# COMPARISON OF LEVEL OF ENDOTHELIAL PROGENITOR CELLS (EPCs) IN ASTROCYTOMA TO ADJACENT NORMAL BRAIN TISSUE

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**PEMBANDINGAN TAHAP SEL-SEL PROGENITOR ENDOTELIA (EPCs) DALAM GLIOMA ASTROSITIK DENGAN TISU OTAK BERSEBELAHAN YANG NORMAL**

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**Pengenalan:** Glioma astrositik merupakan ketumbuhan otak yang paling biasa, mencakupi sehingga dua pertiga daripada semua ketumbuhan otak asal glial dan terutamanya glioma astrositik gred tinggi diketahui sebagai sangat vaskular. Kajian terdahulu menunjukkan bahawa vaskular dalam glioma astrositik terutamanya yang gred tinggi, disumbangkan oleh beberapa cara yang menjelaskan, rintangan glioma astrositik kepada terapi anti-angiogenik. Sel-sel progenitor endotelia dipercayai memainkan peranan yang penting dalam daripada astrocytoma, secara langsung dengan pembentukan salur darah (Vasculogenesis) atau tidak langsung dengan menghasilkan pelbagai faktor proangiogenik dan pada masa akan datang mungkin jadi sasaran terapi.

**Objektif:** Untuk membandingkan tahap EPCs di antara sampel tisu glioma astrositik dengan tisu otak bersebelahan yang normal dan sub analisis, membandingkan tahap EPCs dalam glioma astrositik gred tinggi dengan gred rendah.

**Kaedah:** Sebanyak 17 pesakit dengan glioma astrositik, 11 pesakit dengan glioma astrositik gred tinggi (WHO Gred III dan IV) dan 6 pesakit dengan gred rendah (WHO Gred I dan II), telah diambil dari Hospital Universiti Sains Malaysia. Semua pesakit menjalani pembedahan untuk astrositik glioma, tisu ketumbuhan dan tisu otak bersebelahan yang normal disampelkan. Tisu ketumbuhan dan tisu otak bersebelahan yang normal telah dihiris dan ditandakan dengan CD 133 dan VEGFR-2 dan diikuti oleh analisis immunohistokimia untuk menentukan EPCs dalam tisu ketumbuhan dan tisu otak bersebelahan yang normal.

**Keputusan:** Tahap sel-sel progenitor endotelia adalah lebih tinggi dalam tisu glioma astrositik berbanding dengan tisu otak bersebelahan yang normal. Analisis sub kajian, berbanding tahap sel-sel progenitor endotelia dalam glioma astrositik gred tinggi dengan gred rendah, tidak menunjukkan perbezaan yang signifikan.

**Kesimpulan:** Kajian ini menunjukkan tahap sel sel progenior endotelia yang lebih tinggi dalam astrositik glioma berbanding dengan tisu otak bersebelahan biasa

mencadangkan kemungkinan EPCs memainkan peranan dalam pembentukan salur darah dalam ketumbuhan astrocytoma.

Dato’ Dr Abdul Rahman Izaini bin Ghani: Supervisor

Dr Yvonne-Tee Get Bee: Supervisor

Prof Dr Jafri Malin bin Datuk Abdullah: Co-Supervisor

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**Introduction:** Astrocytomas are the most common primary brain tumors which account for up to two third of all tumors of glial origin and especially the high grade astrocytoma are known to be highly vascularized. Previous studies suggest that vascularization of astrocytoma especially high grade astrocytoma, is contributed by several mode of vascularization which explained, the resistance of the tumor to anti-angiogenic therapy. Endothelial progenitor cells are believed to play an important role in vascularization of astrocytoma, directly by vasculogenesis or indirectly by secreting various proangiogenic factors and possible next target of therapy.

**Objective:** To compare the EPCs level in between the astrocytoma tissue samples to the adjacent normal brain tissue and subanalysed, by comparing the EPCs level in high grade astrocytoma to low grade astrocytoma.

**Methods:** A total of 17 patients with astrocytoma, 11 patients with high grade astrocytoma (WHO Grade III and IV) and 6 patients with low grade astrocytoma (WHO Grade I and II), were recruited from Hospital Universiti Sains Malaysia. All patients underwent surgery for astrocytoma, tumor and normal adjacent brain tissues were collected. Tumor and normal adjacent brain tissues were sliced and stained with CD 133 and VEGFR-2 marker and followed by immunohistochemical analysis to determine the EPCs in the tumor and normal adjacent brain tissue.

**Results:** The levels of EPCs were significantly higher in the astrocytoma compared to adjacent normal brain tissue. Sub analysis of the study, compared the level of EPCs in the high grade astrocytoma to the low grade astrocytoma, do not showed significant difference.

**Conclusions:** This study showed higher level of EPCs in the astrocytoma as compared to the normal adjacent brain tissue suggest the possibility of EPCs play a role in astrocytoma vascularization.

Dato' Dr Abdul Rahman Izaini bin Ghani: Supervisor

Dr Yvonne-Tee Get Bee: Supervisor

Prof Dr Jafri Malin bin Datuk Abdullah: Co-Supervisor

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GLOSSARY AND ABBREVIATIONS:

| | |
|---|---|
| bFGF | Basic Fibroblast Growth Factor |
| BUSE/Crea | Blood urea, serum electrolyte/ creatine |
| CCNU | Carmustine |
| CMC | Chloromphenicol ointment |
| CRF | Case Report Form |
| CT scan | Computed Tomography scan |
| CXR | Chest X-ray |
| DTI | Diffusion Tensor Imaging |
| ECG | Electrocardiogram |
| eNOS | endothelial nitric oxide synthase |

| | |
|---|---|
| EORTC | European Organization of Research and Treatment of Cancer |
| EPCs | Endothelial Progenitor Cells |
| EPGF | Epidermal Growth Factor |
| EPGR | Epidermal Growth Factor Receptor |
| FBC | Full Blood Count |
| GBM | Glioblastoma Multiforme |
| GCP | Good Clinical Practice |
| gm | Gram |
| GSH | Group Screen and Hold |
| HREC | Human Research Ethic Committee USM |
| ICP | Intra-cranial Pressure |

| | |
|---|---|
| IL-6 | Interleukin-6 |
| IL-8 | Interleukin-8 |
| IV | Intravenous |
| IQR | Inter Quartile Range |
| JEPeM | Jawatankuasa Etika Penyelidikan Manusia USM |
| MAPK | Mitogen-Activated Protein Kinase |
| MEG | Magnetoencephalography |
| mg | Milligram |
| mL | Milliliter |
| MRI | Magnetic Resonat Imaging |
| P | Procarbazine |

| | |
|---|---|
| PDGF | Platelet Derived Growth Factor |
| PDGFR | Platelet Derived Growth Factor Receptor |
| PI3K | phosphastidylinositol 3-kinase |
| PT/PTT/INR | Prothrombin Time, Partial Thromboplastin Time/International Normalized Ratio |
| SD | Standard Deviation |
| SPSS | Statistical Package for Social Science |
| V | Vincristine |
| VEGF | Vascular Endothelial Growth Factor, |
| VEGFR | Vascular Endothelial Growth Factor Receptor |
| WHO | World Health Organization |

# CHAPTER 1.0: Introduction

Astrocytic gliomas are the most common primary brain tumors which account for up to two third of all tumors of glial origin (Sclag P.M. et al. 2009). According to WHO classification of central nervous system tumors, astrocytomas are typically classified as pilocytic (grade I- less aggressive), diffuse (grade II), anaplastic (grade III), or glioblastoma multiforme (grade IV- most aggressive) in order of increasing anaplasia (Ohkada H. et al. 2009). Glioblastoma multiforme is the most common type of astrocytomas, highly invasive and almost uniformly fatal tumor. Glioblastoma miltiforme is among the most highly vascularized of all malignancies and relies upon angiogenesis for growth and histological progression (Cloughesy T.F. et al. 2009).

When tumors reached a certain size, the requirements for oxygen and nutrients lead to the growth of new blood vessels. Recent studies have demonstrated the existence of additional angiogenic and vasculogenic mechanisms associated with tumor growth, such as intussusceptive angiogenesis, vessel co-option, vasculogenic mimicry, lymphangiogenesis and the recruitment of endothelial progenitor cells (EPCs) (Janis B. et al. 2010, Hillen F. et al. 2007, Dome B. et al. 2007)

EPCs are the vascular precursor cells recruited from the bone marrow to form new blood vessels. It has been shown that the recruitment of bone-marrow derived EPCs to the site of tumor induced abnormal differentiation of these cells into mature endothelial cells which then incorporate into a subset of sprouting tumor neovessels (Bussolati B. et al. 2010, Nolan N.J. et al. 2007, Dome B. et at. 2007, Charamlabous C. et al. 2006). The newly

formed vessels from this process were less disorganised, tortuous, dilated, leaky and hemorrhagic (Bussolati B. et al. 2010, Chi A. S. et al. 2009, Rafat N. et al. 2009, Greenfield et al. 2009). Moreover, it is recently showed that EPCs are present abundantly in the peripheral blood of patients with glioblastoma multiforme (Greenfield et al. 2009) and the numbers of EPCs were higher in glioblastoma multiforme compared to other nervous system disease (Zheng P. P. et al. 2007). The ability of circulating EPCs to homing the sites of tumor shows their capacity to be used as a biomarker for the diagnosis and monitoring tumor aggressiveness as well as measuring tumor angiogenicity (Nolan N.J. et al. 2007, Chi A. S. et al. 2009, Rafat N. et al. 2009).

## RATIONALE OF RESEARCH

Until recently, few treatment options were available for high grade astrogliomas. However, despite significant advances in surgery, radiotherapy, and chemotherapy over the past 3 decades, the median patient survival remains < 2 years from lesion diagnosis (Dash S. et al. 1997, Laperriere N. et al. 2002, Law ER Jr et al. 2003). Although extensive works have been conducted, the treatment options for these tumors remain limited. No significant advancement in the treatment of glioblastoma has occurred in the past 25 years except for chemotherapy with Temozolomide combined with radiotherapy, which has demonstrated a limited prolongation (approximately 3 months, compared with radiotherapy only) of patients' survival (Okada H. et al. 2009). Nevertheless, all the standard treatments for gliomas are not totally efficient to diminish the tumor cells and there is a possibility for the tumor to recur. Hence, a very effective alternative form of treatment is needed to overcome the problems.

The induction of angiogenesis in tumor progression has insight the set stage for the development of anti-angiogenic (anti-vascular endothelial growth factor, VEGF) therapy. However, the clinical benefits of anti-angiogenic therapies have been modest and it has been demonstrated to associate with various adverse effects such as hypertension, thrombotic events, delayed wound healing, and congestive heart failure (Alluwalia M. S. et al. 2010).

a. Patient First. The development and optimization of biomarkers to measure angiogenesis in tumors, such as quantitation of the numbers of tumour EPCs, will potentially help us identify patients that may responding to and benefit from the anti-angiogenic therapy.

b. Data. This study not only provides us with local data regarding endothelial progenitor cell in astrocytoma but also justify and validate previous published data.

c. New Biomarker. Knowledge of endothelial progenitor cells in astrocytoma has a vast potential in aiding the future of treatment strategies especially in patient selection for anti-angiogenic therapy.

d. Value-added study. This study procedure is actually a standard operating procedure for patient with astrocytoma, using the routinely collected tumor tissue and biopsy of normal brain tissue at the adjacent area for endothelial progenitor cells analysis. In other words, this study does not directly alter or change any standard treatment for astrocytoma but offering value added service and maximizing the opportunity from a single operating procedure.

Hence, our general aim is to add valuables new information that may be useful in improving the efficiency of anti-angiogenic therapy for astroglioma thus limit the adverse effects among patients.

# Chapter 2.0 Literature Review

## 2.1.0: Historical perspective of astrocytoma

In 1829, Cruveilhier published the first macroscopic descriptions of brain tumors. Later in 1860, Sir Virchow described the brain consists of interstitial matrix in which individual cells are suspended, which provide clearer view and lead to subsequent discovery of various brain tumors. He was the first to use the term glioma to describe a lesion which is diffusely involved the brain parenchyma but did not destroy it.

In 1926, Bailey and Cushing published their classification for gliomas, they proposed 14 tumor types by comparing the morphological features of the tumor cells to those of normal cells at each defined stage of histogenesis.

The Bailey and Cushing classification was dominated until 1949, when James Kernohan and colleagues come out with a simpler classification. Kernohan believed the glial tumors develop from differentiated cell, and different histological appearance does not represent different tumor rather different degrees of de-defferenciation of one tumor type. He classified glial tumors to five types: astrocytoma, ependymoma, oligodendroglioma, neuroastrocytoma and medulloblastoma. And he also introduced a four tier grading system to grade astrocytoma and ependymomas, which based on the degree of anaplasia and differentiation of the tumor. Kernohan classification has laid a platform to the development of modern classification of glioma.

2.1.1: Historical perspective of angiogenesis

Angiogenesis is defined as a physiological process, which formation of new blood vessels from the pre existing vessels, while vasculogenesis is defined as the in situ differentiation of vascular endothelial cell from precursor cells.

The important of angiogenesis in the tumor growth was proposed by Judah Folkman in 1971, he hypothesized the solid tumor growth is dependent on the angiogenesis. By blockage of angiogenic factor can render the tumors harmless, which lead to discovery of series of stimulator and inhibitor of angiogenesis (Folkman J. et al. 1971). Napoleone Ferrara developed a monoclonal antibody to VEGF (Vascular Endothelial Growth factor), bevacizumad, by inhibition of VEGF signaling hence successfully halts the growth of gliomas (Ferrara et al. 2002)

Angiogenesis was previously thought as the only mechanism through which new blood vessel form from proliferation and migration of preexisting endothelial cell and contribute to tumor vascularization, until recently few studies shown endothelial progenitor cell play an important role in tumor vascularization through vasculogenesis, a process where endothelial progenitor cells are recruited and differentiated into mature endothelial cell.

## 2.2 World Health Organization (WHO) classification

The WHO classification of brain tumor was first published in 1979 and latest revised in year 2007, although imperfect but it is one of the most widely used classification system for astrocytoma.

- WHO grade I
    - pilocytic astrocytoma 9421/11 - WHO grade I
    - subependymal giant cell astrocytoma 9384/1 - WHO grade I
- WHO grade II
    - pilomyxoid astrocytoma 9425/3* - WHO grade II
    - pleomorphic xanthoastrocytoma 9424/3 - WHO grade II
    - diffuse astrocytoma 9400/3 - WHO grade II
        - fibrillary astrocytoma 9420/3
        - protoplasmic astrocytoma 9410/3
        - gemistocytic astrocytoma 9411/3
- WHO grade III
    - anaplastic astrocytoma 9401/3 - WHO grade III
- WHO grade IV
    - glioblastoma 9440/3 - WHO grade IV
    - giant cell glioblastoma 9441/3 - WHO grade IV
    - gliosarcoma 9442/3 - WHO grade IV
- gliomatosis cerebri 9381/3

## 2.3 Epidemiology

Astrocytoma are the most common primary brain tumors which account for up to two third of all tumors of glial origin (Schlag P. M. et al. 2009).

Low grade astrocytoma which include WHO grade I and grade II are usually diagnosed in young patient between 20-45 years with mean age of 35 years old. In fact, there is a biphasic distribution, with first peak in childhood with age from 6 to 12 years old and the second peak in early adulthood with age from 26 to 46 years old. (Tonn J. et al. 2006)

High grade astrocytoma which include WHO grade III and grade IV are the most common primary brain tumor which can occur at any age, but usually occur after the age of 40 with a peak incidence between 65 and 75 years of age. There is a slight male preponderance with a 3:2, male to female ratio. (Ohgaki et al. 2005)

## 2.4 Aetiology

Multiple aetiologic factors have been shown inconsistent in relation to astrocytoma, which include the dietery, tobacco smoking, alcohol consumption, genetic syndrome and exposure to radiation.

### 2.4.1 Diet

Epidemiologic studies between diet and astrocytoma remain weak and inconsistent, N-nitroso compounds and their precursor is believed it might associated with increased risk of glioma as reported by Boeing et al. 1993 and intake of vegetable and fruit have protective effects against glioma (Chen et al. 2002).

### 2.4.2 Alcohol consumption and tobacco smoking

So far there was no evidence linking alcohol consumption and association with glioma. direct and passive smoking associated with increased risk of childhood CNS tumor (Preston Martin et al.1982) and increased risk of CNS tumor in adult especially meningioma in female, but not glioma. marijuana user have 2.8 fold increase risk for glioma. (Efird J.T. et al. 2004)

### 2.4.3 Radiation

Exposure to radiation is an important risk factor which associated with glioma and other CNS tumor. Shintani et al. reported the survivor of the Hiroshima bombing has higher rate of glioma. and another example is children who had exposed to radiation for treatment of tinea capitis and skin hemangioma has been associated with higher risk of glioma. (Sadetszki et al. 2005)

### 2.4.4 Genetic syndrome

The genetic syndrome that associated with glioma includes neurofibromatosis type 1 and 2, Turcot syndrome, tuberous sclerosis, retinoblastoma and Li fraumeni syndrome. These syndromes are associated with tumor suppressor gene dysfunction which makes the patient prone to the development of tumor.

## 2.5 Clinical presentation and natural history

Clinical presentation of astrocytoma is divided mainly in accordance to the WHO grade of the tumor and location.

### 2.5.1 Low grade astrocytoma (WHO grade I and grade II)

Low grade astrocytoma most commonly arise in frontal lobe followed by temporal and parietal lobe. Most of the patient present with seizure as an initial symptoms some studies report as high as 80% and usually with an intact neurological examination. Other signs and symptoms depend on the size and location of the tumor which produces raised intracranial symptoms and focal neurological deficit.

### 2.5.2 High grade astrocytoma (WHO grade III and grade IV)

High grade astrocytoma frequently arise in cerebral hemisphere, they can occur as primary tumor (de novo) or arise from low grade astrocytoma through malignant transformation as secondary tumor. Anaplastic astrocytoma WHO grade 3 has a innate tendency to transform into glioblastoma multiforme WHO grade 4. Commonly, patient with high grade astrocytoma will present with raised intracranial pressure.

## 2.6 Genetic

Alteration in cell DNA is a basis of cancer formation and it can be classified into loss of function mutation, gain of function mutation and mutator gene.

### 2.6.1 Loss of function mutation

Loss of function mutation involves mutation of tumor suppressor gene which lead to increase the mutation rate in the cell's DNA. The common tumor suppressor gene mutation in low grade astrocytoma is p53 gene. p53 gene is located at the chromosome 17p13.1 and plays an important role in the regulation of apoptosis and cell cycle progression. Mutation of p53 gene lead to acceleration of cell growth and malignant transformation of the astrocytes (Bogler et al. 1995). About 50% - 60% of grade II, grade III astrocytoma, and secondary GBM. But significant subset of primary GBM does not express p53 gene mutation.

The PTEN (phosphatase and tensin homology) gene, is located at 10q23.3 encodes a protein which play an important role in regulating cell growth by inhibiting the PIP3 signal pathway. PTEN mutation is common in primary GBM about 15 to 40% (Knobbe CB et al. 2002) and rare in secondary GBM.

### 2.6.2 Gain of function mutation

Gain of function mutation is mutation lead to increase normal gene function or add a new function, or change of proto oncogene to oncogene and eventually contributes to tumor progression.

EPGR (Epidermal Growth Factor Receptor) is located on the chromosome 7 which encode a transmembrane receptor responsible for transducing proliferation signal. The EGFR gene is the most common amplified gene in GBM. EFGR amplification is the most common

cause of over expression of EFGR in about 70 -90% and occurs in about 40% of primary GBM.

## 2.7 Mechanisms of vessel formation.

Without blood supply the to rapidly growing tumor cells, tumor would be self limited. As tumor reaching certain size, tumor requires additional blood supply for survival (Folkman J. et al 2007). The four mechanisms brain tumors can use to acquire new blood vessels.

1) Angiogenesis 2) Vessel Co – option 3) Intussusception 4) Vasculogenesis.

### 2.7.1 Angiogenesis

Angiogenesis play an important role in the progression of low grade glioma to high grade glioma, with the onset of angiogenic switch which allow logarithmic growth of low grade glioma into large vascularized tumor such as GBM. Angiogenesis not only affect the growth of glioma but also can cause cancer cell undergoes oncogenes activation for example myc and ras oncogene activation is angiogenesis dependent (Folkman J. et al 2007).

Angiogenesis in glioma involve few key step, 1) in response to hypoxia or gene mutation, glioma cells release growth factors, one of the most important growth factor is VEGF, others include Interleukin-6 (IL-6), Interleukin-8, basic fibroblast growth factor and hypoxia inducible factor 1 alpha . 2) Growth factors will bind to correspond receptors and activate signal transduction pathway which lead to proliferation of endothelial cell. 3) Sprouting vessel will growth toward the tumor.

2.7.1.1 Vascular Endothelial Growth Factor

The family of Vascular Endothelial Growth Factors and receptors are the dominant pathway in glioma angiogenesis. The VEGF family are refer to six glycoproteins which include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor. There are 2 types of VEGF receptors, referred to as VEGFR-1 and VEGFR-2. Among the 6 VEGFs and 2 types of receptors, VEGF-A and VEGFR-2 are generally agreed play an important role in tumor angiogenesis.

VEGF have a diverse biological effect on the endothelial cell through different signaling pathway. VEGF can activate the MAPK signaling pathway which leads to endothelial proliferation and also increase the vascular permeability by rearranging cadherin/caterin complexes and break down of the blood brain barrier by loosening the adhering junction between the endothelial cells. VEGF also activate the Akt and endothelial nitric oxide synthase (eNOS) pathway which lead to increase vascular permeability.

VEGFR-2 activation on the endothelial cells will result in signaling through the phosphastidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway which promote endothelial cell survivor and lead to proliferation, migration of endothelial cell and extracellular matrix remodeling.

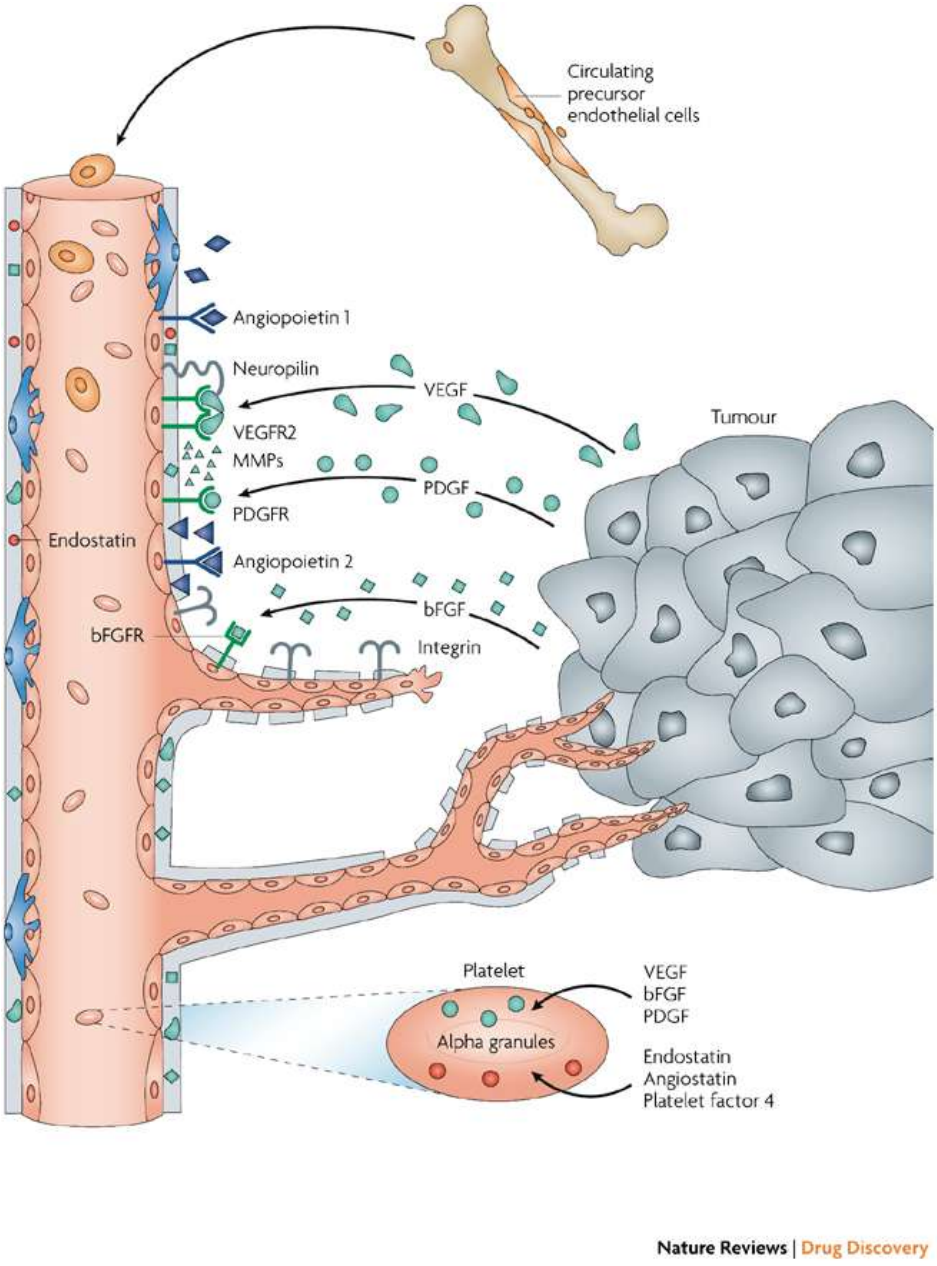


Figure 2.1: Mechanism of tumor angiogenesis. (Source: Folkman J. et al 2007)

Growth factors secreted by tumor cells bind to corresponding receptors, which lead to induction of intracellular signaling. VEGF: Vascular Endothelial Growth Factor, bFGF: Basic Fibroblast Growth Factor, PDGF: Platelet Derived Growth Factor, VEGFR: Vascular Endothelial Growth Factor Receptor, bFGFR: Basic Fibroblast Growth Factor Receptor, PDGFR: Platelet Derived Growth Factor Receptor.

### 2.7.2 Vessel co – option

Vessel co – option is a mechanism in which tumor cell take control of the existing blood vessels, it was first describe d in the brain tumor. It is one of the alternative way to get blood supply without the need of angiogenic switch which explained the blood supply will not be affected by inhibition of angiogenesis (Karl H et al 2012).

### 2.7.3 Intussusception

Intussusception is a mechanism in which the pre existing blood vessels can split and give rise to a new vessel. Both irradiation and anti angiogenic therapy cause a switch from angiogenesis to intussusception and accounting for the resistance to above mentioned treatment (Karl H et al 2012).

### 2.7.4 Vasculogenesis

Vasculogenesis is refers to the de novo blood vessels formation from the endothelial progenitor cells, which previously thought only occur in the embryo. Until 1997, Asahara et al reported the presence of bone marrow – derived endothelial progenitors in tumor vasculature.

Other than EPCs, cancer stem cell can contribute to vasculogenesis by integrating into the vascular wall andtransdiffentiation into endothelial cell and give rise to new blood vessel. Ricci-Vitiani et al 2010 reported that the genetic alterations found in the GBM vascular cell are typically found in the tumor cell, ans conclude that cancer stem cell can contribute to tumor vasculogenesis.

2.7.5 Vasculogenic mimicry

Vasculogenic mimicry is defined as a process where cancer cell form a vessel with lumen. Francescone et al (2012) reported that vasculogenic mimicry can contribute to vascularization of GBM. But otherwise currently, there is a still very sparse study on vasculogenic mimicry to confirm it contribution to tumor vasculogenesis.

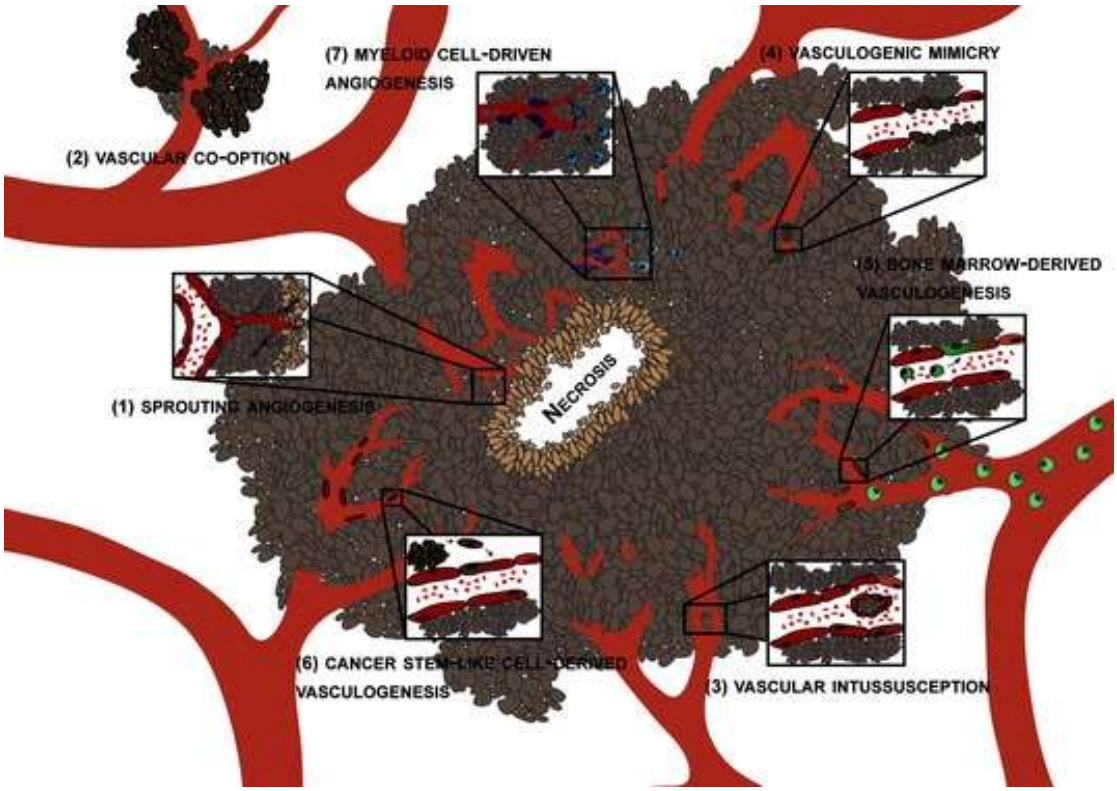


Figure 2.2 mode of vascularization in glioma (Source: Karl H. et al. 2012). (1) sprouting angiogenesis, (2) vascular co-option, (3) vascular intussusecption, (4) vasculogenic mimicry, (5) bone marrow-derived vasculogenesis, (6) cancer stem-like cell derived vasculogenesis and (7) myeloid cell-driven angiogenesis are all considered to contribute to tumor angiogenesis.

## 2.8 Endothelial Progenitor cell markers

In 1997, Asahara et al indentified and isolated endothelial progenitor cell based on the coexpression of CD 34 and VEGFR-2 of these cells. Recently with emergence of specific surface markers have facilitated in identify and purify these cells. Endothelial progenitor cells and haemotopoetic stem cells share many other surface markers apart from the aforementioned, so up to date there is no simple definition of EPC exists, but it is widely accepted that endothelial progenitor cells are CD133 +/ CD34 +/VEGFR-2 +cells.

Several other important markers typical (figure) for the endothelial progenitor cell, for example c-kit and CXCR-4. C-kit is a transmembrane glycoprotein which is a tyrosine kinase receptor and it ligand stem cell factor (SDF), it plays an important role in mediating cell survival differentiation, homing, migration and proliferation. CXCR-4 is a receptor for the chemokine stromal cell-derived factor-1 and highly expressed on the endothelial progenitor cell.

### 2.8.1 Origin of endothelial progenitor cell

Currently origin of endothelial progenitor cell is believed to arise from bone marrow and has been confirmed in few studies (Bertolini et al 2003, Pasqueir et al 2010). Mononuclear cell was isolated from the peripheral blood or cord blood have shown to transform into endothelial cells 2 – 3 weeks later which expressed vascular endothelial cell marker and able to form new blood vessel in vivo. Further analysis, suggesting these cells are stem cells from myeloid lineage as EPC also expressed various hematopoietic markers, and have pro – angiogenic properties when stimulated.

### 2.8.2 Physiological role of endothelial progenitor cells

Endothelial progenitor cells have the ability to circulate, migrate, proliferate and differentiate into mature endothelial cell hence they play an important role in maintaining the vascular integrity. Few studies have shown impaired function or reduced number of endothelial progenitor cell have associated with increased risk of cardiovascular diseases and it was found in a variety of vascular beds. Endothelial progenitor cells may represent a reservoir which can be mobilized during vascular injury. However different tissues have different rate of endothelial progenitor integration for example brain has very low level incorporation rate of endothelial progenitor cell as compared to skin with high rates of incorporation up to 2.5% of blood vessels (Crosby et al. 2000, B. Larrivee et al. 2003 )

### 2.8.3 Contribution of endothelial progenitor cells to tumor vascularization

The contribution of endothelial progenitor cell to tumor vascularization first proof by Layden et al (2001),by using an angiogenesis-defective mice Id-mutant mice model, with defective Id gene which cannot support angiogenesis when challenge with tumor. Bone marrow transplantation from the wild type mice into the Id mutant mice restored the tumor growth.

This was followed by many publications, using various animal models and clinical samples, showed that endothelial progenitor cells contribute to human tumor vascularization, for examples colorectal cancer (Gunsilius et al. 2002), breast carcinoma (Sussman et all. 2003),hepatocellular carcinoma (Decai Yu et al. 2007) and including glioma (Zheng et al. 2007).

In glioma, endothelial progenitor cells contribute to tumor vascularization by mean of formation of new blood vessel or vasculogenesis, just like other malignant tumor. Hua Rong Zhang et al 2008 showed circulating endothelial progenitor cells was recruited to malignant glioma xenograft, incorporate into tumor neovessel and differentiated into mature endothelial cell, which account for 18% endothelial cells of the tumor blood vessels.

Other than vasculogenesis, endothelial progenitor cells can contribute to tumor vascularization by releasing various pro angiogenic factor in a paracrine manner improve neovascularization which reported by Gao et al 2008, who found endothelial progenitor cells contribute only 12% of new blood vessels in lung tumor and further gene analysis of endothelial progenitor cells showed upregulation of various pro angiogenic genes including VEGF, fibroblast growth factor receptor 1.

2.8.4 Endothelial progenitor cells mobilization

In order to support tumor vascularization, EPC must mobilized from the bone marrow to the tumor site and differentiate into mature endothelial cells. VEGF is one of the most important mediators which stimulate the quiescent endothelial progenitor cell into a proliferative state and it also upregulate the stromal cell derived factor 1 and CXCR4 which is a chemotactic for EPC and recruit EPC to the neovascularization site.

## 2.9 Diagnostic studies

### 2.9.1 Neuroimaging studies

#### 2.9.1.1 Computed Tomography (CT) scan

CT was the important diagnostic neuroimaging in the past; MRI is now the gold standard imaging study for brain tumor. However, CT is useful in the emergency situation to exclude malignant infarct or hemorrhage.

Low grade astrocytomas typically appear as isodense or hypodense regions of positive mass effect, often without any enhancement. Calcification is may occur in about 20 -30% of cases [1] and may be related to calcification in the oligodendroglial components. Cystic component are also encountered, particularly in pilocytic, gemistocytic and protoplasmic varieties (Riemenschneider et al 2009) .

CT scan findings of high grade glioma may include a heterodense mass with thick irregular margin which enhance with contrast; internal areas of hypodense represent the foci of necrosis; internal areas of hyperdense represent the foci of hemorrhage or, rarely, calcifications and it is more common in secondary GBM or after therapy; and a significant mass effect and vasogenic edema (Riemenschneider et al 2009).

2.9.1.2 Magnetic Resonat Imaging (MRI)

MRI brain enable better characterize the brain tumor as it has multiplanar capability and superior soft tissue contrast with contrast agent it can enhance the soft tissue contrast and delineate the tumor margin (Hsu et al 2002)

Table 2.1 MRI, MRS and Perfusion weighted imaging finding of high grade and low grade astrocytoma.

| | Low Grade Astrocytoma | High Grade Astrocytoma |
|---|---|---|
| T1 weighted image | Isointense to hypointense compared to white matter | hypo to isointense mass within white matter with central heterogenous signal which represent area of necrosis and hemorrhage |
| T2 weighted and FLAIR images | Mass - like hyperintense signals which follow the white matter distribution and cause expansion of the surrounding cortex. | Hyper intense surrounded by mark vasogenic oedema and flow voids signal occasionally seen. |
| DWI | No diffusion restriction. | No diffusion restriction, however, lower measured ADC than low grade gliomas. |
| T1 with contrast | No enhancement | Enhancement is variable, typically peripheral and irregular. |

| | | |
|---|---|---|
| MR spectroscopy | elevated choline peak, low NAA peak , elevated choline : creatine ratio, lack of the lactate peak. | Elevated choline peak, low NAA peak, elevated choline : creatine ratio, typically > 1.5, elevated lactate and lipid peak. |
| Perfusion-weighted imaging | no elevation of rCBV | Elevation of rCBV |

2.9.1.3 Positron emission tomography (PET)

The PET scan uses radioactive isotope fluorodeoxyglucose (FDG) for assessing energy metabolism which is helpful in differentiate high grade glioma from low grade glioma, in high-grade gliomas contained areas of high FDG uptake and low grade gliomas lacked of these. Obtaining sample from these areas with improves accuracy of diagnosis and PET scan also useful to assess response to therapy (Hanson et al 1991).

2.10 Treatment

Treatment of astrocytoma remains a challenge to the neurosurgeons even though great advancement or achievement had been made over the course of medical history. The current treatment up to this point and time has been focused to delay the disease progression and improve the patient outcome.

2.10.1 Observation

Nowadays it is uncommon to follow up patient diagnosed to have low grade astrocytoma based on the clinical presentation and imaging characteristics without histologic diagnosis. The rationale behind is very slowly progressive behavior of these tumors.

### 2.10.2 Medical management

The most common signs and symptoms of patient with astrocytoma are attribute to peritumoral edema and seizures which can be manage medically. Peritumoral edema is treated with corticosteroid for example dexamethasone. However, prolonged and high dose of corticosteroid is associated with multiple side effect include Cushing's syndrome, immunosuppression and osteoporosis.

For patient presented with seizure, the selection of anti epileptic drugs is crucial as some of the antiepileptic such as phenytoin and carbamazepine, can induce hepatic cytochrome P-450 enzymes and increase metabolism of chemotherapeutic drugs.

Patients with glioma have a latent hypercoagulable state that predisposes to thromboembolism, with a cumulative incidence of 20% - 30% (Jame et al. 2012). It is reasonable to start anticoagulant for these patients unless contraindicated.

Depression is very common among patient with glioma, antidepressants and psychiatric support must take into consideration for patient with brain tumors.

### 2.10.3 Biopsy

Stereostatic biopsy can obtains tumor tissue for histologic diagnosis. This is indicated in case where open surgery is declined or carries unacceptable risks, it allows identification of patients with high grade glioma for which observation is inappropriate and required adjunct therapy.

The only drawback about stereostatic biopsy is sometime associated with misdiagnosis and inaccurate diagnosis due to heterogeneity of the tumor and limited tissue sampling.

### 2.10.4 Surgical Resection

Because of infiltrative nature of the glioma which cannot be cured with surgery, the surgical goals are alleviate the mass effect, cytoreduction and to establish a pathological diagnosis. Cytoreduction which reduce the number of tumor cells and reduce the risk of accumulation of genetic aberration and thereby decreasing the chances of malignant transformation for low grade glioma. It also reduces cerebral edema and improves radiosensitivity and chemosensitivity.

The role of surgical resection for low grade glioma at the initial presentation is proven to increase the survival for these patients. Smith et al (2008) has confirmed a more aggressive surgical resection is associated with improvement of overall survival compared to a simple debulking procedure.

The role of extensive surgical resection of high grade glioma and association with improve survival is less clear. With gross total resection (GTR) which allow at best 99% resection of tumor, with the 1 % remaining tumor which allow the tumor to recur. The nature of the high grade glioma which is widely infiltrative and often involved the eloquent area, gross total excision is almost impossible. However, Boon et al. (2007) reported with resection of 98% or more associated with improved survival.