartyl (eg: cathepsin D) and matrix metalloproteinase (MMPs) (eg: MMP-2, MMP-9). In addition the endo and exoglycosidases contribute to ECM degradation by selectively hydrolyzing GAGs and amino sugar moieties of proteoglycans [Turk et al., 2006]. The ADAMs (a disintegrin and metalloproteinase) are a family of multifunctional membrane proteins that are similar to snake-venom metalloproteinases. Structurally, they show a complex domain organization that consists of signal sequence, metalloproteinase domain, disintegrin-like domain, cysteine-rich region, epidermal-growth-factor-like domain, transmembrane region and cytoplasmic tail. So far, 24 ADAMs have been described in human tissues. Some ADAMs are overexpressed in malignant tumors from diverse origin, and their proteolytic activity might be blocked by endogenous and synthetic matrix metalloproteinase inhibitors (MMPIs) [Amour et al., 2000; Kashiwagi et al., 2001]. The ADAMTSs (ADAMs with thrombospondin motifs) are ADAM-related proteases that contain several thrombospondin type I repeats in their carboxy-terminal region, but lack the transmembrane domain that is present in ADAMs. So far, 18 ADAMTSs have been identified in human tissues and, in some cases, their specific functions in normal or pathological processes have been described.
ECM provides structural support to the animal cells in addition to performing various other functions. It includes the interstitial matrix and the basement membrane. Gels of polysaccharides and fibrous proteins fill the interstitial space and act as a compression buffer against the stress. (Lodish et al., 2000; Alberts et al., 2002).
A. Matrix Metalloproteinases and Their Types

It was first shown that diffusible enzymes produced by skin from the resorbing tail of the metamorphosing frog could degrade the triple helix of collagen [Gross et al., 1962]. Since then the matrix metalloproteinase (MMP) family, including the collagenases described in the frog has grown to comprise 23 members in the human [Woessner et al., 1991; Nagase et al., 1992]. MMPs are classified as the matrixin subfamily of zinc metalloprotease family M10 in the MEROPS database (http://www.merops.sanger.ac.uk/) [Nagase et al., 1992]. MMPs are a class of closely related zinc-dependent, neutral endopeptidases which once activated degrades the major components of the ECM (Fig. 3). Members of this family are the only enzymes known to denature and digest fibrillar collagens. Collectively, they are capable of degrading essentially all ECM components. Based on domain organization and substrate specificities, MMPs are grouped into collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs and others (Table 2). However, due to some overlap with substrate specificities in the different MMPs, a numerical nomenclature (eg., MMP-1, MMP-2) is currently preferred. Three enzymes reported earlier (MMP-4, -5, -6) were later found to correspond to known enzymes, so these three numbers have been discontinued and remain vacant. Also MMP-18 is known only as a *Xenopus* enzyme. It is not yet known whether MMPs- 21 and -22, have ECM degrading activity, as their functions are not yet fully understood [Nagase et al., 2006].

1 Collagenases:

MMP-1, MMP-8, MMP-13 and MMP-18 in (*Xenopus*) cleave interstitial collagens I, II and III into characteristic 3/4 and 1/4 fragments but they can digest other ECM molecules and soluble proteins. Two other matrixins, ie, MMP-2 and MMP-14 (MT1-MMP), have collagenolytic activity, but they are classified into other subgroups because of their domain compositions [Woessner et al., 1991; Nagase et al., 1992].

2 Stromelysins:

MMP-3, MMP-10 and MMP-11 have a domain arrangement similar to that of collagenases, but they do not cleave interstitial collagens. MMP-3 and MMP-10 are similar in structure and substrate specificity, but MMP-11 (stromelysin 3) is distantly related. MMP-3 and MMP-10 digest a number of ECM molecules and participate in proMMP activation. MMP-11, on the other hand, has very weak activity toward ECM molecules, but cleaves serpins more readily. MMP-11 has a furin recognition motif RX[R/K]R at the C-terminal end of the propeptide and therefore it is activated intracellularly.

3 Matrilysins:

MMP-7 and -26 lack a hemopexin domain. MMP-7 is synthesized by epithelial cells and is secreted apically. Besides ECM components it processes cell surface molecules such as pro-α-defensin, Fas-
ligand, pro-tumor necrosis factor-α, and E-cadherin. MMP-26 is expressed in normal cells such as those of the endometrium and in some.

4 Gelatinases:
MMP-2 and MMP-9 readily digest gelatin with the help of their three fibronectin type II repeats that binds to gelatin/collagen. They also digest a number of ECM molecules including type IV, V and XI collagens, laminin, aggrecan core protein, etc. MMP-2, but not MMP-9, digests collagens I, II and III in a similar manner to the collagenases. The collagenolytic activity of MMP-2 is much weaker than MMP-1 in solution, but because proMMP-2 is recruited to the cell surface and activated by the membrane-bound MT-MMPs, it may express reasonable collagenolytic activity on or near the cell surface [Woessner., 1991; Nagase et al., 1992].

5 MT-MMPs:
In mammals includes four type I transmembrane proteins (MMP-14, -15, -16, and -24) and two glycosylphosphatidylinositol-anchored proteins (MMP-17 and -25). They all have a furin recognition sequence RX[R/K]R at the C-terminus of the propeptide. They are therefore activated intracellularly and active enzymes are likely to be expressed on the cell surface. All MT-MMPs, except MT4-MMP (MMP-17) can activate proMMP-2. MT1-MMP (MMP-14) has collagenolytic activity on collagens I, II, and III. Seven MMPs is not grouped in the above categories although MMP-12, MMP-20 and MMP-27 have similar structures and chromosome location as stromelysins. Metalloelastase (MMP-12) is expressed primarily in macrophages. It digests elastin and a number of ECM molecules. It is essential in macrophage migration [Nagase et al., 1992].

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>MMP</th>
<th>Name</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenase</td>
<td>MMP-1</td>
<td>Collagenase-1</td>
<td>Col I, II, III, VII, VIII, X, Gelatin</td>
</tr>
<tr>
<td></td>
<td>MMP-8</td>
<td>Collagenase-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMP-13</td>
<td>Collagenase-3</td>
<td></td>
</tr>
<tr>
<td>Gelatinase</td>
<td>MMP-2</td>
<td>Gelatinase-A</td>
<td>Gelatin, Col I, II, III, IV, VII, X</td>
</tr>
<tr>
<td></td>
<td>MMP-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stromelysin</td>
<td>MMP-3</td>
<td>Stromelysin-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMP-10</td>
<td>Stromelysin-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMP-11</td>
<td>Stromelysin-3</td>
<td></td>
</tr>
<tr>
<td>Matrilysins</td>
<td>MMP-7</td>
<td>Matrilysins-1</td>
<td>Fibronecin, Laminin, ColIV, Gelatin, Fibronectin, Fibrinogen</td>
</tr>
<tr>
<td></td>
<td>MMP-26</td>
<td>Matrilysins-2</td>
<td></td>
</tr>
<tr>
<td>MT-MMP</td>
<td>MMP-14</td>
<td>MT1-MMP</td>
<td>Gelatin, Fibronectin, Laminin</td>
</tr>
<tr>
<td></td>
<td>MMP-15</td>
<td>MT2-MMP</td>
<td>Gelatin, Fibronectin, Laminin</td>
</tr>
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<td></td>
<td>MMP-16</td>
<td>MT3-MMP</td>
<td>Gelatin, Fibronectin, Laminin</td>
</tr>
<tr>
<td></td>
<td>MMP-17</td>
<td>MT4-MMP</td>
<td>Fibrinogen, Fibrin, Gelatin, fibrinonectin Laminin</td>
</tr>
<tr>
<td></td>
<td>MMP-24</td>
<td>MT5-MMP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMP-25</td>
<td>MT6-MMP</td>
<td>Gelatin</td>
</tr>
</tbody>
</table>

Table 2. Types of MMPs and their substrate specificity [Woessner et al., 2000]
B. Protein Structure of MMPs:
There are now over 28 members of the MMP family and they can be sub grouped based on their structures. The minimal domain structure consists of a catalytic domain, prodomain, and signal peptide. The propeptide domain contains a conserved cysteine residue (the “cysteine switch”) that coordinates to the catalytic zinc to maintain inactivity. The most common structures for secreted MMPs, including stromelysins and collagenases, have an additional hemopexin-like domain connected by a hinge region to the catalytic domain (MMP-1, -3, -8, -10, -12, -13, -19, and -20). The gelatinases (MMP-2 and -9) contain inserts that resemble collagen-binding type II repeats of fibronectin within their catalytic domains in addition to the simple hemopexin domain structure. The addition of a furin-recognized cleavage site on the carboxyl side of the cysteine switch is found in MMP-11 and -28. Both of these MMPs are activated by furin in the trans- Golgi network and are secreted in their active form. The MT-MMPs are membrane bound rather than secreted, and all have furin-cleavage recognition sites between their pro- and catalytic domains. Four MT-MMPs (MT1-, MT2-, MT3-, and MT5- MMP) have a transmembrane domain and a short cytoplasmic tail, whereas MT4-and MT6-MMP bind to the membrane via a glycosylphosphatidylinositol moiety at their COOH termini and MMP-23 has an NH2-terminal signal anchor and a cysteine-rich, proline-rich, interleukin-1-like domain rather than the hemopexin domain.

Matrix metalloproteinase-2
MMP-2 (Gelatinase A, 72kDa type IV collagenase) is an extensively studied matrix metalloproteinase. It was first purified and described from murine tumor models [Liotta et al., 1979; Salo et al., 1985] and cultured human melanoma cells [Höyhtyä et al., 1990]. MMP-2 is copiously expressed in endothelial, tumor cells and epithelial cells [Vartio et al., 1982; Salo et al., 1985; Hipps et al., 1991] and secreted as proenzymes where they need to be activated for catalytic activity. Overexpression of MMP-2 have been seen in many neoplasms including breast [Pacheco et al., 1998; Talvensaari et al., 2001], ovarian [Afzal et al., 1998; Davidson et al., 1999; Sakata et al., 2000], cervical [Nuovo et al., 1995] and gastric cancers [Mori et al., 1997]. Previous findings have shown [Ward et al., 1994] that most MMPs can be activated extracellularly by other already activated MMPs or serine proteinases. MMP-2 is activated at the cell surface and the activation is mediated by the membrane-type metalloproteinase-1 (MT1-MMP) [Strongin et al., 1995; Visse & Nagase., 2003] and participates in extracellular matrix degradation (ECM) having a wide range of substrates ( Table 2). MMP-2 is able to degrade type I, IV, V, VII and X collagens, laminin, elastin, fibronectin and proteoglycans [Nagase et al., 1999; Woessner., 1999; Sternlicht et al., 2001]. Besides its direct proteolytic actions, MMP-2 are also capable of releasing
Fig. 3 The structure of MMPs: MMPs can be divided into eight distinct structural groups, five of which are secreted and three of which are membrane-type MMPs (MT-MMPs). The minimal-domain of MMPs contain an amino-terminal signal sequence (Pre), a propeptide (Pro) with a zinc-interacting thiol (SH) group and a catalytic domain with a zinc-binding site (Zn). The first and the last of the four repeats in the hemopexin-like domains are linked by a disulphide bond (S–S) [Woessner et al., 2000]
growth factors from ECM and/or inactive complexes, cleaving growth factor receptors and activating growth factors excreted as pre-pro-enzymes, like transforming growth factor (TGF)-α & β. In 1999 Stetler-Stevenson have shown that degradation of ECM components by MMPs expose the integrin binding sites, triggering integrin activation and regulate the downstream signaling. Direct interaction

between αvβ3 to MMP-2 initiate integrin signaling and thus contribute to endothelial cell proliferation and survival. and MMP-2 activates another major gelatinase called MMP-9.

Matrix metalloproteinase-9

MMP-9 or Gelatinase B are also known as 92kDa gelatinase/ type IV collagenase respectively and was first purified from human macrophages by Vartio et al in 1982. MMP-9 is secreted as an inactive precursor form, proMMP-9 is found in endothelial cells, neutrophils, and other inflammatory cells. Like MMP-2, MMP-9 has the ability to hydrolyse gelatins, type III, IV, V, and XIV collagens and have three repeat domains that are homologous to the type II domain of fibronectin. Type IV collagen is one of the major macromolecular constituent of basement membranes [Timpl et al., 1996] and is synthesized as six distinct α-chains, namely, α1 to α6 [Prockop et al., 1995]. These α-chains are assembled into triple helices and are composed of three domains, the N-terminal domain, the middle triple helical domain, and the C-terminal domain [Timpl et al., 1996]. Type IV collagen is thought to be important in endothelial cell proliferation and migration during the angiogenic process [Kamphaus et al., 2000; Colorado et al., 2000; Madri et al., 1997]. MMP-9 is required for intravasation in an intravasation model. It forms a tight complex with TIMP-1 and TIMP-3. The complex of proMMP-9 and TIMP-1 is a potential inhibitor of MMPs.

C. Tissue Inhibitor of Metalloproteiase:

Tissue Inhibitor of Metalloproteiases (TIMPs) are naturally occurring known inhibitors of matrix metalloproteiases that bind MMPs, they also have a role in the activation of certain MMPs. Proteolytic and biologic activity of MMP is partially regulated by the expression of TIMP. MMP often exist complexed with TIMP, and (previous evidence suggest that this complex forms after their secretion [Nguyen et al., 1998]. In-vivo murine tumor models have shown that invasiveness of certain tumors may be inversely related to tumor cell expression of TIMP-1 [Soloway et al., 1996] and that overexpression of TIMP-3 by tumor cells reduces tumor growth, possibly by its angiostatic activity [Anand-Apte et al., 1996]. In vitro migration of ECs through gelatin is significantly inhibited by overexpressed TIMP-1. TIMPs inhibit MMP activity by a common mechanism involving interaction of the amino-terminal cysteine residue with the zinc atom at the MMP active site. The mechanism of MMP activity associated with tumor invasion and angiogenesis is inhibited by TIMPs, and these
observations led to the development of synthetic MMP inhibitors for potential therapeutic application in cancer.

**TIMP-1:**
TIMP-1 has complex roles in physiological and pathological tissue remodeling. TIMP-1 inhibits endothelial migration but not proliferation by MMP dependent stimulation of endothelial adhesion molecules and MMP-independent dephosphorylation of the focal contact molecules, FAK and paxillin, associated with cytoskeletal changes [Akahane et al., 2004]. While, evidence demonstrates that TIMP-1–blocking antibody enhances endothelial cell migration *in vitro* and angiogenesis *in vivo.*

**TIMP-2:**
Baker et al 2002 reported that TIMP-2, inhibit EC migration and invasion. TIMP-2 is a 21-kDa non-glycosylated protein. TIMP-2 has been shown to suppress tumor growth and metastatic potential in many cell model systems. In addition, TIMP-2 modulates other relevant aspects of the metastatic phenotype including cell proliferation and, most interestingly, cell survival [Valente et al., 1998]. They consist of 184–194 amino acids and have an N-and C-terminal domain. Each containing three conserved disulfide bonds and inhibits the MMPs proteolytic activity by forming non-covalent 1:1 stoichiometric complexes. TIMP-2 bind to the catalytic site of activated MMPs, and coordination of the zinc atom of the MMP active site by the amino group of the NH2-terminal cysteine and the antiangiogenic activity to the COOH-terminal domain of the TIMP results in protease inhibition [Baker et al., 2002] and Several reports have demonstrated that TIMP-2 can directly inhibit the proliferation of endothelial cells in response to angiogenic stimuli such as fibroblast growth factor 2 or VEGF-A [Fernandez et al., 2003]; this effect is independent of MMP inhibitory activity and is not observed with other members of the TIMP family or synthetic MMP inhibitors. Recently it has demonstrated that the growth-inhibitory activity of TIMP-2 for hMVECs is mediated through binding to α3β1 integrin and induction of protein tyrosine phosphatase activity [Seo et al., 2003; Junseo et al., 2004].

**TIMP-3:**
TIMP-3 inhibits TNF-α converting enzyme and induces apoptosis in several cell culture systems including rat vascular smooth muscle cells, as well as tumor cell lines [Baker et al., 1999]. Similarly like TIMP-2 in 2003Spurbeck et al has been demonstrated that TIMP-3 inhibits capillary morphogenesis *in vivo* and endothelial cell migration *in vitro* mediated by inhibition of MMP activity. However, TIMP-3 has also been shown that it function as an antagonist for the vascular endothelial growth factor receptor (VEGFR)-2, and inhibits binding of vascular endothelial growth factor (VEGF)-A to there receptor.
**TIMP4:**
Like all the above TIMPs, TIMP-4 has been shown to be potential antiangiogenic agent because it is able to inhibit tumor growth, metastasis and angiogenesis in a of mammary tumor genesis in mice xenograft model [Wang et al., 2001].

**Role of Adhesion Molecules in Glioma Angiogenesis**

Beside cell-matrix receptors (MMPs), cell-cell contact mediating by adhesion receptors play a crucial role in cell invasion and especially facilitate transendothelial migration of tumor cells as well as leukocytes and passages of macromolecules. Migration of EC as well as leukocytes is a critical step in the tumor angiogenic process and is dependent on coordinated interactions at cell–cell junctions and cell–ECM adhesion sites. During this process one of the first events that probably occurs is the deterioration of stable cell-cell contacts between endothelial cells in the blood vessels and the transition of a quiescent stationary to a dynamic migratory endothelial cell [Folkman et al., 1977]. Furthermore, areas of replicating endothelial cells exhibit increased vascular permeability [Schwartz et al., 1978]. Cell-cell adhesion involves a variety of molecules, including the immunoglobulin superfamily member platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31), CD34, cadherin-catenin complex and CD44 (trans-membrane glycoproteins). It has been reported that αvβ3 integrin as well as CD24 (glycosylphatidylinositol-anchored) protein facilitates tumor cell transmigration and enhances tumor cell invasion in *in vitro* condition [Mierke et al., 2008]. Endothelial cells expressed PECAM-1 and L1-CAM have been described as counter-receptors that interact with cancer cell αvβ3 integrin [Montgomery et al., 1996; Buckley et al., 1996] and also a loss of E-cadherin is important for cell invasion and has recently been discovered as a tumor suppressor gene [Humphries et al., 2007]. The α4β1 integrin interacts either with VCAM-1 enhance tumor cell migration or invasion [Klemke et al., 2007]. However, their role in tumor progression is not always fixed and till date there were several adhesion molecules so far have been discovered which known as angiogenic regulator. These are

**I. Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1)/CD31:**
Among the several critical steps of angiogenesis losing of cell-cell junctions is one of the most critical steps which enabling cell migration and invasion through the underlying and surrounding matrix followed by cell proliferation, matrix deposition and again formation of stable cell-cell contacts. These activities are strictly controlled to ensure normal development of blood vessels. The adhesive interactions of endothelial cells by PECAM-1 which is constitutively expressed in most vessels types in pathological angiogenesis such as tumor dissemination and diabetic retinopathy. CD31 is used as a
phenotypic protein expression marker that can be used to label and distinguish the endothelium in complex cell population. While, with the used of antibody CD31, angiogenesis can be quantified in tumor tissue using direct counts of the vessel surface area including small and large vessels or of the microvessel density after immunostaining the tissue section. Expression of CD31 plays a key role in cellular interactions, particularly in adhesion between ECs during angiogenesis and polymorphonuclear leukocytes, monocytes, as well as on lymphocytes during inflammatory condition. Its localization at the endothelial cell junctions helping in transendothelial cellular migration and hold cells together. In Bogen et al 1994; Muller et al 1986; have shown that CD31 only localized cell-cell contacts and to apical, lumen-facing, cell areas, where it participates in mediating immune-cell transmigration through the endothelium. In 1995 Whitehead et al reported that PECAM-1 induced phosphorylation of β-catenin on their tyrosine residues give endothelium stability which is required during the transmigration. In addiation in 1997 Lu et al reported that PECAM-1 exhibits an immunoreceptor tyrosine based activation (ITAM) motif.

II. CD34:
CD34 is a cell surface transmembrane glycoprotein and is a novel marker to select EC. Apart from quiescent EC, CD34 is also expressed by hematopoietic stem and progenitor cells [Andrews et al., 1986; Krause et al., 1996; Nielsen et al., 2009]. CD34 triggering the functional shift of quiescent endothelial cells into tip cells and helps in migration and proliferation of EC during sprouting angiogenesis which is key event in glioma angiogenesis. In order to promote vascular network expansion, sprouting angiogenesis requires a subset of specialized endothelial cells while the majority of cells remain quiescent in the pre-existing blood vessel. Among these highly specialized cells are tip cells, which coordinate multiple critical processes in a hierarchical way. Located at the leading edge of the vascular sprout, tip cells form cellular protrusions or filopodia to guide migration towards a source of angiogenic growth factors [Gerhardt et al., 2003]. Simultaneously, they signal to adjacent endothelial cells via Delta-like ligand (DLL)-Notch interactions not to adapt the tip cell phenotype, but to maintain the proliferative stalk cell phenotype and to form a vascular lumen. Because tip cells comprise a distinct subpopulation of endothelial cells with a unique molecular signature, they constitute an attractive target for pro- and anti-angiogenic therapy. CD34-positive human umbilical vein endothelial cells show low proliferation activity and increased mRNA expression of all known tip cell markers, as compared to CD34-negative cells. Genome-wide mRNA profiling analysis of CD34-positive endothelial cells demonstrated enrichment for biological functions related to angiogenesis and migration, whereas CD34-negative cells were enriched for functions related to proliferation.
III. Integrin:

Integrins are heterodimeric cell surface receptors (Fig. 4) belonging in immunoglobulin superfamily and mediate adhesion to the ECM [Hynes et al., 1992, 2002; Humphries et al., 2006] molecules. At least 24 distinct integrin have been discovered in heterodimer conditions that are formed by the combination of 18 α-subunits and 8β subunits. The binding of integrins to extracellular matrix (ECM) proteins or, to counter-receptors on adjacent cells, provide cell adhesion strength which is crucial for physiological as well as pathological development, tissue repair, and host defense. Recent findings indicate that other cell types, such as endothelial cells and tumor cells, can also regulate their interaction with extracellular matrix proteins by integrin expression/activation [Byzova et al., 1998; 2006; Dormond & Felding-Habermann et al., 2001]. This regulation may help to control angiogenesis and tumor metastasis. The importance of integrins in tumor progression has made them an appealing target for cancer therapy. The role of integrins in cell migration and invasion is one of their most studied functions in tumor biology by directly binding to the components of the extracellular matrix (ECM) and providing the traction necessary for cell motility and invasion. However, recent study have shown that integrin αvβ3 can bind the MMP-2 in a non–RGD-dependent manner and causes to localize the active form of the enzyme on the surface of EC in growing angiogenic blood vessels. This enables quiescent/angiogenic ECs to degrade and remodel the ECM during their invasion. However, after MMP-dependent proteolytic cleavage of collagen, these RGD sites are exposed and become ligated to αvβ3. Apart from invasion integrins have the ability to either enhance cell survival through several mechanisms, including increased expression of BCl-2 [Matter et al., 2001; Uhm et al., 1999] activation of the PI3K–AKT pathway [Aoudjit et al., 2001] or nuclear factor-κB (nF-κB) [Scatena et al., 1998; Courter et al., 2005] inactivation of p53 [Bao et al., 2000] and also unligated integrins can promote anti-apoptotic cascades and inhibit apoptosis.
Figure 4: αv Integrin expressed on multiple cell types contribute to angiogenesis and tumor progression. Sprouting ECs express a unique profile of integrins that can be targeted to suppress vascular proliferation. Proteolytic degradation of extracellular matrix proteins expose integrins binding site expressed by angiogenic ECs. Binding of Integrin yo Matrix protein enhance their (ECs) ability to migrate, invade metastasize and survive in hostile environments.
## Table 3: Integrin in cancer Progression

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Integrins expressed</th>
<th>Associated phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliobastoma</td>
<td>αvβ3 and αvβ5</td>
<td>Both are expressed at the tumor cell and have a role in cell invasion</td>
</tr>
<tr>
<td>Melanoma</td>
<td>αvβ3 and α5β1</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Breast</td>
<td>αvβ3 and α6β4</td>
<td>Increased tumor size, grade and decreased survival</td>
</tr>
<tr>
<td>Prostate</td>
<td>αvβ3</td>
<td>Increased bone metastasis</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>αvβ3</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Ovarian</td>
<td>α4β1 and αvβ3</td>
<td>Increased potential metastasis and tumor proliferation</td>
</tr>
<tr>
<td>Cervical</td>
<td>αvβ3 and αvβ6</td>
<td>Decreased patient survival</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>α5β1</td>
<td>Decreased patient survival with lymph node-negative tumors</td>
</tr>
<tr>
<td>Colon Cancer</td>
<td>αvβ6</td>
<td>Reduced patient survival</td>
</tr>
</tbody>
</table>

Desgrosellier et al., 2010
IV. VE-Cadherin /CD144:
Vascular Endothelial Cadherin (VE-cadherin) is homophilic transmembrane adhesion molecule that bridges adjacent endothelial cells. The cytoplasmic tail of VE cadherin associate with beta and gamma catenin which belongs to armadillo proteins family [Dejana et al., 1999; Vestweber et al., 2007]. This association holds the endothelial cells tightly together; the endothelial layer functions as a barrier for macromolecules and leukocytes and thereby protects the underlying tissue from damage in pathological condition such as inflammation and tumor development. However, during inflammation and angiogenesis redistribution of VE cadherin regulate the initiation and maturation of newly formed vessels, this initiate the traffic of leukocytes across the endothelial layer through the cell-cell junctions and simultaneously, the initiation of angiogenesis occurs when the continuity of the endothelial layer is interrupted due to the loosening of the cell-cell contacts enabling the endothelial cells to proliferate and migrate to the free area [Ozawa et al., 1998; Allport et al., 2000]. During maturation phase or later stage of angiogenesis it is now essential that the endothelial cells establish the intercellular contacts in order to maintain the morphological integrity and quiescence of the newly formed vessel. It thus appears that the molecular mechanisms that govern formation and stabilization of cell-cell contact and inflammation are suitably coordinated and regulated according to their need and time to time. In support to this hypothesis recent efforts have shown that functional link between cell-cell adhesion and phosphorylation of VE cadherin at their cytoplasmic residues by inflammatory molecules such as TNF-α provided the information about VE-cadherin which regulates the vascular permeability.

V. β-catenin:
The catenins are thought to play an important role in the regulation of VE-cadherin function and in their strength. Lampugnani and co-workers showed that the expression VE cadherin and catenins was increased in loosely confluent endothelial monolayers after the phosphorylation while in tightly confluent monolayers the phosphorylation is diminished. Followed by these results several other investigations on the signaling pathways of downstream of VE-cadherin revealed that during initial stages the expression/ level of β-catenin in the nucleus of endothelial cells in culture was increased and decrease in its levels in the nucleus, associated with an increase in the cytosol during later stages of culture.VE-cadherin was initially considered as a constitutive protein with downregulated expression in angiogenic transition. However, this issue has to be reconsidered in view of several studies in few years. In 2004 first time Parker et al challenging the previous assumption and showed that the mRNA expression of VE cadherin was elevated in endothelial cells of human breast cancer compared to normal vasculature. While other results recently reported that increased VE cadherin promoter activity
was also observed in mouse tumors and in matrigel plugs impregnated with bFGF [Prandini et al., 2005], suggesting that bFGF might be the angiogenic factor responsible of VE-cadherin transcription enhancement. Apart from VE cadherin stabilization, expression of β-catenin increases the transcription of the Notch ligand Delta-like (Dll)4, which, in turn, activates Notch-1 and -4 [Dejana et al., 2010].

VII. CD44:
CD44 is a transmembrane glycoprotein which is used as tumor marker for progression and certain types of cancer. The expression of CD44 is highly regulated phenomenon and is usually not expressed in normal condition [Mackay et al., 1994; Naor et al., 1997]. CD44 is played great role in cell adhesion with proposed functions in extracellular matrix binding, leukocyte homing and activation, and metastasis formation [Aruffo et al., 1990; Jalkanen et al., 1986]. It has been found that after post translational modification CD44 has shown great molecular heterogeneity on their cell surface. HA is the principal ligand for CD44 and binding of CD44 to HA is dependent on the glycosylation state [Bartolazzi et al., 1996; Bennett et al., 1995] and activational processes [Lesley et al., 1992].

Previously it has been shown that CD44 and its ligand mediate rolling of lymphocytes on the EC layer under pathological flow conditions, which potentiates a novel lymphocyte-EC adhesion pathway [DeGrendel et al., 1996]. Earlier studies about the cellular distribution of CD44 on EC are contentious and have been cited to lack this marker [Alho et al., 1989; Picker et al., 1989]. However, In recent years a number of reports on human endothelium studies have revealed that EC expressed some standard CD44 isoform (CD44s) on their membrane [Mackay et al., 1993; Bennett et al., 1995] which might be of advantage for leukocyte infiltration in inflamed tissue [Bennett et al., 1995]. Recently the effects of CD44 ligation on cell activation have been clarified and indicated that CD44 signaling involves tyrosine kinases which directly regulate cell activation steps [Taher et al., 1996] and poor prognosis in cancer in a variety of different systems. The upregulation of different isoforms of CD44 such as CD44v and CD44s that expressed on human endothelial during angiogenesis, triggering the downstream signaling leading to a synergistic stimulation of EC proliferation, migration, and differentiation.
ROLE OF INFLAMMATORY MOLECULES/CYTOKINE IN TUMOR ANGIOGENESIS

Angiogenesis and inflammation are frequently coupled in pathological situations such as arthritis, atherosclerosis, diabetes and cancer. One of the hallmarks of inflammation, resulting from either local injury or, systemic stimuli is an increase in vascular permeability and induces endothelial cell activation, frequently driven by an excess of nitric oxide, VEGF, or other mediators which, when persistent, results in capillary sprouting. This inflammation-induced angiogenesis and the subsequent remodeling steps are in large part mediated by extracellular matrix (ECM) proteins and proteases. The focal increase in capillary permeability is an early consequence of inflammation, and results in the deposition of a provisional fibrin matrix. Subsequently, ECM turnover by proteases permits an invasive program by specialized endothelial cells whose phenotype can be regulated by inflammatory stimuli. ECM activity also provides specific mechanical forces, releases biologically active fragments (matrikines) and exposes cryptic adhesion sites, and matrix-sequestered growth factors, all of which are critical for vascular morphogenesis. Two of the chief consequences of this are the formation of a provisional matrix from plasma proteins and the exit of leucocytes to the subendothelial space, initiating and sustaining the inflammatory response. The resulting acute inflammation can induce an angiogenic response, producing a highly vascularized granulation tissue, as occurs during wound healing.

In the tumor microenvironment, inflammatory cells, namely microglia and lymphocytes regulating the tumor angiogenesis by secreting pro- (TNF-α, IL-1 α, IL-6, & IL-8) and anti-inflammatory (IL-4 & IL10) cytokines, that could control endothelial cell (EC) proliferation, their survival and apoptosis, as well as their migration and activation [Lingen et al., 2001]. Generally speaking, pro-inflammatory mediators promote angiogenesis mediated by some stimulus like IL-1 and TNF-α [Wilson et al., 2002]. However, there are exceptions: indeed, it has been extensively reported that other pro-inflammatory cytokines, such as interferon IL-12 and (IFN)–γ are associated with an anti-angiogenic properties [Naldini et al., 2003]. Whereas IL-4 and IL-10 has the pleiotropic ability of influencing negatively and positively the function of innate and adaptive immunity in different experimental animal models have been reported [Simone et al., 2005].

1. TNF-α:
TNF-α is a low molecular weight of (17 kDa) polypeptide and is a potent pro-inflammatory cytokine, shown to be secreted by macrophage and other cells also with a major role in initiating a cascade of activation of other cytokines and growth factors in tumor and in inflammatory responses [Li et al., 2002]. In the CNS system TNF- α is synthesized by astrocytes, microglia and some populations of
other cells [McCoy et al., 2008]. Previous reports have shown that in tumor microenvironment TNF-α promotes angiogenesis via ephrin A1 expression in EC [Cheng et al., 2001] and it also triggers NF-κB activation and translocation via phosphorylation of IκB, a critical step for NF-κB activation and results in expression of genes involved in inflammation and cellular proliferation. Systemic spillover of TNF-α may account for unwanted inflammatory effects like fever and wasting. However, previously it had been found that this cytokine synthesis is necessary for healing of wounds, but excessive amounts of this cytokine exerted in non-healing wounds such as ulcers and/or inflammatory diseases [Cooney et al., 1997; Garner et al., 1993; Trengove et al., 2000]. Taken together, accumulated data suggest that, during normal healing of wound it requires some involvement of inflammatory cytokines while increased amounts of these mediators cause some abnormal wound healing. Different animal experiments have shown that differing roles of cytokines depending on the dosage. For example, high concentrations of TNF-α impair angiogenesis [Fajardo et al., 1992] and provide the evidence that TNF-α might stimulate tumor development by promoting vessel growth and participate in tumor development by changing the endothelial layer function. In 2000 Han et al have shown that TNF-α may exhibit matrix remodeling through NF-κB mediated induction of MMP-2 expression during pathological processes.

2. Nuclear Factor-κB:

Nuclear factor-κB (NF-κB) is a proinflammatory transcription factor that has emerged as an important player in the development and progression of malignant cancers and was first identified by Sen and Baltimore in 1986. NF-κB is a cytoplasmic protein which binds to IκB. Binding of IκB to NF-κB causes the cytoplasmic retention by blocking nuclear localization of NF-κB. Generally activation of NF-κB has shown to be stimulus dependent, which when activated upregulates its target genes via transcription. Most agents that activate NF-κB activity causes the phosphorylation of IκB followed by degradation. Once the phosphorylation occurs at two conserved serine residues (S32 and S36) of IκB in its N-terminal regulatory domain, immediately it undergoes post-translational modification called polyubiquitination and release the NF κB for its activation and translocation to nucleus. Activation of NF-κB protects the tumor cell from apoptosis [Duffey & Bentires et al., 1999]. Previous reports showing that in in vitro model inhibition of NF-κB in cancer cells is not only restricted by their inhibitors, even it also occur by other mechanisms, such as inhibition of cell adhesion [Pajonk et al., 1999], inhibition of proinflammatory cytokine production [Higgins et al., 1993], or inhibition of plasminogen activator and matrix metalloproteinase [Wang et al., 1999], which contribute to neoplastic angiogenesis, growth and metastasis.
3. Interleukine-8:
Il-8, a member of the cytokine family, has been shown to play an important role in pathological angiogenesis, tumor growth and metastasis. Recent reports suggested that in gliomas condition, IL-8 is expressed and secreted at high levels both in vitro and in vivo and is critical to glial tumor neovascularity and progression. Generally, Interleukin-8 is a member of the chemokine family with important CXC amino acid motif. The chemokines are kind of specialized cytokines secreted by a variety of normal and neoplastic cell types, which have been defined by their ability to cause directed migration of leukocytes. They are generally secreted in response to growth factors, inflammatory cytokines, and pathophysiologic conditions [Matsushima et al., 1989; Walz et al., 1987; Yoshimura et al., 1987]. Previously it has been found that it plays an important role in inflammatory, infectious, and autoimmune diseases [Smyth et al., 1991; Koch et al., 1992; Harada et al., 1994]. Because of its potent pro-inflammatory properties, expression of IL-8 is either low or undetectable in normal tissues have been found. The receptors which is present on EC for IL-8 are CXCR1 and CXCR2, [Murdoch et al., 1999; Salcedo et al., 2000] and have been shown to play a important role in endothelial cell proliferation [Koch et al., 1992] and capillary tube organization in a concentration-dependent manner, which can be blocked by neutralizing anti-IL-8 Abs, suggesting that IL-8 promotes angiogenesis by directly interacting with endothelial cells. Transcriptional regulation of IL-8 synthesis incorporates signals from diverse intracellular signaling pathways and appears to differ according to whether the stimulus for upregulation is a cytokine or cellular stress [Holtmann et al., 1999; 2001]. Transcriptional stimulation of the IL-8 gene by the cytokines TNF-α or IL-1 involves a fragment of the promoter (-1 to -133) that includes the NF-κB and NF-IL-6 binding sequences in promoter region. Even, transcription of the IL-8 gene is also upregulated by oxidative stress, and this type of stimulation appears to depend largely on the AP-1 binding site [Lakshminarayanan et al., 1998].

4. Interleukin -6:
Interleukin 6 is another multifunctional cytokine which belong to the family of inflammatory cytokines. In human glioblastoma cell line IL-6 acts as a growth and survival factor which plays an important role in malignant progression [Liu et al., 2010]. This cytokine is produced by a variety of cells after stimulation in certain environment such as trauma, immunological challenge and infection. The main sources of IL-6 in vivo are monocytes, fibroblasts and endothelial cells. Even macrophages, T-cell and B-Cells also produce IL6 after stimulation. Several groups have reported that in pathological conditions, stress released stimuli such as TNF-α and lipopolysaccharide induce the activation of the IL-6 gene through the phosphorylation of NF-κB [Yamaguchi et al., 2009; Shimizu., Libermann., Zhang et al., 1990]. The IL6 gene promoter contains many different regulatory elements
allowing the induction of expression by various stimuli, including glucocorticoids and cAMP. In non-lymphoid cells the NF-kappa-B binding site is responsible for the induction of the IL6 gene expression by IL1 or TNF. In 2001 Faruqi et al proposed a theory about the IL6 autocrine signalling. He proposed an ‘inside-out’ rather than an ‘outside in’ signalling model for NF-kB activation initiated by Rac1-protein that leads to IL-6 gene expression. Rac1 is activated by hypoxia and required for HIF-1a expression and transcriptional activity [Hirota and Semenza et al., 2001], a condition commonly found in GBM. In their model, the secreted IL-6 then binds to its receptor and activates the JAK/STAT pathway. Tyrosine-phosphorylated STAT3 proteins dimerize, translocate to the nucleus and bind to DNA target sites of responsive genes inducing a genetic programme of oncogenic transformation. Whereas, in lymphoid cells a factor related to the reloncogene (IL6kB binding factor II) functions as a repressor that prevents the interaction of transcription factors with the IL6-kappa-B binding site. Evidence from previous research has shown that IL-6 promotes cellular invasion and migration in glioblastoma cell lines such as U251 and T98G by both protease-dependent and -independent manners. The mechanism involves invasion and migration of tumor cells after phosphorylation of STAT3 at ser 727 site and deterred the invasion and migration after use of pathway blocker (JSI-124), indicating that the IL-6 induce activation of JAK/STAT3 pathway, mediating signal transduction and promoting vascular endothelial cell migration and invasion, thus facilitating tumor angiogenesis [Liu et al., 2010]. Furthermore, this IL-6 trans signaling also enhance the expression of some endothelial leukocyte adhesion molecules such as ICAM-1 and VCAM-1, and promoting leukocyte accumulation [Ni et al., 2004; Hurst et al., 2001] at the site of inflammation.

5. **Transforming growth factor-β1:**

Transforming growth factor-betas (TGF-βs) are multifunctional polypeptides that regulate cell growth and differentiation, ECM deposition, cellular adhesion properties, angiogenesis and immune functions. In mammalian cells, there are three subtypes of TGF-β ligands, β1, β2, and β3. They are encoded by separate genes, but signal through the same signaling cascade. TGF-β binds to a transmembrane, heteromeric complex of serine/threonine kinases, which are comprised of “type I” and “type II” receptors. Once the TGF-β ligand binds to the receptor complex, TGF-β type II receptor (TβRII) phosphorylates the GS domain of the type I receptor (TβRI). This in turn activates TβRI, which then autophosphorylates itself and phosphorylates downstream target proteins [Roberts et al., 1990; Derynck et al., 2001]. TGF-β1 is a potent inducer of pathological angiogenesis by their pleiotropic activity secreted by macrophages and microglia cells during tumor microenvironment. It can induce capillary formation of endothelial cells cultured on collagen matrix [Fransvea et al., 2009; Madri et al., 1988] and it creates favorable environmental condition for upregulation of MMP-2 and MMP-9 in ECs
[Behzadian et al., 2001; Derynck et al., 2001]. TGF-β1 acts through the TGF-β type I and type II receptors to activate intracellular mediators, such as Smad proteins, the p38MAPK, and the ERK pathway [Laping et al., 2002]. TGF-β secreted by most cultured cells is in biologically inactive form, and cannot bind TGF-β receptors; the latent TGF-β is activated by proteases such as plasmin and cathepsin D, low pH, chaotropic agents such as urea, and heat [Lawrence et al., 1985; Lyons et al., 1988]. Several studies suggested that VEGF increases plasminogen activator (PA) activity in vascular ECs and that plasmin is able to activate latent TGF-β1 which decreases Flk-1 expression and thereby negatively regulates the VEGF/Flk-1 signal transduction pathway in EC, raise the possibility that a complex self-regulating mechanism of VEGF signal transduction may exist during angiogenesis. In addition, TGF-β1 inhibits the generation of the anti-angiogenic molecule angiostatin by human pancreatic cancer cells in a time- and dose-dependent manner, and this effect is mediated through modulation of the plasminogen/plasmin system. Furthermore, TGF-β indirectly stimulates angiogenesis by the recruitment of inflammatory mediators that secrete angiogenic factors. Thus, TGF-β regulates vascular remodeling through its pleiotropic effects on different cell types.

6. Interleukin-4:

IL-4 is a crucial modulator of the immune system secreted by Th2 cells, mast cells and an active antitumor agent. Its role as an anti-inflammatory agent in the inflammatory condition has emerged recently. The receptor/ligand which mediates the IL-4 signaling called IL-4R α-chain (IL-4Rα) expressed on hematopoietic cells and nonhematopoietic cells [Nelms et al., 1999; Callard et al., 1996]. Once IL-4 binding to its ligand, causes the IL-4Rα dimerization either with the common γ-chain to produce the type 1 signaling complex, located mainly on hematopoietic cells, or with the IL-13Rα1 to produce the type 2 complexes, which is expressed on nonhematopoietic cells. In hematopoietic cells IL-4 signaling upregulate JAK/STAT pathway which promote survival and growth through the PI3/AKT, PKB/mTOR, and other pathways [Luzina et al., 2012]. In contrast, in 1997 Saleh et al have shown that tumors which express IL-4 have a reduced the level of vascular density. The type 2 receptors also signal through JAK family kinases (JAK1 and TYK2) but are expressed by nonhematopoietic cells such as fibroblasts and endothelial cells. Binding of IL-4 on their respective receptor produces the familiar signaling cascade like type 1. While, in some other studies on IL-4 in macrophages revealed that it act as an anti-inflammatory agent against inflammatory stimulus TNF-α [Hart et al., 1989; Wong et al., 1992; Suzuki et., 1993] and decreasing subsequent production of nitric oxide (NO) from astrocytes cells after LPS stimulation. In in vivo when IL-4 incorporated at concentrations of 10ng/ml or more into the rat cornea or when delivered systemically to the mouse by intraperitoneal injection, it blocked the induction of corneal neovascularization by basic fibroblast
growth factor [Volpert et al., 1998]. In glioma inflammatory condition some interleukins such as IL-6 which found abundantly in inflamed tissues sites induce vascular cell adhesion molecule 1 (VCAM-1) in the microvascular cells that give rise to new vessels during tumor angiogenesis where as IL-4 exerts revere effects [Swerlick et al., 1992. Haraldsen et al., 1996; Petzelbauer et al., 1993], and deprees the expression of VCAM-1 in the microvessels [Bergese et al., 1995]. In support these above findings other group also have shown that in in vitro IL-4 inhibits angiogenesis by acting directly on endothelial cells [Bouck et al., 1996] and inhibit the proliferation, chemotaxis, and tube formation of HUVEC that were stimulated by VEGF or bFGF [Lee et al., 2002]. IL-4 inhibitory function exhibited in the two critical steps of angiogenesis 1) inhibit the migration of endothelial cells to the inflamed site and 2) inhibit the differentiation of migrated cells to the organized vessel structure [Lee et al., 2002]. Despite its effects on migration, IL-4 did not appear to have any effect on the growth of endothelial cells. While, at very low doses, IL-4 was able to induce a weak neovascularization response in vivo this occurs with the inhibitor of angiogenesis thrombospondin-1 and can be explained by the sequential activation of two distinct receptors. In addition, now the abundance of data strongly suggests that IL-4 has an anti-inflammatory function [Röcken et al., 1996] and it act as modest mitogen for both large vessel endothelial cells and microvascular [Toi et al., 1991].So these results suggest that IL-4 exhibits antiangiogenic properties under certain conditions.

7. Interleukin-10:
IL-10 is another anti-inflammatory cytokine; it plays an important role in scheming tumor growth and metastasis. Via indirect effects on the immune system the effects of IL-10 on tumor growth have varied from modulating tumor growth to inhibiting tumor angiogenesis and metastasis. In 1996 Huang et al evidence that IL-10 transfected cDNA in melanoma cells (A375P) exhibited reduction of tumor growth and with a significant inhibition of neovascularity in growing tumor vessels. Moreover, in similar kind of studies by Richter et al in 1993 reported that induction of IL-10 blocked tumor growth by blocking angiogenesis and macrophage penetration of the tumor tissue. In in vitro model treatment of melanoma cells by IL-10 was down-regulate expression of VEGF, TNF-α, IL-6, IL-1b, and MMP-9 and not only that production of pro-inflammatory cytokines such as IL-6 and TNF-α was low in macrophages cells stimulated in the presence of IL-10, IL-4, or IL-13 [Dace et al., 2008]. In addition results from the other studies presented that IL-10 upregulated TIMP-1and down regulated MMP-2 to block, angiogenesis, growth and metastasis of tumor.
ANTI-ANGIOGENIC TREATMENT OF CANCER

The observation that angiogenesis occurs around tumors was made nearly 100 years ago. The hypothesis that tumors produce a diffusible ‘angiogenic’ substance was put forward in 1968. In 1971, Folkman proposed that tumor growth and metastasis are angiogenesis-dependent, and hence, blocking angiogenesis could be a good strategy to arrest tumor growth. This possibility stimulated an intensive search for pro- and anti-angiogenic molecules. In 1976, Gullino showed that cells in pre-cancerous tissue acquire angiogenic capacity on their way to becoming cancerous. He proposed that this concept be used to design strategies to prevent cancer, a hypothesis later confirmed by genetic approaches and later on treatment of tumors by anti-angiogenic agent is a highly promising therapeutic avenue shown by Fidler et al in 1994. Thus, for over last couple of years, there has been a robust activity aimed towards the discovery of angiogenesis inhibitors [Ruegg et al., 2007; Verhoef et al., 2006]. Since then, more or less about forty anti angiogenic drugs are being tested in clinical trials all over the world in human cancer patients. These are:

I. Bevacizumab (Avastin):
Bevacizumab is a neutralizing monoclonal antibody that received first FDA approval as angiogenesis inhibitor in 2004 a drug that slows the growth of new blood vessels. It is licensed to treat various cancers, including colorectal, lung, breast (outside the USA), glioblastoma (USA only), kidney and ovarian. It prevents the interaction between VEGF to VEGFR on endothelial cells and thereby causes the inhibition of ECs proliferation and angiogenesis. It has been found that during the clinical trial in phase I studies it helps in improvement of metastatic colorectal cancer and non-small cell lung cancer when administered in combination with chemotherapy [Arkenau et al., 2009; Herbst et al., 2007]. Whereas, in combination with irinotecan Bevacizumab significantly increase median progression-free survival (PFS) in glioma patients. Bevacizumab, in glioma patients imparts deleterious side effects including hypertension, bleeding and gastrointestinal perforation [Fong et al., 2003]. Resistance to bevacizumab leads to rapid deterioration of patients and recurrent glioma arising after bevacizumab failure is far more aggressive [Gupta et al., 2007].

II. Cetuximab (Erbitux):
Cetuximab is a monoclonal antibody preventing cell proliferation and angiogenesis by hindering the ligand binding and activation of epidermal growth factor receptor (EGFR), and causing internalization and degradation of the receptor. Previously it has been found that in human colorectal carcinoma (CRC) cell line and in human CRC mouse xenografts models Cetuximab causes the downregulation of EGFR expression in a dose-dependent manner [Petit et al., 1997]. This treatment is approved for metastatic CRC and head and neck cancer in patients who are refractory to irinotecan-based
chemotherapy. Cetuximab has also been shown to sensitize cells to radiation and chemotherapy, potentially through blocking EGFR.

One of the more side effects of cetuximab therapy is the incidence of acne-like rash. This rash rarely leads to dose reductions or termination of therapy. It is generally reversible. Further, severe infusion reactions include fevers, chills, rigors, urticaria, pruritis, rash, hypotension, N/V, HA, bronchospasm, dyspnea, wheezing, angioedema, dizziness, anaphylaxis, and cardiac arrest. Therefore, pretreatment with diphenhydramine 30-60 min before administration is standard care. Other common side effects include photosensitivity, hypomagnesemia due to magnesium wasting and less commonly pulmonary and cardiac toxicity.

III. Trastuzumab (Herceptin):
Trastuzumab is a humanized monoclonal antibody that binds the extracellular domain of HER-2, which is overexpressed in 25-30% of invasive breast cancer tumors [Nichols et al., 2002] with high recurrence rate, poorer prognosis with decreased survival compared with HER2-negative breast cancer [Dean-Colomb et al., 2008]. This is the first humanized antibody for Breast cancer patients approved by the FDI in 1998 [Nahta et al., 2007]. The previous studies of trastuzumab showed that it improved overall survival in late-stage (metastatic) breast cancer from 20.3 to 25.1 months. In early stage breast cancer, it reduces the risk of cancer returning after surgery by an absolute risk of 9.5%, and the risk of death by an absolute risk of 3% however increases serious heart problems by an absolute risk of 2.1% which may recur if treatment is stopped. Trastuzumab is also being studied in the treatment of other types of cancers such as lung, pancreatic endometrial, cervical and ovarian cancer.

One of the significant complications of trastuzumab is its effect on the heart. Trastuzumab is associated with cardiac dysfunction in 2-7% of cases. As a result, regular cardiac screening with either a MUGA scan or echocardiography is commonly undertaken during the trastuzumab treatment period. Trastuzumab downregulates neuregulin-1 (NRG-1), essential for the activation of cell survival pathways in cardiomyocytes and the maintenance of cardiac function. NRG-1 activates the MAPK pathway and the PI3K/AKT pathway as well as focal adhesion kinases (FAK). These are all significant for the function and structure of cardiomyocytes. Trastuzumab can therefore lead to cardiac dysfunction. Approximately 10% of patients are unable to tolerate this drug because of pre-existing heart problems; physicians are balancing the risk of recurrent cancer against the higher risk of death due to cardiac disease in this population. The risk of cardiomyopathy is increased when trastuzumab is combined with anthracycline chemotherapy (which itself is associated with cardiac toxicity).
IV. Sunitinib (Sutent):
Sunitinib inhibits multiple tyrosine kinases receptor such as VEGFR-1, VEGFR-2, VEGFR-3, and PDGFR-β [Izzedine et al., 2007]. It is approved by FDA as Sunitinib malate for treating advanced renal cell carcinoma [Rock et al., 2007] and also for gastrointestinal stromal tumor (GIST) in patients whose disease has progressed or who are unable to tolerate treatment with imatinib [Rock et al., 200; Goodman et al., 2007]. In Phase II trials it has been found that Sunitinib possess as an anti-tumor activity in metastatic hepatocellular carcinoma in US, European and Asian countries of the patients. According to the NCI clinical trial results, Sunitinib is currently evaluated for breast [Park et al., 2008], ovarian [Taran et al., 2008], and non small cell lung cancer [Socinski et al., 2008].
Sunitinib has been generally well tolerated. Adverse events were considered somewhat manageable and the incidence of serious adverse events low. The most common adverse events associated with sunitinib therapy are fatigue, diarrhea, nausea, anorexia, hypertension, a yellow skin discoloration, hand-foot skin reaction, and stomatitis. In the placebo-controlled Phase III GIST study, adverse events which occurred more often with sunitinib than placebo included diarrhea, anorexia, skin discoloration, mucositis/stomatitis, asthenia, altered taste, and constipation. In grade 3 or 4 serious adverse events occur in ≤10% of patients including hypertension, fatigue, asthenia, diarrhea, and chemotherapy-induced acral erythema. Lab abnormalities associated with sunitinib therapy include lipase, amylase, neutrophils, lymphocytes, and platelets. Hypothyroidism and reversible erythrocytosis have also been associated with sunitinib.

V. Erlotinib (Tarceva):
Erlotinib is an orally available small molecule which inhibits the EGFR tyrosine kinase activity. Erlotinib hydrochloride is a drug used to treat non-small cell lung cancer, pancreatic cancer and several other types of cancer. Erlotinib specifically targets the adenosine triphosphate (ATP) of the epidermal growth factor receptor. For the signal to be transmitted, two EGFR molecules need to come together to form a homodimer. These then use the molecule of ATP to trans-phosphorylate each other on tyrosine residues, which generates phosphotyrosine residues, recruiting the phosphotyrosine-binding proteins to EGFR to assemble protein complexes that transduce signal cascades to the nucleus or activate other cellular biochemical processes. By inhibiting the ATP, formation of phosphotyrosine residues in EGFR is not possible and the signal cascades are not initiated. It has been approved by US FDA for treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen [Cohen et al., 2005, Erlotinib et al., 2003]. Erlotinib has shown a survival benefit in the treatment of lung cancer in phase III trials. Interesting recent studies have demonstrated that Erlotinib and Bevacizumab act on two different pathways critical to tumor growth and
dissemination, administering these drugs concomitantly may confer additional clinical benefits to cancer patients with advanced disease. Erlotinib induction is also being helpful in the treatment of other types of cancers such as breast cancer, hepatocellular carcinoma and in metastatic renal cancer in phase II trials when given in combination with Bevacizumab.

The side effects that occur after Erlotinib therapy in the majority of patients is rashes, Diarrhea, Loss of appetite, Fatigue. Rarely, interstitial pneumonitis, which is characterized by cough and increased dyspnea. This resembles acne and primarily involves the face and neck. It is self-limited and resolves in the majority of cases, even with continued use. Interestingly, some clinical studies have indicated a correlation between the severity of the skin reactions and increased survival, though this has not been quantitatively assessed. This may be severe and must be considered among those patients whose breathing acutely worsens. It has also been suggested that erlotinib can cause hearing loss.

VI. Bortezomib (Velcade):
Bortezomib was originally synthesized in 1995 (MG-341) in a company called Myogenics, which soon changed its name to ProScript. After promising preclinical results, the drug (PS-341) was tested in a small Phase I clinical trial on patients with multiple myeloma cancer. After that it has characterized as an indirect anti-angiogenic agent [Roccaro et al 2006] that disrupts signaling of cancer cells ultimately leading to cell death. In addition it also induces the high quality responses as third line salvage therapy with acceptable toxicity in a significant proportion of homogeneously pre-treated myeloma patients with progressive disease after autologous transplantation and thalidomide [Musto et al., 2006]. In Phase 3 trail Bortezomib increased median improved overall survival and increased response rate. While in cutaneous T cell Lymphomas (CTCL) it was found to be very effective when it supplied in combination with doxorubicin and gemcitabine [Horwitz et al 2008]. The drug is an N-protected dipeptide and can be written as Pyz-Phe-boroLeu, which is forpyrazinoic acid, phenylalanine and Leucine with aboronic acid instead of a carboxylic acid. Peptides are written N-terminus to C-terminus, and this convention is used here even though the "C-terminus" is a boronic acid instead of a carboxylic acid. The boron atom in bortezomib binds the catalytic site of the 26S proteasome with high affinity and specificity. In normal cells, the proteasome regulates protein expression and function by degradation of ubiquitylated proteins, and also cleanses the cell of abnormal or misfolded proteins. Clinical and preclinical data support a role in maintaining the immortal phenotype of myeloma cells, and cell-culture and xenograft data support a similar function in solid tumor cancers. While multiple mechanisms are likely to be involved, proteasome inhibition may prevent degradation of pro-apoptotic factors, permitting activation of programmed cell death in neoplastic cells dependent upon suppression of pro-apoptotic pathways. Recently, it was found that bortezomib caused a rapid and dramatic change in the levels of intracellular peptides that are produced
by the proteasome. Some intracellular peptides have been shown to be biologically active, and so the effect of bortezomib on the levels of intracellular peptides may contribute to the biological and/or side effects of the drug.

Bortezomib is associated with peripheral neuropathy in 30% of patients; occasionally it can be painful. This can be worse in patients with pre-existing neuropathy. In addition, myelosuppression causing neutropenia and thrombocytopenia can also occur and be dose limiting. However, these side effects are usually mild relative to bone marrow transplantation and other treatment options for patients with advanced disease.

VII. Panitumumab (Vectibix):
Panitumumab is another monoclonal antibody that bind specifically to the human EGFR, preventing ligand binding and activation of its receptor resulting in internalization and degradation of the receptor culminating in inhibition of cell proliferation and angiogenesis. This treatment is approved for metastatic CRC and head and neck cancer in patients who are refractory to irinotecan-based chemotherapy.

VIII. Thalidomide (Thalomid):
Thalidomide is an anti-nausea and sedative drug that was introduced in the late 1950s to be used as a sleeping pill, and was quickly discovered to help pregnant women with the effects of morning sickness. It was sold from 1957 until 1962, when it was withdrawn after being found to be a teratogen, which caused many different forms of birth defects. In 1994 Harvard Professor Robert D’Amato at Boston Children's Hospital discovered that thalidomide was a potent inhibitor of new blood vessel growth. Since then, many studies have shown that thalidomide, in combination with dexamethasone, has increased the survival of multiple myeloma patients. The combination of thalidomide and dexamethasone, often in combination with melphalan, is now one of the most common regimens for patients with newly diagnosed multiple myeloma, with an improved response rate of up to 60–70%. Thalidomide has been characterized as an anti-inflammatory, and immunomodulatory agent, although the precise mechanisms of action are not fully understood. It was found to be minimally effective and moderately tolerated in patients with histologically proven advanced hepatocellular carcinoma [Pinter et al., 2008]. Thalidomide provided no survival benefit for patients with multiple, large, or midbrain metastases when combined with WBRT (whole-brain radiation therapy) [Knisely et al., 2008].
Fig. 5: Molecules targeted by angiogenesis inhibitors currently in clinical trials.
Most of the anti-angiogenic agents currently in phase I/II trials for brain tumors are blockers of the vascular endothelial growth factor (VEGF) pathway (see also Table 1). Green boxes highlight molecules known to be targeted by drugs that are currently in clinical trials. VEGF is targeted by bevacizumab, whereas tyrosine kinase inhibitors such as cediranib target mainly VEGF receptors 1–3 (VEGFR1–3), TIE2 (as well as TIE1, which interacts with TIE2) and platelet-derived growth factor receptors α and β (PDGFRα and PDGFRβ). Agents targeting other pathways, such as inhibitors of mammalian target of rapamycin (mTOR) (for example, temsirolimus), SRC or integrins (such as αvβ3 and αvβ5), are also in clinical development for brain tumors. ANG1/2, angiopoietin 1/2; CKII, casein kinase II; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase 1; GSK3β, glycogen synthase kinase-3β; MEK, mitogen-activated protein kinase; NRP1, neuropilin 1; PI3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; PLCγ, phospholipase-Cγ. [Jain et al., 2007]
IX. Thrombospondin-1 (TSP-1):
Thrombospondin-1 (TSP-1) is an extracellular anti-angiogenic factor that is synthesized and secreted by multiple normal cells and tissues, such as platelets, endothelial cells, and smooth muscle cells [Adams et al., 2004; Lawler et al., 2002]. The antiangiogenic effects of TSP-1 treatment have been studied extensively in dermal microvessel ECs propagated in vitro. TSP-1 inhibits the migration and proliferation of these cells, and also induces apoptosis [Jimenez et al., 2000; Volpert et al., 2002; Nor JE et al., 2000]. The most potent anti-angiogenic effects of TSP-1 are induced through the binding of TSP-1 to CD36, which is expressed on the surface of microvessel ECs. This interaction is mediated by the type 1 repeat domain (TSR) of TSP-1 and the CLESH-1 domain of CD36 [Simantov et al., 2003]. Other proteins can modify the TSP-1 response by cooperating with or modulating CD36 function; for example, integrin αvβ3 modulates CD36 function in macrophages [Bottcher et al., 2006].

X. Thrombospondin-2:
TSP-2 is highly homologous to TSP-1 and also exhibits anti-angiogenic properties [Adams et al., 2004; Simantov et al., 2003; Streit et al., 1999]. TSP-2 is expressed by ECs and other cells in the normal mouse brain [Fears et al., 2005], and its expression is downregulated in GBM tumors as compared to normal brain [Kazuno et al., 1999]. Furthermore, mouse malignant glioma tumors propagated orthotopically in the TSP-2-null mouse showed an increased tumor size and an increased microvessel density as compared to tumors propagated in the wild-type mouse [Fears et al., 2005], supporting a host anti-tumor and anti-angiogenesis role for TSP-2 in the brain.

XI. Endostatin and Tumstatin:
Endostatin is naturally occurring anti-angiogenic molecule which can be produced during the proteolytic cleavage of the basement membrane in GBM tumors [Strik et al., 2001; Morimoto et al., 2002]. It is produced by by elastase, cathepsin-L and an MMP at C-terminal cleavage of collagen type XVIII. [Folkman et al., 2006; Heljasvaara et al., 2005]. Interaction of Endostatin with several different receptors presented on the ECs inhibit the angiogenesis through multiple mechanisms, including blockade of VEGFR2, binding to integrin α5β1, blockade of focal adhesion kinase (FAK)-mediated leading to inhibition EC migration and survival [Folkman et al., 2006; Shi et al., 2007; Sudhakar et al., 2003]. Endostatin also can reduce expression of anti-apoptotic Bcl2 family members [Folkman et al., 2006]. In addition, endostatin binds the pro-angiogenic protease MMP-2 and blocks its activation and catalytic activity [Folkman et al., 2006]. Multiple studies in rodent model have shown that when recombinant endostatin is delivered to tumors propagated in an ectopic or an orthotopic manner, or when the endostatin gene is delivered to orthotopic tumors [Barnett et al., 2004; Sorensen et al., 2002] it inhibit the growth of malignant glioma. Another anti-angiogenic molecule derived from the
proteolytic cleavage of the basement membrane is tumstatin. Tumstatin is generated by the cleavage of collagen type IV by MMPs, and is thought to inhibit neo-vessel formation through an interaction with integrin αvβ3 resulting in blockade of FAK, and the PI3K signaling pathway [Sudhakar et al., 2003; Maeshima et al., 2000; Mundel et al. 2007].

XII. Angiostatin:

Among the angiogenesis endogenous inhibitor Angiostatin is one of the best known of the endogenous anti-angiogenic protein which is made up of the first four Kringle (K1-4) domains of plasminogen [O'Reilly et al., 1994]. Plasminogen is primarily synthesized and secreted by the liver and is present in high concentrations in the plasma; it can be proteolytically digested by proteases, such as cathepsin-D and MMPs, to generate angiostatin [Wahl et al., 2005; Kirsch et al., 1998]. The anti-angiogenic activity of angiostatin was first demonstrated in mice bearing subcutaneous Lewis lung carcinoma tumors [O'Reilly et al., 1994]. Multiple studies have now shown that angiostatin inhibits malignant glioma growth in tumor induced animal models [Kirsch et al., 1998; Joe YA et al., 1999]. Angiostatin mediate its antiangiogenic effects through binding to several different receptors presented on the EC, including binding to integrin αvβ3, and ATP synthase [Wahl et al., 2005], [Tarui et al., 2001; Chekenya et al., 2002]. Angiostatin also upregulate pro-apoptotic proteins in activated or proliferating endothelial cells [Wahl et al., 2005] as well as it causes the cell cycle arrest, inhibition of migration, and induction of apoptosis, in non-brain EC propagated in vitro [Cao et al., 1998; Zhang et al., 1990].
## Summary of Therapies for Glioma Antiangiogenic Agent Trialed in High-Grade Gliomas

### 1) Antibody Therapies

<table>
<thead>
<tr>
<th>Antibody Therapies</th>
<th>Major Molecular Targets</th>
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</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>Free VEGF-A</td>
</tr>
<tr>
<td>Ramucirumab</td>
<td>VEGFR-2</td>
</tr>
<tr>
<td>Nimotuzumab</td>
<td>EGFR</td>
</tr>
<tr>
<td>VEGF-trap</td>
<td>Decoy receptor for VEGF</td>
</tr>
<tr>
<td>IMC-3G3</td>
<td>PDGFR alpha</td>
</tr>
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### 2) Tyrosine Kinase Inhibitors

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<thead>
<tr>
<th>Tyrosine Kinase Inhibitors</th>
<th>Major Molecular Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cediranib</td>
<td>VEGFR/PDGFR/c-Kit</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>VEGFR/PDGFR/c-Kit/Raf</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>VEGFR/PDGFR</td>
</tr>
<tr>
<td>AE788</td>
<td>EGFR/VEGFR</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>EGFR</td>
</tr>
<tr>
<td>Imatinib</td>
<td>PDGFR/Bcr-abl/c-Kit</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>Src kinases</td>
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### 3) Signal Pathway Inhibitors

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<tr>
<th>Signal Pathway Inhibitors</th>
<th>Major Molecular Targets</th>
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</thead>
<tbody>
<tr>
<td>Enzastaurin</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>Rapamycin/Temsirolimus</td>
<td>mTOR</td>
</tr>
<tr>
<td>Tipifarnib</td>
<td>Ras</td>
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</table>

### 4) Other Agents

<table>
<thead>
<tr>
<th>Other Agents</th>
<th>Major Molecular Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalidomide/Lenalidomide</td>
<td>NO/TNF alpha/IL-6</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>Endostatin</td>
</tr>
<tr>
<td>Cilengitide</td>
<td>Integrins αβ3 and αβ5</td>
</tr>
<tr>
<td>2-methoxyestradiol</td>
<td>HIF1α</td>
</tr>
<tr>
<td>Prinomastat</td>
<td>MMPs 2,9,13 and 14</td>
</tr>
<tr>
<td>SAHA(Vorinostat)</td>
<td>Histone deacetylase</td>
</tr>
</tbody>
</table>

Table 4: Ref: Rahman et al., 2009
During the progression of gliomas conventional cytotoxic chemotherapies control them by blocking the expansion and proliferation steps, but leave angiogenesis and invasion unchecked. However, long term exposure of chemotherapy has significant side effects including interstitial lung diseases, mouth ulcer, cardiovascular diseases and tumor lysis syndrome. So development of nontoxic, affordable, multi-level targeted agents with sustained efficacy in inhibiting angiogenesis is the best strategy for glioma antiangiogenesis therapy.

**T11target Structure (T11TS) – A Novel Antineoplastic Agent**

T11-Target Structure (T11TS) or sheep form of LFA- 3 (S-LFA3) is a 42 KD SRBC membrane glycoprotein. Initially in 1976, Kitao et al successfully isolated T11TS from sheep red blood cells (SRBC) membrane and later it was characterized as an important costimulator of the immune system [Hunig et al., 1987; Sarkar et al., 2004]. The molecule T11TS belongs to immunoglobulin superfamily which binds with the CD2 receptor present on the surface of T lymphocyte that forms erythrocyte rosetting complex through a ligand receptor interaction [Hunig et al., 1987; Ogasawara et al., 1995]. The homologue of this protein in human is known as CD58 and murine counterpart is CD48 [Hunig et al., 1987]. Recently Chatterjee et al in 2012 further detailed the secondary structure of this protein (Fig. 6). The Insilico analysis of the T11TS highlights that the intermolecular hydrogen bonding and electrostatic force are the responsible for the receptor-ligand interactions. Whereas analysis of the primary structure of the glycopeptides reveals that serine is the principle amino acid with significant amounts of lysine and glycine and to a lesser extent aspartic acid, alanine and threonine were found. While, the carbohydrate counterpart contains galactose as the principal monosaccharide with sialic acid, galactosamine and glucosamine are present in about half the amount. The protein consists of two immunoglobulin like extracellular domains, of which the first N-terminal V domain lacks disulfide bond and the second C2 domain has a disulfide bond [Dustin et al., 1987]. Two types of mechanisms anchor these chains to the cellular membrane; transmembrane anchored form and glycosylphosphatidylinositol (GPI)-anchored form and that are generated by alternate splicing. In 2002 Sarkar et al have shown that T11TS acts as an immune potentiating agent stimulating the peripheral immunocytes including macrophage, PMN and lymphocytes in glioma induced rat brain. Although, different research conducted on structural information, physiological and immunological roles of the glycoprotein but no reports on disease relevance of such molecule on glioma animal model were done.
**Fig. 6: Mode of T11TS and CD2 Interaction:**

A. Human CD2, Rat CD2 and Mouse CD2 are presented in superposed ribbon diagram in interacting mode with T11TS. Human CD2, Rat CD2 and Mouse CD2 are green, violet and pink colored respectively. T11TS is shown in surface mode where blue color and orange red color highlight the positive charge and negative charge environment. The residues in AGFCC’C” face is interacting with three CD2 molecules.

B. Positioning of CD2 Binding Sites and Glycosylation Sites of T11TS: CD2 binding domain and N–glycosylation sites (N12 and N62) are shown in the ribbon diagram of the model [Chatterjee et al., 2011].
Complete abrogation of tumor mass and rejuvenation of immune system (down regulated by immunosuppressive factors from the tumor mass) were achieved for the first time when this novel glycopeptide molecule was administered intraperitoneally (i.p.) in chemical carcinogen (ethyl nitrosourea) induced brain tumor rat model. T11TS activated T lymphocytes enter brain parenchyma by crossing the blood brain barrier (BBB) and cross talk with microglia the chief immunomodulatory cell of the brain and kill the glioma cells apoptotically [Begum et al., 2004; Bhattacharjee et al., 2008]. Histological observations depicted involvement of infiltrated immunocytes in brain and termination of glioma, which was supported by GFAP expression study [Ghosh et al., 2007]. Next, the effect of T11TS mediated activation was observed on the resident microglia, the chief immunomodulator of brain and brain infiltrated lymphocytes (BIL). T11TS administration causes the modulation of MHC class II and CD2 receptors, which facilitate proper ‘immunological synapse’ formation, hence better antigen presentation to lymphocytes and associated costimulation [Sarkar et al., 2004; Begum et al., 2004]. Simultaneously T11TS also causes the microglial activation markers CD4 and CD25 which were found to be activated in first two T11TS doses whereas third dose acts as regulatory dose [Begum et al., 2004]. In 2005 Mukherjee et al have shown that T11TS mediated infiltration of CD4+ and CD8+ T cells was increased in CNS tissue which balances for co-ordination of the effector function properly and resulted in destruction of the glioma cells [Mukherjee et al., 2005]. Moreover, macrophage mediated phagocytosis was found to be activated with the administration of T11TS. This is partly dependent on ROS, but mainly on RNI production. This glycoprotein, as an immunotherapeutic probe, also potentially regulates the cytokine milieu in the CNS tissue of glioma animals. It facilities the IL-12 production and depresses IL-10 production in interacting glioma, microglia and BIL to promote inflammatory Th1 response during first two doses of T11TS, but the trend was reversed in the final dose. Similarly, high IL-4 production in glioma environment diminished with the application of T11TS, but increased in BIL and microglia to suppress the inflammatory response as a homeostatic regulation in the third T11TS dose. Our toxicological investigation of T11TS in rodent species was performed as per norms of Govt. of India (www.cdsco.nic.in) and has evidence that T11TS act as non toxic agent [Sarkar et al., 2007].

Further, the specific role of T11TS as apoptotic inducer via immune cross talk with the two intracranial immune competent cells such as microglia and the brain-infiltrating lymphocytes has been documented in glioma animal models [Bhattacharjee et al., 2008]. T11TS mediated apoptotic results have shown that maximum death of the glioma cells occurred after T11TS administration by both pathways.
Further studies elucidated that T11TS-mediated cell cycle arrest occurred at G1 phase by inhibiting the CDK4 expression via their association factor such as downregulation of cyclin D (1 and 3) and simultaneous upregulate p21<sup>Cip1</sup> and p27<sup>Kip1</sup> followed by increasing levels of pRb/p107 and pRb/130. Flow cytometry based DNA content assay with propidium iodide revealed that T11TS-exerts its effect on cell cycle progression of glioma cells by accumulating them at G1-phase and further PCNA (Proliferating Cell Nuclear Antigen) assay showed sharp downregulation after T11TS administration proving decisively cessation of cell cycle progression through G1-phase of the cell cycle [Acharya et al., 2010].

Furthermore, our preclinical studies on human sample indicate that T11TS regulates the activation of cytotoxic T-cells in grade I and grade II glioma [Kumar et al., 2012]. The experiments conducted on CTL assay in different grades of human sample reveals that T11TS induced CTL death occurred via the cellular immune response which orchestrates the death of target tumor cell by regulating the CD8<sup>+</sup> and NK cell activity. The results of perforin granzyme mediated death of target tumor hint that T11TS incubated lymphocytes significantly increased the expression perforin and granzyme compared to non T11TS incubated lymphocytes in grade I and grade II samples. Moreover, the phagocytic assay reveals that T11TS enhances the phagocytic activity of macrophages in grade I & II of human glioma. Significant downregulation of macrophage secreted VEGF in grade I and upregulation of TNF-α in grade I &II substantiate the immunomodulatory potential of T11TS. Furthermore, investigation on TGF-β1 assay hints that the upregulation of TGF-β1 expression from grade I to grade IV sample causes the immune suppression. While, significant downregulation of TGF-β1 in grade I and II tumor, after T11TS induction strengthen the T11TS immunopotentiating activity in in vitro glioma model.

Our previous histopathological study reveals that T11TS induction causes brain endothelial cell apoptosis and disruption of microvessels in glioma induced rat brain section. Similarly findings in our lab had demonstrated that T11TS inhibits the production of key angiogenic molecule vascular endothelial growth factor (VEGF) from ECs [Bhattacharaya et al., 2013] and can also cause the down regulation of TGFβ-1 in glioma cells in human sample [Kumar et al., 2012]. Therefore this was indicative of the fact that T11TS possibly acts as an inhibitor of the tumor angiogenic potential, by inhibiting other glioma angiogenic proteins such MMPs, TIMPs, Integrin, VE cadherins and the relates cytokines.