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Review

Understanding the role of endothelial cells in brain tumor formation and metastasis: a proposition to be explored for better therapy

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ABSTRACT

Glioblastoma is one of the most devastating central nervous system disorders. Being a highly vascular brain tumor, it is distinguished by aberrant vessel architecture. This lends credence to the idea that endothelial cells (ECs) linked with glioblastoma vary fundamentally from ECs seen in the healthy human brain. To effectively design an antiangiogenic treatment, it is crucial to identify the functional and phenotypic characteristics of tumor-associated ECs. The ECs associated with glioblastoma are less prone to apoptosis than control cells and are resistant to cytotoxic treatments. Additionally, ECs associated with glioblastoma migrate more quickly than control ECs and naturally produce large amounts of growth factors such as endothelin-1, interleukin-8, and vascular endothelial growth factor (VEGF). For designing innovative antiangiogenic drugs that particularly target tumor-related ECs in gliomas, it is critical to comprehend these distinctive features of ECs associated with gliomas. This review discusses the process of angiogenesis, other factors involved in the genesis of tumors, and the possibility of ECs as a potential target in combating glioblastoma. It also sheds light on the association of tumor microenvironment and ECs with immunotherapy. This review, thus gives us the hope that neuro endothelial targeting with growth factors and angiogenesis regulators combined with gene therapy would open up new doorways and change our traditional perspective of treating cancer.

1. Introduction

Brain tumors are among the most aggressive neurological disorders with their highest-grade neoplasm resulting in a very short life expectancy. Based on the catastrophic potential of brain tumors, the World Health Organization (WHO) has divided brain tumors into four categories varying from benign to metastatic tumors. Grade I and II (pilocytic astrocytoma, oligodendroglioma) are considered to be of low grade with improved prognosis, while grades III and IV (anaplastic astrocytoma, glioblastoma) are considered to be malignant in nature with worse prognosis.[1] *Ostrum* et al.[2] carried out an epidemiological analysis of primary brain and other central nervous system tumors in the United States between 2011 and 2015. The most frequent malignancies were of the brain and other primary tumors with an average annual age-adjusted incidence rate of 5.65 per 100,000 people between the age group of 0 to 14 years while above the age group of 40 years, it was 44.47 per 100,000 population. Primary brain tumors are classified by the types of cells involved, such as glioma, meningioma, astrocytoma, etc., while secondary brain tumors arise due to metastasis of other cancers to the brain.[1,3]

The fifth edition of the WHO classification of central nervous system (CNS) tumors, which was published in 2021, essentially classified CNS tumors based on molecular diagnostics along with established approaches of histopathology and immunohistochemistry. For instance, gliomas, glioneuronal tumors, and neuronal tumors have been classified into 6 families, viz., adult-type diffuse gliomas, pediatric-type diffuse low grade gliomas, pediatric-type diffuse high-grade gliomas, circumscribed astrocytic gliomas, glioneuronal and neuronal tumors and ependymomas.[4] Glioblastoma multiforme (GBM) is the most serious grade IV tumor, which comes under adult-type diffuse gliomas, and may reduce the survival time to less than 5 years. The GBM exhibits either as an isocitrate dehydrogenase (IDH) mutant or an IDH wild-type tumor.[5,6] On the basis of its origin, GBM is further classified into primary and secondary GBM. The former emerges from de novo brain stem cell precursors, while the latter develops from the progression of low-grade diffuse astrocytoma or anaplastic astrocytoma. Overall survival

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Table 1
Nano formulations for the treatment of glioblastoma multiforme.

Drug	Technology	Remark	Reference
Zoledronate (2021)	Microglia membrane coated zoledronate nanoparticles	Apoptosis of temozolomide resistant GBM cells and increased proportion of M1 type GBM associated macrophages	13
Doxorubicin (2020)	Natural pH responsive tripeptide (Lys-Phe-Gly) capped gold nanoparticle	Increased apoptic response with various cell lines and decreased cell proliferation, tumor growth in BT 474 cell line xenograft model in nude mice	14
Docetaxel (2018)	Angiopeptide-II surface conjugated nanoparticle	Increased cytotoxicity, cellular internalization and prominent apoptosis than unconjugated nanoparticles	15
Etoposide (2016)	Melanotransferrin conjugated antibody on the surface of solid lipid nanoparticles	Potent nanocarrier for transporting etoposide across BBB and enhanced internalization across glioblastoma cells	16
Cetuximab (2017)	I^{131} radiolabeled anti-epidermal growth factor receptor binding bovine serum albumin nanoparticles	Enhanced uptake and accumulation of nanoparticles in xenograft model of nude mouse	17
Lenalidomide (2020)	Lactoferrin protein matrix used for loading titanocene enclosing zeolitic imidazolate framework (ZIF-8) along with 5-fluorouracil and ZIF-8 and further coated with lenalidomide-hyaluronic acid conjugate linked via hydrazone linkage	Superior cell growth suppressing ability and pH-responsive sustained release of 5-fluorouracil and lenalidomide (multimodal therapy)	18
Doxorubicin (2018)	Myristic acid modified ^{D}A7R peptide Pegylated liposome	Enhanced internalization in glioma, tumor neovascular, and brain capillary endothelial cells	19
Vincristine and Tetrandrine (2017)	Distearoylphosphatidylethanolamine-polyethylene glycol modified liposomes (DSPE-PEG2000)	Enhanced transport across BBB and counteracted multidrug resistance, blocking the cancer cell invasion through in vitro study. In vivo result showed prolonged circulation time.	20
Temozolomide (2018)	Cationic liposomes with potential of recruitment of protein and natural targeting capacity in the biomolecular corona layer that form around cationic liposomes	Greater uptake of temozolomide in human umbilical vein endothelial cells	21
miR155 (2021)	Virus mimicking nucleic acid nanogel	Reprogrammed microglia and macrophage from a pro-invasive M2 phenotype to an anti-tumor M1 phenotype by mimicking the virus infection process	22

Abbreviations: BBB, blood-brain barrier; GBM, glioblastoma multiforme.

in GBM is influenced by three factors, viz., tumor factors, patient factors, and treatment factors. The GBM manifests itself as a diverse collection of genetic and epigenetic changes. Primary GBM presents mutations in cyclin-dependent kinase inhibitors 2A (CDKN2A), phosphatase tensin homolog (PTEN) gene, and epidermal growth factor receptor (EGFR). Molecular indicators for secondary GBM comprise IDH-1 and TP53 which closely correlate with O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation.[7] The first line of therapy for GBM starts with surgery, and radiotherapy and chemotherapy are followed subsequently. This therapy is limited in recurrent GBM cases as even a slight residual tumor gives prompt recurrence resulting in the death of patients. Thus, there is a pressing requirement for identifying new targets to facilitate the development of novel targeted therapy. The current standard of care for GBM rests upon the safe surgical resection utilizing 5-aminolevulinic acid, followed by radiotherapy (60 Gray) with temozolomide for 6–12 months.[8] Immunotherapy and the Food and Drug Administration (FDA)-approved tumor targeting field (TTF) are emerging therapies for newly diagnosed and recurrent cases of GBM.[9] The TTF is a non-invasive anticancer therapy that makes use of alternating electric fields of precise frequencies and intensities and is used to break down the cycle of mitosis in cancerous cells and cause apoptosis.[10,11] Besides this, numerous nanoformulations such as liposomes, dendrimers, polymeric nanoparticles, and micelles have been explored in the treatment of cancer.[12] Table 1 provides a brief overview of different nanoformulations used for the treatment of GBM. So far, treatment strategies have been proven suboptimal. We believe that a better treatment plan will emerge from a sound understanding of the processes involved in the genesis of the tumor. In this review, therefore, we underscore the process of angiogenesis, metastasis, other factors involved in tumorigenesis and the potential of using angiogenetic regulators and other growth factors as target for GBM treatment.

2. Angiogenesis: the key process in tumor formation

The term angiogenesis was first used by a British surgeon named John Hunter in 1787. Angiogenesis refers to the process of forming blood vessels from prevailing blood vessels. It is required for tumor growth and metastasis and is driven by chemical signals supplied by tumor cells for rapid growth and development.[23] Because of a lack of blood and oxygen, tumor cells located distant from capillaries suffer hypoxia. A hypoxic environment causes cancer stem cells (CSCs) to develop into endothelial progenitor cells and mature endothelium, resulting in the formation of new blood vessels inside the tumor. Pathological angiogenesis occurs when tumors create aberrant and functionally immature blood vessels as a result of unregulated variables such as angiogenic growth factors, angiogenesis inhibitors, and other hereditary factors. Moreover, the blood vessels present in the tumor site differ significantly from normal vessels in terms of lumen diameter, permeability, and shape. Due to dilation and hyperpermeability of tumor blood vessels, the interstitial pressure gets built up which further cause alteration of blood flow and local edema. Thus, the tumor microenvironment is drastically altered by this irregularly-grown tumor vasculature, which also influences the tumor's growth, and its ability to metastasize to distant sites. This emphasizes the relevance of angiogenesis in tumor growth and calls for treatment strategies that limit tumor angiogenesis.[24] Some of the notable examples of United States FDA-approved drugs belonging to the anti-angiogenic class include cetuximab, trastuzumab, tyrosine kinase inhibitors like sunitinib, imatinib, immunomodulatory agents like thalidomide, lenalidomide, etc. The mechanism by which such drugs elicit their therapeutic effects on tumor cells can be viewed as three steps: depletion of vessels, normalization of vessels and activation of the immune system.[25] These mechanisms are illustrated in Fig. 1.

The development of brain tumor is directed by several angiogenic factors during angiogenesis. Some of the angiogenic factors controlling GBM development include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), transforming growth factor (TGF-β), matrix metalloproteinase (MMP), basic fibroblast growth factor (bFGF), etc. Different mechanisms have been put forth for the upregulation of these angiogenic factors during angiogenesis, viz., hypoxic tumor microenvironment, loss of tumor-suppressing gene, and activation of oncogene function. The expression of angiogenic factors can be well linked with the progression of a brain

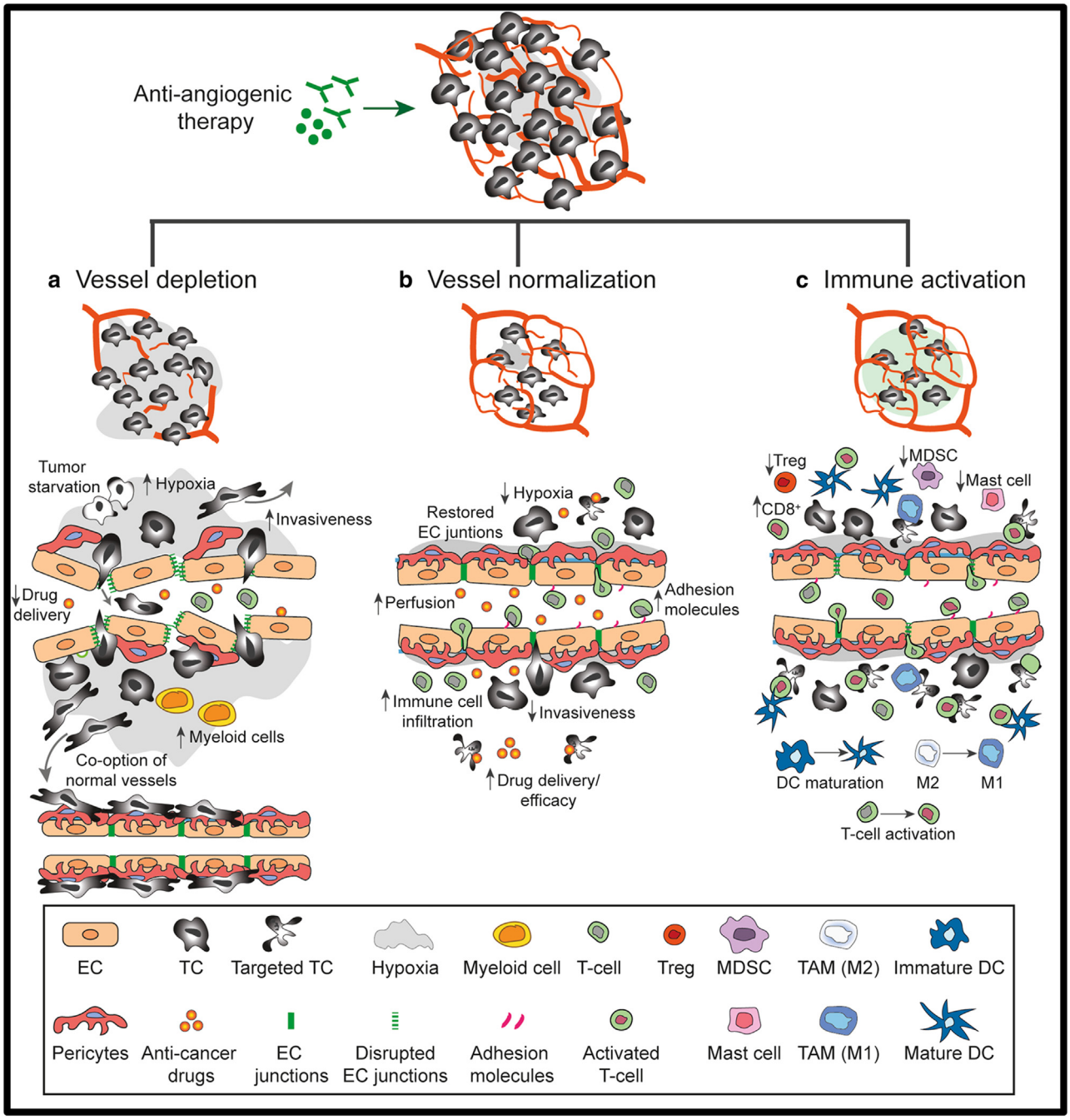

Fig. 1. Mechanism of action of antiangiogenic therapy. It is classified into three categories. a. Vessel depletion: Tumor cells are starved and tissue hypoxia is enhanced as a result of vessel loss. Further, it may encourage recruitment of proangiogenic myeloid cells and tumor cells are mobilized from hypoxic tissues to normal tissues leading to inefficient delivery of anticancer drugs. b.Vessel normalization: By restoring endothelial cell junctions, the extent of tissue hypoxia is decreased along with increased expression of adhesion molecules. Increased immune cells would further lead to increase in drug delivery efficacy. c. Immune activation: Anti-angiogenic drugs result in dendritic cell maturation, T cell activation combined with decreased regulatory T cells, myeloid derived suppressor cells and mast cells. Polarization of tumor associated macrophages has also been approved after anti-angiogenic therapy (Reprinted with permission from R.Lugano, M. Ramchandran, A. Dimberg, Tumor angiogenesis: causes, consequences, challenges and opportunities, Cellular and Molecular Life Sciences (2020) 77:1745–1770.[25]). EC, endothelial cell; DC, dendritic cell; MDSC, Myeloid-derived suppressor cell; TAM, tumor-associated macrophage; TC, tumor cell.

tumor.[24] In the next section, these factors are elaborated in correlation with the process of angiogenesis. Fig. 2 represents the process of angiogenesis. This process is further controlled by positive and negative regulators. Interestingly, as the name suggests both types of regulators work contrary to each other i.e., positive regulators promote angiogenesis while negative regulators arrest the growth. The specific role of positive and negative regulators in angiogenesis is explained in the following section.

2.1. Positive regulators

2.1.1. Vascular endothelial growth factors

The VEGF and its receptors (VEGFR) are found to have a principal role in tumor neovascularization. The VEGF is the most effective angiogenic agent in tumor and normal cell neovascularization. Hypoxia can induce the VEGF and its receptors through hypoxia-induced factor 1-α (HIF 1-α), and HIF 1-α gene plays a vital role in the process of

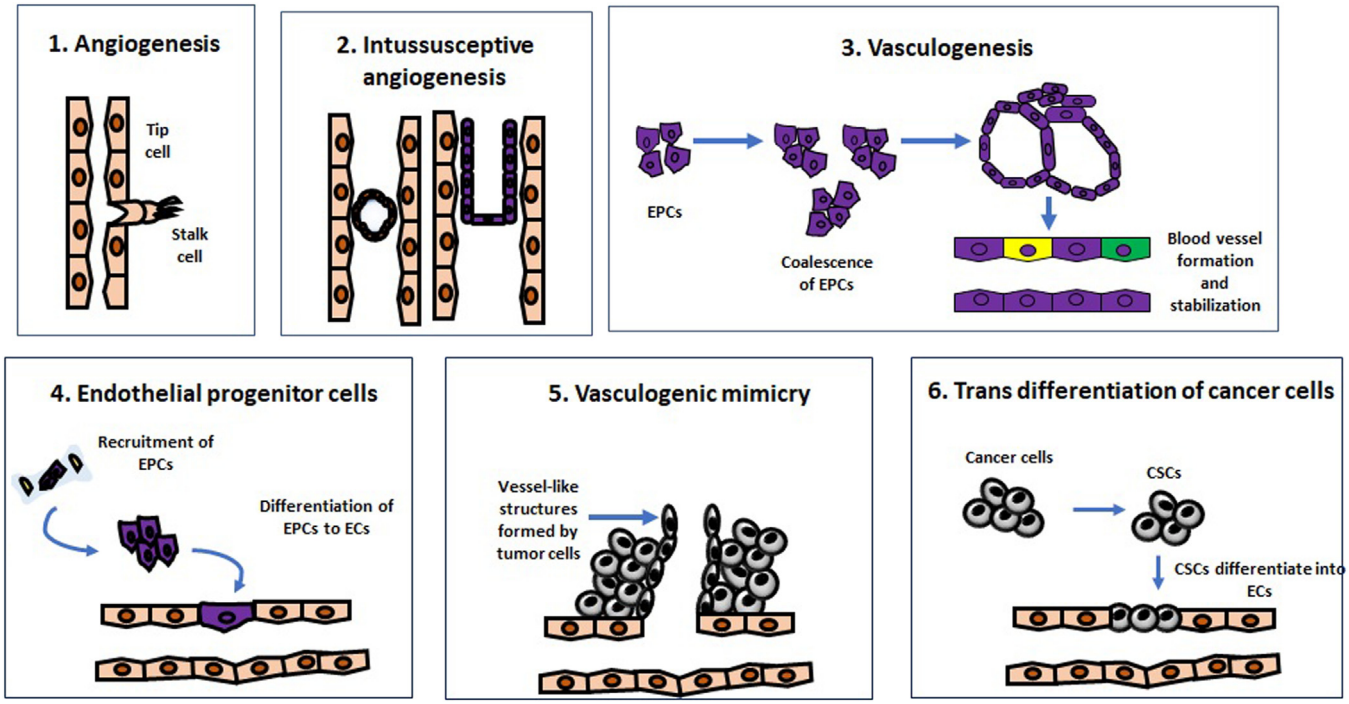

Fig. 2. The angiogenesis process. The process occurs through following mechanisms. 1. Initiation of angiogenesis: In this process, tip cells originate and proliferate followed by fusion with an existing vessels. 2. Intussusceptive angiogenesis: In this process, existing vessels split and new vasculature is formed. 3. Vasculogenesis: Proliferation of endothelial progenitor cells and lumen formation leading to new vasculature formation. 4. Recruitment of endothelial progenitor cells: In this process, progenitor cells are recruited leading to vessel formation in tumor. 5. Vascular mimicry: Tumor cells form vessel like structures. 6. Trans differentiation of cancer cells: Cancer stem cells are differentiated into endothelial cells. CSC, cancer stem cell; EC, endothelial cell; EPC, Endithelial progenitor cell.

angiogenesis. Hypoxia promotes blood vessel growth by upregulation of proangiogenic pathways and influences the aspects of angiogenesis maturation and functioning of blood vessels.[26] The VEGF binding to its receptors activates the endothelial mitogen-activated protein kinase (MAPK) signal transduction pathways that stimulate the nucleus to activate genes for new blood vessel formation. The VEGF induces proteins matrix metalloproteinase (MMP), urokinase-type tissue plasminogen activator (uPA), and tissue-type plasminogen activator that break down the basement membrane to allow endothelial cells to migrate and invade. When MMP goes to extracellular fluid (ECF), it degrades the extracellular matrix (ECM) to allow VEGF to reach VEGFRs on endothelial cells that stimulate angiogenic signals in the vessels.[27] VEGF mRNA expression was shown to be high in necrotic areas of glioblastoma tumors, promoting vascular proliferation and tumor growth in human glioblastoma.[24] There are a few drugs like bevacizumab and ramucirumab that block VEGF. Bevacizumab, a monoclonal antibody selectively binds to VEGF and inhibits interaction with its receptors on the surface of blood vessels. This results in the reduction of the tumor microvascular growth and further reduces the tumor blood supply. This antibody can also be used with temozolomide for GBM and with irinotecan for glioma treatment.[28,29] Ramucirumab is a VEGFR-2 antagonist and it inhibits the binding of ligands like VEGF-A, C, and D to VEGFR-2. Thus, ramucirumab has a potential role in inhibiting the ligand-receptor interaction, thereby inhibiting the proliferation and migration of endothelial cells.[30]

2.1.2. Matrix metalloproteinase

The MMPs can be stimulated by several factors, like bFGF, TGF-α and β, VEGF, angiogenin, and interleukin-8 (IL-8). The TNF-α is known to stimulate the MMP-2, 8, and 9 in the endothelial cells and promotes angiogenesis.

MMP-1 elevates the expression of VEGFR-2 and endothelial cell proliferation. The elevated MMP-2 level is associated with VEGF expression, which emphasizes the crucial part of MMPs in angiogenesis.[31] The bFGF and FGF2 are also known to stimulate the expression of MMPs. The bFGF has been reported for its angiogenic activity and it interacts with receptors like tyrosine of endothelial cells. The FGF2 which is produced from tumor cells or endothelial cells goes and binds to FGF2 receptors on the endothelial cells, which further induces angiogenesis by a cascade of events like cell proliferation, migration, and invasion.[32,33]

2.1.3. Platelet-derived growth factors

The PDGF-β plays a vital role in glioma genesis. Overexpression of PDGF-β and its receptor are seen in glioma and known to act through autocrine signaling pathways. It has been shown by the stimulatory action of PDGF-β on U87MG tumor angiogenesis. The PDGF-β also stimulates the VEGF expression in tumor endothelium.[34,35] Glioblastoma progression has been linked with the overexpression of PDGF receptors. *Cenciarelli* et al.[36] identified the role of isoforms of PDGF receptors in attenuating PDGFR-α signaling which resulted in apoptosis of glioblastoma stem cells.

2.1.4. Transforming growth factor-β

The TGF-β controls proliferation, differentiation, and apoptosis and promotes angiogenesis by modulating the thrombospondins-4 (TSP-4) levels. The TGF-β induces alterations in the vascular matrix, which further enhances angiogenesis. It also alters the ECM composition which favors the pro-angiogenic TSP-4, which is an ECM specific factor.[37] The elevated level of TGF-β was reported to be linked with a meager prognosis of GBM through elevating the expression of other factors like VEGF, FGF, and PDGF-β.[24] TGF-β and VEGF signaling cross-talk in glioblastoma has been demonstrated to exhibit both pro- and anti-angiogenic actions in both human brain-derived microvascular endothelial cells (hCMECs) and glioblastoma-derived endothelial cells (GMECs). TGF-β increases VEGF mRNA and protein expression in glioma cells, resulting in pro-angiogenic effects. Exogenous TGF-β, on the other hand, inhibits endothelial characteristics and causes endothelium-mesenchymal transition (EndoMT) in hCMEC and GMEC.[38]

2.1.5. Interleukin-8

IL-8 has proangiogenic and tumorigenic properties and its concentration increases in response to cytosolic Ca^{2+}, death receptor activation, IL-1, and various kinds of cellular stresses. The IL-8 exerts its effect on the receptors such as CXCR1, and CXCR2. IL-8 also is associated with glioma. It was reported that IL-8 has been proven to be an alternative option to VEGF for glioma angiogenesis.[39]

2.1.6. Miscellaneous factors

Klotho gene, discovered in 1997 and known to have an anti-aging role, manufactures two protein forms, viz., transmembrane and secreted form. There has been enough evidence reports that strengthened the role of Klotho in carcinogenesis. The reports suggested that Klotho expression was decreased in breast tumor cells compared to healthy cells. Apart from this, Klotho gene has also been associated with an anti-tumor effect in terms of the methylation status of gene promoter and the active role played by insulin-like growth factor-1 (IGF-1). The research findings also showed the possible involvement of Klotho gene in glioblastoma cells, particularly A-172 cells by PI3K/AKT pathway.[40,41] There have been reports which corroborated that Klotho gene is responsible for changing the expressions of Bax, Bcl-2, and Wnt expression and thus opening up a new mechanistic channel for GBM research.[41] Another important factor that has been associated with angiogenesis in tumor cells is exosomal miR-194 which can activate pulmonary vascular endothelial cells (PMVECs) causing tumor cells to repopulate. The dying tumor cells that had been exposed to radiation show considerable intracellular signaling alterations and release a lot of chemicals. According to a study, PGE2, a lipid molecule released by tumor cells that are on the verge of dying, encourages tumor repopulation and accelerates angiogenesis through the secretion of HMGB1 and VEGF.[42] Small, non-coding RNA molecules called microRNAs (miRNAs) control post-transcriptional mRNA translation and gene expression, which has an impact on a variety of biological activities. The miRNA-21 is known to transcribe from a gene present in chromosome 17q23 containing two STAT3 sites, which are responsible for brain tumor growth and angiogenesis. Research has shown that miRNA-21 is often elevated in gliomas and that it participates in a range of biological processes that support tumor cell survival and invasiveness. Additionally, it has been linked to treatment resistance to radiation as well as chemotherapy.[43]

2.2. Negative regulators

2.2.1. Angiostatin

Angiostatin promotes apoptosis in endothelial cells of normal and tumor blood vessels and inhibits the migration and formation of blood vessel tubules in endothelial cells. Some tumors activate proteases that produce the angiostatin from plasminogen. Angiostatin also shows an inhibitory effect on sprouting angiogenesis. This decreases the expression of messenger RNA (mRNA) for bFGF and VEGF. Angiostatin was the first isolated molecule and it is a tumor-derived inhibitor.[44]

2.2.2. Interferons

Interferons can inhibit endothelial cell migration as it is a crucial step in angiogenesis, and it blocks the synthesis of angiogenic factors. Alpha-interferon is found to be the first successful molecule at the clinical stage in the treatment of proliferating hemangiomas. It was proved to be nontoxic and had good efficacy in hepatitis C.[45]

3. Angiogenesis and tumor metastasis

Angiogenesis has direct potential to augment the process of metastasis. Cancer cells can spread in two ways, viz., hematogenous spread (spread through blood vessels) and lymphogenous spread (spread through lymph vessels). The spreading of a tumor from a primary tumor to different body parts through the blood vessels and lymph vessels is known as tumor metastasis. The newly formed metastatic tumor is the same as that of the primary tumor. Because of these leaky blood vessels, there are more chances of metastasis.[46] It is suggested that increased production of bFGF may lead to an increased potential of metastasis, and the survival chances will be decreased. A study on breast cancer suggested that increased density of vasculature of tumor tissues increased the metastasis and subsequently decreased the survival rate of patients.[47] This study was not only limited to breast cancer but also cervical, prostate, lung, stomach, and ovary cancer.[48] Additionally, circulating tumor cells (CTC) are responsible for worsening breast cancer survival chances. The key factor involved is plakoglobin which regulates cell adhesion, clustering, and signaling. It can perform dual functions, viz., loss of plakoglobin expression encourages epithelial-mesenchymal transition causing metastasis and high expression of it leads to tumor cells clustering with breast cancer metastasis.[49] On a similar note, *Aceto* et al. demonstrated that CTC clusters derived from oligoclonal tumor cells have more metastatic potential in breast cancer when combined with elevated plakoglobin levels.[50] The shedding of primary tumor cells is allowed by neovascularization.[51] It is reported that tumor cell growth is limited where the blood supply is less; however, the growth drastically increased after vascularization in mice.[52] Slow and linear growth was observed initially in the mice, but after vascularization exponential growth was seen.[53]

Due to the tumor's permeable vasculature, the generation of new vasculature surrounding the primary tumor allows cells to leave the primary tumor and enter the bloodstream.[54] The growth of a primary tumor needs angiogenesis to grow beyond a certain size causing metastasis.[47] The tumor size is restricted in isolated perfused tumors in the absence of neovascularization. However, a drastic change in size was observed when implanted in mice.[51]

3.1. Role of endothelial cells in intra and extravasation during metastasis

Although understanding of metastatic progression has advanced markedly, still it remains one of the most enigmatic components in the pathogenesis of cancer. Metastatic progression has been described as a cascade that includes a sequence of distinct steps. In metastatic dissemination, primary cancer cells tend to attack the adjoining tissues either collectively or as a single cell.[55] This single cancer cell migration takes place in two interdependent modes; one is rounded migration and the other is elongated migration.[56] However, the mode of migration depends on the environmental condition.[57] This step includes local destruction, intravasation, arrest in a vital new organ, and extravasation into the nearby tissues, followed by initiation and maintenance of growth at the distant organ site. To cross the endothelium, cancer cells require both extravasation and intravasation. This process is called trans endothelial migration.[58,59] Fig. 3 represents the process of intravasation and extravasation with respect to metastasis.

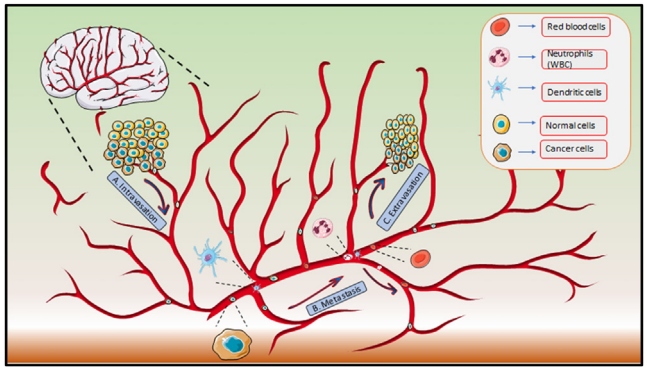

Fig. 3. The combined process of intravasation, extravasation and metastasis A. Intravasation: Crossing of tumor cells through the endothelial barrier. B. Metastasis: The spreading of tumor from primary tumor to different parts of body through blood vessels. C. Extravasation: Crossing of tumor cells from blood vessels to other parts through the endothelial barrier.

3.1.1. Intravasation

Cancer metastasis involves the dissemination of the cells to another part of the body by gaining entry into the blood circulation known as intravasation.[55,59,60] The rate-limiting step against the dissemination of tumors during metastatic progression is crossing the endothelial barrier.[61] During the intravasation, tumor cells invade the blood vessels through the tissues thereby promoting local angiogenesis. These newly formed blood vessels usually have a weak cell-cell junction by which cancer cells can easily enter the vasculature.[62] If cancer cells are able to survive the shear stress and the immune protective cells present in circulation, they tend to attach to the endothelial cells. Leukocytes and platelets are involved in this process. Two routes have been reported for tumor cells to cross the endothelial barrier; the paracellular route and the transcellular transendothelial migration.[60] Paracellular intravasation involves disruption of endothelial cell junction between neighboring endothelial cells, allowing tumor cells to enter in between them.[63] Tumor cells secrete several factors which promote the opening of endothelial cell junctions. TGF- β[64] and VEGF are responsible for the reduction in endothelial barrier function disrupting the VE-cadherin-β-catenin complex and inducing the opening of endothelial cell junctions.[65] Additionally, endothelial junction opening can be induced by tumor-associated macrophages (TAMs). Secretion of pro-apoptotic factors by several tumor cells may result in permanent damage to the endothelium.[66] TAMs by secreting TNF alpha can promote the transendothelial migration (TEM) of tumor cells which also induces the opening of endothelial junctions.[67] The transcellular route refers to tumor cell migration through the endothelial cells which often uses mechanisms other than the paracellular migration mechanism. The transcellular pathway often constitutes the breaking down of tight junction proteins as well as adherens junctions, cadherin proteins, in particular. In the study conducted by *Herman* et al., it was observed that breast cancer cells were migrated into cerebral endothelium from the apical side of the endothelium to the basolateral side of the endothelial cells. N-cadherin proteins were found to play a vital role in the process of transmigration.[68]

It is complex to define the protein/factors that are particularly involved in the intravasation process. For instance, the neural Wiskott Aldrich syndrome protein, an actin nucleation promoter, plays an imperative role in the breast cancer cell invasion and subsequent intravasation process.[69] The expression of Notch receptors occurs on cancer cells, whereas its ligands get expressed on the endothelial cells.[70]

3.1.2. Extravasation

In the extravasate stage, cancer cells attach to the endothelial cells lining the blood vessels as well as the vessel walls of various organs. Extravasation of tumor cells typically occurs in small capillaries. The extravasation process induces dynamic changes to the tumor cell shapes and results in the formation of specific protruding structures that promote migration.[71] It is reported that successful extravasation of tumor cells demands the presence of cancer-specific protrusions.[69] Cancer cells reduce the function of the capillaries with the same diameter as that of cells, suggesting that it tends to become physically restricted and then forms a stable attachment.[72,73]

Initially, it was considered that vascular endothelium participates in tumor cell extravasation. However, proinflammatory cytokines-mediated endothelium activation plays a crucial role in regulating the tumor cell adhesion that further facilitates TEM.[74] The endothelial cell adhesion necessitates the expression of receptors and cognate linkages on both endothelial as well as tumor cells. Several ligands and receptors play a vital role in this process including selectin, cadherins, integrins, and the immunoglobulin superfamily of receptors.[75,76]

A tumor cell has several adhesion receptors and facilitates the adhesion process. Several events were reported during the extravasation of cancerous cells. Similar to the leukocytes, cancer cells roll-on endothelium and initiate a more stable attachment. However, rolling has not been proven for cancer cells in vivo. Selectins are vital receptors for leukocytes present on the endothelium in vivo and often have been associated with cancer cells adhesion to endothelial cells. It has been found that the extravasation of melanoma cells to the brain takes place with the help of endothelial selectin (E-selectin).[77]

Tumor cells tend to be connected with the endothelium, and further interact with various cell types found in the circulating bloodstream such as neutrophils, monocytes, and natural killer (NK) cells. All of these cells possess the ability to alter the efficiency and rate of the extravasation process.[76] In vivo study demonstrated that the interaction between $\beta-2$ integrin on neutrophils and tumor cell intercellular adhesion molecule (ICAM)-1 showed a rise in the level of melanoma cell anchoring to the endothelium.[78] The opening of endothelial cell junctions can be activated by several factors which are produced by tumor cells. Distinct adhesion molecules are also observed to promote the migration of tumor cells to the brain. For instance, increased expression of integrin $\alpha 3\beta 1$ in highly brain metastatic subclone of a human non-small cell lung cancer cell line associated with lung cancer tends to promote brain metastasis.[79] Another adhesion molecule $\alpha\beta$-crystallin which is expressed in aggressive tumors is associated with the breast cancer cell adhesion to the brain endothelium via a $\alpha 3\beta$ integrin-dependent mechanism. Similarly, another receptor cadherin-2 is also involved in the attachment and rolling of cancer cells. This receptor promotes the breast cancer cells rolling on endothelial cells.

In addition to the receptors that are involved in rolling, several other receptors also play a major role in the stabilization of cancer cells and endothelial cell adhesion. These include the integrins CD44 and MUC1.[80,81] Integrins interact with cell surface receptors and extracellular matrix ligands. This receptor has also a key role in metastasis. The adhesion of cancer cells to the endothelial cells that result in cancer metastasis is correlated with CD44 expression on tumor cells. Chemokines and the associated seven-transmembrane spanning G protein-coupled receptors also play a crucial role in cancer metastasis.[34] Chemokines derived from cancer cells can attract the leukocytes which promote the cancer cells' extravasation in various malignancies.

The brain-derived neurotrophic factor (BDNF) is crucial in cell growth, development, and differentiation. Through tropomycin receptor kinase B (TrkB) signaling, BDNF plays a key role in brain cancer by promoting antiapoptotic signaling, the proliferation of cells, and the phosphorylation of EGFR.[82] In a study performed by Garofalo and coworkers, it has been found that an enriched environment that promotes the synthesis of BDNF reduced proliferation, invasion, and growth of cancerous cells in brain tumors in mice through direct or indirect methods. Direct mechanism encompasses stimulation of NK cells while indirect mechanism entails binding of BDNF to TrkBT1 on cancerous cells leading to stimulation of BDNF. In another study, it was also proven that BDNF was responsible for the stimulation of IL-15 leading to interferon-γ production and NK cells.[83,84]

4. Key players involved in tumorigenesis

Apart from the role of angiogenesis and endothelial cells in the spread of tumors as explained hitherto, there are other factors i.e. cell adhesion molecules, selectins, neuronal cadherins, etc., that are involved in the genesis of tumors. Such factors are explained in this section.

4.1. Cell adhesion molecules

Molecules involved in cell adhesion are categorized into five groups, namely, integrins, cadherins, selectins, and the immunoglobulin superfamily (IgSF), which includes nectins and mucins. Apart from the conventional adhesion molecules, an enzyme like vascular adhesion protein-1 is also involved in cell adhesion. Selectin, cadherin, and IgSF are involved in cell-cell adhesion while integrins are found to bind with the extracellular matrix.[85]

4.1.1. Selectin

Selectins are known to attach to carbohydrates in a calcium-dependent manner. They are classified into the P, E, and L types depending on their origin. However, P selectins are also present on the endothelial cells. Based on their kinetics, selectins differ from each other. Expression of P-selectins occurs within minutes, while E-selectins require hours to get expressed.[86] The most important function of selectins is related to the initial stage that comprises the rolling cell adhesion cascade.[87] The inflammatory cytokines secreted by cancer cells or cancer-associated leukocytes induce the release of E selectins. However, normally they are not expressed on quiescent endothelial cells. The main ligand of all three selectins, P selectin glycoprotein ligand-1 (PSGL-1) is dependent on carbohydrates for proper functionality.

4.1.2. Neuronal cadherins

Neuronal cadherin (N-cadherin) is a receptor that plays an important function in cell rolling and in cancer cells' attachment to the endothelium. They are classified into type E, type P, and type N cadherins. N-cadherin is mainly expressed by endothelial cells as well as other types of cancer cells. To date around 10 different types of cadherins have been reported. It has been said that after crossing the cell membrane, cadherin binds to the actin through vinculin and mediates adhesive properties.[88] It also exerts a critical role in the development of the central nervous system. E-cadherin expresses strongly during the initial period of ectodermal development. Similarly, N-cadherin is also expressed in central nervous systems and other organs and plays an important role in their development.

4.2. Transforming growth factor

Several cellular processes such as differentiation, adhesion, proliferation, and apoptosis are affected by TGF.[89] It binds to serine/threonine receptors kinase (type I and II) which activates Smads, the signaling proteins. Research suggested that the cells of glioblastoma produce the active forms of TGF-1β and TGF- β2.[90] It has also been observed that these two forms (TGF-β1 and TGF-β2) are strongly expressed in human gliomas.[91] Smad activity induced by high TGF-β has been shown to confer poor prognosis in patients with glioblastoma.[92]

4.3. Fibroblast growth factor

FGFs are comprised of 19 members which constitute a large family of growth factors. FGF2 is the most crucial one in the regulation of brain tumor angiogenesis.[93] It attaches to the FGFR1, a tyrosine kinase receptor most commonly expressed on endothelial cells. FGF2 upregulates the uPA and also shows the expression of collagenase on the endothelial cells.[94] This facilitates the migration of endothelial cells through the ECM and supports tumor growth. Additionally, FGF2 can also act as a chemotactic agent for endothelial cells and assists in the formation of capillary-like tubes.[95] It also induces VEGF expression and its production.[96]

4.4. Vascular endothelial growth factor

Brain tumor-associated VEGF can be both endothelial cell-derived and tumor-derived that exerts its effects in an autocrine and paracrine manner through high binding to the tyrosine kinase receptors and VGFR1/FIK-1 and VGFR2 /FIK-1.[97,98] VEGF induces vasculogenesis, angiogenesis, blood vessel permeability, and extravasation of proteins. These events result in vasogenic edema which is observed in brain tumors.[99] This edema results in blood-brain barrier (BBB) leakage that increases intracranial pressure. In fact, angiogenesis is a prerequisite for the growth and colonization of cancer cells in the brain. One report stated a decrease in the metastatic growth of brain tropic tumor cells upon inhibition of the activity of VEGF.[100] Overexpression of VEGF in melanoma cells induces brain metastasis progression. Additionally, phosphatidylinositol 3-kinase and mammalian target of rapamycin signaling pathways mediated by VEGF impart an important role in brain tumor metastasis.[101] Therefore, researchers in the oncology field found the usefulness of VEGF antibodies like bevacizumab in brain metastasis.

4.5. Platelet-derived growth factor-β

Human gliomas express the PDGF-β. There have been a lot of controversies regarding the role of this factor in tumorigenesis and depends on the origin of the cell.[102] Particularly, PDGF-β is derived from glioblastoma and promotes angiogenesis by encouraging the production of VEGF in endothelial cells that overexpress the PDGF receptors-β.[103] After all, when PDGF-β is overexpressed and derived from the vascular endothelial cells surrounding the tumor mass, it shows contrasting effects.[104]

4.6. Pleitrophin

Pleiotrophin and its receptors are expressed in human glioblastoma.[105] Pleiotrophin is a small heparin-binding cytokine that is expressed in the CNS during progression; however, it is not profoundly expressed in CNS during pathological processes.[106] This activates the anaplastic lymphoma kinase 1 in a murine glioblastoma model, which acts on its receptors and results in VEGF deposition and vascular abnormalities.[106]

4.7. Integrins

Integrins are heterodimers comprising α- and β-chains that form intact receptors on the plasma membrane. Integrins attach to several types of ligands in the ECM, other cell surfaces, and soluble proteins. Leukocyte expresses several types of integrins and has a central role in metastasis. Integrins promote the release of key mediators like focal adhesion kinase, which is thought to play a crucial role in migration and proliferation by initiating abnormal signals for survival, invasion, and angiogenesis. Preclinical studies in animal models of non-small cell lung cancer (NSCLC) have demonstrated that blocking of $\alpha 3\beta 1$ integrin significantly reduces brain metastasis.[79]

5. Brain tumor, endothelial targeting, and gene therapy

Understanding the process of angiogenesis and tumorigenesis opens novel avenues of treatment of glioblastoma. Gene therapy holds a significant place in the treatment of glioma. To attain high-level efficacy, viral and nonviral vectors need to be chosen shrewdly with less immunogenicity and enhanced biosafety.[107] Dr. Folkman proposed that there would be a requirement for angiogenesis if the tumor size reaches beyond 1 mm.[3] This opened new revenue for the treatment of cancer by targeting endothelial cells rather than cancer cells themselves.[108] Brain endothelial cells remain to be attractive targets for scientists working on the treatment of brain tumors. Gene therapy has the capability of administering genes at a high concentration to a localized site for a prolonged period, overcoming the problem of continuous administration. Modified C6 glioma cells expressing mouse endostatin, implantation of modified embryonic cells in solid tumors, and transduced bone marrow stem cells by retroviral vectors secreting antiangiogenic factors are some of the reported methods within the gene therapy context in the mitigation of CNS dysfunctionalities.[109]

Endothelial cells (EC) associated with GBM are phenotypically and functionally different than normal endothelial cells. It has been hypothesized that the origin of tumor EC could be from normal EC and the recruitment of angiogenic growth factors to tumor cells. Another hypothesis talked about the progression of endothelial progenitor cells like CD34 into tumor EC. Some of the biomarkers like vWF, CD31, and CD105 give

rise to a stark contrast between GBM EC and normal EC. It has been further evidenced by the fact that in tumor EC there is a loss of CD144 (VE-cadherin), a protein responsible for maintaining the integrity of brain vasculature. Apart from this, reports showed that there have been increased levels of growth factors like VEGF, IL-8, Flt-1, and Flk-1 in tumor EC which aid in enhancing proliferation.[110] *Moore* et al. assessed the potential of endothelial progenitor cells (EPC) for site-specific gene delivery on two types of mice models, viz., glioma severe combined immunodeficiency (SCID) mice and non-tumor SCID mice. Through fluorescence study, it was shown to have greater specificity towards the EPC.[111]

Blood-brain barrier being a primary hurdle for transporting drugs across endothelial cells of the brain, novel nanocarrier systems of dendrimers PAMAM-PEG-SRL were formulated for the delivery of gene. The experimentation was done by *Zarebkohan* et al. on C6 glioma cell lines and the results were promising enough to show higher transfection efficacy when entrapped inside PAMAM dendrimers. It was further investigated that low-density lipoprotein receptor protein (LRP), which is largely expressed on glioma cells, could serve as a better therapeutic target and SRL peptide could be easily conjugated to it. This facilitated the internalization process into brain endothelial cells by the caveolin clathrin mechanism.[112] A similar polymer-based gene delivery system was established by *Green* et al.[113] using poly (beta-amino esters) for endothelial targeting. They concluded that such systems provided both safe and efficacious delivery systems. *Suh* et al.[114] showed increased transfection efficiency for DNA against endothelial cells by incorporating DNA in polyethyleneimine (PEI) polymeric complexes.

One of the strategies used by scientists is the use of adeno-associated virus (AAV) through which gene delivery would be possible. Scientists have evaluated engineered AAVBR1 vectors for which the target was brain endothelial cells. This vector proved to have low immunogenicity and increased transduction efficacy with the capacity for brain homing to treat different CNS disorders. AAV vectors are not only found to be effective against neurological disorders but also in the treatment of other organs' dysfunctions as these vectors can exhibit a multitude of peptide libraries.[115] *Merkel* et al. evaluated the transduction capability of varied viral vectors on primary human brain microvascular endothelial cells (BMVECs). BMVECs was developed with optimum resistance, permeability, adhesion molecular expression, and tight junction protein localization. The results indicated that AAV9 had reduced efficiency but more penetrating power in crossing barriers through cell-mediated transduction than AAV2. By performing luciferase assay, trans-endothelial assay, permeability assay, flow cytometry, and western blotting, it was found that AAV9 outperformed AAV2 in penetrating across the monolayer barrier that is BMVEC. This could be viewed as a further prospect for gene delivery across brain endothelial cells in the mitigation of CNS disorders and brain tumors.[116] Apart from AAV vectors, lentiviral vectors (HIV-1) are better suited for expressing silenced RNA and GBM antigen receptors. It was corroborated by promising results on GBM cell lines in vitro and human GBM xenograft models.[107]

In recent years, immense progress has been done in the biomedical field, especially in cancer diagnosis and treatment. The functionalized nanoplatforms are widely explored in conjunction with 3D printing.[117] These platforms are superior in terms of cellular uptake, biocompatibility, stability, and overcoming cancer resistance.[118] *Carlson* et al. investigated molecular pathways of tumor-associated vessels and tumor-derived endothelial cells under the purview of glioblastoma treatment. With the utilization of in-utero electroporation and CRISPR/Cas9 engineering in a glioma mouse model, three-dimensional (3D) vascular tumor dynamics were studied and vessel function and morphological characteristics were found to be altered over a period of time. It was also found that tumors produced their own endothelium and a heterogeneous population of endothelial cells was seen eliciting insights into the molecular understanding of GBM.[119] The tumor microenvironment plays a crucial role in tumorigenesis with an impact on glioma stem cells (GSC) and tumor-endothelial cell communication. *Li* et al. demonstrated that glioma-associated endothelial cells were responsible for releasing extracellular vesicles (EVs), thereby increasing the GSC population. They further explored the possible role of CD9 in mediating GSC functions on tumorigenesis and confirmed that EC-mediated EVs migration of CD9, and through activation of BMX /STAT3 pathway, had a key role to play in GBM progression.[120] On a similar note, brain endothelial cells-derived EVs have been explored as a platform to mediate the entry of photosensitizers across BBB. Cao and team tried to link chlorin e6 with triphenylphosphonium (TPP), which is a mitochondrial-specific moiety and then this was entrapped into EVs. This facilitated stability and cellular internalization improving photodynamic therapy (PDT) efficiency, both in vitro (U87MG cells) and in vivo (orthotopic GBM-xenograft mice). The findings of this research indicate mitochondria as an effective target for GBM PDT treatment.[121] It is widely documented that tumor development and metastasis rely on angiogenesis factors inside the tumor vasculature. Delivering genes that are responsible for angiogenesis inhibition could be a feasible option in treating cancer and suppressing tumor growth. *Kudo* et al. used the brain-specific angiogenesis inhibitor 1 (BAI1) gene and the effect was assessed on mouse renal carcinoma cell lines. BAI1 gene is the *P53* gene that regulates cell division. *BAI1* gene is known for encoding amino acid residue consisting of thrombospondin type 1 repeats and its overexpression results in the inhibition of angiogenesis in pancreatic carcinoma.[122]

Chimeric antigen receptor (CAR) T cell therapy has been quite effective in treating leukemia with the first FDA-approved therapy emerging in the year 2017. Thereafter, several attempts have been made under the same purview of genetically engineered T cells in the amelioration of GBM. Interleukin-13 receptor alpha 2, human EGF-II, EGFR variant III (EGFRvIII) are a few targets that are overexpressed on cancerous cells but not on healthy tissues.[123] T cells upon binding to T cell receptors start stimulating signaling molecules like CD28, OX40 which can bind to antigen-presenting cells (APCs) on major histocompatibility complex (MHC), thereby taking part in a cell-mediated immune response. On the other hand, CAR being a synthetic molecule has improved tumor-killing efficacy and facilitates releasing a particular chemokine independent of MHC levels. Several instances of clinical trials of CAR T cell therapy have been reported and gained a lot of pace in the care treatment of GBM. For the improvement of T cell therapy, efforts are being put towards overexpressing transgenic proteins such as IL-15, and IL-7R and stimulating and proliferating the T cells. Some of the promising tumor-associated antigens (TAA) include CD70, CD133, GD2, and B7-H3 and are explored under clinical trials.[124] Table 2 represents an overview of different clinical studies making use of CAR T cell therapy for the treatment of brain tumors.

6. Interplay of endothelial cells, brain tumor heterogeneity, and immunotherapy

As has been previously discussed, the tumor microenvironment comprising of APCs, T cells, B cells, cancer-associated fibroblasts, and tumor endothelial cells (TECs) plays a crucial role in terms of tumor development and metastasis.[125,126] TECs are different from normal endothelial cells in terms of genetic, phenotypic, and metabolic levels. They can further induce tumor angiogenesis and alter genetic expressions which have direct implications in antibody checkpoint treatment and immunotherapy in the quest for counteracting cancer.[126]

With breakthroughs in bulk and single-cell RNA sequencing (scRNAseq), it is now clear that intratumoral heterogeneity, where various subpopulations of cells occur inside a single tumor, complicates intertumoral heterogeneity. As far as GBM is concerned, sampling was taken from different parts of the same tumor site that exhibits multiple genetic expressions. This could be due to genetic alterations getting accrued over a period of time. This spatial heterogeneity in association with temporal heterogeneity makes things complicated from a treatment point of view and this gets reflected in patients getting varied responses to the same treatment modality. Apart from the tumor's genetic evolu-

Table 2
Overview of clinical studies pertaining to CAR T cell therapy for the treatment of brain tumor.

Name of clinical study (study initiated year)	Objective of study	Study design	No. of participants	Outcome measures	Remarks	Clinical trial ID
T cells expressing HER2-specific CAR for patients with HER2-positive CNS tumors (2015)	To find the largest safe dose of HER2-CAR T cells and to see any improvement in patients with brain tumor	Phase I, interventional, non-randomized, open-label, treatment (2015-ongoing)	28 (estimated) recruiting	Primary: number of patients with dose-limiting toxicity after CAR T cell administration; Secondary: number of patients with tumor response	Estimated to complete in 2036. Participants having tumor resection of either sex and 3 years and older are included.	NCT02442297
C7R-GD2.CAR T cells for patients with GD2-expressing brain tumors (GAIL-B) (2019)	To find the largest safe dose of GD2-C7R T cells and duration of it in blood for detection purpose	Phase I, interventional, a single group assigned, open-label, treatment (2020-ongoing)	34 (estimated) recruiting	Primary: dose-limiting toxicity rate (4 weeks); Secondary: response rate according to standard criteria (6 and 12 weeks post T cell infusion)	Estimated to complete in 2039. Participants with tumor size less than 5 cm of either sex and between 12 months to 18 years of age are included.	NCT04099797
CAR T cell receptor immunotherapy targeting EGFRVIII for patients with malignant gliomas expressing EGFRVIII (2011)	To determine a safe number of cells to infuse and to evaluate whether treatment (cyclophosphamide and fludarabine + aldesleukin) along with CAR of EGFRVIII is safe and effective. To determine 6-month progression-free survival receiving regimen	Phase I/II, interventional, sequentially assigned, non-randomized open-label, treatment (2012–2018)	18	Primary: number of adverse events related to treatment (4 weeks-77 days) and progression-free survival (up to 6 months); Secondary: several participants with serious and non-serious adverse events. Circulating CAR cells in peripheral blood at 1-month post-treatment	Completed study. EGFR VIII expression promotes oncogenesis and lacks in normal tissues. Participants with recurrent GBM of either sex and 18–70 years of age were included.	NCT01454596
VB-111 in surgically accessible recurrent/progressive GBM (2020)	To study new viral cancer therapy for recurrent GBM and test safety and effectiveness of this drug	Phase II, randomized, controlled, double-blinded, parallel, surgical trial, treatment (2020-ongoing)	15	Primary: density of tumor-infiltrating T cells; Secondary: 6-month progression-free survival and overallsurvival (up to 6 years)	Estimated to be completed in 2023. VB-111 targets and damages blood vessels causing tumor cells to starve. Participants having GBM of either sex and older than 18 years were included.	NCT04406272
An investigational immuno-therapy study of temozolomide plus radiation therapy with nivolumab or placebo, for newly diagnosed patients with glioblastoma (2016)	To evaluate patients with GBM that is MGMT methylated and to compare TMZ and radiation with nivolumab combined with TMZ and radiation.	Phase III, interventional, randomized, parallel, triple masking, treatment (2016-ongoing)	716	Primary: assessment of progression-free survival (35 months) and overall survival (69 months); Secondary: overall survival (up to 24 months)	Estimated to complete in 2023. Participants suffering from GBM of either sex and older than 18 years are included.	NCT02667587

(*continued on next page*)

Table 2 (*continued*)

Name of clinical study (study initiated year)	Objective of study	Study design	No. of participants	Outcome measures	Remarks	Clinical trial ID
A pilot study of B7-H3 CAR-T in treating patients with recurrent and refractory Glioblastoma (2020)	To evaluate the safety and tolerability of intratumoral injection of B7-H3 CAR T in between TMZ cycles and to assess PKPD of CAR T	Phase I, interventional, non-randomized, open-label, treatment (2020-ongoing)	12 (estimated) recruiting	Primary: incidence and type of adverse events, maximum tolerable dose, overall survival and progression free survival; Secondary: PK of B7-H3 disease response	B7-H3, not found in normal tissues being attractive target for GBM. Estimated to complete in 2024, Participants having GBM of either sex and older than 18 years are included.	NCT04385173
CAR T cells with chlorotoxin tumor-targeting domain for the treatment of MPP2+recurrent or progressive GBM (2020)	To determine max tolerated dose schedule and phase 2 dosing plan and to assess feasibility and safety of dual delivery of chlorotoxin-CD28-CD19 expressing CAR T lymphocytes	Phase I, interventional, single group assignment, open-label, treatment (2020-ongoing)	36 (estimated) recruiting	Primary: dose-limiting toxicity; Secondary: CAR T cell and endogenous T cells, cytokine levels, progression-free survival time and overall survival	Estimated to complete in 2024. Dose escalation study. Participants having GBM of either sex and older than 18 years are included.	NCT04214392
CAR T-EGFRvIII+pembrolizumab in GBM (2018)	To assess safety and tolerability of EGFRvIII T cells with PD-1 inhibitor with MGMT unmethylated GBM	Phase I, interventional, single group assignment, open-label, treatment	7	Primary: number of subjects with treatment-related adverse events; Secondary: overall survival rate, PFS, ORR	Completed in 2021. Participants having GBM of either sex and older than 18 years were included.	NCT03726515
Genetically modified T cells in treating patients with recurrent or refractory malignant glioma (2015)	To assess the feasibility and safety of cellular immunotherapy utilizing ex vivo expanded T cells that are modified to express IL13Rα−2 specific CAR and CD19 in glioma patients	Phase I, interventional, non-randomized, parallel, treatment	82	Primary: incidence of grade 3 toxicity for 15 years and dose-limiting toxicity; Secondary: changes in the largest length of tumor, cytokine levels, CAR T cells level, PFS time, CAR T cell detection and IL13Rα−2 antigen expression levels for 1 year	Estimated to complete in 2023. Participants having a prior diagnosis of grade III or IV glioma with life expectancy of more than 4 weeks and KPS $\geq$ 60% were included.	NCT02208362

Abbreviations: CAR, chimeric antigen receptor; CNS, central nervous system; EGFRVIII, epithelial growth factor receptor variant III; GBM, glioblastoma; HER2, epidermal growth factor receptor 2; KPS, Karnofsky performance scale; MGMT, O6-methylguanine-DNA methyltransferase; PD-1, programmed cell death-1; PK, pharmacokinetic; PKPD, pharmacokinetic and pharmacodynamics; PFS, progression-free survival; ORR, objective response rate; TMZ, temozolomide.

tion, treatment modality may result in the selection of resistant clones, further making it complicated and tumor evolution may be accelerated further by the acquisition of treatment-induced hypermutations. This is exemplified in a large number of recurrences which are caused by clone-specific divergent mutations that were not present in the parent tumor. As a result, intratumoral heterogeneity is fundamental to therapy failure, and personalized studies of subclones using genomic and RNA sequencing are critical for predicting clone response to various treatment modalities and guiding the selection of appropriate combination therapy.[127]

Immunotherapy among the potential brain tumor treatment modalities has been associated with several components like altered cytokines, oncolytic viruses, cancer-targeted vaccines, and checkpoint inhibitors, to name a few.[128,129] These therapies have shown a great deal of promise in preclinical settings; however, their clinical translational ability has been put into question and further research is warranted on the same issue.[129] One of the major hurdles to immunotherapy efficacy is the development of treatment resistance. It is evident with the fact that primary resistance can be developed to the extent of 10–40% towards programmed cell death-1 (PD-1)/PD-ligand 1 (PD-L1), which is one of the most successful immunotherapies in clinical settings.[130]

Endothelial reprogramming has become a popular choice under immunotherapy wherein, abnormal vascularity is achieved via genetic reprogramming of tumor endothelial cells and also characterized by the endothelial mesenchymal transition. Efforts are being made toward reversing the abnormal vasculatures within the TME framework.[131] On a similar note, PAK4 was identified as a new target to reprogram ECs in glioblastoma, which was the outcome of the efforts put in by *Ma* et al. by performing kinome-wide genetic screening of mesenchymal-like transcriptional activity in tumor ECs. PAK4 genetic ablation or pharmacological suppression increased adhesion molecule expression in tumor ECs, decreased vascular irregularities, and enhanced T cell infiltration, making tumors more prone to CAR T immunotherapy.[132] In addition to this, TME-transformed ECs are also known to generate an immunosuppressive vascular niche by releasing IL-6, which promotes M2 phenotypes in TAMs and limits T cell infiltration and activation at the TME.[133] Thus, endothelial reprogramming is responsible for creating a TME endowed with anti-tumor immunity which is conducive to immunotherapy success. Another major complication in effective immunotherapy is the lack of infiltration of T cells. A research finding supported the notion that tumor ECs transformed into high endothelial venules, which required signals from NK and $CD8^+$ cells and facilitated the expansion of $PD1^+TCF^+$ progenitor $CD8^+$T cells into effector T cells.[134] In recent years, the major focus of research has been on elevating the extent of infiltration of cytotoxic immune cells in brain tumors, and at the same time, efforts need to be channelized into selectively inhibiting immunosuppressive immune response which can interfere with cytotoxic immune cells by exposing TME.[135] Thus, improving immunotherapy by playing around with endothelial cells within the TME framework could bring better prospects in terms of clinical outcomes for patients suffering from brain tumors.

7. Conclusion and future perspectives

In summary, this review discusses the pathogenesis of glioblastoma as well as presently known therapeutics to enhance patient survival rates. It also critically examines the several regulators that regulate the angiogenesis process. Various diagnostic methods and in vivo models are urgently required for correctly displaying the metastatic process. Understanding and designing therapeutics based on tumor vasculature at the molecular, functional, and anatomical levels is critical. This review offers insight into the role of endothelial cells in tumor metastatic intra and extravasation, as well as current clinical studies of gene therapy in the treatment of brain malignancies. The current standard of care for the treatment of glioblastoma is not sufficient, thus opening new avenues of treatment, with neuro endothelial cells being one of them. Key players like TGFs, PDGFs, and BDNFs regulate the process of angiogenesis and could be utilized as diagnostic markers which are generally overexpressed on the tumor cells. Biophysical tools for assessing the mechanical properties of endothelial cells would be required to completely understand the process of angiogenesis and metastatic progression of the tumor. As compared to current treatment options comprising surgery, radiation therapy, and chemotherapy in terms of temozolomide administration, novel delivery systems encapsulating drugs like docetaxel, etoposide, and cetuximab into solid nanoparticles or liposomes or polymeric drug delivery systems have attracted more attention from researchers recently. However, this needs to be critically formulated and analyzed through clinical phases and an economical point of view. Along with nanoformulations, neuro endothelial cell targeting has been reflected in gene therapy. The defective genes can either be deleted or substituted with corrected genes. In the recent decade, CAR T cell therapy has become popular as a potential option to treat brain tumors. As US FDA has already approved the first CAR T cell therapy in the treatment of leukemia, efforts are being made on the same front to tackle glioblastoma and have been evidenced in many more clinical trials currently undergoing using CAR T cell therapy. CAR T cell therapy provokes a cell-mediated immune response and cytokines signaling, regulating the expression of the MHC. In the future, it could serve as dual-purpose theranostics applications in the treatment of brain tumors. The designing of a suitable delivery system that would enable corrected gene-evoking immune responses would be the need of the hour.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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Author contributions

T.A. and A.J. designed the structure of the article. T.A., S.S., T.S., and I.S. did the literature search and wrote the draft. T.A. and S.S. made the tables and T.S. and I.S. made all the figures. V.G., J.H., and A.J. edited and reviewed the draft. All authors have read and agreed to the published version of the manuscript.

References

1. Abolanle AAK, Amina S, Muhammad A, et al. Brain tumor: an overview of the basic clinical manifestations and treatment. *Glob J Cancer Ther*. 2020;6:038–041. doi:10.17352/2581-5407.000034.
2. Ostrom QT, Gittleman H, Truitt G, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2011-2015. *Neuro Oncol*. 2018;20:iv1–iv86. doi:10.1093/neuonc/noy131.
3. Roth P, Pace A, Le Rhun E, et al. Neurological and vascular complications of primary and secondary brain tumours: EANO-ESMO clinical practice guidelines for prophylaxis, diagnosis, treatment and follow-up. *Ann Oncol*. 2021;32(2):171–182. doi:10.1016/j.annonc.2020.11.003.
4. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021;23(8):1231–1251. doi:10.1093/neuonc/noab106.
5. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131(6):803–820. doi:10.1007/s00401-016-1545-1.
6. Tykocki T, Eltayeb M. Ten-year survival in glioblastoma. A systematic review. *J Clin Neurosci*. 2018;54:7–13. doi:10.1016/j.jocn.2018.05.002.
7. Verdugo E, Puerto I, Medina MÁ. An update on the molecular biology of glioblastoma, with clinical implications and progress in its treatment. *Cancer Commun*. 2022;42(11):1083–1111. doi:10.1002/cac2.12361.
8. Ibrahim O, Hafez MA, Haleem HA, et al. Recent advances in the treatment of gliomas: the multimodal care therapy. *Open Access Maced J Med Sci*. 2021;9(B):503–508. doi:10.3889/oamjms.2021.6229.

9. Liu S, Zhao Q, Shi W, et al. Advances in radiotherapy and comprehensive treatment of high-grade glioma: immunotherapy and tumor-treating fields. *J Cancer*. 2021;12(4):1094–1104. doi:10.7150/jca.51107.
10. Huang B, Zhang H, Gu L, et al. Advances in immunotherapy for glioblastoma multiforme. *J Immunol Res*. 2017;2017:3597613. doi:10.1155/2017/3597613.
11. Mun EJ, Babiker HM, Weinberg U, et al. Tumor-treating fields: a fourth modality in cancer treatment. *Clin Cancer Res*. 2018;24(2):266–275. doi:10.1158/1078-0432.CCR-17-1117.
12. Rana D, Salave S, Longare S, et al. Nanotherapeutics in tumour microenvironment for cancer therapy. *Nanosci Nanotechnol-Asia*. 2021;11:1–16. doi:10.2174/2210681211666210908144839.
13. Qiao S, Cheng Y, Liu M, et al. Chemoattractants driven and microglia based biomimetic nanoparticle treating TMZ-resistant glioblastoma multiforme. *J Control Release*. 2021;336:54–70. doi:10.1016/j.jconrel.2021.06.015.
14. Kumar K, Moitra P, Bashir M, et al. Nanoparticles for efficacious doxorubicin delivery both in vitro and in vivo. *Nanoscale*. 2020;12(2):1067–1074. doi:10.1039/c9nr08475d.
15. Kadari A, Pooja D, Gora RH, et al. Design of multifunctional peptide collaborated and docetaxel loaded lipid nanoparticles for antiglioma therapy. *Eur J Pharm Biopharm*. 2018;132:168–179. doi:10.1016/j.ejpb.2018.09.012.
16. Kuo YC, Chao IW. Conjugation of melanotransferrin antibody on solid lipid nanoparticles for mediating brain cancer malignancy. *Biotechnol Prog*. 2016;32(2):480–490. doi:10.1002/btpr.2214.
17. Li C, Tan J, Chang J, et al. Radioiodine-labeled anti-epidermal growth factor receptor binding bovine serum albumin-polycaprolactone for targeting imaging of glioblastoma. *Oncol Rep*. 2017;38(5):2919–2926. doi:10.3892/or.2017.5937.
18. Pandey A, Kulkarni S, Vincent AP, et al. Hyaluronic acid-drug conjugate modified core-shell MOFs as pH responsive nanoplatform for multimodal therapy of glioblastoma. *Int J Pharm*. 2020;588:119735. doi:10.1016/j.ijpharm.2020.119735.
19. Ying M, Wang S, Zhang M, et al. Myristic acid-modified DA7R peptide for whole-process glioma-targeted drug delivery. *ACS Appl Mater Interfaces*. 2018;10(23):19473–19482. doi:10.1021/acsami.8b05235.
20. Song XLi, Liu S, Jiang Y, et al. Targeting vincristine plus tetrandrine liposomes modified with DSPE-PEG2000-transferrin in treatment of brain glioma. *Eur J Pharm Sci*. 2017;96:129–140. doi:10.1016/j.ejps.2016.09.024.
21. Arcella A, Palchetti S, Digiacomo L, et al. Brain targeting by liposome-biomolecular corona boosts anticancer efficacy of temozolomide in glioblastoma cells. *ACS Chem Neurosci*. 2018;9(12):3166–3174. doi:10.1021/acschemneuro.8b00339.
22. Gao X, Li S, Ding F, et al. A virus-mimicking nucleic acid nanogel reprograms microglia and macrophages for glioblastoma therapy. *Adv Mater*. 2021;33(9):1–9. doi:10.1002/adma.202006116.
23. Ziyad S, Iruela-Arispe ML. Molecular mechanisms of tumor angiogenesis. *Genes Cancer*. 2011;2(12):1085–1096. doi:10.1177/1947601911432334.
24. Ahir BK, Engelhard HH, Lakka SS. Tumor development and angiogenesis in adult brain tumor: glioblastoma. *Mol Neurobiol*. 2020;57(5):2461–2478. doi:10.1007/s12035-020-01892-8.
25. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci*. 2020;77(9):1745–1770. doi:10.1007/s00018-019-03351-7.
26. Krock BL, Skuli N, Simon MC. Hypoxia-induced angiogenesis: good and evil. *Genes Cancer*. 2011;2(12):1117–1133. doi:10.1177/1947601911423654.
27. Nishida N, Yano H, Nishida T, et al. Angiogenesis in cancer. *Vasc Health Risk Manag*. 2006;2(3):213–219. doi:10.2147/vhrm.2006.2.3.213.
28. Von Baumgarten L, Brucker D, Tirniceru A, et al. Bevacizumab has differential and dose-dependent effects on glioma blood vessels and tumor cells. *Clin Cancer Res*. 2011;17(19):6192–6205. doi:10.1158/1078-0432.CCR-10-1868.
29. Butowski N. Anti-angiogenic therapy in glioma. *Clin Transl Oncol*. 2011;13(5):294–300. doi:10.1007/s12094-011-0657-2.
30. Vennepureddy A, Singh P, Rastogi R, et al. Evolution of ramucirumab in the treatment of cancer – a review of literature. *J Oncol Pharm Pract*. 2017;23(7):525–539. doi:10.1177/1078155216655474.
31. Quintero-Fabián S, Arreola R, Becerril-Villanueva E, et al. Role of matrix metalloproteinases in angiogenesis and cancer. *Front Oncol*. 2019;9:1–21. doi:10.3389/fonc.2019.01370.
32. Presta M, Mitola S, Dell'Era P, et al. Fibroblast growth factor-2 in angiogenesis. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev*. 2005;16(2):159–178. doi:10.1007/978-0-387-71518-6_7.
33. IF D, O H, PM B. Growth factors in glioma angiogenesis: FGFs, PDGF, EGF, and TGFs. *J Neurooncol*. 2000;50(1–2):121–137. doi:10.1023/A:1006436624862.
34. Sarvaiya PJ, Guo D, Ulasov I, et al. Chemokines in tumor progression and metastasis. *Oncotarget*. 2013;4(12):2171. doi:10.18632/ONCOTARGET.1426.
35. Guo P, Hu B, Gu W, et al. Platelet-derived growth factor-B enhances glioma angiogenesis by stimulating vascular endothelial growth factor expression in tumor endothelia and by promoting pericyte recruitment. *Am J Pathol*. 2003;162(4):1083–1093. doi:10.1016/S0002-9440(10)63905-3.
36. Cenciarelli C, Marei HE, Felsani A, et al. PDGFRα depletion attenuates glioblastoma stem cells features by modulation of STAT3, RB1 and multiple oncogenic signals. *Oncotarget*. 2016;7(33):53047–53063. doi:10.18632/oncotarget.10132.
37. Muppala S, Xiao R, Krukovets I, et al. Thrombospondin-4 mediates TGF-β-induced angiogenesis. *Oncogene*. 2017;36(36):5189–5198. doi:10.1038/onc.2017.140.
38. Krishnan S, Szabo E, Burghardt I, et al. Modulation of cerebral endothelial cell function by TGF-β in glioblastoma: VEGF-dependent angiogenesis versus endothelial mesenchymal transition. *Oncotarget*. 2015;6(26):22480–22495. doi:10.18632/oncotarget.4310.
39. Brat DJ, Bellail AC, Van Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro Oncol*. 2005;7(2):122–133. doi:10.1215/S1152851704001061.
40. Peshes-Yeloz N, Ungar L, Wohl A, et al. Role of klotho protein in tumor genesis, cancer progression, and prognosis in patients with high-grade glioma. *World Neurosurg*. 2019;130:e324–e332. doi:10.1016/j.wneu.2019.06.082.
41. Melekhin VV, Ponomarev AI, Desyatova MA, et al. Investigation of the role of induced overexpression of the isolated secreted Klotho on the A-172 human glioblastoma cells. *J Mol Neurosci*. 2022;72(2):401–409. doi:10.1007/s12031-021-01960-1.
42. Jiang MJ, Chen YY, Dai JJ, et al. Dying tumor cell-derived exosomal miR-194-5p potentiates survival and repopulation of tumor repopulating cells upon radiotherapy in pancreatic cancer. *Mol Cancer*. 2020;19(1):1–15. doi:10.1186/s12943-020-01178-6.
43. Aloizou AM, Pateraki G, Siokas V, et al. The role of MiRNA-21 in gliomas: hope for a novel therapeutic intervention? *Toxicol Rep*. 2020;7:1514–1530. doi:10.1016/j.toxrep.2020.11.001.
44. Folkman J. Angiostatin and endostatin: angiogenesis inhibitors in blood and stroma. *Angiogenes Integr Approach Sci Med*. 2008:129–146. doi:10.1007/978-0-387-71518-6_12.
45. Sun JK, Uehara H, Karashima T, et al. Expression of interleukin-8 correlates with angiogenesis, tumorigenicity, and metastasis of human prostate cancer cells implanted orthotopically in nude mice. *Neoplasia*. 2001;3(1):33–42. doi:10.1038/sj.neo.7900124.
46. Paduch R. The role of lymphangiogenesis and angiogenesis in tumor metastasis. *Cell Oncol*. 2016;39(5):397–410. doi:10.1007/s13402-016-0281-9.
47. Liu M, Xing L. Basic fibroblast growth factor as a potential biomarker for diagnosing malignant tumor metastasis in women. *Oncol Lett*. 2017;14(2):1561–1567. doi:10.3892/ol.2017.6335.
48. Zetter BR. Angiogenesis and tumor metastasis. *Annu Rev Med*. 1998;49(1):407–424. doi:10.1146/annurev.med.49.1.407.
49. Lu L, Zeng H, Gu X, et al. Circulating tumor cell clusters-associated gene plakoglobin and breast cancer survival. *Breast Cancer Res Treat*. 2015;151(3):491–500. doi:10.1007/s10549-015-3416-1.
50. Aceto N, Bardia A, Miyamoto DT, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell*. 2014;158(5):1110–1122. doi:10.1016/j.cell.2014.07.013.
51. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst*. 1990;82(1):4–7. doi:10.1093/jnci/82.1.4.
52. Ogawa Y, Chung YS, Nakata B, et al. Microvessel quantitation in invasive breast cancer by staining for factor VIII-related antigen. *Br J Cancer*. 1995;71(6):1297–1301. doi:10.1038/bjc.1995.251.
53. Guex N, Crespo I, Bron S, et al. Angiogenic activity of breast cancer patients' monocytes reverted by combined use of systems modeling and experimental approaches. *PLoS Comput Biol*. 2015;11(3):1–28. doi:10.1371/journal.pcbi.1004050.
54. Rak JW, Croix BD, Kerbel RS. Consequences of angiogenesis for tumor progression, metastasis and cancer therapy. *Anticancer Drugs*. 1995;6(1):3–18. doi:10.1097/00001813-199502000-00001.
55. van Zijl F, G Krupitza, Mikulits W. Initial steps of metastasis: cell invasion and endothelial transmigration. *Mutat Res*. 2011;728(1–2):23. doi:10.1016/J.MRREV.2011.05.002.
56. Sanz-Moreno V, Gadea G, Ahn J, et al. Rac activation and inactivation control plasticity of tumor cell movement. *Cell*. 2008;135(3):510–523. doi:10.1016/J.CELL.2008.09.043.
57. Paul CD, Mistriotis P, Konstantopoulos K. Cancer cell motility: lessons from migration in confined spaces. *Nat Rev Cancer*. 2017;17(2):131. doi:10.1038/NRC.2016.123.
58. Mierke CT. Role of the endothelium during tumor cell metastasis: is the endothelium a barrier or a promoter for cell invasion and metastasis? *J Biophys*. 2008:1–13 2008. doi:10.1155/2008/183516.
59. Chiang SPH, Cabrera RM, Segall JE. Tumor cell intravasation. *Am J Physiol - Cell Physiol*. 2016;311(1):C1–C14. doi:10.1152/AJPCELL.00238.2015.
60. Reymond N, D'Água BB, Ridley AJ. Crossing the endothelial barrier during metastasis. *Nat Rev Cancer*. 2013;13(12):858–870. doi:10.1038/nrc3628.
61. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell*. 2011;147(2):275–292. doi:10.1016/j.cell.2011.09.024.
62. Weis SM, Cheresh DA. αV integrins in angiogenesis and cancer. *Cold Spring Harb Perspect Med*. 2011;1(1):a006478. doi:10.1101/cshperspect.a006478.
63. Wrobel JK, Toborek M. Blood–brain barrier remodeling during brain metastasis formation. *Mol Med*. 2016;22:32–40. doi:10.2119/molmed.2015.00207.
64. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci*. 2020;77(9):1745–1770. doi:10.1007/s00018-019-03351-7.
65. Duffy A.M., Bouchier-Hayes D.J., Harmey J.H. Vascular endothelial growth factor (VEGF) and its role in non-endothelial cells: autocrine signalling by VEGF. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2013; 2000-2013. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6482/.
66. Rajendran P, Rengarajan T, Thangavel J, et al. The vascular endothelium and human diseases. *Int J Biol Sci*. 2013;9(10):1057–1069. doi:10.7150/ijbs.7502.
67. Chen Y, Tan W, Wang C. Tumor-associated macrophage-derived cytokines enhance cancer stem-like characteristics through epithelial–mesenchymal transition. *Onco Targets Ther*. 2018;11:3817–3826. doi:10.2147/OTT.S168317.
68. Herman H, Fazakas C, Haskó J, et al. Paracellular and transcellular migration of metastatic cells through the cerebral endothelium. *J Cell Mol Med*. 2019;23(4):2619–2631. doi:10.1111/jcmm.14156.
69. Gligorijevic B, Wyckoff J, Yamaguchi H, et al. N-WASP-mediated invadopodium formation is involved in intravasation and lung metastasis of mammary tumors. *J Cell Sci*. 2012;125(Pt 3):724–734. doi:10.1242/jcs.092726.

70. Li D, Masiero M, Banham AH, et al. The Notch Ligand Jagged1 as a target for anti-tumor therapy. *Front Oncol*. 2014;4:254. doi:10.3389/FONC.2014.00254.
71. Miles FL, Pruitt FL, van Golen KL, et al. Stepping out of the flow: capillary extravasation in cancer metastasis. *Clin Exp Metastasis*. 2008;25(4):305–324. doi:10.1007/s10585-007-9098-2.
72. Wilhelm I, Molnár J, Fazakas C, et al. Role of the blood-brain barrier in the formation of brain metastases. *Int J Mol Sci*. 2013;14(1):1383–1411. doi:10.3390/ijms14011383.
73. Ito S, Nakanishi H, Ikehara Y, et al. Real-time observation of micrometastasis formation in the living mouse liver using a green fluorescent protein gene-tagged rat tongue carcinoma cell line. *Int J Cancer*. 2001;93(2):212–217. doi:10.1002/ijc.1318.
74. Wrobel JK, Toborek M. Blood–brain barrier remodeling during brain metastasis formation. *Mol Med*. 2016;22(1):32–40. doi:10.2119/molmed.2015.00207.
75. Khuon S, Liang L, Dettman RW, et al. Myosin light chain kinase mediates transcellular intravasation of breast cancer cells through the underlying endothelial cells: a three-dimensional FRET study. *J Cell Sci*. 2010;123(Pt 3):431–440. doi:10.1242/jcs.053793.
76. Reymond N, d'Água BB, Ridley AJ. Crossing the endothelial barrier during metastasis. *Nat Rev Cancer*. 2013;13(12):858–870. doi:10.1038/nrc3628.
77. Schuldes H, Bade S, Knobloch J, et al. Loss of in vitro cytotoxicity of cisplatin after storage as stock solution in cell culture medium at various temperatures. *Cancer*. 1997;79(9):1723–1728. doi:10.1002/(SICI)1097-0142(19970501)79:9.
78. Huh SJ, Liang S, Sharma A, et al. Tumor and stem cell biology transiently entrapped circulating tumor cells interact with neutrophils to facilitate lung metastasis development. *Cancer Res*. 2010;70(14):6071–6082. doi:10.1158/0008-5472.CAN-09-4442.
79. Yoshimasu T, Sakurai T, Oura S, et al. Increased expression of integrin $\alpha 3\beta 1$ in highly brain metastatic subclone of a human non-small cell lung cancer cell line. *Cancer Sci*. 2004;95(2):142–148. doi:10.1111/j.1349-7006.2004.tb03195.x.
80. Orian-Rousseau V. CD44, a therapeutic target for metastasising tumours. *Eur J Cancer*. 2010;46(7):1271–1277. doi:10.1016/J.EJCA.2010.02.024.
81. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer*. 2010;10(1):9–22. doi:10.1038/NRC2748.
82. Amato LC, Speranza L, Volpicelli F. Neurotrophic factor BDNF, physiological functions and therapeutic potential in depression, neurodegeneration and brain cancer. *Int J Mol Sci*. 2020;21(20):7777.
83. Garofalo S, Porzia A, Mainiero F, et al. Environmental stimuli shape microglial plasticity in glioma. *Elife*. 2017;6:e33415. doi:10.7554/eLife.33415.
84. Garofalo S, D'Alessandro G, Chece G, et al. Enriched environment reduces glioma growth through immune and non-immune mechanisms in mice. *Nat Commun*. 2015;6:6623. doi:10.1038/ncomms7623.
85. Jalkanen S, Karikoski M, Mercier N, et al. The oxidase activity of vascular adhesion protein-1 (VAP-1) induces endothelial E- and P-selectins and leukocyte binding. *Blood*. 2007;110(6):1864–1870. doi:10.1182/blood-2007-01-069674.
86. Kappelmayer J, Nagy Jr B. The interaction of selectins and PSGL-1 as a key component in thrombus formation and cancer progression. *Biomed Res Int*. 2017:6138145 2017. doi:10.1155/2017/6138145.
87. McEver RP, Zhu C. Rolling cell adhesion. *Annu Rev Cell Dev Biol*. 2010;26:363–396. doi:10.1146/ANNUREV.CELLBIO.042308.113238.
88. Takeichi M, Hirano S, Matsuyoshi N, et al. Cytoplasmic control of cadherin-mediated cell-cell adhesion. *Cold Spring Harb Symp Quant Biol*. 1992;57:327–334. doi:10.1101/SQB.1992.057.01.037.
89. J M. The transforming growth factor-beta family. *Annu Rev Cell Biol*. 1990;6:597–641. doi:10.1146/ANNUREV.CB.06.110190.003121.
90. Wesolowska A, Kwiatkowska A, Slomnicki L, et al. Microglia-derived TGF-β as an important regulator of glioblastoma invasion - an inhibition of TGF-β-dependent effects by shRNA against human TGF-β type II receptor. *Oncogene*. 2008;27(7):918–930. doi:10.1038/SJ.ONC.1210683.
91. Lange D, Persson U, Wollina U, et al. Expression of TGF-β related Smad proteins in human epithelial skin tumors. *Int J Oncol*. 1999;14(6):1049–1056. doi:10.3892/IJO.14.6.1049.
92. Bruna A, Darken RS, Rojo F, et al. High TGFbeta-Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. *Cancer Cell*. 2007;11(2):147–160. doi:10.1016/j.ccr.2006.11.023.
93. Miraux S, Lemière S, Pineau R, et al. Inhibition of FGF receptor activity in glioma implanted into the mouse brain using the tetracyclin-regulated expression system. *Angiogenesis*. 2004;7(2):105–113. doi:10.1007/S10456-004-1037-0.
94. Mignatti P, Mazzieri R, Rifkin DB. Expression of the urokinase receptor in vascular endothelial cells is stimulated by basic fibroblast growth factor. *J Cell Biol*. 1991;113(5):1193–1201. doi:10.1083/JCB.113.5.1193.
95. Montesano R, Vassalli JD, Baird A, et al. Basic fibroblast growth factor induces angiogenesis in vitro. *Proc Natl Acad Sci USA*. 1986;83(19):7297–7301. doi:10.1073/PNAS.83.19.7297.
96. Plate KH, Breier G, Weich HA, et al. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. *Nature*. 1992;359(6398):845–848. doi:10.1038/359845A0.
97. de Vries C, Escobedo JA, Ueno H, et al. The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. *Science*. 1992;255(5047):989–991. doi:10.1126/SCIENCE.1312256.
98. Terman BI, Dougher-Vermazen M, Carrion ME, et al. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. *Biochem Biophys Res Commun*. 1992;187(3):1579–1586. doi:10.1016/0006-291X(92)90483-2.
99. Tate MC, Aghi MK. Biology of angiogenesis and invasion in glioma. *Neurotherapeutics*. 2009;6(3):447–457. doi:10.1016/J.NURT.2009.04.001.
100. Lee SK, Huang S, Lu W, et al. Vascular endothelial growth factor expression promotes the growth of breast cancer brain metastases in nude mice. *Clin Exp Metastasis*. 2004;21(2):107–118. doi:10.1023/B:CLIN.0000024761.00373.55.
101. Criscuoli ML, Nguyen M, Eliceiri BP. Tumor metastasis but not tumor growth is dependent on Src-mediated vascular permeability. *Blood*. 2005;105(4):1508–1514. doi:10.1182/blood-2004-06-2246.
102. Shih AH, Holland EC. Platelet-derived growth factor (PDGF) and glial tumorigenesis. *Cancer Lett*. 2006;232(2):139–147. doi:10.1016/j.canlet.2005.02.002.
103. Guo P, Hu B, Gu W, et al. Platelet-derived growth factor-B enhances glioma angiogenesis by stimulating vascular endothelial growth factor expression in tumor endothelia and by promoting pericyte recruitment. *Am J Pathol*. 2003;162(4):1083–1093. doi:10.1016/S0002-9440(10)63905-3.
104. Reis M, Czupalla CJ, Ziegler N, et al. Endothelial Wnt/β-catenin signaling inhibits glioma angiogenesis and normalizes tumor blood vessels by inducing PDGF-B expression. *J Exp Med*. 2012;209(9):1611–1627. doi:10.1084/JEM.20111580.
105. Zhang L, Kundu S, Feenstra T, et al. Pleiotrophin promotes vascular abnormalization in gliomas and correlates with poor survival in patients with astrocytomas. *Sci Signal*. 2015;8(406):ra125. doi:10.1126/scisignal.aaa1690.
106. González-Castillo C, Ortuño-Sahagún D, Guzmán-Brambila C, et al. Pleiotrophin as a central nervous system neuromodulator, evidences from the hippocampus. *Front Cell Neurosci*. 2015;8:443. doi:10.3389/fncel.2014.00443.
107. Banerjee K, Núñez FJ, Haase S, et al. Current approaches for glioma gene therapy and virotherapy. *Front Mol Neurosci*. 2021;14:621831. doi:10.3389/fnmol.2021.621831.
108. Dong Z, Nör JE. Transcriptional targeting of tumor endothelial cells for gene therapy. *Adv Drug Deliv Rev*. 2009;61(7–8):542–553. doi:10.1016/j.addr.2009.02.006.
109. Demeule M, Régina A, Annabi B, et al. Brain endothelial cells as pharmacological targets in brain tumors. *Mol Neurobiol*. 2004;30(2):157–183. doi:10.1385/MN:30:2:157.
110. Charalambous C, Chen TC, Hofman FM. Characteristics of tumor-associated endothelial cells derived from glioblastoma multiforme. *Neurosurg Focus*. 2006;20(4):E22. doi:10.3171/foc.2006.20.4.e22.
111. Moore XL, Lu J, Sun L, et al. Endothelial progenitor cells' "homing" specificity to brain tumors. *Gene Ther*. 2004;11(10):811–818. doi:10.1038/sj.gt.3302151.
112. Zarebkohan A, Najafi F, Moghimi HR, et al. SRL-coated PAMAM dendrimer nano–carrier for targeted gene delivery to the glioma cells and competitive inhibition by lactoferrin. *Iran J Pharm Res*. 2016;15(4):629–640.
113. Green JJ, Shi J, Chiu E, et al. Biodegradable polymeric vectors for gene delivery to human endothelial cells. *Bioconjug Chem*. 2006;17(5):1162–1169. doi:10.1021/bc0600968.
114. Suh W, Han SO, Yu L, et al. An angiogenic, endothelial-cell-targeted polymeric gene carrier. *Mol Ther*. 2002;6(5):664–672. doi:10.1006/mthe.2002.0721.
115. Marchiò S, Sidman RL, Arap W, et al. Brain endothelial cell-targeted gene therapy of neurovascular disorders. *EMBO Mol Med*. 2016;8(6):592–594. doi:10.15252/emmm.201606407.
116. Merkel SF, Andrews AM, Lutton EM, et al. Trafficking of adeno-associated virus vectors across a model of the blood-brain barrier; a comparative study of transcytosis and transduction using primary human brain endothelial cells. *J Neurochem*. 2017;140(2):216–230. doi:10.1111/jnc.13861.
117. Khalilov R. A comprehensive review of advanced nano-biomaterials in regenerative medicine and drug delivery. *Adv Biol Earth Sci*. 2023;8(1):5–18.
118. Eftekhari A, Kryschi C, Pamies D, et al. Natural and synthetic nanovectors for cancer therapy. *Nanotheranostics*. 2023;7(3):236–257. doi:10.7150/ntno.77564.
119. Carlson JC, Cantu Gutierrez M, Lozzi B, et al. Identification of diverse tumor endothelial cell populations in malignant glioma. *Neuro Oncol*. 2021;23(6):932–944. doi:10.1093/neuonc/noaa297.
120. Li D, Tian Y, Hu Y, et al. Glioma-associated human endothelial cell-derived extracellular vesicles specifically promote the tumourigenicity of glioma stem cells via CD9. *Oncogene*. 2019;38(43):6898–6912. doi:10.1038/s41388-019-0903-6.
121. Nguyen Cao TG, Kang JH, Kang SJ, et al. Brain endothelial cell-derived extracellular vesicles with a mitochondria-targeting photosensitizer effectively treat glioblastoma by hijacking the blood–brain barrier. *Acta Pharm Sin B*. 2023. doi:10.1016/j.apsb.2023.03.023.
122. Kudo S, Konda R, Obara W, et al. Inhibition of tumor growth through suppression of angiogenesis by brain-specific angiogenesis inhibitor 1 gene transfer in murine renal cell carcinoma. *Oncol Rep*. 2007;18(4):785–791.
123. Chen Q, Lu L, Ma W. Efficacy, safety, and challenges of CAR T-cells in the treatment of solid tumors. *Cancers*. 2022;14(23):1–30. doi:10.3390/cancers14235983.
124. Transl J, Land CA, Musich PR, et al. Chimeric antigen receptor T-cell therapy in glioblastoma : charging the T cells to fight. *J Transl Med*. 2020;18(1):428. doi:10.1186/s12967-020-02598-0.
125. Li SJ, Chen JX, Sun ZJ. Improving antitumor immunity using antiangiogenic agents: mechanistic insights, current progress, and clinical challenges. *Cancer Commun*. 2021;41(9):830–850. doi:10.1002/cac2.12183.
126. Nagl L, Horvath L, Pircher A, et al. Tumor endothelial cells (TECs) as potential immune directors of the tumor microenvironment-new findings and future perspectives. *Front Cell Dev Biol*. 2020;8:1–18. doi:10.3389/fcell.2020.00766.
127. Habashy KJ, Mansour R, Moussalem C, et al. Challenges in glioblastoma immunotherapy: mechanisms of resistance and therapeutic approaches to overcome them. *Br J Cancer*. 2022;127(6):976–987. doi:10.1038/s41416-022-01864-w.
128. Polivka J, Holubec L, Kubikova T, et al. Advances in experimental targeted therapy and immunotherapy for patients with glioblastoma multiforme. *Anticancer Res*. 2017;37(1):21–33. doi:10.21873/anticanres.11285.
129. Ratnam NM, Gilbert MR, Giles AJ. Immunotherapy in CNS cancers: the role of immune cell trafficking. *Neuro Oncol*. 2019;21(1):37–46. doi:10.1093/neuonc/noy084.

130. Zhang T, Jia Y, Yu Y, et al. Targeting the tumor biophysical microenvironment to reduce resistance to immunotherapy. *Adv Drug Deliv Rev*. 2022;186:114319. doi:10.1016/j.addr.2022.114319.
131. Lamplugh Z, Fan Y. Vascular microenvironment, tumor immunity and immunotherapy. *Front Immunol*. 2021;12:1–12. doi:10.3389/fimmu.2021.811485.
132. Ma W, Wang Y, Zhang R, et al. Targeting PAK4 to reprogram the vascular microenvironment and improve CAR-T immunotherapy for glioblastoma. *Nat Cancer*. 2021;2(1):83–97. doi:10.1038/s43018-020-00147-8.
133. Yang F, He Z, Duan H, et al. Synergistic immunotherapy of glioblastoma by dual targeting of IL-6 and CD40. *Nat Commun*. 2021;12(1):1–15. doi:10.1038/s41467-021-23832-3.
134. Hua Y, Vella G, Rambow F, et al. Cancer immunotherapies transition endothelial cells into HEVs that generate TCF1+ T lymphocyte niches through a feed-forward loop. *Cancer Cell*. 2022;40(12) 1600-1618.e10. doi:10.1016/j.ccell.2022.11.002.
135. Duru G, van Egmond M, Heemskerk N. A window of opportunity: targeting cancer endothelium to enhance immunotherapy. *Front Immunol*. 2020;11:1–23. doi:10.3389/fimmu.2020.584723.