Migratory factors influencing the invasive character of GBM

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ABSTRACT

Glioblastoma multiforme (GBM) is one of the most common and lethal brain tumors with a low median survival rate for patients. Due to the aggressive character of GBM, the World Health Organization (WHO) has categorized it as the highest grade brain tumor. The prognosis for GBM patients is poor, due to its invasive character combined with high resistance to treatment. The migration of GBM cells is instigated by hypoxia and is influenced by both paracrine and autocrine factors of wich HIFS and VEGF are the most important, other factors include TGF-β, TWIST, cadherin, integrin, matrix-metalloproteinases and CXCL12. These factors and the tumor cell propagation are both involved in an invasive display. Recently, a small group of GBM cells with stem cell properties have been identified, glioblastoma stem cells (GSCs), which drive tumor formation and have been implicated in resistance to treatment. Current research regarding new treatment is concentrated on inhibiting migration and tumor resistance.
The aim of this paper is to identify the most influential factors in inducing migration in GBM, and discuss potential targets to attenuate the invasive nature of GBM.

CONTENTS

1. GLIOBLASTOMA MULTIFORME

GBM is the most common and malignant form of a primary brain tumor. Primary brain tumors have a low incidence rate but are renowned for their high mortality. GBM has a highly invasive character, although it rarely shows metastasis outside the brain. The median survival period for GBM patients is approximately 1 year. The world health organization (WHO) has a grading system to categorize brain tumors in 4 grades: grade 1 being benign and grade 2 to 4 malignant. With each ascending grade, the aggressiveness and invasiveness of the tumor increases. Grade 1 tumors can be removed by surgery, grade 2 tumors exhibit infiltration of neighboring tissue and grade 3 tumors show an increase in proliferation and anaplasia. Grade 4 tumors (such as GBM) are the most malignant brain tumors as they also display necrosis, resistance to radiation and chemotherapy and induction of angiogenesis (Grzmil and Hemmings, 2010).

There are two major challenges in battling GBM, one is its inclination to migrate and form new tumors, and the other is its tendency to become resistant to conventional therapy (Park and Rich, 2009).
GBM exhibits a highly invasive character and mostly invades the adjacent brain parenchyma, impairing functional brain structures. Other properties ascribed to GBM include its necrotic disposition, aptness to apoptotic resistance, capacity to induce angiogenesis and genomic instability. The symptoms accompanying GBM, caused mainly by the impaired brain structures, include seizures, nausea and hemipareses. (Denysenko, 2010)

The suffix multiforme refers to the inter and intra heterogeneity that is characteristic for GBM. (Krakstad and Chekenya, 2010) Inter heterogeneity can be attributed to the fact that GBM can be divided in primary or secondary GBM, which arises from a preexisting low grade tumor (see figure 1). Intra heterogeneity can be ascribed to the differences between cells within the tumor, seen as a hierarchical structure within a tumor, such as the undifferentiated tumor initiating cells and the differentiated mature cells.

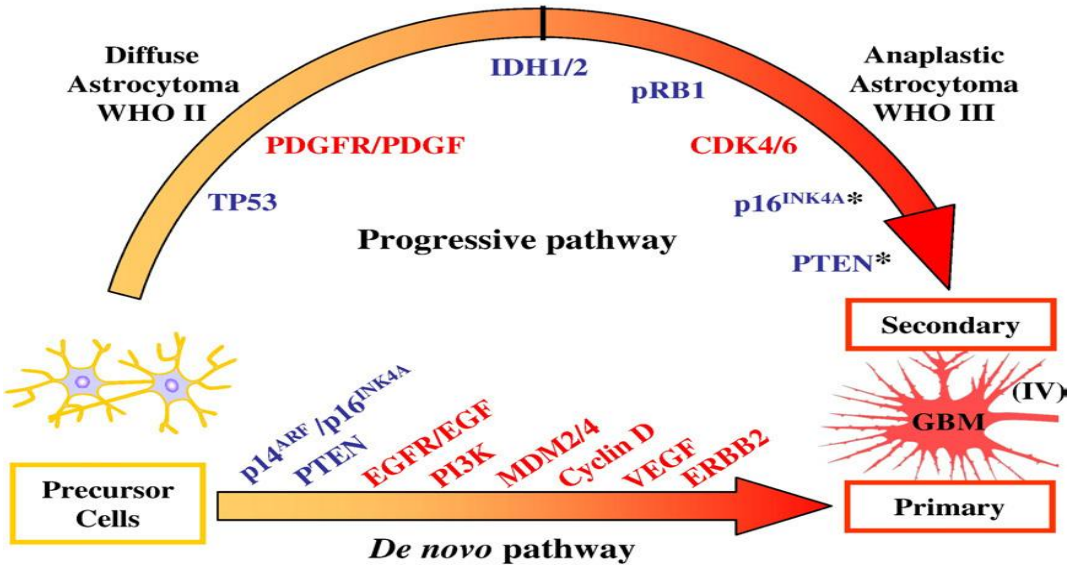

Fig. 1. A schematic overview of primary and secondary GBM. *The common mutations are depicted for both types of GBM. Speculations on the cell of origin tend towards stem cells or progenitor cells. As is shown, primary GBM occurs de novo and secondary GBM develops gradually from low grade brain tumors (Grzmil and Hemmings, 2010).*

As mentioned the inter heterogeneity is based on the division of GBM into primary and secondary GBM, which can be achieved based on their clinical characteristics (See table 1). A primary GBM arises de novo whereas secondary GBM gradually develops from a preexisting and less aggressive brain tumor. The primary GBM is the most common variant observed and is mostly seen in older patients, whereas secondary GBM mostly afflicts the younger patients. They are similar in prognosis but they deviate in their molecular profiles (Grzmil and Hemmings, 2010). In primary GBM mutations are often seen in the epidermal growth factor, cyclin-dependent kinase inhibitor 2A and loss of heterozygosis on chromosome 10q23 which is where PTEN is located. Secondary GBM shows mutations in platelet derived growth factor and P53 (Krakstad and Chekenya, 2010).

Current treatment of GBM entails surgical resection combined with radiation and chemotherapy. These treatments are mainly aimed at prolonging survival as they rarely cure the cancer. Complete surgical resection is near impossible because GBM is too diffuse and gross resection will likely impair brain function. Therefore, additional treatment with radiation and chemotherapy is opted for. The chemotherapeutic drug, Temozolomide (TMZ), has shown the most promising results when administered concomitantly with radiation (Fukushima, et al., 2009). Initially a good response is often seen to these treatments, however this is followed by a recurrence of treatment resistant tumor cells. This resistance is thought to be elicited by cancer stem cells (CSCs) (Eyler and Rich, 2008).

	Common mutations	Type mutation	Function
Primary GBM	EGFR	amplification / over-expression	Proliferation
	Mdm2	amplification / over-expression	P53 inhibitor
	CDKN2A, also known as p16INK4A	Deletion	Tumor suppressor, cell cycle control
	PTEN	Inactivating	Tumor suppressor
Secondary GBM	P53		Tumor suppressor
	PDGFR	amplification / over-expression	proliferation, migration, and angiogenesis
	RB	Deletion	Tumor suppressor
	17p, 19q, and 10q	loss of heterozygosity	
Zhong J. et al., 2010			

Table 1. Common mutations observed in both types of GBM. *The type of mutation and the function of these genes give an indication as to the aggressiveness of the cancer. These mutations are not mandatory for either type. Mutations in EGFR and P53 are the most common observed in primary GBM respectively secondary GBM.*

2. CANCER STEM CELL THEORY

There is much speculation as to the cells of origin in GBM. The presence of neural stem cell-like or progenitor cells in GBM have recently been identified as the likely culprits (Fomchenko and Holland, 2005). It has been shown that the human forebrain contains ample amounts of neural stem cells (NCS) that can migrate and differentiate into different cell types (astrocytes, etc). Similarly, it has also been shown that GBM contains cells with stem cell-like properties and stem cell markers (Singh, et al., 2003). This could indicate stem cells or progenitor cells as possible cells of origin (Denysenko, 2010). If the stem cells or progenitor cells are the instigator of a GBM tumor mass than it is not a farfetched notion to consider that these tumors harbor stem cells to maintain the proliferation of its cells.

The cancer stem cell (CSC) theory states that tumors often harbor a small number of undifferentiated cells that contain stem cell properties such as self renewal, multi-lineage differentiation and tumor initiating capabilities (Sengupta, 2010).
There is growing evidence to support the CSC theory. First, CSCs are thought to be a scarce population within the tumor with an ability to give rise to progeny in the form of transient amplifying cells or mature cells. Rare populations of cells have been found in vivo with tumor initiating capacity (Sengupta, 2010). Second, with the use of flow

cytometry and certain markers, distinctive rare populations of cells, within tumors, were found with stem cell-like properties (Sengupta, 2010). Third, cells within a tumor are heterogenous in both morphology and phenotype, which suggests a certain hierarchy. This hierarchy is to be expected if a tumor were to be formed with CSCs.

Tumors exhibit heterogeneity in their cellular structure that is also observed in clonal tumors. All cells within a tumor share a genetic makeup of their clonal origin, however genetic and epigenetic abnormalities have been observed among various cells. It is possible that all cancer cells within a tumor are similar and that the heterogeneity is accounted for by influences, intrinsic or extrinsic, which results in a random patterning. This is according to the stochastic model (see figure 2), which indicates that every cell should be capable of tumor initiation. Observations have been made, however that could contradict this model. It was shown that a large amount of cancer cells were needed for tumor transfer (Sengupta, 2010), if all cells are capable of tumor initiation then the expectation would be that only a small amount of cells would be ample for tumor transference. According to this observation it is more conceivable that the heterogeneity is achieved by a few CSCs at the base of the tumor formation which would inevitably lead to a hierarchical organization, as is according to the hierarchical model (Dick, 2008).

Despite the growing evidence towards the CSC theory there is still a reluctance to embrace this theory. For instance there are only a few surface markers of which the specificity to bind CSCs is uncertain and therefore none of them are widely accepted. There is also the possibility that a lineage committed progenitor cell can still acquire stem cell properties (Sengupta 2010; Jamieson et al.,2004). The purification of CSCs in GBM remain a challenge, partly due to the debated selectivity of the stem cell markers (Gilbert and Ross, 2009).The CSC theory remains a theory and should therefore be regarded as such, until their presence has been proven. However, based on the observations of the tumor behavior, the presence of CSCs is highly likely and the CSC theory will therefore be upheld in this paper.

CSCs were first observed in leukemia however the first proposed CSCs in solid tumors were observed in GBM (Dick, 2008). GBM has a highly invasive character meaning that at least a few of the cells in the tumor have the propensity to migrate and establish tumor formation. Newly formed tumors within the brain indicate a migrating property of the cancer stem cells or progenitor cells (Cheng, 2010).

Markers can be used to identify different cell types, for example CSCs in different cancer types. CD133, A2B5 and SSEA-1 are commonly used markers in identifying GBM CSCs, however none of these are widely accepted. CD133 is a cell surface marker for neural cancer stem cells. A2B5 is a cell surface ganglioside marking neural precursor cells. SSEA-1 is a carbohydrate antigen (Gilbert and Ross, 2009).

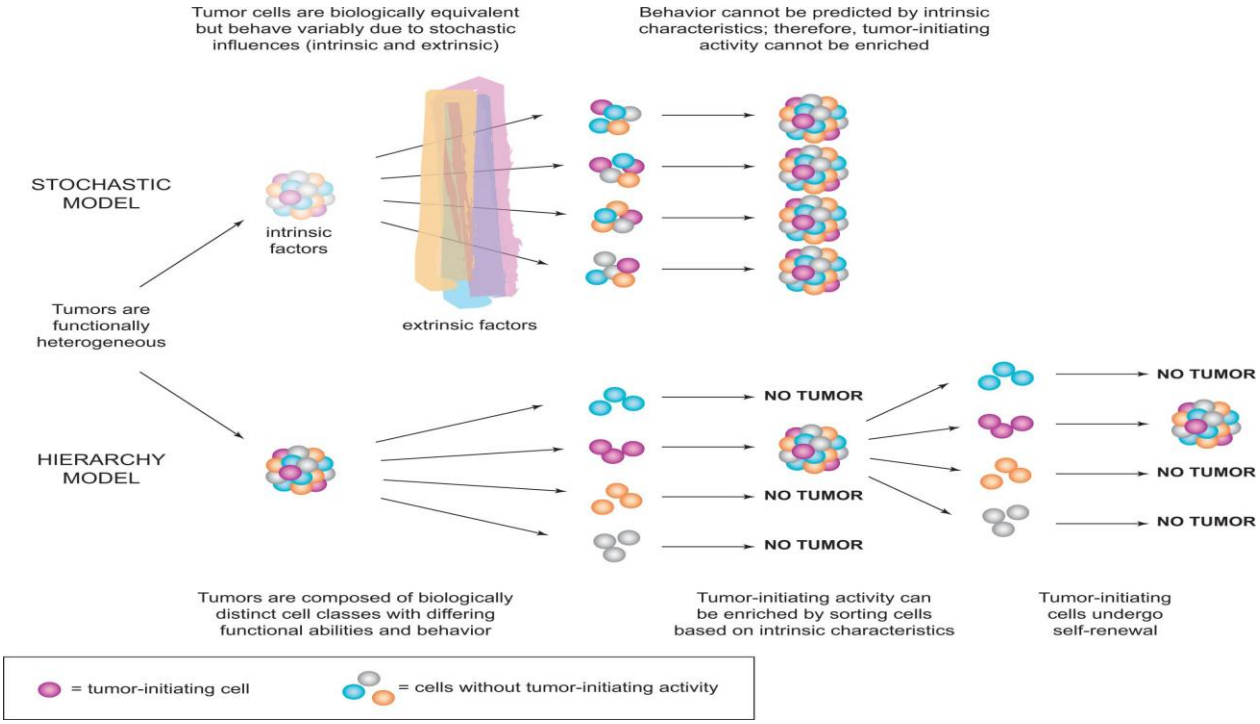

Figure 2. Two proposed models for tumor heterogeneity. C*ells within a tumor are observed as being heterogenous both in function and morphology, these models propose an explanation for their differences. There is no dispute that all these cells contain the same genetic composition, there is however much dispute as to how these differences in functions arise. The stochastic model states that all cells posses the ability to perform the same functions. According to this model all cells posses the ability to initiate a tumor but only if the required extrinsic and intrinsic factors are involved. The hierarchy model states that there are only a few cells within the tumor that possess the ability to initiate tumor formation, the rest of the cells in a tumor mass constitute of more differentiated or mature cells (Dick, 2008).*

The presence of CSCs is likely to be a hindrance to current conventional treatment as the CSCs are known to be more resistant to radio- and chemotherapy. These treatments are mainly focused on inducing DNA damage and consequently target the rapidly proliferating cells. CSCs have low proliferating rates making them a poor target for these treatments. They also possess elaborate DNA repair mechanisms, efflux pumps and they express anti-apoptotic proteins (Gilbert and Ross, 2009).

3. MIGRATION

GBM has a tendency to migrate within the brain, forming a highly infiltrating tumor. Many factors are involved in inducing migration.
Metastasis is a highly inefficient process as most cells do not survive to reach their target. The migrating cell must be capable of successfully completing all steps prior to migration, which is explained hereafter (see figure 3), as well as being capable of tumor growth and maintenance. The cells responsible for colonizing distant sites are therefore likely to be CSCs (Hunter, et al., 2008). It is unlikely that fully differentiated cancer cells are capable of tumor initiation, as they must dedifferentiate or reprogram in order to propagate tumor formation and maintenance. Therefore the migrating cancer cells, responsible for metastasis, must have stem cell properties or be capable of acquiring them (Schiffer, et al., 2010).
The invasive nature of GBM influences the success of GBM treatment. GBM tumors can be surgically removed however the highly invasive character that accompanies GBM makes complete resection of all the tumor cells near impossible and additionally the migrating cells can escape the focus area of chemotherapy and radiation.
Once a cell has migrated away from the mass it is free to start tumor initiation in a new location, provided that it has the required stem cell- like properties. Many pathways are deregulated in GBM providing the cells with the properties needed for proliferation and migration. Despite the highly invasive nature of GBM, metastases in tissue other than the brain are very scarce. GBM cells have a preferential migratory route along the parenchyma (Louis, 2006).
GBM cells belong to a solid tumor mass and therefore several steps need to occur before migration can be initiated (see figure 3). The migrating cell needs to detach from surrounding tumor and stromal cells and remain attached to the extracellular matrix (ECM). Both the ECM and the migrating cell require alterations to enable the migration of the cell. The ECM will be degraded and the cell will undergo the necessary changes in its cytoskeleton and form extensions, such as lamellipodia and filopodia, in order to move along the ECM (Teodorczyk and Martin-Villalba, 2009). Signaling proteins within these extensions enable contact with the ECM after which the cytoskeleton will contract, allowing the forward movement of the cell (Demuth and Berens, 2004)

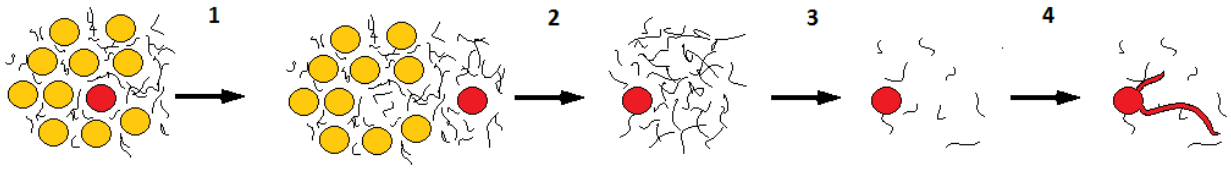

Fig 3. Steps prior to migration. *A schematic overview of the required processes of migration.* ***1*** *the cell separates from the tumor mass.* ***2*** *the migrating cell attaches to the ECM.* ***3*** *the ECM is degraded.* ***4*** *the migrating cell forms filopodia (Teodorczyk and Martin-Villalba, 2009).*

The interaction of the migrating cell with its environment is important for migration to occur, for example in the case of hypoxia, where the requirements of the vast proliferating cells are too extensive for the environment to sustain with regards to oxygen. The cells can respond to this shortage by inducing factors needed for migration. Figure 4 gives a schematic overview of a migratory pathway in which several of these factors are included.
Migration and invasion are linked processes, as invasion cannot occur without migration. Factors that stimulate migration promote the motility of the cell. Factors that stimulate invasion can promote the movement of the cell through different tissues.

a. Hypoxia

It is not clear how many and exactly which conditions can instigate migration, however hypoxia has been proposed as one of the possible triggers. High proliferation of cells require a high metabolism with a demand for abundant supply of oxygen. At a certain point the tumor extends to such a volume that oxygen becomes scarce and hypoxia can occur (Louis, 2006). In the same line of thought it is conceivable that nutrition could also be an instigating factor as high metabolism demands sufficient nutrition.
Hypoxia plays an important role in the migration of GBM both directly and indirectly. Directly by reducing the degradation rate of hypoxia induced factors (HIF). Indirectly hypoxia has been implicated in migration by inducing the retention of the pleuripotency of stem cells and progenitor cells (Bar, et al., 2010).
HIFs are important in migration as they can induce the expression of many factors involved in the migratory pathway, most importantly VEGF. To survive a hypoxic environment cells require an adaptive response which can be induced by certain genes in response to transcription factor HIF1α and HIF-2α. There are three HIFs, HIF-1α, HIF-2α and HIF-3α, whereby HIF-1α and HIF-2α are the most similar in function and both bind to HIF-1β, but they regulate different target genes and HIF-2α is more restricted in its tissue expression. HIF-3α is different as it acts as an inhibitor with a negative regulation of transcription. HIF-1α and HIF-2α can bind to HIF-1β by dimerization, thereby mediating the expression of various genes involved in migration (Oliver, et al., 2009). Both physiological and pathological activation of growth factor pathways can activate HIFs (Kaur, et al., 2005).
The physiological hypoxic response relies on oxygen sensors that regulate the HIFs, which is achieved by the prolyl-hydroxylase domain proteins (PHD) 1-3. Both HIF-1α and HIF-1β are continuously being transcribed however they rarely bind under normal conditions as HIF-1α is continuously being degraded. HIF-1α is quickly hydroxylated by the PHDs, followed by several reactions, resulting in the degradation of HIF-1α. To hydroxylate the HIFs, the PHDs use oxygen as substrate, therefore the activity of the PHDs correlates with the oxygen concentration. A decrease in oxygen concentration concurs with a decrease of PHD activity resulting in a stabilization of HIF which can then mediate their transcriptional responses. An interesting observation regarding the PHDs

was made in a normoxic condition. Only a decrease of PHD-2 leads to an increase of HIF-1α in normal oxygen levels. The function of PHD-1 and PHD-3 in this context remains unclear (Oliver, et al., 2009).

The pathological activation of HIFs relies on the activation of receptor tyrosine kinases (RTKs). Growth factors can induce the activation of RTKs, after which it can activate phosphatidylinositol- 3- kinase (PI3K), which is a lipid kinase. The activated PI3K can trigger a cascade that will result in the activation of AKT, which is a serine/ threonine protein kinase. The activation of AKT can cause an increase of HIF. Inhibition of PI3K and downstream targets of the PI3K pathway can therefore lead to a reduced HIF induction (Kaur, et al., 2005)

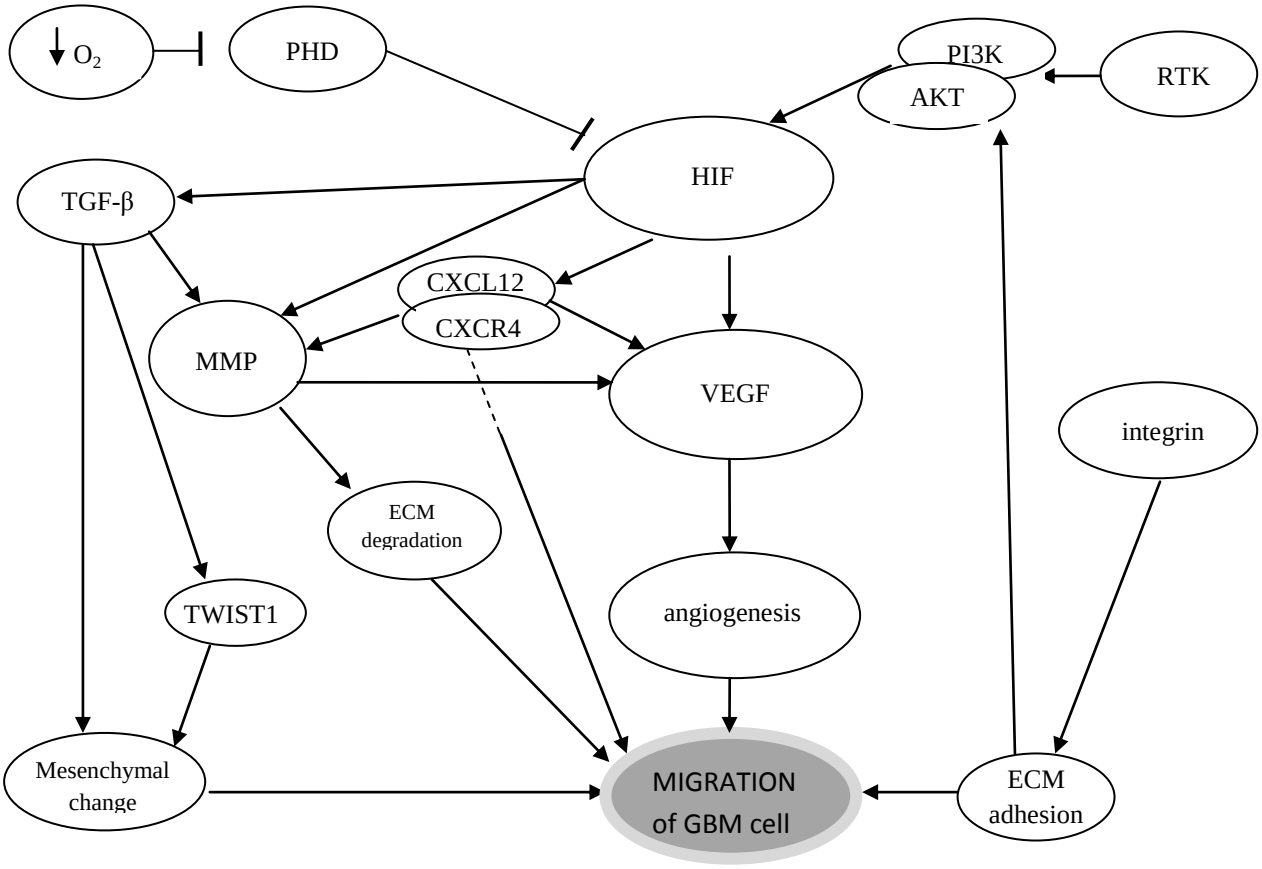

Fig 4. Migratory pathway in GBM. *This schematic overview highlights several factors that are important in the induction of migration in GBM cells.*

b. Angiogenesis

Angiogenesis is an extremely important regulating factor in the development of glioma malignancy. Angiogenesis leads to the formation of blood vessels along which GBM cells can migrate (Dützmann, et al., 2010). Angiogenesis can be regulated by paracrine and autocrine mechanisms. Both mechanisms rely on the expression of vascular endothelial growth factor (VEGF)-A and other angiogenic factors. In paracrine

mechanisms these factors are expressed by non-endothelial cells, whereas in autocrine mechanisms these factors are expressed by the endothelial cells themselves. VEGF-A can interact with the cell surface receptors on endothelial cells, thereby initiating angiogenesis (Fong, 2009).

The expression of VEGF can be induced by several genetic factors but it is mainly induced by HIFs in response to hypoxia. Vast proliferating cells demand an extensive provision of nutrients and oxygen which is delivered to the cells by blood vessels. If the tumor grows large enough the existing vasculature can become inadequate to sustain the high demands of all the tumor cells and hypoxia can occur. HIFs are induced as an adaptive response, which in turn promotes angiogenesis by inducing VEGF. In a hypoxic environment the vascular smooth muscle cells can become less adhesive to the ECM in the basement membrane. The dissociation from the basement membrane is deemed necessary for the protrusions of the endothelial cells to form new sprouts (Fong , 2009)

There are five receptors to which VEGF can bind, vascular endothelial growth factor receptor (VEGFR) 1-3 and neuropilin (NRP) 1-2. Endothelial cells mainly express VEGFR-1 and VEGFR-2. The VEGF/ VEGFR-2 pathway is thought to be the principal mitogenic signaling pathway for VEGF which is probably why this pathway is the most prevalent angiogenic pathways found in gliomas (Argyriou, et al.,2009; Kaur, et al., 2005).

c. Mesenchymal properties of GBM

Mesenchymal transition in GBM is implicated as an important event associated with a more aggressive phenotype. Acquiring a mesenchymal phenotype causes a disruption of cell-cell contacts, resulting in the dissemination of the cell from the tumor mass, which is an imperative for metastasis. The acquisition of a mesenchymal phenotype can therefore lead to an enhancement of cell motility (Guarino, et al., 2007). The activation of molecular and cellular mesenchymal changes is similar to the mechanism that carcinomas use, known as the epithelial to mesenchymal transition (EMT). EMT is implicated in the development of aggressive and invasive carcinoma tumors. The EMT concept is partially based on a cadherin switch, where the epithelial marker, E-cadherin, is suppressed and the mesenchymal marker, N-cadherin, is activated. The cadherin switch has not been observed in GBM even though it uses a similar mechanism to acquire a mesenchymal phenotype for migration (Mikheeva, et al., 2010). There are several fundamental factors involved in the activation of mesenchymal changes. TGF-β, which is a growth factor, and TWIST1, which is a transcription factor, have both been implied as crucial factors in this process.

TGF-β is a protein that plays an important role in cell cycle regulation. Its involvement, amongst others, is in growth and differentiation. TGF-β has both tumor inhibiting and tumor promoting properties. TGF-β can inhibit proliferation by inhibiting the expression of cyclin- dependent kinases (cdks) and induce activity of cdk inhibitors (Golestaneh and Mishra, 2005). TGF-β can also promote migration. In response to TGF-β, the TGF-β

receptor 1 (TβR 1) can activate Smad2 and Smad3 through phosphorylation. Smad2 and Smad3 form a complex with Smad4 which then migrates to the nucleus to take part in the activation of target genes (Golestaneh and Mishra, 2005). In carcinomas TGF-β has been implicated as an inducer of EMT (Brabletz et al., 2005). An up-regulation of TGF- β has been observed in GBM. It is most likely that TGF-β plays a similar crucial role in the mesenchymal change in GBM as it does in EMT in carcinomas.
TGF-β can induce TWIST1, which is a helix-loop-helix protein, involved in the activation of EMT in embryonic development as well as cancer metastasis. An up-regulation of TWIST1 has been observed in GBM, which suggests an important role in obtaining a mesenchymal phenotype. TWIST1 regulates similar genes in GBM and carcinomas for the mesenchymal change. Mikheeva et al. showed that TWIST1 has a crucial role in promoting the mesenchymal molecular and cellular changes in GBM. Therefore TWIST1 can induce invasion through mesenchymal changes that are independent of the cadherin switch (Mikheeva et al., 2010).

d. Adhesion molecules

Adhesion molecules play an important role in the invasiveness of GBM, they can either promote or inhibit migration of the cells.
Cadherins are a group of adhesion molecules responsible for adherens junctions which are important for the maintenance of cell structures. Cadherins can establish cell-cell contact in two adjacent cells through the interaction of their extracellular Ca^{2+} binding domains. To enforce this contact, multiple cadherins will cluster together thereby inhibiting migration (Demuth and Berens, 2004).
Integrins are another group of adhesion molecules which can facilitate migration by allowing the migrating cell to attach to the ECM and altering the contact location by changing the internal signaling for affiliation to bind (Demuth and Berens, 2004). The integrin receptors are expressed on glioma cells and are activated by an interaction with extracellular ligands. This interaction can lead to an alteration of the cytoskeleton of the cell which could facilitate migration (Louis, 2006).
ECM adhesion facilitated through integrins can also activate the PI3K/AKT pathway. Binding of integrins can activate integrin- linked kinase which in turn leads to an increase of HIF-1α and the production of VEGF. In gliomas this increase of integrin- linked kinase activity has been observed (Kaur, et al., 2005)

e. Matrix-metalloproteinases

Matrix-metalloproteinases (MMPs) are involved in degrading the ECM and thereby create a favorable route for migrating cells. MMPs belong to a zinc- binding endopeptidase family and are expressed as inactive peptidases, only after proteolytic cleavage do the MMPs become active (Kaur, et al., 2005). MMP-2 and MMP-9 are the most commonly implicated MMPs in migration (Demuth and Berens, 2004). MMPs can be regulated by both HIF-1α and CXCL12. Both induce an up-regulation of MMP9 and

HIF-1α can additionally up-regulate MMP1, MMP2, and MMP7 (Kaur, et al., 2005). An altered environment, through degradation of the ECM due to proteases, is also implicated in tumor growth as it allows for spatial expansion (Louis, 2006). In addition it can further up-regulate MMP1, MMP2 and MMP9 by inducing TGF-β.
Serine protease urokinase-type plasmogen activator and its receptor together with cystein protease cathepsin B are also implicated in the process of ECM degradation. Studies have revealed that a decreased function of these proteases concurs with a decreased occurrence of migration (Louis, 2006).

f. CXCL12/ CXCR4 pathway

Chemokine (C-X-C motif) ligand 12 (CXCL12, also known as stromal derived factor-1, SDF-1), can bind to the receptors CXCR4 and CXCR7. Upon binding to CXCR4 expressing cells, CXCL12 can induce several important biological effects which are all involved in the migration. These effects constitute motility, chemotactic response, adhesion and secretion of matrix-metalloproteinases (MMPs) and angiogenic factors such as VEGF (Kucia, et al., 2004). HIF-1α and integrin-linked kinase leads to an up-regulation of CXCL12 which indicates that the expression of chemokines can be triggered by hypoxia (Petit, et al., 2007).
Chemokines are chemo attractant cytokines which amongst others can have an involvement in steered migration. The CXCR12/ CXCL12 pathway is involved in retaining and homing hematopoietic stem cells (HSCs). The function of CXCL12 is in the regulation of the trafficking of HSCs. Functional CXCR4 is expressed on embryonic stem cells and on certain tissue committed stem cells, for example on neural tissue stem cells. Cells with a functional CXCR4 expression can migrate along a CXCL12 gradient. CXCL12 is expressed in several organs, including the brain (Teicher and Fricker, 2010). CXCL12 can recruit and retain CXCR4 pro-angiogenic cells to a niche where it can support angiogenesis for tumor growth (Petit, et al., 2007). This explains the important role that chemokines play in the “seed and soil” theory, which explains different metastasis patterns of tumors in different tissues. This theory states that tumor cells migrate to a location where they are attracted and halted by locally secreted chemo-attractants, and where they will find an adequate niche for survival and proliferation (Kucia, et al., 2004)
The mechanism by which migrating cancer cells use the CXCL12/ CXCR4 gradient is in concurrence with the “seed and soil” theory. A cell is detached from the tumor mass and has started migration. The tumor cell can sense a chemo-attracting gradient if it is along its migratory path. This gradient will then direct the tumor cell to an adequate niche for tumor growth (Kucia, et al., 2004).

4. TARGETS FOR FUTURE INTERVENTION

As far as the medical advances in research are concerned, the therapeutic means for treating GBM fall short. For future intervention, a better understanding in the progression of GBM is warranted, especially towards the migratory tendency that GBM exhibits, in order to extend life expectancy of GBM patients as well as improving quality of life.

As mentioned before, there are two major challenges in battling GBM, the inclination to become resistant to current treatment and its migratory tendency. Current research is focused on addressing these challenges.
There is an up-regulation of VEGFR2 in GBM, therefore several studies have been performed in targeting these receptors to inhibit angiogenesis. Monoclonal antibodies and tyrosine kinase inhibitors (TKIs) are currently tested as inhibitors of VEGFR2 with promising results. Monoclonal antibodies have difficulties passing the blood brain barrier therefore the use of TKIs are preferred (Argyriou, et al., 2009).
By inhibiting HIFs, their downstream signaling can be attenuated. Narita et al., have identified KC7F2 as a potent HIF-1α inhibitor, which can prevent acitivation of HIF downstream signaling such as MMPs (Narita, et al., 2009). The silencing of HIF-1α can result in decreased cell survival and vascularization, however proliferation and tumorigenesis were found unaffected. HIF-2α could be a more important factor for the autonomous proliferation for cancer cells as it can activate important receptor tyrosine kinases (RTKs), such as EGFR and their signaling pathways. Indeed the inhibition of HIF-2α resulted in the deactivation of EGFR (Franovic, et al., 2009).

To address the challenge of treatment of resistant GBM cells, studies could focus on anti-CSC therapies or on sensitizing the cancer cells.
The development of anti-CSC therapies is burdened by the fact that normal stem cells should remain as unaffected by the treatment as possible, thus targeting factors which are only crucial for cancer stem cells (Eyler and Rich, 2008). In leukemia, rapamycin has been shown to only affect the cells with PTEN deletions, which were CSCs and leukemia cells, whilst maintaining the normal hematopoietic stem cells (Yilmaz, et al., 2006). These results indicate CSCs as possible targets and its use should be explored in other cancers bearing stem cell subpopulations. The identification of the specific markers for GSCs could lead to interesting observations with regards to anti-CSC therapies

A better response to treatment can be achieved by sensitizing the tumor cells. A recent study has shown that GBM cells can be sensitized by targeting the DNA repair mechanism. WEE 1 is an important kinase in the regulation of cell cycle control. GBM was found to have an up regulation of WEE1. This up-regulation was found to coincide with efficient DNA repair. Researchers were able to successfully inhibit WEE1 using small interference RNA. Inhibiting WEE1 can lift the cell cycle control at checkpoint G2, terminating the process of DNA repair prematurely. The cell cycle will continue but without proper repair of the DNA damage inflicted by the chemo- and radiation therapy, the cell could be forced into apoptosis, provided that the DNA damage is too extensive (Mir, et al., 2010).

SUMMARY

- GBM is a highly malignant brain tumor, with no current effective treatment as GBM cells are highly prone to migration and resistance to therapy.
- GBM stem cells maintain tumor growth and invasion and have mechanisms to promote resistance to treatment.
- Hypoxia is the main instigator of migration, where many factors are involved in the downstream signaling
- Current research is focused inhibiting both migration and therapy resistance

5. DISCUSSION

A promising option towards improving treatment is focusing research on migration factors. If the migratory tendency of cancer cells can be halted then the cells will be restricted to a location. Surgical resection could prove to be more efficient, as will chemotherapy and radiation, which could then be administered with the focus on that location with a higher probability that the treatment reaches as much of the cancer cells as possible. Several factors play a role in stimulating migration in the GBM cells. A prerequisite for the development of new treatments for GBM patients is better understanding of these factors.

Focusing research on hypoxia and its induced factors should give promising results, with respect to inhibiting migration. Many factors influence migration and all of them could be targeted, however hypoxia is at the top of the chain inducing HIFs, which regulate many downstream events leading up to migration. This suggests that this would make an ideal target for intervention of invasion of GBM cells.

An imperative for migration is angiogenesis. Inhibiting angiogenesis could decrease invasion of GBM and several studies have shown promising results, however reports on this matter regarding the success of inhibiting invasion are conflicting. A decrease of angiogenesis could induce hypoxia resulting in its downstream signaling. It might therefore be promising if angiogenesis could be steered and artificially regulated within the tumor.

It is most likely that subpopulations of CSCs are present in GBM. The presence of cancer stem cells could potentially have a major impact on the invasive character of GBM. If GBM cells migrate within the brain, they could only form a new tumor if they have tumor initiating properties. The CSC theory provides a candidate for these tumor initiating cells, if these cells are capable of migration it could prove to be an eligible target for future intervention. The challenge here would be to target only the cancer stem cells and not the healthy stem cells. The anti-CSC therapy could be promising in battling GBM, however, research on this subject is still in its infancy. For now the challenge remains to purify cancer stem cells in GBM for further investigation.

Another option for improvement of GBM treatment is to sensitize the tumor cells to the therapies which are currently given. The recent success in inhibiting WEE1 might have important implications for improving therapy, however it is still dependant on the effectiveness of current treatment.

Studies on migration and GSCs should provide much insight into possible targets for treating GBM and therefore more research is warranted on this matter . The HIFs could be considered the most influential migratory factor and VEGF the most influential invasive factor, which implicates them as the most viable target to attenuate the invasive character that accompanies GBM.

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